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Detection of Pyrethroid Resistance Mutations in *Aedes aegypti* and *Aedes albopictus* from Ho Chi Minh City, Vietnam

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Abstract

Vietnam is one of the Southeast Asian countries most heavily affected by dengue, where *Aedes aegypti (L.)* and *Aedes albopictus (Skuse)* serve as the primary vectors. The increasing prevalence of pyrethroid resistance in these two mosquito species, particularly in Ho Chi Minh City, poses a significant challenge to vector control efforts. This study aimed to evaluate resistance to 0.75% permethrin and to investigate knockdown resistance (kdr) mutations in IIS6 and IIIS6 domains of the voltage-gated sodium channel (VGSC) gene among resistant *Ae. aegypti* and *Ae. albopictus* individuals that survived bioassay exposure. The results showed that moderate levels of permethrin resistance were detected in both mosquito populations, *Ae. aegypti* and *Ae. albopictus*, with mortality rates ranging from 80.43% to 86.96% in *Ae. aegypti* and from 84.45% to 88.89% in *Ae. albopictus*. The Ser989Pro, Ile1011Met, and Val1016Gly mutations were not detected. In contrast, the Phe1534Cys mutation was observed at high frequencies, occurring in 80% (20/25) of *Ae. aegypti* and 92% (23/25) of *Ae. albopictus* specimens analyzed. The 1534C allele frequency was 0.62 in *Ae. aegypti* and 0.68 in *Ae. albopictus*, indicating comparable levels of the mutant allele in both species. Allele frequencies were calculated within the resistant group, and no statistically significant differences were detected between the two species. This study focused on resistant phenotypes without assessing haplotype structure or comprehensive genotype-phenotype associations. Nevertheless, its findings provide updated data on VGSC mutations linked to permethrin resistance in Ho Chi Minh City, thereby contributing to ongoing insecticide resistance surveillance and management within vector control programs.

Keywords

Aedes aegypti; Aedes albopictus; knockdown resistance (kdr); permethrin; pyrethroids; voltage-gated sodium channel (VGSC).

Introduction

Ae. aegypti and *Ae. albopictus* are the primary vectors for dengue virus transmission. *Ae. aegypti* plays a dominant role in urban areas, whereas *Ae. albopictus* is more common in suburban regions. Both species are highly competent vectors and play a critical role in sustaining year-round dengue transmission, particularly in Southeast Asia (Bhatt *et al.*, 2013; Kawada *et al.*, 2009a; Yanola *et al.*, 2011a). Dengue hemorrhagic fever is currently one of the most serious mosquito-borne infectious diseases worldwide, posing a severe public health, economic, and social burden. It is estimated that approximately 390 million dengue infections occur annually, of which about 96 million show clinical symptoms, and 3.9–5.6 billion people in more than 128 countries are at risk of infection (Bhatt *et al.*, 2013). The disease is primarily endemic in tropical and subtropical regions and is showing rapid geographic expansion into new areas, including Europe and the Eastern Mediterranean. In Vietnam, dengue fever remains a major public health issue, with an estimated average of approximately 100,000 cases reported annually. Ho Chi Minh City represents an epidemiological hotspot, regularly recording tens of thousands of cases (Huynh & Minakawa, 2022; Thi *et al.*, 2016).

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Despite the availability of a dengue vaccine, chemical vector control remains one of the most effective measures. Pyrethroids account for approximately 60–70% of total insecticide use, with permethrin being one of the most widely used active ingredients due to its high efficacy and low toxicity (Amelia-Yap *et al.*, 2018). However, prolonged use has exerted strong selection pressure, leading to the rapid spread of pyrethroid resistance in Aedes mosquitoes, thereby significantly reducing the effectiveness of dengue control programs. This phenomenon has been reported in more than 80 countries and represents a major challenge to current vector control strategies (Amelia-Yap *et al.*, 2018).

