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UNRAVELING THE PHENOTYPIC AND GENOTYPIC RESISTANCES ASSOCIATED WITH PYRETHROID AND ORGANOPHOSPHATE IN *Aedes aegypti* (DIPTERA: CULICIDAE)

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ABSTRACT

The widespread emergence of insecticide resistance in *Aedes aegypti* threatens the effectiveness of dengue vector control programmes in Malaysia. This study investigated the phenotypic susceptibility and the underlying genotypic resistance mechanisms associated with pyrethroid and organophosphate insecticides in *Ae. aegypti* populations collected from four localities in Penang, Malaysia, namely Apartment Asoka (AA), Taman Bukit Jambul (TBJ), Taman Machang Bubok (TMB), and Flat Sri Pauh (FSP). Adult susceptibility bioassays were conducted following World Health Organization (WHO) protocols using deltamethrin (0.03%), permethrin (0.4%), pirimiphos-methyl (60 mg/m²), and malathion (5%). Target-site resistance was assessed by sequencing the voltage-gated sodium channel (VGSC) domains II and III. Bioassay results revealed severe and widespread pyrethroid resistance across all populations. Deltamethrin mortality ranged from 5% in Taman Bukit Jambul (TBJ) to 65% in Flat Sri Pauh (FSP), while permethrin mortality ranged from 3% (TBJ) to 85% (FSP), indicating operational failure of both insecticides. Pirimiphos-methyl showed moderate resistance, with mortality ranging from 30% to 65%, whereas malathion remained largely effective, achieving 100% mortality in most populations, except TBJ, which showed possible resistance (95%). Genotypic analysis identified widespread knockdown resistance (*kdr*) mutations, including S989P, V1016G, T1520I, and F1534C. The most resistant population (TBJ) exhibited complex mutation profiles, including triple-mutation genotypes (V1016G/T1520I/F1534C) at frequencies up to 20% under permethrin exposure. Notably, the T1520I mutation was detected predominantly in deltamethrin-exposed populations, with frequencies ranging from 14.3% to 25%, and rarely occurred as a single substitution. These findings indicate advanced resistance development in *Ae. aegypti* populations in Penang, underscoring the urgent need to revise pyrethroid-dependent control strategies and implement evidence-based insecticide resistance management.

Keywords: *Aedes aegypti*; bioassay; *kdr;* mosquito; resistance

ABSTRAK

Kemunculan rintangan insektisid secara meluas dalam *Aedes aegypti* mengancam keberkesanan program kawalan vektor denggi di Malaysia. Kajian ini mengkaji kerentanan fenotipik dan mekanisme rintangan genotip yang mendasari mekanisme insektisid berkaitan kumpulan piretroid dan organofosfat dalam populasi *Ae. aegypti* yang dikumpulkan dari empat lokaliti di Pulau Pinang, Malaysia iaitu Apartment Asoka (AA), Taman Bukit Jambul (TBJ), Taman Machang Bubok (TMB) dan Flat Sri Pauh (FSP). Bioesei kerentanan dewasa dijalankan mengikut protokol Pertubuhan Kesihatan Sedunia (WHO) menggunakan deltametrin (0.03%), permetrin (0.4%), pirimifos-metil (60 mg/m²) dan malation (5%). Rintangan pada tapak sasaran dinilai melalui penjujukan domain II dan III gen saluran natrium berpagar voltan (VGSC). Keputusan bioesei menunjukkan rintangan piretroid yang teruk dan meluas di semua populasi. Kadar kematian deltametrin berjulat daripada 5% di Taman Bukit Jambul (TBJ) hingga 65% di Flat Sri Pauh (FSP), manakala kadar kematian permetrin berjulat daripada 3% (TBJ) hingga 85% (FSP), menunjukkan kegagalan operasi kedua-dua insektisid tersebut. Pirimifos-metil menunjukkan rintangan sederhana, dengan kadar kematian antara 30% hingga 65%, manakala malation kekal berkesan dengan kadar kematian 100% di kebanyakan populasi, kecuali TBJ yang menunjukkan kemungkinan rintangan (95%). Analisis genotip mengenal pasti mutasi rintangan knockdown (*kdr*) yang meluas, termasuk S989P, V1016G, T1520I dan F1534C. Populasi paling rintang (TBJ) menunjukkan profil mutasi yang kompleks, termasuk genotip tiga mutasi (V1016G/T1520I/F1534C) dengan frekuensi sehingga 20% di bawah pendedahan permetrin. Mutasi T1520I didapati terutamanya dalam populasi yang terdedah kepada deltametrin, dengan frekuensi antara 14.3% hingga 25% dan jarang berlaku sebagai mutasi tunggal. Dapatan ini menunjukkan kemajuan perkembangan kerintangan dalam populasi *Ae. aegypti* di Pulau Pinang, sekali gus menekankan keperluan mendesak untuk menyemak semula strategi kawalan bergantung piretroid dan melaksanakan pengurusan rintangan insektisid berasaskan bukti.

Kata kunci: *Aedes aegypti*; bioesei; *kdr*; nyamuk; rintang

INTRODUCTION

Aedes aegypti (Linnaeus 1762) is the principal urban vector responsible for the transmission of several medically important arboviruses, including dengue, Zika, chikungunya, and yellow fever. These diseases pose significant public health challenges globally, with dengue alone affecting approximately 390 million people annually across tropical and subtropical regions (Ullah 2024). The mosquito's close association with human habitats, day-biting behavior, and widespread geographic distribution have made it a primary target for vector control interventions aimed at reducing arboviral transmission and preventing epidemic outbreaks (Bhatt et al. 2013).

