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RESEARCH ARTICLE

Molecular surveillance of insecticide resistance: High frequency of *kdr* mutations in permethrin-exposed *Aedes* mosquitoes from Kuala Lumpur and Selangor, Malaysia

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ABSTRACT

Despite ongoing vector control programs, dengue incidence in Malaysia continues to rise. A significant contributing factor is the emergence of insecticide resistance in *Aedes* mosquitoes, particularly against pyrethroids such as permethrin. This study aimed to evaluate permethrin resistance in *Aedes aegypti* and *Aedes albopictus* from Kuala Lumpur and Selangor and to investigate the prevalence of knockdown resistance (*kdr*) mutations in the voltage-gated sodium channel (Vgsc) gene. Due to limited sample quantity and quality, molecular assessment of *kdr* mutations was performed only for *Ae. aegypti*. Ovitraps were deployed in three dengue hotspot areas to collect *Aedes* eggs. Eggs were reared to adulthood under controlled laboratory conditions. Adult female mosquitoes were subjected to the WHO tube bioassay using permethrin to assess phenotypic resistance. Genomic DNA was extracted from individual *Ae. aegypti*, and polymerase chain reaction (PCR) was conducted to amplify target regions of the Vgsc gene using published *kdr*-associated primers. Direct sequencing of PCR products was performed to identify point mutations associated with pyrethroid resistance. Both *Ae. aegypti* and *Ae. albopictus* populations showed high levels of permethrin resistance, with mortality rates of <5% and <50%, respectively, indicating confirmed resistance. Sequencing of *Ae. aegypti* revealed the presence of pyrethroid resistance-associated *kdr* mutations, including S989P, A1007G, V1016G, and F1534C. These findings suggest strong selective pressure on local *Ae. aegypti* populations, reflecting ongoing adaptation to insecticidal interventions. The identification of these mutations emphasizes the challenges faced by current vector control strategies relying predominantly on chemical control.The detection of both phenotypic permethrin resistance and *kdr* mutations in *Ae. aegypti* populations from Kuala Lumpur and Selangor highlights the urgent need to revise and diversify vector control strategies. Improved sampling and molecular surveillance of *Ae. albopictus* are recommended to provide a more comprehensive understanding of insecticide resistance dynamics in Malaysian dengue vectors.

Keywords: *Aedes*; insecticide resistance; knockdown resistance (*kdr*); voltage-gated sodium channel (*Vgsc*) gene.

INTRODUCTION

In Malaysia, the number of dengue cases significantly declined in 2021 compared to 2020. The number of cases reported in 2020 was 90,304, but by October 2021, it decreased to 21,455 cases (Ahmad Zaki & Xin, 2022). However, the recent trend of dengue cases in Malaysia rose to a total of 96,443 cases by 31 October 2023 (OCHA, 2023). Dengue is a viral illness caused by the dengue virus (DENV), which spreads to humans through mosquito bites, specifically the *Aedes* mosquitoes. The two primary species of *Aedes* responsible for transmitting this virus are the *Ae. aegypti* and *Ae. albopictus*. Apart from dengue, *Aedes* mosquitoes are also known to transmit other arboviruses, including Zika, Chikungunya, and yellow fever virus (Ogunlade *et al.*, 2021).

There have been various methods implemented by the government and non-governmental organizations to control the dengue cases in Malaysia. One of them is through vector control, with the main control technique being fogging, handled by qualified practitioners from the Ministry of Health, local municipalities, and Pest Control Operators (PCO) from private companies. The four common classes of insecticides used to control *Aedes* mosquitoes in Malaysia are pyrethroid, organophosphate, carbamate, and organochlorine (Rohaizat Hassan *et al.*, 2021). Additional control strategies involve indoor residual spraying, the use of physical

barriers, and public awareness campaigns. However, even with these measures in place, dengue cases continue to rise due to the growing resistance of mosquitoes to commonly used insecticides, which unexpectedly cause resistance in these mosquitoes, resulting in biological changes in the target population and behavioral changes (VDCI, 2022). *Aedes* mosquito shows resistant traits when there is a genetic mutation in the voltage-gated sodium channel (*Vgsc*) gene, also known as the knockdown resistance (*kdr*) mutation (Spadar *et al.*, 2024), which mainly causes resistance to pyrethroids. This mutation can be passed down to the next generation and increase the survivability of this mutated population compared to the wild type. As a result, the ones left surviving and reproducing in that area will be the mutated mosquitoes, while the wild type will die out (Knols *et al.*, 2007). Hence, it will pose challenges to the vector control efforts.