One of the most important pyrethroid resistance mechanisms is knockdown resistance (kdr) mutations in the voltage-gated sodium channel (VGSC) gene. These mutations alter the target protein's structure, thereby reducing pyrethroid binding affinity and leading to decreased neuroparalytic efficacy in mosquitoes (Kasai *et al.*, 2011, 2019; Plernsub *et al.*, 2016; Saavedra-Rodriguez *et al.*, 2007; Zhao *et al.*, 2023). Common kdr mutations reported in Aedes mosquitoes include Val1016Gly (V1016G), Ser989Pro (S989P), and Phe1534Cys (F1534C) (Brengues *et al.*, 2003). In addition to these, the L982W mutation has also been reported in Aedes aegypti populations in Vietnam (Kawada *et al.*, 2023), further highlighting the diversity of resistance-associated mutations in this species. The frequencies may exceed 70–90% in highly resistant populations across Asia, Latin America, and Africa (Amelia-Yap *et al.*, 2018). Among these, the F1534C mutation located in domain IIIS6 of the VGSC gene is one of the most prevalent in Asia and is strongly associated with resistance to permethrin and deltamethrin (Kawada *et al.*, 2009a; Plernsub *et al.*, 2016; Thi *et al.*, 2016).

In *Ae. aegypti*, common kdr mutations such as V1016G and F1534C occur either individually or in combination. The combined V1016G + F1534C genotype has been shown to increase survival following permethrin exposure by more than 500-fold compared to susceptible individuals. In *Ae. albopictus*, F1534C is the most frequently reported mutation; however, new mutations such as I1532T and V1016G have recently emerged in several Asian countries, raising concerns about potential cross-resistance (Kasai *et al.*, 2011; (Kasai *et al.*, 2019). Southeast Asia is a key dengue epidemiological region and a hotspot for pyrethroid resistance in Aedes mosquitoes. Studies conducted in Thailand, Cambodia, and Malaysia have recorded kdr mutation frequencies exceeding 80% in *Ae. aegypti*, accompanied by reduced residual spraying efficacy (Vontas *et al.*, 2012). In Vietnam, the presence of V1016G and F1534C in *Ae. aegypti* has been reported in Hanoi, Da Nang, and Khanh Hoa (Kawada *et al.*, 2023). However, data on the level of permethrin resistance combined with molecular analysis of kdr mutations in both *Ae. aegypti* and *Ae. albopictus* in Ho Chi Minh City remain limited. This data gap fails to fully reflect the current situation in one of the country's highest epidemiological risk areas (Kawada *et al.*, 2009a; Thi *et al.*, 2016).

Therefore, this study aimed to: (1) assess permethrin susceptibility in *Ae. aegypti* and *Ae. albopictus* populations in Ho Chi Minh City; (2) investigate knockdown resistance (kdr) mutations in the VGSC gene to provide experimental scientific evidence supporting optimal insecticide use and sustainable dengue vector control strategies.

Materials and Methods

Materials

Aedes mosquitoes were collected from five military facilities and surrounding areas in Ho Chi Minh City between March and August 2025, including the Eastern People Military Hospital (designated as MuMD), 7A Military Hospital (Mu7A), 2nd Infantry Battalion/Gia Dinh Regiment (MuCC), Military Command of Binh Thanh District (MuBT), Military Command of Can Gio District (MuCG). Study samples included: (1) adult mosquitoes directly collected in the field; and (2) larvae collected in the field and subsequently transported to the laboratory for rearing to obtain F1-generation adult mosquitoes.

Methods

Collection of Adult Mosquitoes and Larvae at The Study Sites

Collection of Adult Mosquitoes

Adult mosquitoes resting on clothing, bed nets, curtains, and indoor surfaces in military barracks, administrative offices, kitchens, storage areas, livestock shelters, and gardens of military facilities, as well as inside houses and on surrounding vegetation in nearby residential areas, were collected using sweep nets. Captured mosquitoes were transferred into sterile 50-mL Falcon tubes using a hand-held aspirator. After collecting 5–10 individuals, mosquitoes were placed into rearing cages and supplied with food consisting of cotton pads soaked in 10% glucose solution. Collected mosquitoes were used for species identification and insecticide susceptibility assays.

Collection of Larvae

Larvae were collected from water-holding containers in and around households using dippers, ladles, pipettes, or fine-mesh nets, depending on container size. Samples were transferred into prepared bottles or containers and transported to the laboratory. In the laboratory, larvae were reared in trays filled with clean water and fed either commercial fish food or a mixture of dried yeast and fish food at a ratio of 3:1. Rearing conditions were maintained at 26 ± 2°C and 70 ± 10% relative humidity. Emerged adult mosquitoes were morphologically identified and transferred to new cages, where they were fed daily with 10% glucose solution on cotton. Healthy F1 female mosquitoes aged 2–5 days were selected for insecticide resistance bioassays.

Identification of Aedes Mosquito Species

Morphological Identification:

Ae. aegypti and *Ae. albopictus* were identified based on external morphological characteristics according to the guidelines of the World Health Organization (WHO) and the National Institute of Malariology, Parasitology and Entomology (Vietnam).