Chemical control through insecticide application remains the cornerstone of *Ae. Aegypti* management programs worldwide. Pyrethroids, particularly deltamethrin and permethrin, are the most commonly deployed adulticides due to their rapid knockdown effect, low mammalian toxicity, and cost-effectiveness (Liu et al. 2005; Moyes et al. 2017). These compounds are extensively used in space-spraying operations, indoor residual spraying, and treated materials. Organophosphates, such as malathion, temephos, and pirimiphos-methyl, serve as important alternatives or complementary tools, applied both as adulticides and larvicides in many vector control programs (Collins et al. 2025; Rubio-Palis et al. 2023). The intensive and sustained use of these insecticide classes over several decades has created strong

selective pressure on *Ae. aegypti* populations, driving the evolution and spread of resistance mechanisms (Wang et al. 2023).

At the genotypic level, insecticide resistance in *Ae. aegypti* is driven by two principal mechanisms, which are target-site modifications and metabolic detoxification. Target-site resistance to pyrethroids primarily involves mutations in the voltage-gated sodium channel (VGSC) gene, commonly referred to as knockdown resistance (*kdr*) mutations (Du et al. 2016; Yanola et al. 2011). The most extensively characterized *kdr* mutations include F1534C, V1016G, V1016I, V410L, and S989P, which occur individually or in complex haplotypes across different geographic populations (Linss et al. 2014; Kawada et al. 2009). The F1534C mutation, in particular, has reached near-fixation in many populations, with allele frequencies exceeding 0.80 in regions such as Burkina Faso, Sri Lanka, and parts of Brazil (Fernando et al. 2020; Stenhouse et al. 2013; Zongo et al. 2005). Multiple *kdr* mutations often coexist within populations, forming complex resistance haplotypes that confer enhanced levels of pyrethroid resistance. For example, the double mutant NaVR2 haplotype (V1016I+F1534C) has been detected at high frequencies in Brazilian and Venezuelan populations, correlating with elevated phenotypic resistance (Brito et al. 2018).

Given the escalating challenge of insecticide resistance and its heterogeneous distribution, unravelling the phenotypic and genotypic resistances associated with pyrethroid and organophosphate use in *Ae. aegypti* is essential for designing effective and sustainable vector control interventions. Therefore, this study aims to examine the current resistance of *Ae. aegypti* on the phenotypic expression and genetic determinants in response to pyrethroid and organophosphate in Penang, Malaysia.

MATERIALS AND METHODS

Mosquito Collections

Populations of *Ae. aegypti* were collected from four localities identified as dengue hotspots distributed across Penang, Malaysia (Figure 1). The selection of sampling sites was based on dengue hotspot classifications provided by the Ministry of Health Malaysia (MOH), following a comprehensive analysis of the national distribution of dengue cases. The identified sites encapsulated geographically diverse regions within Malaysia, which included (1) Apartment Asoka (AA) (5°21′N, 100°18′E); and (2) Taman Bukit Jambul (TBJ) (5°33′N, 100°28′E) situated on Penang Island (3) Taman Machang Bubok (TMB)(5°30′02″N, 100°25′70″E) and (4) Flat Sri Pauh (FSP)(5°33′92″N, 100°50′46″E) located on Penang Mainland.

In each of the aforementioned localities, 60 ovitraps were systematically deployed at random intervals for a duration of five consecutive days. Hardwood paddles were utilized as oviposition substrates, which were subsequently retrieved and transported to the Medical Entomology Laboratory at Universiti Sains Malaysia. The collected eggs were then reared to adulthood under meticulously controlled insectary conditions (27±2°C; 70–80% relative humidity).