According to Leong *et al.* (2019), the Allele-specific PCR (AS-PCR) for the detection of the common *kdr* mutations (V1016G, F1534C, and S989P) in the nine districts in Selangor shows the presence of these three mutations in the resistant *Ae. aegypti*, which significantly contributes to the resistance towards pyrethroids. All nine districts include Gombak (Batu Caves), Hulu Selangor (Batang Kali), Hulu Langat (Cheras), Klang (Pulau Ketam), Kuala Langat (Banting), Kuala Selangor (Kuala Selangor), Sabak Bernam (Sabak Bernam), Petaling (Bandar Sunway), and Sepang (Salak Tinggi). The paper shows that while the adult female mosquitoes in these locations are susceptible to insecticides like malathion and propoxur, there are various levels of resistance against insecticides like dichlorodiphenyltrichloroethane (DDT) and pyrethroids. Hence, it is important to study the *kdr* mutations that cause the resistance and the distribution of the resistance haplotypes of *Aedes* mosquitoes in Kuala Lumpur and Selangor regions to improve the vector control strategies.

MATERIALS AND METHODS

Ethical approval

Mosquito rearing and protocols used in the study were approved by Universiti Malaya Institutional Biosafety & Biosecurity Committee (IBBC), IBBC No:. UMIBBC/PA/R/FOM/PARA-030/2024.

Study sites

Mosquito sampling was conducted at three dengue hotspot localities within the Kuala Lumpur and Selangor regions. Site selection was based on data from the iDengue portal, a monitoring platform developed by the Ministry of Health (MOH) in collaboration with the Malaysian Space Agency (MYSA) and the Ministry of Science, Technology, and Innovation (MOSTI). The selected sites include Program Perumahan Rakyat Lembah Sungai 2 (PPR LS2), Taman Harmonis, Gombak (THG), and PPR Taman Wahyu 2 (TW2), which were identified as hotspots due to sustained dengue case reports exceeding 30 consecutive days.

Mosquito samplings

Mosquito sampling in this study was conducted using ovitraps. At each study site, 100 ovitraps were strategically deployed, and their placement locations were recorded. Egg collection was prioritized over adult mosquito sampling due to the low likelihood of vertical dengue virus (DENV) transmission from infected females to their offspring (Gonçalves *et al.*, 2020). The retrieval was done after five days of exposure, paddles were sealed in plastic bags and transported to the Vector Control Technology Laboratory, Department of Parasitology, Universiti Malaya, for hatching. Any residual water in the ovitraps was also collected in plastic bottles and returned to the laboratory for further examination for the presence of eggs and larvae.

Mosquito rearing

The eggs and larvae collected from the sites were reared under controlled laboratory conditions. The temperature was set at 27±2°C with a relative humidity of 70-80% and a 12h:12h light-dark cycle. A 10% sucrose solution with vitamin B was placed as a food source for the adult mosquitoes. 1-2 post-emergence adult mosquitoes were morphologically identified and classified according to their species. The mosquitoes were blood-fed and cultured for up to 3 generations to obtain samples for the adult insecticide resistance bioassay test.

Tested insecticides

The insecticide chosen in this study is made up of pyrethroid type-I, using 0.4% permethrin. The concentration is the discriminating concentration at which the wild-type mosquito strain normally dies when exposed to it (WHO, 2022a).

Adult insecticide resistance bioassay

For every test, four bottles treated with insecticide were utilized, and two control bottles (pyrethroid (PY) oil control) were used. According to WHO (2022b) guidelines, insecticide-impregnated papers with known concentrations of insecticide (0.4% permethrin) were placed in separate exposure chambers. Each bottle contained 25 females *Ae. aegypti* or *Ae. albopictus* mosquitoes that have been fed with sucrose for 3-5 days.

Firstly, the mosquitoes were starved for at least 1 hour before being transferred to 6 holding tubes (green dot) (25 mosquitoes each). They were left inside the holding tubes for 1 hour to observe the condition of the mosquitoes to ensure they were fit and healthy. In addition, it was also to ensure that none of the mosquitoes were injured or dead due to handling and transferring. The mosquitoes were then transferred to the 4 insecticide tubes (red dot) (4 replicates) and 2 control tubes (yellow dot) and let exposed for 1 hour. The number of knocked-down mosquitoes was observed and recorded at 5-minute intervals up to 1 hour. The mosquitoes were transferred back into the holding tube, and sucrose pads were placed on top of the tube. After 24 hours, the mortality rate was recorded. Mosquitoes that were unable to fly or maintain an upright posture were assumed to be knocked-down or dead (after 24 hours). Laboratory strain of both species (susceptible to 0.4% permethrin) was used as a control in the assay.

Data analysis

The insecticide resistance status is classified as susceptible, possible resistance, and confirmed resistance based on the mortality rates.

$$\text{Mortality percentage} = \frac{\text{Total number of dead mosquitoes}}{\text{Total number of exposed mosquitoes}} \times 100\%$$

A mortality rate of 98% to 100% suggests susceptibility. If the mortality is more than 90% but less than 98%, it suggests possible resistance. If the mortality is < 90%, it suggests confirmed resistance given that at least 100 mosquitoes per species were tested.