Molecular Identification:

Molecular identification was conducted to confirm species identity using ten pooled samples, each pool consisting of ten adult mosquitoes morphologically identified as *Ae. aegypti*, and ten pooled samples of *Ae. albopictus*. Species identification was performed by PCR targeting the internal transcribed spacer 2 (ITS2) region. The assay employed a universal forward primer (Aedes-F) in combination with species-specific reverse primers: ALB-R (Bang *et al.*, 2021) for *Ae. albopictus* and AEG-R (Menegon *et al.*, 2025) for *Ae. aegypti*. This primer set amplifies fragments of 438 bp and 252 bp for *Ae. albopictus* and *Ae. aegypti*, respectively.

Aedes Mosquito Resistance Test to permethrin (Type I Pyrethroid)

Female *Ae. aegypti* and *Ae. albopictus* were tested for susceptibility to 0.75% permethrin following WHO guidelines (World Health Organization, 2016). A total of 1,000 mosquitoes (500 per species) were collected from five study sites, with 100 individuals per species sampled at each site.

At certain sites, the number of field-collected adult mosquitoes was insufficient to meet the recommended sample size. Therefore, F1 females (2–5 days old) reared from larvae collected at the same locations were included to ensure an adequate sample size and consistency of the experimental design. At each site, 100 individuals per species were tested, comprising 60 F1 females (age-standardized, non-blood-fed, maintained under laboratory conditions at 26 ± 20°C and 70 ± 10% relative humidity) and 40 field-collected adult females. Both groups originated from the same local natural populations. Bioassays were conducted in five replicates, each consisting of 20 mosquitoes exposed to 0.75% permethrin-impregnated papers and 20 control mosquitoes exposed to untreated papers. After 1 hour of exposure, mosquitoes were transferred to holding tubes and provided with 10% glucose solution. Mortality was recorded after 24 hours. Results were considered valid when control mortality was ≤ 5%; mortality rates between 5% and 20% were corrected using Abbott's formula; and tests were discarded and repeated if control mortality exceeded 20%.

Mosquitoes that survived the bioassay were preserved for subsequent analysis of knockdown resistance (kdr) mutations in the VGSC gene.

Sample Selection and VGSC Gene Sequencing Design

Mosquitoes that survived exposure to 0.75% permethrin were selected for analysis of kdr mutations in the VGSC gene. Resistant specimens collected from Ho Chi Minh City were pooled by species to determine the overall prevalence of kdr mutations in *Ae. aegypti* and *Ae. albopictus*. A total of 50 resistant individuals per species were sequenced, with 25 analyzed in domain IIS6 and 25 in domain IIIS6. As these domains were sequenced from different individuals, the co-occurrence of multiple kdr mutations within the same mosquito was not evaluated.

Genomic DNA was individually extracted from 50 *Ae. aegypti* and 50 *Ae. albopictus* specimens using the TopPURE® Genomic DNA Extraction Kit (ABT, Vietnam), following the manufacturer's instructions. The domains IIS6 and IIIS6 were amplified by PCR using primer sequences based on H. Kawada *et al.* (2009) (Thi *et al.*, 2016). For the domain IIS6, the primer pairs AaSCF1 (5'-AGACAATGTGGATCGCTTCC-3') and AaSCR4 (5'-GGACGCAATCTGGCTTGTTA-3') were used, yielding an amplicon of approximately 750-800 bp. The domain IIIS6 was amplified using AaSCF7 (5'-GAGAACTCGCCGATGAACTT-3') and AaSCR7 (5'-GACGACGAAATCGAACAGGT-3'), producing an amplicon of approximately 650-700 bp. PCR amplification was performed with an initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 30 s, and extension at 72°C for 5 min, with a final hold at 4°C indefinitely. PCR products were separated by electrophoresis on 2% agarose gels at 110 V for 40–60 min. Amplified amplicons were purified using the TopPURE® PCR/Gel DNA Purification Kit (ABT, Vietnam), according to the manufacturer's protocol.

Nucleotide sequences were determined by Sanger sequencing using primers AaSCF3 (5′-GTGGAACTTCACCGACTTCA-3′) for analysis of kdr mutations in domain IIS6, and primer AaSCR8 (5′-TAGCTTTCAGCGGCTTCTTC-3′) for the domain IIIS6. Sequencing reactions were performed on an ABI 3130/3130 XL Genetic Analyzer (Thermo Fisher Scientific). Sequence chromatograms were generated in .ab1 format. Raw sequences were edited and preliminarily aligned using BioEdit v7.2.6.1. The processed sequences were compared with reference sequences available in GenBank (accession number: NC_035109). Nucleotide and corresponding amino acid substitutions associated with knockdown resistance in *Ae. aegypti* and *Ae. albopictus* were identified using MEGA X software.