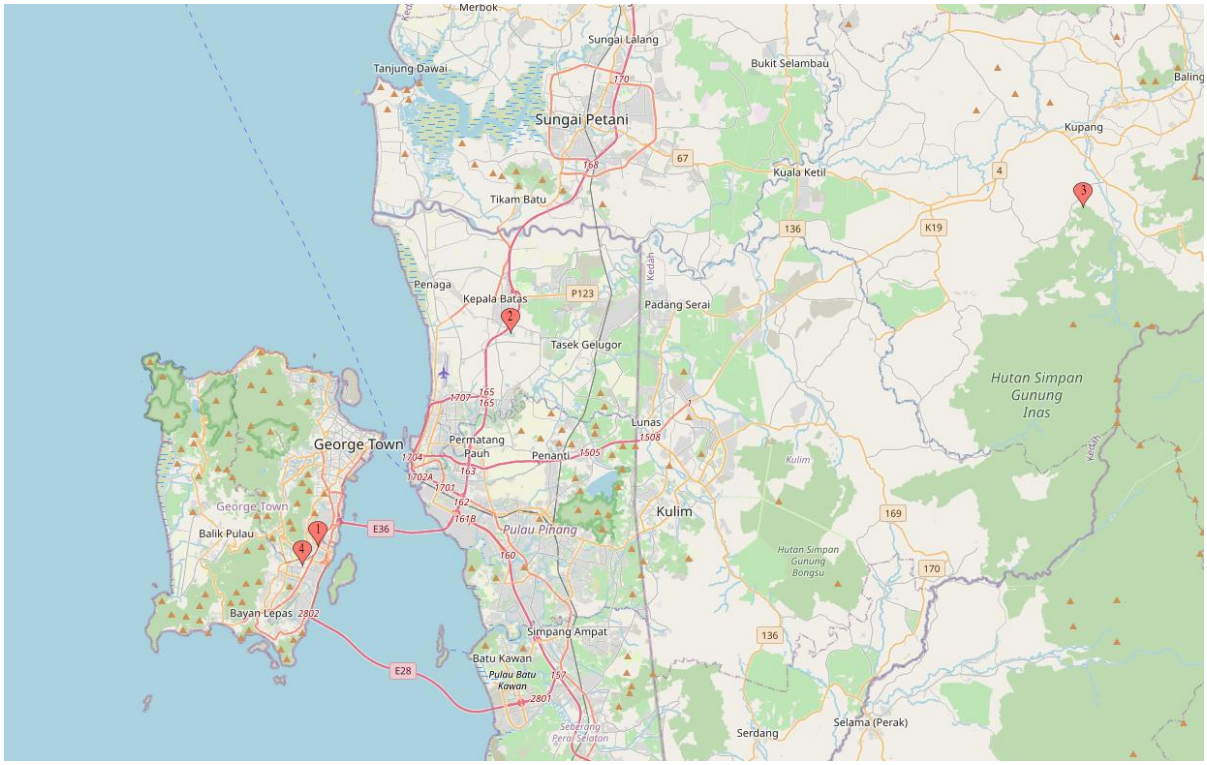

Figure 1. Sampling sites of *Aedes aegypti* across Penang Island and Seberang Perai, Malaysia, 1- Apartment Asoka (AA), 2- Taman Sri Pauh (FSP), 3- Taman Machang Bubok (TMB), and 4- Taman Bukit Jambul (TBJ)

Adult Bioassay

Adult susceptibility bioassays were conducted according to the World Health Organization (WHO 2022) standard protocol. Four replicates comprising 25 non-blood-fed female *Aedes aegypti* (2–5 days post-emergence) from each respective field population were subjected to exposure using insecticide-impregnated filter papers formulated by the Vector Control Research Unit (VCRU) at Universiti Sains Malaysia. The insecticides evaluated included: (1) 0.03% deltamethrin, (2) 0.4% permethrin, (3) pirimiphos-methyl at a dosage of 60 mg/m², and (4) 5% malathion.

Initially, the mosquitoes were placed into holding tubes and allowed to acclimatize for one hour. Any specimens that exhibited damage, injury, or mortality were substituted prior to the commencement of the testing. Subsequently, a total of 25 female mosquitoes were transferred into exposure tubes lined with insecticide-impregnated paper. Knockdown data were meticulously recorded at 5-minute intervals until one hour. Following the exposure period, the mosquitoes were relocated into clean paper cups containing a 10% sucrose solution and were maintained under controlled conditions of 27±2°C and 60-80% relative humidity. Mortality rates were evaluated 24 hours post-exposure, and four replicates were conducted for each insecticide tested. Two control replicates were prepared utilizing silicone oil for the pyrethroids, olive oil for malathion, and acetone for pirimiphos-methyl. A laboratory-susceptible strain of *Ae. aegypti*, maintained at VCRU (Penang, Malaysia; 5°21′N, 100°18′E) and reared for over 300 generations since the 1960s, was employed as the reference baseline for comparison.

Genomic DNA Extraction

Genomic DNA was extracted from individual adult *Ae. aegypti* mosquitoes that survived susceptibility bioassays using the PrimeWay Genomic II DNA Extraction Kit (Apical Scientific Sdn. Bhd., Malaysia), following the manufacturer's protocol with minor modifications to improve yield. The elution buffer was preheated to 60°C prior to extraction. Each mosquito was homogenized in 100 µL of GL1 buffer using a sterile pestle, which was rinsed with an additional 100 µL of GL1 buffer. Protein digestion was performed by adding 20 µL of Proteinase K and incubating at 60°C for 3 h with occasional inversion. After incubation, samples were centrifuged at 12,000 × g for 2.5 min at room temperature, and the supernatant was transferred to a new 1.5 mL microcentrifuge tube. A total of 200 µL of GL2 buffer was added, thoroughly mixed, followed by 4 µL of RNase A and incubation at room temperature for 5 min. DNA precipitation was induced by adding 200 µL of absolute ethanol and vortexing until homogeneous. A volume of 750 µL of the mixture was loaded onto a PrimeWay silica spin column and centrifuged at 12,000 × g for 1.2 min. The column was washed sequentially with 400 µL of Wash Buffer G1 and 600 µL of Wash Buffer G2, each followed by centrifugation at 12,000 × g for 35 s. An additional centrifugation at 12,000 × g for 3.5 min was performed to remove any residual ethanol.

DNA was eluted by adding 50 µL of preheated (60°C) elution buffer directly to the membrane, incubating for 3 min, and centrifuging at 12,000 × g for 35 s. DNA concentration and purity were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Extracted DNA was stored at −20°C until further analysis.

Detection of Knockdown Resistance (*kdr*) Mutations

Fragments of the voltage-gated sodium channel (VGSC) gene corresponding to domains II and III were amplified to detect *kdr* mutations at positions 989, 1007, 1011, 1016, 1520, and 1534. Each 30 µL PCR reaction contained 15 µL of OneTaq Quick-Load 2× Master Mix (domain II) or EconoTaq Plus Green 2× Master Mix (domain III), 3 µL each of forward and reverse primers, and 3 µL of genomic DNA. Reaction mixtures were briefly centrifuged prior to amplification. Domain II was amplified using primers AaSCF1 and AaSCR4, while domain III was amplified using primers AaSCF7 and AaSCR7 (Kawada et al. 2016a). PCR cycling conditions consisted of an initial denaturation at 94°C for 5 min, followed by 36 cycles of 94°C for 30 s, annealing at 50°C (domain II) or 60.4°C (domain III) for 30 s, and extension at 72°C for 1 min. A final extension at 72°C for 10 min was performed.