Genomic DNA extraction

DNA from the mosquito that shows possible and confirmed resistance from the bioassay was extracted for molecular detection of *kdr* mutation. The method used to extract the DNA is the Livak gDNA extraction technique (Livak, 1984; Lynd *et al.*, 2005).

For the gDNA extraction, one mosquito was frozen per tube, which then ground in 100 µL preheated Livak grind buffer in 1.5 mL Eppendorf tube using a pestle. To maximize total yield, the mosquito was first ground in 50 µL Livak grind buffer, then the remaining 50 µL was used to rinse the pestle (100 µL total in the tube), which was then immediately transferred to 65°C. It was left incubated at 65°C for approximately 30 minutes. Then, 14 µL 8 M potassium

acetate was added and mixed using vortex. It was then incubated on ice for approximately 30 minutes. After that, the tube was centrifuged at 4°C and 13000 rpm for 20 minutes. The supernatant was then carefully transferred to a new 1.5 mL Eppendorf tube without any debris. Optionally, the tube can be re-spun for 20 minutes and the new supernatant transferred to the new tube. Next, 200 µL of 100% ethanol was added to the tube and mixed using vortex. It was then spun for 15 minutes at 4°C and 13000 rpm. Subsequently, the supernatant was removed and discarded, while the pellet was rinsed carefully using approximately 100 µL ice-cold 70% ethanol, not to dislodge the pellet, then left at room temperature (RT) for 1 minute. The tube was then shaken gently and spun for 5 minutes at 4°C and 13000 rpm. The 70% ethanol was then discarded carefully so as not to remove the DNA pellet. Finally, the remaining ethanol was left to dry overnight. To re-suspend the pellet, 30 µL nuclease free water was added into the Eppendorf tube containing the pellet and incubated at 65°C for 10 minutes. The extracted DNA tube was stored at -20°C until needed for PCR.

Molecular detection of knockdown resistance (*kdr*)

The *Vgsc* gene was detected and amplified using PCR. A fragment of the coding region of the *Vgsc*, which spans exon 19 to exon 31, was used to detect the *kdr* mutations. This includes domain II (codons 989, 1011, and 1016) and domain III (codon 1534). Positive controls for the PCR are the lab strains while non-template control (NTC) used is nuclease free water. Due to limited sample quantity and quality, molecular assessment of *kdr* mutations was performed only for *Ae. aegypti*. Table 1 shows the primers for *kdr* mutation detection in *Ae. aegypti.* Primers for the amplification of the VGSC domain II and III regions were designed based on previously published primer sets AaSCF1/AaSCR4 and AaSCF7/AaSCR7 (Zuharah & Sufian, 2021; Akhir *et al*., 2022). The PCR protocol in this study, which includes initial denaturation at 1 cycle, 95°C at 3 minutes, denaturation at 40 cycles, 95°C at 30 seconds, annealing at 40 cycles, 58°C at 1 minute, extension at 40 cycles, 72°C at 1 minute, final extension at 1 cycle, 72°C at 5 minutes.

After PCR was completed, agarose gel electrophoresis (AGE) was done to visualize the amplified DNA fragments. 1.5% agarose gel was prepared by mixing 0.525 g agarose powder in 35 mL 1X TAE buffer. The mixture was then microwaved for 1 minute and stained with 1 µL SYBR^{TM}Safe DNA gel stain (Thermo Fisher Scientific, USA). After that, the solution was poured into cast with comb and left harden for 30 minutes. Next, 2 µL 100 bp DNA ladder was loaded into the first well followed by 5 µL of positive control. The remaining wells were loaded with 5 µL PCR products and NTC. The Bio-Rad Mini-Sub Cell GT system machine was set at 100 V, and the samples were run for 30 minutes. After that, the gel was observed using Gel Doc^{TM} EZ Imager.

DNA sequencing

The PCR amplicons were sent to a sequencing service provider (Apical Scientific Sdn Bhd) for direct nucleotide sequencing.

Statistical analysis

The bioassay results were analyzed using statistical software by the International Business Machines Corporation (IBM) called Statistical Package for the Social Sciences (SPSS) version 27. After the sequence was received from the sequencing service provider, the mutation sites on the *Vgsc* gene were detected using the MEGA12 software and Chromas. The gene sequence of the resistant mosquitoes was compared with the reference strain obtained from the National Center for Biotechnology Information (NCBI) and the lab strains.