Due to financial and logistical constraints, sequencing was performed only on phenotypically resistant individuals to maximize the probability of detecting resistance-associated mutations. Therefore, the study design does not allow for the estimation of allele frequencies at the whole-population level or genotype–phenotype association analysis.

Nucleotide Sequence Accession Numbers: The nucleotide sequences obtained in this study have been deposited in the GenBank database under the accession numbers PZ225565-PZ225570.

Ethics Statements: Mosquito collection was conducted with permission from local authorities. The study involved insects only and did not require institutional ethical approval.

Results

Collection and Identification of Ae. aegypti and Ae. albopictus Mosquitoes

Results of mosquito collection at study sites

- Adult mosquito surveillance conducted at five sites in Ho Chi Minh City yielded 2,854 specimens. The collections were co-dominated by the genera *Aedes* (47.7%) and *Culex* (45.7%), which together accounted for 93.4% of the total specimens collected.

Table 1. Number of mosquitoes collected at five locations in Ho Chi Minh City

No	Locations	*Aedes* sp.	*Culex* sp.	*Anopheles* sp.	Other species	Total
1	MuMD	404	191	0	0	595
2	MuBT	281	226	0	0	507
3	Mu7A	232	383	0	46	661
4	MuCC	362	167	0	0	529
5	MuCG	83	336	137	6	562
Total		**1,362 (47.72 %)**	**1,303 (45.66%)**	**137 (4.8%)**	**52 (1.82%)**	**2,854**

Aedes species were most prevalent at sites MuMD and MuCC, whereas *Culex* species predominated at Mu7A and MuCG. Notably, *Anopheles* mosquitoes were collected exclusively at site MuCG, accounting for 4.8% of the total catch (Table 1). The remaining 1.8% comprised other mosquito species detected only at sites Mu7A and MuCG.

Table 2. Number of adult Aedes mosquitoes emerged from larvae

No	Sampling site	Larvae	Adult Aedes emerged from larvae	Rate (%)
1	MuMD	980	541	23.21
2	MuBT	730	442	18.96
3	Mu7A	870	464	19.91
4	MuCC	930	509	21.84
5	MuCG	840	375	16.08
	Total	**4,350**	**2,331**	**100**

In addition, a total of 2,331 adult Aedes mosquitoes were reared to adulthood under laboratory conditions from more than 4,350 field-collected larvae. Of these emerged adults, 541 individuals (23.21%) originated from site MuMD, 442 (18.96%) from MuBT, 464 (19.91%) from Mu7A, 509 (21.84%) from MuCC, and 375 (16.08%) from MuCG (Table 2).

Identification of Ae. aegypti and Ae. albopictus Mosquitoes Based on External Morphological Characteristics.

Based on morphological identification (Figure 1), a total of 3,713 adult Aedes mosquitoes were collected from the five sites, comprising 1,757 (47.3%) *Ae. aegypti* and 1,956 (52.7%) *Ae. albopictus*. The relative abundance of each species varied among sites (Table 3). *Ae. aegypti* was most abundant at site MuMD (n = 407, 23.2% of the total *Ae. aegypti* collection), followed by MuBT (n = 396, 22.5%) and Mu7A (n = 373, 21.2%). In contrast, *Ae. albopictus* was most prevalent at site MuMD (n = 556, 28.4% of total *Ae. albopictus* collection) and MuCC (n = 519, 26.5%). Overall, site MuMD contributed the largest proportion of the total Aedes collection (n = 963, 25.9%).

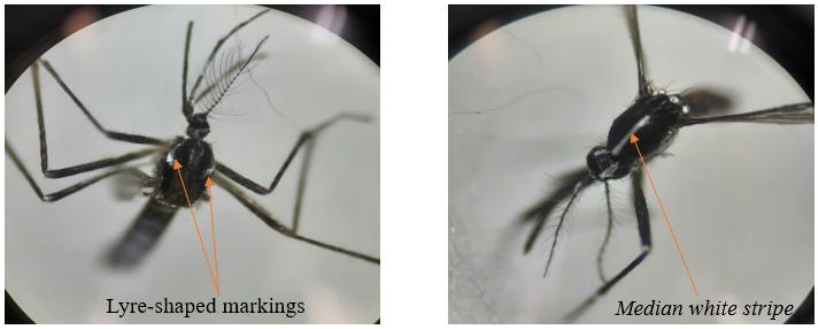


Figure 1. Characteristic features of *Aedes* mosquitoes: a) *Ae. aegypti* (lyre-shaped markings on the thorax) and b) *Ae. albopictus* (median white stripe along the thorax). Arrows indicate key morphological differences. (Photo by Nguyen Van Hiep).