PCR products were separated on 1.7% agarose gels stained with FluoroSafe in 1× TAE buffer and electrophoresed at 140 V for 50 min. Amplicons were visualized under UV illumination, purified, and sent to MyTACG DNA Sequencing Services (Malaysia). Sequencing was performed using primers AaSCR8 for domain II and AaSCR9 for domain III (Kawada et al. 2016b).

RESULTS

Based on the mortality percentages and WHO susceptibility classifications in Figure 2, the highest level of resistance in *Ae. aegypti* from all localities was observed against the pyrethroid insecticides, deltamethrin and permethrin. Among these, deltamethrin showed the greatest overall resistance, particularly in Taman Bukit Jambul (TBJ), with the mortality being approximately 5%, indicating extreme resistance. Taman Machang Bubok (TMB) exhibited approximately 35% mortality (resistance), while Flat Sri Pauh (FSP) showed approximately 65% mortality (resistance). Overall, deltamethrin resistance was widespread, with no

population fully susceptible. Similarly low mortality values were also recorded for permethrin at the same site from TBJ (3%), confirming pronounced pyrethroid resistance. TMB recorded moderate mortality of approximately 45% (resistance), and Flat Sri Pauh showed high but still resistant-level mortality of about 85% (resistance), while Apartment Asoka (AA) was approximately 80% (resistance). Across all four locations, both deltamethrin and permethrin consistently failed to meet the WHO susceptibility threshold, indicating widespread, operationally significant resistance.

In comparison, pirimiphos-methyl showed moderate to high resistance in all localities, with mortality values of 30–65%, higher than those of the pyrethroids. However, no population of *Ae. aegypti* reached the WHO susceptibility threshold for pirimiphos-methyl. In contrast, malathion remained the most effective insecticide tested, which remained largely effective, except for possible resistance (95%) detected in TBJ. Malathion has largely retained its high efficacy compared to other insecticides.

The summary pattern of this study showed that pyrethroids, particularly deltamethrin, followed closely by permethrin, represent the insecticides with the highest levels of resistance among the studied *Ae. aegypti* Penang populations. The finding has critical implications for vector control programmes that continue to rely heavily on pyrethroid-based interventions.

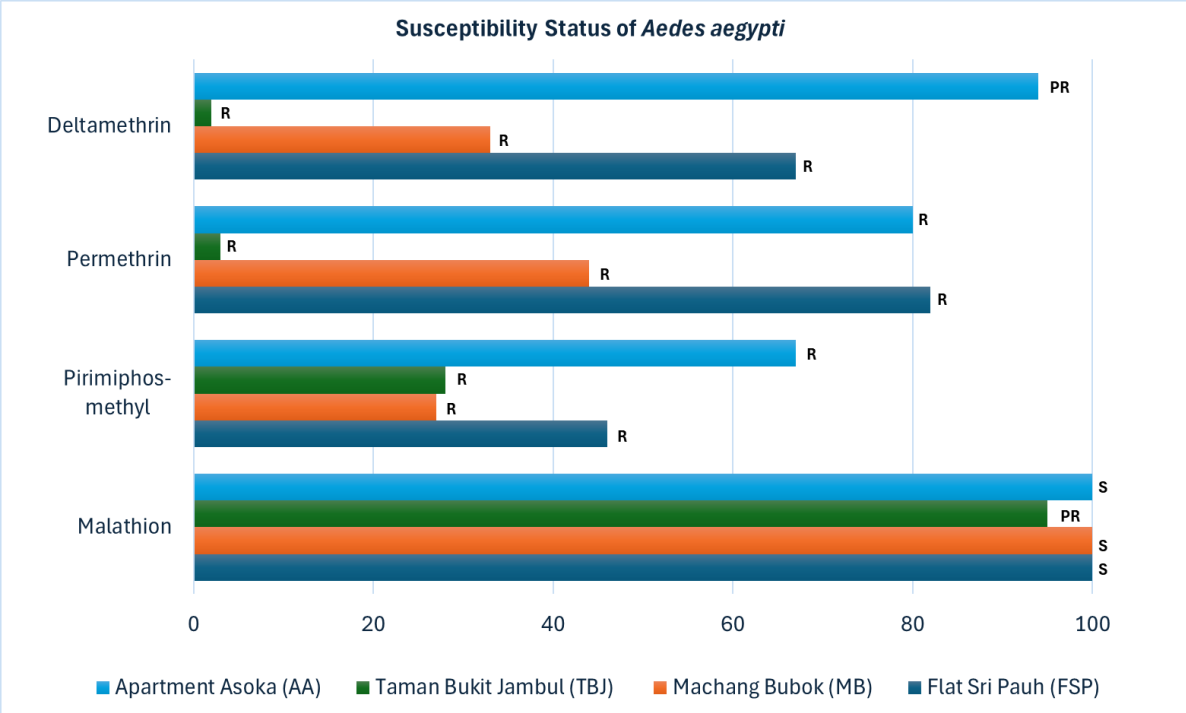

Figure 2. Insecticide susceptibility and resistance patterns of A*edes aegypti* populations from four study locations in Penang based on the WHO adult bioassay mortality; R- resistance, PR- possible resistance, S- susceptible

The distribution of voltage-gated sodium channel (*kdr*) mutations and their combinations differed across localities and between permethrin and deltamethrin, reflecting variation in the Selection pressure exerted by these pyrethroids, as shown in Figure 3.