Table 1. Primer for *kdr* mutation detection in *Ae. aegypti*

Domain	Direction	Primer	Sequence (5'–3')
DII	Forward	AaSCF1	AGA CAA TGT GGA TCG CTT CC
	Reverse	AaSCR4	GGA CGC AAT CTG GCT TGT TA
DIII	Forward	AaSCF7	GAG AAC TCG CCG ATG AAC TT
	Reverse	AaSCR7	GAC GAC GAA ATC GAA CAG GT

RESULTS

Adult insecticide resistance bioassay

As shown in Figure 1, exposure to 0.4% permethrin resulted in high susceptibility for both *Ae. aegypti* and *Ae. albopictus* laboratory strains, with a 100% mortality for both strains. In contrast, for the PPR LH2 mosquitoes, both *Ae. aegypti* and *Ae. albopictus* with a mortality percentage of 3 ± 0.5% and 26 ± 2.38% respectively shows a high resistance towards 0.4% permethrin. THG susceptibility varied towards 0.4% permethrin with *Ae. aegypti* being highly resistant (0% mortality) towards the insecticide whereas *Ae. albopictus* shows 50 ± 2.38% mortality. For PPR TW2, the result for *Ae. aegypti* demonstrated high resistance (0% mortality). Due to an insufficient sample size, data for *Ae. albopictus* was unavailable for this area.

As shown in Figure 2, the laboratory strains of *Ae. aegypti* and *Ae. albopictus* demonstrated a high knockdown percentage within a short exposure period, as reflected by the plot's gradient. The *Ae. aegypti* lab strain achieved 100% knockdown within approximately 30 minutes, while *Ae. albopictus* laboratory strain achieved complete knockdown in 45 minutes. This result is followed by *Ae. albopictus* from THG and PPR LS2. *Ae. aegypti* from PPR LS2 exhibited minimal knockdown percentage (<5%) over 60 minutes, whereas *Ae. aegypti* from both THG and PPR TW2 showed 0% knockdown throughout the entire period.

Table 2 presents the predicted knockdown time for 50% of the exposed mosquito population (KT_{50}) and 95% of the exposed mosquito population (KT_{95}) of both *Ae. aegypti* and *Ae. albopictus* strains using SPSS. For the laboratory strains, KT_{50} and KT_{95} for *Ae. aegypti* are 12.231 minutes and 21.418 minutes, respectively, while *Ae. albopictus* lab strain, the KT_{50} and KT_{95} are 16.110 minutes and 31.851 minutes respectively. For *Ae. aegypti* from PPR LS2, the KT_{50}, and KT_{95} for are not significant as the values are too big (KT_{50} > 60 minutes). In contrast, the *Ae. Albopictus* from the same location had a KT_{50} of 35.852 minutes but the KT_{95} is not significant due to being out of the measurable range. For field strains of *Ae. aegypti* from THG and PPR TW2, the KT_{50} and KT_{95} are not applicable as no knockdown was observed. However, the KT_{50} and KT_{95} for *Ae. albopictus* from THG are 31.811 minutes and 47.657 minutes respectively. No KT estimates were obtains for *Ae. albopictus* PPR TW2 strain due to insufficient sample size.

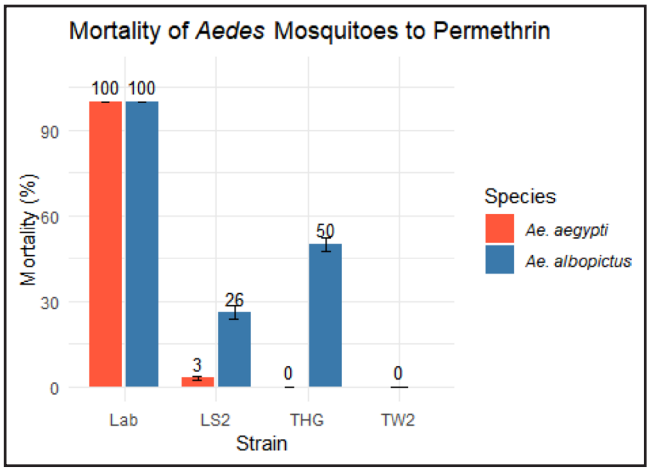

Figure 1. Mortality rate of adult *Ae. aegypti* and *Ae. albopictus* collected from each location 24 hours after exposure to 0.4% permethrin. Value is reported in mean ± SD.
Note: Lab: Lab strain; LS2: PPR Lembah Subang 2; THG: Taman Harmonis, Gombak; TW2: PPR Taman Wahyu 2.