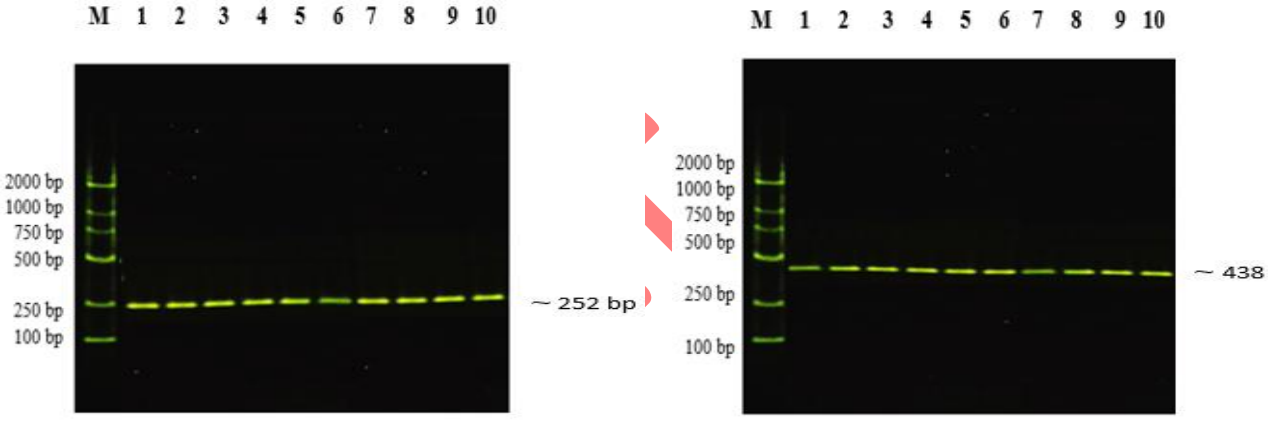
Table 3. Numbers of *Ae. aegypti* and *Ae. albopictus* collected at the study sites

No	Sampling site	*Ae. aegypti* Field + F1 (n, %)	*Ae. albopictus* Field + F1 (n, %)	Total (n, %)
1	MuMD	407 (23.16%)	556 (28.43%)	963 (25.94%)
2	MuBT	396 (22.54%)	327 (16.72%)	723 (19.47%)
3	Mu7A	373 (21.23%)	323 (16.51%)	696 (18.74%)
4	MuCC	352 (20.03%)	519 (26.53%)	871 (23.46%)
5	MuCG	229 (13.04%)	231 (11.81%)	460 (12.39%)
Total		**1,757 (47.32%)**	**1,956 (52.68%)**	**3,713**

Identification of Ae. aegypti and Ae. albopictus Mosquitoes Using Molecular Biological Methods

Electrophoresis of PCR products on a 2% agarose gel showed clear and specific bands of the expected sizes in all samples: 252 bp for the 10 *Ae. aegypti* specimens (Figure 2a) and 438 bp for the 10 *Ae. albopictus* specimens (Figure 2b), as determined by comparison with a DNA ladder.

These molecular results were consistent with morphological identification, confirming the reliability of external morphological characteristics for distinguishing *Ae. aegypti* from *Ae. albopictus*.



a b

Figure 2. Agarose gel electrophoresis (2%) of PCR products: **a)** M: 2000 bp DNA marker; lanes 1–10: ITS2 amplicons of *Ae. aegypti* (expected size: 252 bp); **b)** M: 2000 bp DNA marker; lanes 1–10: ITS2 amplicons of *Ae. albopictus* (expected size: 438 bp).

Permethrin susceptibility bioassay results

According to WHO (2016) criteria, mosquito populations are classified as susceptible when 24-hour mortality is 98–100%, suspected resistant when mortality ranges from 90–97%, and resistant when mortality is below 90%. Classification was based on corrected 24-hour mortality rates using Abbott's formula when necessary.