Aedes aegypti TBJ strain exposed to permethrin showed multiple *kdr* mutations, including S989P, V1016G, T1520I, and F1534C, as well as a triple-mutation combination (1016/1520/1534) at 20%. The accumulation of multiple target-site mutations indicates long-standing and intense pyrethroid selection pressure, consistent with the extremely low mortality observed in bioassays from this area (mortality 3%; Figure 2). This indicates strong and complex target-site resistance. Meanwhile, in the FSP strain, permethrin-exposed populations showed limited mutation diversity, mainly involving S989P and F1534C, with no high-order combinations. Similar to TMB, which showed only detection at S989P, V1016P, and F1534C, with the highest frequency at V1016G (50%).

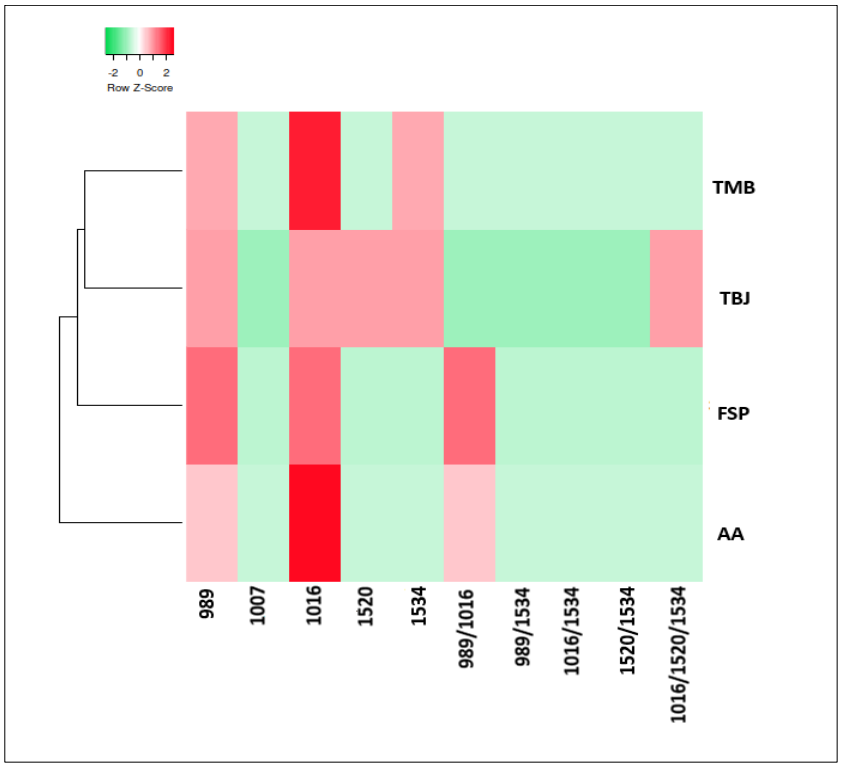

Figure 3. Heatmap and hierarchical clustering of k*dr* mutation frequencies for single and combined genotypes in *Aedes aegypti* permethrin-resistance populations across study localities

Whereas, *Ae. aegypti* strain from FSP exposed to deltamethrin showed the greatest selection of resistance variation (Figure 4). Deltamethrin-exposed mosquitoes exhibited a higher number of mutations, including S989P (14.3%), V1016G (28.6%), T1520I (14.3%), and F1534C (14.3%), as well as double-mutation combinations (989/1016 and 989/1534). Stronger selection pressure from deltamethrin was also observed in the TMB strain. Deltamethrin exposure showed a markedly higher frequency of mutations, particularly at V1016 (30%) and T1520I (20%), and a notable presence of double-mutation combinations (1016/1534 of 30%).

Across all sites, T1520, the newly detected resistance haplotype, was detected exclusively or predominantly in deltamethrin-exposed populations, with frequencies ranging from 14.3% to 25%, except in the TBJ strain, where it also appeared under permethrin (20%). Importantly, T1520 rarely occurred alone; it was often found together with other *kdr* mutations

(1520/1534), which is known to enhance resistance intensity beyond that conferred by single substitutions.

Taman Bukit Jambul showed the most advanced resistance profile, characterised by its high mutation counts and complex genotypes, supported by the low mortality in bioassay (Figure 1), Across localities, both insecticides showed classic pyrethroid *kdr* mutations, particularly S989P, V1016G, and F1534C, which are known to reduce pyrethroid binding to sodium channels. However, permethrin tended to be associated with more complex multi-mutation genotypes in areas with intense historical use, such as in the TBJ strain. In which the data indicate spatially heterogeneous resistance dynamics, with permethrin exerting stronger long-term selection leading to complex *kdr* haplotypes in some sites. Meanwhile, deltamethrin consistently showed higher mutation frequencies and combinations across several localities, including FSP and TMB.