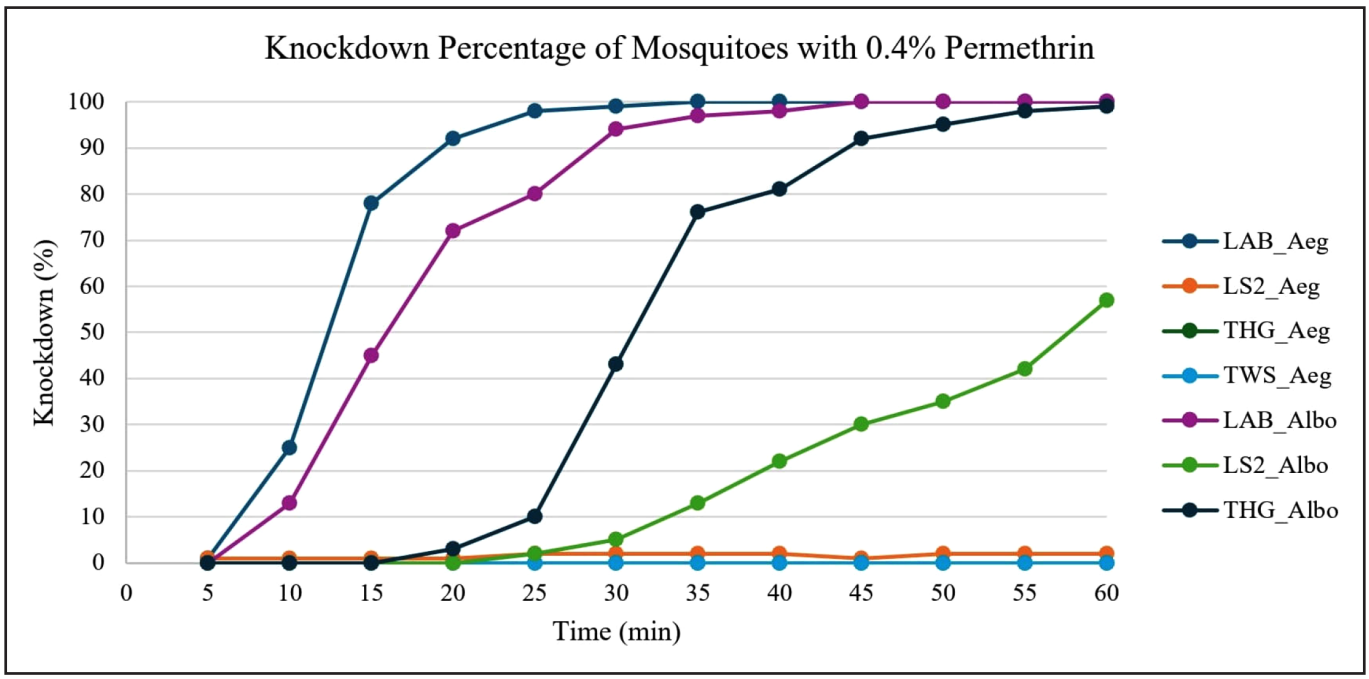

Figure 2. Knockdown percentages for every 5 minutes for 60 minutes.

Table 2. Mortality percentage and knockdown time of each species and strain

Species	Strain	% Mortality (±SD)[a]	Phenotype[b]	KT_{50}[c] (95% C.I.)	KT_{95}[d] (95% C.I.)
Ae. aegypti	Lab	100 (±0.0)	S	12.231 (11.510–12.917)	21.418 (19.944–23.388)
	PPR Lembah Subang 2	3 (±0.5)	R	NS[e]	NS
	Taman Harmonis Gombak	0 (±0.0)	R	NA[f]	NA
	PPR Taman Wahyu 2	0 (±0.0)	R	NA	NA
Ae. albopictus	Lab	100 (±0.0)	S	16.110 (15.206–16.978)	31.851 (29.764–34.505)
	PPR Lembah Subang 2	26 (±2.38)	R	35.852 (25.358–58.965)	NS
	Taman Harmonis Gombak	50 (±2.38)	R	31.811 (30.857–32.739)	47.657 (45.692–50.093
	PPR Taman Wahyu 2	–	–	–	–

[a] Mean ± standard deviation of 4 replicates.
[b] S: susceptible; R: resistant.
[c] KT_{50}: time (in minutes) required to knock down 50% of the exposed mosquito; 95% C.I.: 95% confidence interval of time.
[d] KT_{95}: time (in minutes) required to knock down 95% of the exposed mosquito; 95% C.I.: 95% confidence interval of time.
[e] NS: Not significant.
[e] NA: Not applicable.

Direct sequencing *Ae. aegypti* domain II

The sequences of *Ae. aegypti* domain II field isolates were analyzed and compared to both the laboratory strain and the NCBI reference sequence with the accession number LC557556. 3 non-synonymous mutations were observed. The first mutation observed at codon 989 where substitution from serine (S) to proline (P) had occurred (Figure 3). The same isolates also showed substitution at codon 1016, which had valine (V) substituted to glycine (G). Additionally, another mutation at codon 1007 resulted in substitution of alanine (A) with glycine (G).

These non-synonymous mutations occurred due to the substitution of the first nucleotide at codon 989 from thymine (T) to cytosine (C) which converted serine (TCC) to proline (CCC) and substitution of the second nucleotide at codon 1007 from cytosine (C) to guanine (G) which converted alanine (GCC) substituted to glycine (GGC). At codon 1016, the second nucleotide was substituted from thymine (T) to guanine (G) which converted valine (GTA) to glycine (GGA).