Susceptibility of Ae. aegypti to 0.75% Permethrin

The 0.75% permethrin bioassay showed that all *Ae. aegypti* populations from the five study sites were resistant. Corrected mortality rates ranged from 80.43% to 86.96%, remaining below the 90% threshold for resistance classification (Table 4). Mortality differences between each field population and the control group were statistically significant (Fisher's exact test, $p < 0.0001$). Mortality values were relatively similar across sites, with mortality rates varying by up to 6.5% between sites.

Table 4. Susceptibility of *Ae. aegypti* to 0.75% permethrin at five study sites

Parameter	Control	MuMD	MuBT	MuCC	Mu7A	MuCG
Number tested (n)	100	100	100	100	100	100
Corrected mortality (%)	8.00	80.43	86.96	85.87	81.52	85.87
p-value (Fisher's exact test)	–	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Resistance status	–	Resistant	Resistant	Resistant	Resistant	Resistant

Note: MuMD, MuBT, MuCC, Mu7A, and MuCG represent the study sites. Resistance status was determined according to WHO criteria. Mortality rates were corrected using Abbott's formula when control mortality was between 5% and 20%.

Susceptibility of Ae. albopictus to 0.75% Permethrin

Similarly, all *Ae. albopictus* populations were classified as resistant. Corrected mortality rates ranged from 84.45% to 88.89% (Table 5). Mortality differences between field populations and the control group were statistically significant (Fisher's exact test, $p < 0.0001$). With mortality rates varying by up to 4.4% between sites.

Table 5. Susceptibility of *Ae. albopictus* to 0.75% permethrin at five study sites

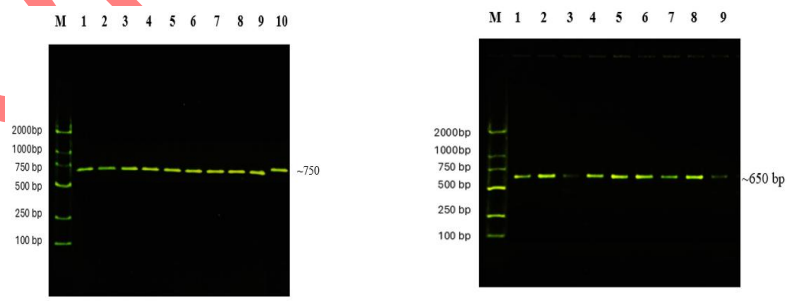
Parameter	Control	MuMĐ	MuBT	MuCC	Mu7A	MuCG
Number tested (n)	100	100	100	100	100	100
Corrected mortality (%)	10.0	85.55	88.89	87.78	84.45	87.78
p-value (Fisher's exact test)	–	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Resistance status	–	Resistant	Resistant	Resistant	Resistant	Resistant

Note: MuMD, MuBT, MuCC, Mu7A, and MuCG represent the study sites. Resistance status was determined according to WHO criteria. Mortality rates were corrected using Abbott's formula when control mortality was between 5% and 20%.

Both *Ae. aegypti* and *Ae. albopictus* exhibited resistance to permethrin at all study sites. Mortality rates were slightly higher in *Ae. albopictus* than in Ae. aegypti at most locations; however, all values remained below the WHO susceptibility threshold.

Investigation of Mutations in the VGSC Gene Encoding the Voltage-Gated Sodium Channel in Ae. aegypti and Ae. albopictus Collected in Ho Chi Minh City

Genomic DNA extracted from individual mosquitoes was used to amplify two target regions of the voltage-gated sodium channel (VGSC) gene: domain IIS6 and domain IIIS6. The expected amplicon sizes were approximately 750 bp (IIS6) and 650 bp (IIIS6). PCR products were resolved on a 2% agarose gel (Figure 3).



a b

Figure 3. Agarose gel electrophoresis (2%) of VGSC amplicons: a) M: 2000 bp DNA marker; lanes 1–5 *(Ae. aegypti)*, 6–10 *(Ae. albopictus)*: IIS6 amplicons generated using primer pair AaSCF1/AaSCR4; b) M: 2000 bp DNA marker; lanes 1–5 *(Ae. aegypti)*, 6–9 *(Ae. albopictus):* IIIS6 amplicons generated using primer pair AaSCF7/AaSCR7.

All samples yielded clear single bands at the expected sizes, indicating specific amplification. The amplicon yield and quality were adequate for subsequent direct Sanger sequencing.