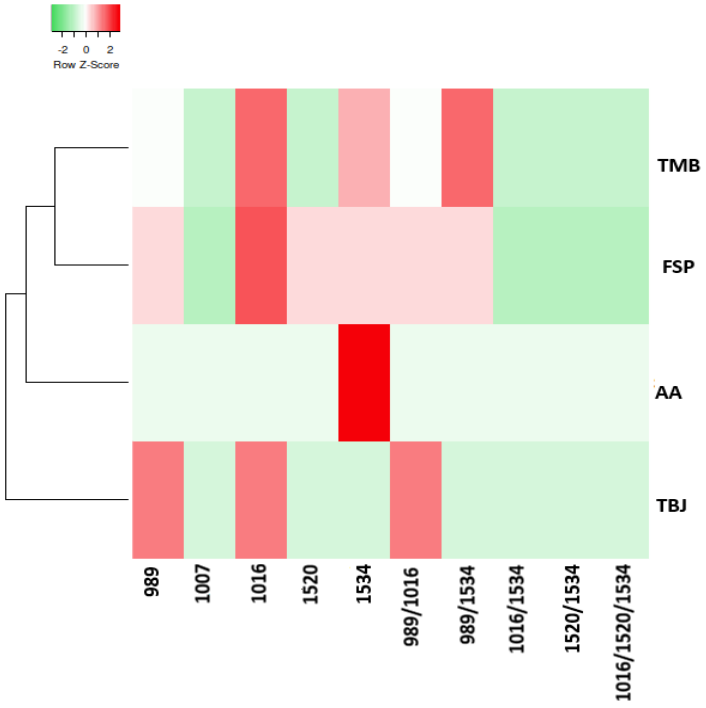

Figure 4. Heatmap and hierarchical clustering of *kd*r mutation frequencies for single and combined genotypes in *Aedes aegypti* deltamethrin-resistance populations across study localities

DISCUSSIONS

The adult WHO bioassay results clearly demonstrate that pyrethroid resistance is widespread and operationally severe in *Ae. aegypti* populations from Penang, with deltamethrin and permethrin consistently failing to reach susceptibility thresholds across all four study sites. The extremely low mortality recorded in the TBJ strain, particularly with deltamethrin (5%) and permethrin (3%), indicates a level of resistance unlikely to be overcome by operational dose adjustments alone. The selection pressure exerted by pyrethroid-based adulticides is a significant factor in the development of resistance among dengue vectors, particularly *Ae. aegypti*. The repeated application of these insecticides favours the survival and reproduction of

resistant individuals, thereby increasing the frequency of resistance over time (Scott 1990). This resistance arises from various mechanisms, including target-site insensitivity and metabolic resistance, which evolve in response to selection pressure (Coleman et al. 2007). In Malaysia, has shown widespread resistance to pyrethroids, particularly permethrin, with a notable increase in resistance rates over the past decade, especially in Selangor (Nurul-Nastasea et al. 2003). The study evaluated resistance levels of *Ae. aegypti* to pyrethroids resulted in 27% mortality with permethrin and 13% with deltamethrin, suggesting high resistance in mosquitoes in Selangor; even the addition of PBO failed to reduce the resistance level (Zuharah & Maryam 2021).

The contrasting performance of organophosphates further supports the presence of multiple resistance phenotypes within the studied populations. Although pirimiphos-methyl showed higher mortality than pyrethroids, none of the populations achieved full susceptibility, suggesting partial loss of efficacy and possible metabolic involvement. Biochemical assays have shown that metabolic enzymes, rather than target-site mutations, are more likely to be responsible for the observed resistance to pirimiphos-methyl in some populations (Wang et al. 2023). Enzymes like monooxygenases (MFOs), esterases, and glutathione S-transferases (GSTs) have been implicated in resistance to both organophosphates and pyrethroids (Namountougou et al. 2020).

In contrast, malathion largely retained effectiveness, with only possible resistance detected in one locality. This pattern is consistent with previous reports showing that malathion resistance often emerges later than pyrethroid resistance and may be more spatially restricted, depending on local application history. In regions where malathion was used extensively, such as in Cuba and India, resistance emerged due to prolonged exposure and selection pressure (Raghavendra et al. 2010). As malathion use has been reduced or discontinued in Penang since 1996, resistance levels have declined, indicating spatial and temporal restriction of resistance. Collectively, the bioassay data indicate a complex resistance landscape in which *Ae. aegypti* populations are no longer uniformly controllable using single-class chemical interventions.

Knockdown resistance (*kdr*) mutations in the voltage-gated sodium channel (VGSC) gene are a significant factor contributing to pyrethroid resistance in *Ae. aegypti* mosquitoes. Classical *kdr* substitutions (S989P, V1016G, and F1534C) were widely detected, with their frequencies and combinations varying markedly among localities and insecticides. The most resistant population (TBJ) exhibited the highest diversity of *kdr* mutations and the presence of triple-mutation genotypes under permethrin exposure, a pattern widely associated with high-intensity resistance and near-complete loss of pyrethroid efficacy. The V1016I and F1534C mutations are prevalent in the Western Hemisphere and are associated with strong pyrethroid resistance, particularly when homozygous (Tognarelli et al. 2025). Population genetic studies have demonstrated that such multi-locus *kdr* combinations substantially reduce the sodium channel sensitivity, leading to strong cross-resistance among pyrethroids. The presence of multiple *kdr* mutations can significantly increase resistance levels, with some combinations leading to 10- to several-hundred-fold increases (Uemura et al. 2024). For example, the 410L+1016I+1534C allele in Colombia conferred varying levels of resistance to different pyrethroids, highlighting the complexity of resistance mechanisms (Silva et al. 2021).

Notably, deltamethrin exposure appeared to exert stronger and more consistent selection pressure across multiple sites, as evidenced by higher mutation frequencies and more frequent double-mutation genotypes in FSP and TMB. This suggests that, despite being a more potent type II pyrethroid, deltamethrin may impose a sharper selective gradient on mosquito

populations when used intensively. This phenomenon is particularly evident in areas with high agricultural activity, where deltamethrin is frequently used (Hien et al. 2017). The study on *Anopheles gambiae* has demonstrated that deltamethrin can rapidly select for resistance, with resistance levels increasing significantly over generations and a notable rise in the frequency of resistance-associated mutations, such as the L1014F *kdr* mutation (Zoh et al. 2023).