Direct sequencing *Ae. aegypti* domain III

The sequenced PCR products of *Ae. aegypti* domain III field isolates were analyzed and compared to both the laboratory strain and the NCBI reference sequence with the accession number ON838300. 1 non-synonymous mutation was identified at codon 153, where phenylalanine (F) was substituted to cysteine (C) (Figure 4). This non-synonymous mutation occurred due to the substitution of the second nucleotide at codon 1534 from thymine (T) to guanine (G) which converted phenylalanine (TTC) to cysteine (TGC) (Figure 5).

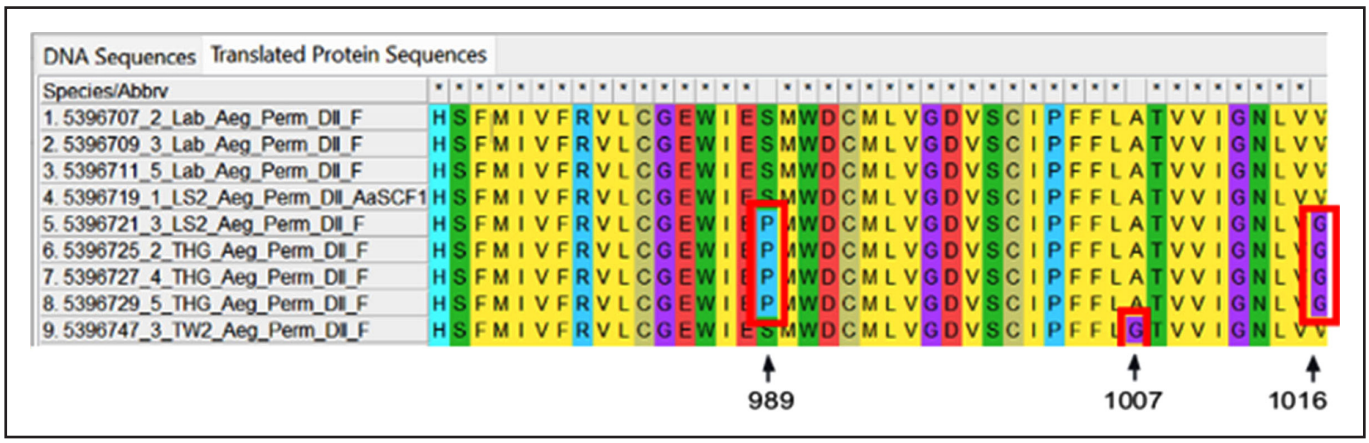

Figure 3. Amino acid sequences alignment of *Aeg*_Perm_DII lab strain, field isolates and reference strain from NCBI with accession number of LC557556. The *Vgsc* gene at domain II showed *kdr* mutations: S989P, A1007G, and V1016G.