Detection of Kdr Mutations in the IIS6 and IIIS6 Fragments

All mutation analyses were performed exclusively on phenotypically permethrin-resistant individuals. For each species, 25 resistant specimens were sequenced in domain IIS6 and an independent set of 25 resistant specimens was sequenced in domain IIIS6.

Sanger sequencing identified wild-type, homozygous mutant, and heterozygous genotypes of the kdr F1534C mutation in domain IIIS6 of the VGSC gene in *Aedes* mosquitoes.

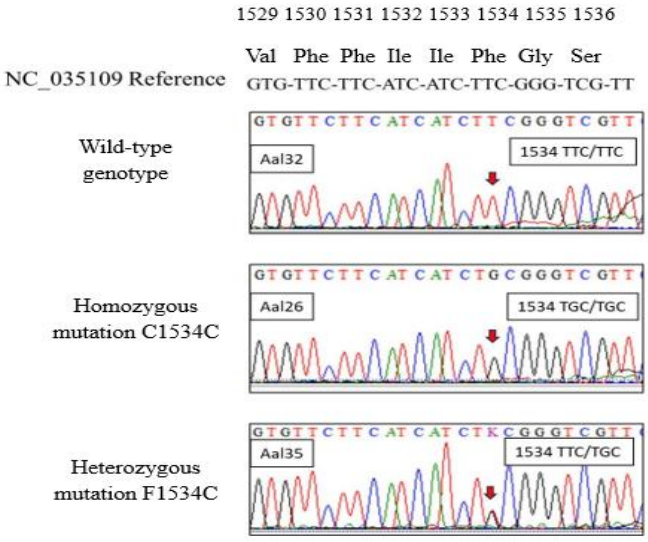


Figure 4. Sequencing chromatograms showing the genotypes at nucleotide position 1534 of the VGSC gene: wild-type (TTC/TTC), homozygous mutant (TGC/TGC), and heterozygous mutant (TTC/TGC) (from top to bottom).

Table 6. Distribution of kdr mutations in domains IIS6 and IIIS6 among permethrin-resistant *Aedes* mosquitoes

Species	IIS6 Mutation n (%)	IIS6 Wild type n (%)	Total	IIIS6 Mutation n (%)	IIIS6 Wild type n (%)	Total
Ae. aegypti (n = 25)	0 (0.0)	25 (100.0)	**25**	20 (80.0)	5 (20.0)	**25**
Ae. albopictus (n = 25)	0 (0.0)	25 (100.0)	**25**	23 (92.0)	2 (8.0)	**25**

No known kdr-associated mutations were detected in domain IIS6 in either *Ae. aegypti* or *Ae. albopictus*, with all specimens exhibiting the wild-type sequence.

In contrast, mutations in domain IIIS6 were detected at high frequencies in both species. The mutation was present in 80.0% (20/25) of *Ae. aegypti* and 92.0% (23/25) of *Ae. albopictus* specimens.

Genotype and Allele Frequencies of the F1534C Mutation

Genotype and allele frequencies of the F1534C mutation in domain IIIS6 are presented in Table 7.

In *Ae. aegypti*, the homozygous mutant (CC) genotype was most frequent (44.0%), followed by heterozygous (FC) (36.0%) and wild-type (FF) (20.0%). The C allele frequency was 0.62 (95% CI: 0.49–0.75).

In *Ae. albopictus*, heterozygous (FC) and homozygous mutant (CC) genotypes predominated (48.0% and 44.0%, respectively), whereas wild-type (FF) was less common (8.0%). The C allele frequency was 0.68 (95% CI: 0.55–0.81).

Table 7. Genotype and allele frequencies of the F1534C mutation in domain IIIS6 of the VGSC gene among permethrin-resistant Aedes mosquitoes

Species	Wild type FF n (%)	Heterozygous mutation FC n (%)	Homozygous mutation CC n (%)	C allele frequency (95% CI)	p-value
Ae. aegypti (n = 25)	5 (20.0)	9 (36.0)	11 (44.0)	0.62 (0.49–0.75)	0.53
Ae. albopictus (n = 25)	2 (8.0)	12 (48.0)	11 (44.0)	0.68 (0.55–0.81)	

Note: Allele frequencies are presented with 95% confidence intervals (Wilson method).

Comparison of C allele frequencies between species revealed no statistically significant difference ($\chi^2 = 0.396$, df = 1, p = 0.53).