A key and novel finding of this study is the detection of the T1520I mutation in Penang, predominantly in deltamethrin-exposed populations and rarely occurring as a single substitution. Instead, T1520I was most often observed in combination with other *kdr* mutations, particularly F1534C. This is supported by Kushwah et al. (2015), who observed that the T1520I mutation is tightly linked to F1534C, suggesting a compensatory role that enhances the resistance conferred by F1534C. Emerging evidence from structural and electrophysiological studies suggests that T1520I may act as a resistance-modifying mutation, enhancing the resistance conferred by primary *kdr* substitutions rather than acting independently (Chen at al. 2019). Its preferential association with deltamethrin exposure in this study supports the hypothesis that T1520I is selected under high-intensity or prolonged pyrethroid pressure.

CONCLUSION

In conclusion, the convergence of bioassay failure, multiple *kdr* mutations, and the emergence of T1520I indicates that, *Ae. aegypti* populations in Penang are undergoing an advanced stage of resistance development. This has significant implications for vector control programmes, particularly those that are still reliant on pyrethroid-based adulticide. The observed spatial heterogeneity in mutation profiles further implies that resistance evolution is shaped not only by insecticide chemistry but also by local operational histories, insecticide rotation practices, and possibly domestic consumer product usage. Without such adaptive management strategies, continued chemical pressure is likely to accelerate the fixation of resistance and further undermine dengue control efforts.

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AUTHORS DECLARATIONS

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Conflict Of Interests
All authors confirm that there are no conflicts of interest that could influence the reported findings in this paper.

Ethics Declarations
No ethical approval was required for this study, as the work did not involve human participants, animal handling/experimentation, or sensitive personal data.

Data Availability Statements
All data generated or analysed during this study are included in this published article.

Authors' Contributions
Wan Fatma Zuharah conceptualized and designed the study, conducted the experiments, analyzed the data, and drafted the original manuscript. She also provided overall scientific direction, contributed to data interpretation, and critically revised the manuscript for intellectual content. Ma Teng, Asfa Nurizzah, and Fatin Nabila helped in data collection and conducted the experiments.

REFERENCES

Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., et al. 2013. The global distribution and burden of dengue. *Nature* 496(7446): 504–507.

Brito, L.P., Carrara, L., Freitas, R.M.D., Lima, J.B.P. & Martins, A.J. 2018. Levels of resistance to pyrethroid among distinct kdr alleles in *Aedes aegypti* laboratory lines and frequency of kdr alleles in 27 natural populations from Rio de Janeiro, Brazil. *BioMed research international* 2018(1): 2410819.

Chen, M., Du, Y., Wu, S., Nomura, Y., Zhu, G., Zhorov, B.S. & Dong, K. 2019. Molecular evidence of sequential evolution of DDT- and pyrethroid-resistant sodium channels in *Aedes aegypti*. *PLoS Neglected Tropical Diseases* 13(6): e0007432.

Coleman, M. & Hemingway, J. 2007. Insecticide resistance monitoring and evaluation in disease-transmitting mosquitoes. *Journal of Pesticide Science* 32(2): 69–76.

Collins, E., Quintana, J., Morales, R., Martínez, K., Barrera, R. & Mackay, A.J. 2025. Profiling insecticide resistance phenotypes and genotypes in *Aedes aegypti* populations across four regions in Puerto Rico. *Scientific Reports* 15: 3709.

Du, Y., Nomura, Y., Zhorov, B.S. & Dong, K. 2016. Sodium channel mutations and pyrethroid resistance in *Aedes aegypti*. *Insects* 7(4): 60.

Fernando, H.S.D., Saavedra-Rodriguez, K., Perera, R. & Black, W.C.IV. 2020. Resistance to commonly used insecticides and underlying mechanisms of resistance in *Aedes aegypti* (L.) from Sri Lanka. *Parasites & Vectors* 13: 393.

Hien, A.S., Soma, D.D., Hema, O., Bayili, B., Namountougou, M., Gnankiné, O., Baldet, T., Diabaté, A. & Dabiré, K.R. 2017. Evidence that agricultural use of pesticides selects pyrethroid resistance within Anopheles gambiae sl populations from cotton growing areas in Burkina Faso, West Africa. *PLoS One* 12(3): e0173098.

Kawada, H., Higa, Y., Komagata, O., Kasai, S., Tomita, T., Nguyen, T.Y., et al. 2009. Widespread distribution of a newly found point mutation in the voltage-gated sodium channel in pyrethroid-resistant *Aedes aegypti* populations in Vietnam. *PLoS Neglected Tropical Diseases* 3(10): e527.

Kawada, H., Oo, S.Z.M., Thaung, S., Kawashima, E., Maung, Y.N.M., Thu, H.M., et al. 2016a. Co-occurrence of point mutations in the voltage-gated sodium channel of pyrethroid-resistant *Aedes aegypti* populations in Myanmar. *PLoS Neglected Tropical Diseases* 10(7): e0004765.

Kawada, H., Higa, Y., Futami, K., Muranami, Y., Kawashima, E., Osei, J.H.N., et al. 2016b. Discovery of point mutations in the voltage-gated sodium channel gene of *Aedes aegypti* populations in Myanmar. *PLoS Neglected Tropical Diseases* 10(7): e0004780.