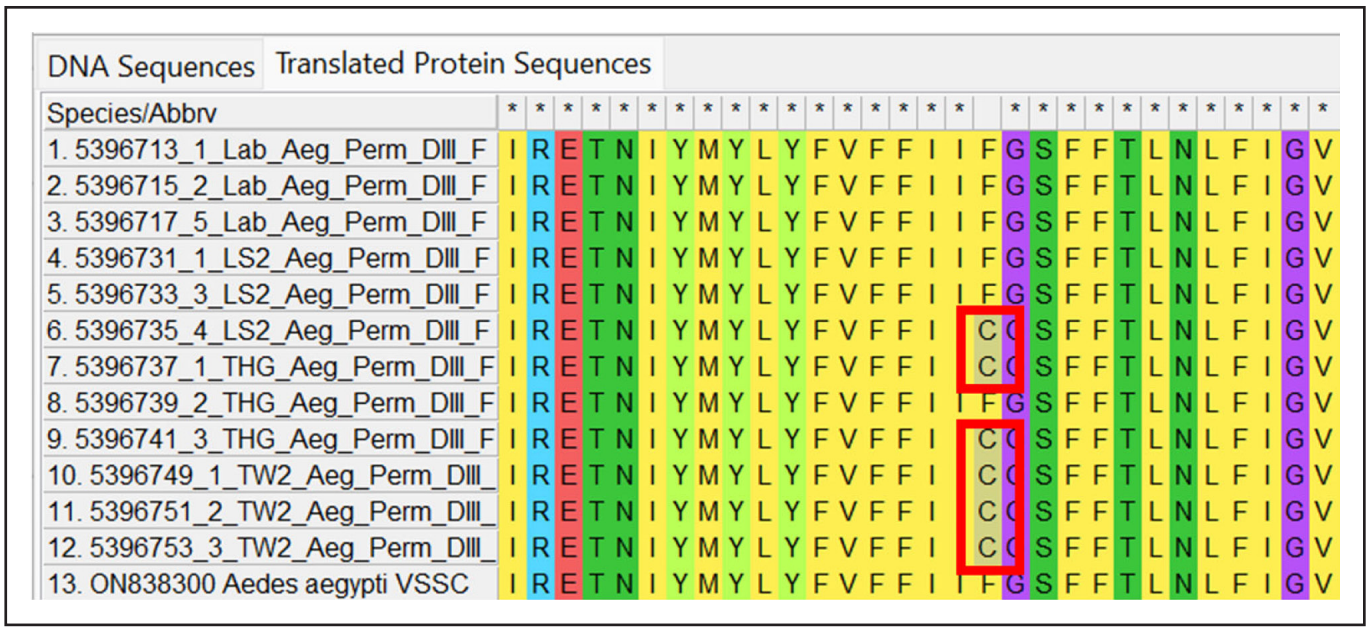

Figure 4. Amino acid sequences alignment of *Aeg*_Perm_DIII lab strain, field strains and reference isolates from NCBI with accession number of ON838300. The *Vgsc* gene at domain II showed *kdr* mutation: F1534C.

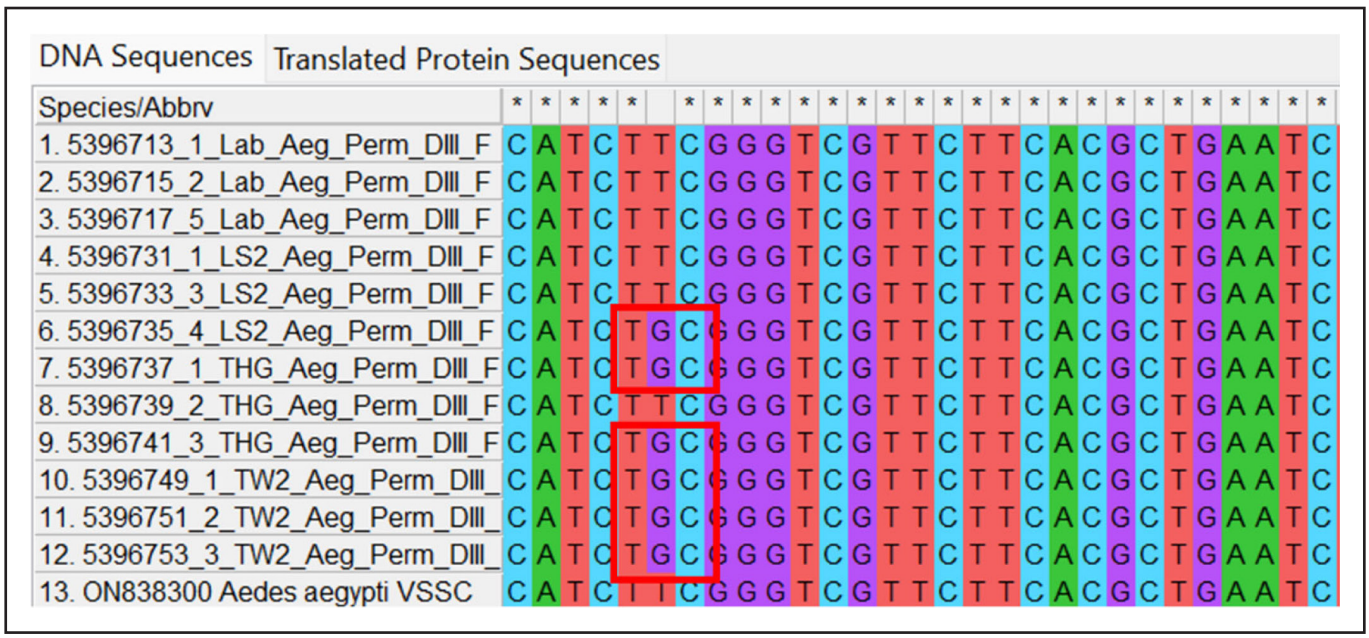

Figure 5. DNA sequence of *Aeg*_Perm_DIII showing *kdr* mutations at codon 1534 (F1534C).

Table 3. Prevalence of *kdr* mutation in *Ae. aegypti* field isolates

Domain II			Domain III
S989P	A1007G	V1016G	F1534C
66.7% (4/6)	16.7% (1/6)	66.7% (4/6)	66.7% (6/9)

Prevalence of *kdr* mutation in *Ae. aegypti* field isolates

Table 3 summarizes the prevalence of *kdr* mutations in field isolates from PPR LS2, THG and PPR TW2. The prevalence of *kdr* mutations in *Ae. aegypti* field isolates from Selangor was notably high for S989P (66.7%), V1016G (66.7%), and F1534C (66.7%), while A1007G was detected at a lower frequency (16.7%). These frequencies are comparably slightly higher than previous reports from Malaysia, where S989P, V1016G, and F1534C have been observed at range of 40% to 60% in local populations, indicating persistent selection pressure by pyrethroid insecticides (Zuharah & Sufian, 2021; Akhir *et al*., 2022).