Vector control remains one of the most effective strategies for preventing mosquito-borne diseases, including dengue fever, chikungunya, and Zika. *Ae. aegypti* and *Ae. albopictus* serve as the principal vectors. The World Health Organization (WHO) recommends pyrethroid insecticides, such as permethrin, for Aedes control because of their rapid knockdown effect and relatively low toxicity to humans. In Vietnam, pyrethroids have been widely used since the mid-1990s following the replacement of DDT. However, prolonged and repeated use may exert selective pressure on mosquito populations, thereby contributing to the development of resistance.

The present study demonstrates that both *Ae. aegypti* and *Ae. albopictus* populations from Ho Chi Minh City are resistant to 0.75% permethrin according to WHO (2016) criteria, with corrected 24-hour mortality rates ranging from 80.43% to 86.96% in *Ae. aegypti* and 84.45% to 88.89% in *Ae. albopictus*. These mortality levels are consistent with previous reports from Vietnam, where permethrin resistance in *Ae. aegypti* has been documented in major urban centers, including Hanoi and Ho Chi Minh City (Huynh & Minakawa, 2022; Thi *et al.*, 2016). Compared with other Southeast Asian countries, the resistance intensity observed in the present study appears lower than that reported in Thailand and Indonesia, where mortality rates below 70% have frequently been documented (Amelia-Yap *et al.*, 2018; Plernsub *et al.*, 2016), but is comparable to findings from parts of Malaysia and Cambodia.

Molecular analysis revealed that the commonly reported kdr mutations in domain IIS6, including V1016G, S989P, and I1011M, were not detected. In contrast, the F1534C mutation in domain IIIS6 was detected at high frequency among phenotypically resistant individuals, with 1534C allele frequencies of 0.62 in *Ae. aegypti* and 0.68 in *Ae. albopictus*. The absence of significant interspecific differences suggests that this mutation is widely established in urban Aedes populations in Ho Chi Minh City. Similar patterns have been reported in southern Vietnam and other parts of Asia, where F1534C is often the predominant kdr mutation associated with pyrethroid resistance (Kawada *et al.*, 2009a; Yanola *et al.*, 2011).

The detection of resistance in both major dengue vector species, together with the relatively high prevalence of the 1534C allele, highlights the importance of ongoing monitoring of pyrethroid effectiveness in Ho Chi Minh City. Strengthened routine resistance surveillance, combined with molecular monitoring of resistance-associated mutations, is therefore essential. In addition, the implementation of integrated resistance management strategies, including insecticide rotation and alternative control approaches, could help delay further resistance development and sustain vector control effectiveness.

Study Limitations

Several limitations should be acknowledged. First, sequencing was restricted to phenotypically resistant individuals; therefore, genotype-phenotype associations and population-level allele frequency estimates could not be determined. Second, Hardy-Weinberg equilibrium was not assessed due to the non-random sampling design. Third, the domains IIS6 and IIIS6 were sequenced in different individuals, which precluded the analysis of mutation co-occurrence and haplotypes. Finally, the inclusion of both F1 progeny and field-collected adults in the bioassays may have introduced variability in resistance estimates. Although this may have influenced the estimation of phenotypic resistance levels, its impact on the molecular detection of kdr mutations is expected to be minimal.

Conclusion

This study provides experimental evidence of permethrin resistance and associated VGSC mutations in *Ae. aegypti* and *Ae. albopictus* collected in Ho Chi Minh City. Bioassay results confirmed that both species are resistant to 0.75% permethrin according to the criteria of the World Health Organization (WHO,

2016), with corrected 24-hour mortality rates ranging from 80.43% to 86.96% in *Ae. aegypti* and from 84.45% to 88.89% in *Ae. albopictus*.

The commonly reported kdr mutations in domain IIS6, including V1016G, S989P, and I1011M, were not detected. In contrast, the F1534C mutation in domain IIIS6 was found at high frequency among phenotypically resistant individuals in both species. The comparable 1534C allele frequencies indicate that this mutation is similarly prevalent in permethrin-resistant populations of both Aedes species in Ho Chi Minh City, reflecting a shared resistance pattern.

Because mutation screening was restricted to resistant individuals, further investigations that include susceptible populations are needed to better define the strength of the association between F1534C and the resistant phenotype. These findings underscore the importance of continued insecticide resistance surveillance and molecular monitoring to support evidence-based vector control strategies in Ho Chi Minh City, and should inform public health policies for sustainable dengue prevention.

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***Data Availability Statement:** The datasets generated and/or analyzed during the current study are available in the GenBank repository under the accession numbers PZ225565–PZ225570.*

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