DISCUSSIONS

WHO tube bioassay

Overall, phenotypic resistance to 0.4% permethrin was observed in both *Ae. aegypti* and *Ae. albopictus* across the 3 dengue hotspot locations in Kuala Lumpur and Selangor. This is evident from the mortality rates for fields strains, all of which were below the 90% threshold, indicating confirmed resistance. The concentration of 0.4% represents the discriminating concentration for permethrin, expected to cause 100% mortality of the susceptible *Aedes* mosquito population but not necessarily the resistant ones (Leong *et al.*, 2019).

Notably, *Ae. aegypti* exhibited more resistance towards permethrin compared to *Ae. albopictus* in PPR LS2 and THG. In PPR LS2, the mortality rates for *Ae. albopictus* was approximately 9 times greater than the value for *Ae. aegypti*, while in THG, *Ae. albopictus* showed 50% mortality and the *Ae. aegypti* is 0%. *Ae. aegypti* behavioral tendency to inhabit indoor environments increases the chance of insecticide exposure to household insecticide (HHI) whereas *Ae. albopictus* typically favours outdoor settings with less HHI contact (Owusu-Asenso *et al.*, 2022). This increased exposure liklely contributes to the higher resistance observed in *Ae. aegypti*.

The WHO tube bioassay results for *Ae. aegypti* in THG showed 0% mortality, a stark contrast to the previous study (70-80%) conducted by Leong *et al.*(2019). This suggests a substantial rise in pyrethroid resistance within 6 years, highlighting the need to continuously monitor the resistance status to ensure the effectiveness of current insecticide-based vector control strategies.

Furthermore, the SPSS prediction on the knockdown time (KT_{50} and KT_{95}) for field isolates (resistant) were significantly higher compared to laboratory strains that often exceeds 60 minutes, indicating slower knockdown in resistant populations. These findings furthur confirm the reduced susceptibility of field strains to permethrin compared to their susceptible counterparts.

DNA analysis

The results indicate that both *Ae. aegypti* and *Ae. albopictus* exhibit resistance toward 0.4% permethrin (phenotypically). Consequently, DNA analysis was conducted to investigate whether this phenotype is associated with *kdr* mutations in the *Vgsc* gene, which are widely known markers of pyrethroid resistance (genotypically) for *Ae. aegypti*.

Analysis of *Ae. aegypti* field isolates revealed the presence of *kdr* mutations, while none were observed in the *Ae. aegypti* laboratory strain. The mutations found are S989P, A1007G, V1016G, and F1534C. S989P, V1016G, and F1534C are well-documented mutations in the *Vgsc* gene of *Aedes* that are known to link with resistance towards pyrethroid. These mutations cause alteration of the target site of pyrethroid (voltage-gated sodium channel), which inhibits the active ingredient in the insecticide from binding to it (Saavedra-Rodriguez *et al.*, 2021). The A1007G, on the other hand, is a mutation specifically found in *Ae. aegypti* population from Malaysia (Akhir *et al.*, 2022; Zhao *et al.*, 2023). These findings are also consistent with the calculated *kdr* mutations prevalence, where S989P, V1016G, and F1534C were observed to have higher prevalence (66.7%) compared to A1007G (16.7%), which proves that these 3 mutations are more common in *Aedes* mosquitoes.

However, it is important to recognize that *kdr* mutations are not the sole mechanism for resistance. It can also occur because of metabolic function, where there is an increase in the rate of metabolism and rapid clearance of the toxic compounds of the insecticides from the mosquitoes' system. To confirm whether resistance is solely due to *kdr* mutations, synergist bioassays using piperonyl butoxide (PBO) may prevent cytochrome P450s in mosquitoes from metabolizing the insecticide (Ng & Zuharah, 2024).

Given the strong correlation between genotypic (*kdr* point mutations) and the phenotyping results (using WHO tube bioassay) in the present study, further evaluation using larger sample sizes should be done to evaluate the potential of molecular detection of *kdr* mutations as a molecular surveillance marker for pyrethroid resistance in *Aedes* populations. Molecular methods offer significant advantages, especially for laboratories lacking in insectary facilities, as they are more feasible and cost-effective and directly detect the *kdr* mutations that have been linked to pyrethroid resistance.

CONCLUSION

In conclusion, adult *Ae. aegypti* and *Ae. albopictus* in the Kuala Lumpur and Selangor regions exhibit a high level of resistance towards permethrin. The detection of *kdr* mutations in many of the resistant individuals supports a strong association between *kdr* and resistance towards pyrethroid in *Aedes* mosquitoes. Although only *Ae. aegypti* was genotypically analyzed , the findings remain valuable and contribute to future studies formulating new and alternative combinations of insecticides and new chemicals to effectively manage resistance in future vector control strategies.

Conflict of Interest Statement

The author declares that they have no conflict of interests.

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