Kushwah, R.B.S., Cherry L.D., Neera K., Tridibes A. & Singh, O.P. 2015. Pyrethroid-resistance and presence of two knockdown resistance (*kdr*) mutations, F1534C and a

novel mutation T1520I, in Indian *Aedes aegypti. PLoS Neglected Tropical Diseases* 9 (1): e3332.

Linss, J.G.B., Brito, L.P., Garcia, G.A., Araki, A.S., Bruno, R.V., Lima, J.B.P., et al. 2014. Distribution and dissemination of the Val1016Ile and Phe1534Cys knockdown resistance mutations in Brazilian *Aedes aegypti* populations. *Parasites & Vectors* 7: 25.

Liu, H., Xu, Q., Zhang, L. & Liu, N. 2005. Chlorpyrifos resistance in mosquito *Culex quinquefasciatus*. *Journal of Medical Entomology* 42(5): 815–820.

Moyes, C.L., Vontas, J., Martins, A.J., Ng, L.C., Koou, S.Y., Dusfour, I., et al. 2017. Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *PLoS Neglected Tropical Diseases* 11(7): e0005625.

Namountougou, M., Soma, D.D., Balboné, M., Kaboré, D.A., Kientega, M., Hien, A.S., et al. 2020. Monitoring insecticide susceptibility in *Aedes aegypti* populations from Burkina Faso: Implication of metabolic resistance. *Tropical Medicine and Infectious Disease* 5(2): 84.

Nurul-Nastasea, S., Yu, K-X., Raza, A., Mohamed Nor, Z., Tengku-Idris, T.I.N., Dianita, R., et al. 2023. Insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* in Malaysia (2010–2022): A review. *Asian Pacific Journal of Tropical Medicine* 16(10): 434–445.

Raghavendra, K., Verma, V., Srivastava, H., Gunasekaran, K., Sreehari, U. & Dash, A.P. 2010. Persistence of DDT, malathion and deltamethrin resistance in *Anopheles culicifacies* after their withdrawal from indoor residual spraying in India. *Indian Journal of Medical Research* 132(3): 260–264.

Rubio-Palis, Y., Dzuris, N., Sandi, C., Vizcaino-Cabarrus, R.L., Corredor-Medina, C., González, J.A. & Lenhart, A.E. 2023. Insecticide resistance levels and associated mechanisms in three *Aedes aegypti* populations from Venezuela. *Mem Inst Oswaldo Cruz* 118: e220210.

Scott, J.G. 1990. Investigating mechanisms of insecticide resistance: Methods, strategies, and pitfalls. In Roush, R.T. & Tabashnik, B.E. (eds.). *Pesticide Resistance in Arthropods*, pp. 39–57. New York: Springer,

Silva, J.J., Kouam, C.N. & Scott, J.G. 2021. Levels of cross-resistance to pyrethroids conferred by the Vssc knockdown resistance allele 410L+1016I+1534C in *Aedes aegypti*. *PLoS Neglected Tropical Diseases* 15(7): e0009549.

Stenhouse, S.A., Plernsub, S., Yanola, J., Lumjuan, N., Dantrakool, A., Choochote, W. & Somboon, P. 2013. Detection of the V1016G mutation in the voltage-gated sodium channel gene of *Aedes aegypti* and its effect on deltamethrin resistance in Thailand. *Parasites & Vectors* 6: 253.

Tognarelli, J., Moya, P.R., González, C.R. & Collao-Ferrada, X. 2025. Global distribution and impact of knockdown resistance mutations in *Aedes aegypti* on pyrethroid resistance. *Parasites & Vectors* 18(1): 382.

Uemura, N., Itokawa, K., Komagata, O. & Kasai, S. 2024. Recent advances in the study of knockdown resistance (*kdr*) mutations in *Aedes* mosquitoes. *Current Opinion in Insect Science* 63: 101178.

Ullah, M.A. 2024. Breaking the fever: Global strategies for sustainable dengue recovery and prevention. *Borneo Medical Journal* 10(1): 1–3.

Wang, Y., Wang, X., Brown, D.J., An, M., Xue, R-D. & Liu, N. 2023. Insecticide resistance: Status and potential mechanisms in *Aedes aegypti*. *Pesticide Biochemistry and Physiology* 195: 105577.

World Health Organization (WHO). 2022. *Manual For Monitoring Insecticide Resistance in Mosquito Vectors and Selecting Appropriate Interventions*. Geneva: World Health Organization.

Yanola, J., Somboon, P., Walton, C., Nachaiwieng, W., Somwang, P. & Prapanthadara, L.A. 2011. High-throughput assays for detection of the F1534C mutation in the voltage-gated sodium channel gene in permethrin-resistant *Aedes aegypti* and the distribution of this mutation throughout Thailand. *Tropical Medicine & International Health* 16(4): 501-509.

Zoh, M.G., Bonneville, J.M., Laporte, F., Tutagata, J., Sadia, C.G., Fodjo, B.K., Mouhamadou, C.S., McBeath, J., Schmitt, F., Horstmann, S. & Reynaud, S. 2023. Deltamethrin and transfluthrin select for distinct transcriptomic responses in the malaria vector Anopheles gambiae. *Malaria Journal* 22(1): 256.

Zongo, S., Toe, H.K., Guelbeogo, M.W., Sanou, A., Traore, A. & Dabiré, R.K. 2025. Spatial distribution of insecticide resistance in *Aedes aegypti* across Burkina Faso highlights strengthened resistance management strategies. *Acta Tropica* 271: 107847.

Zuharah, W.F. & Sufian, M. 2021. The discovery of a novel knockdown resistance (*kdr)* mutation A1007G on *Aedes aegypti* (Diptera: Culicidae) from Malaysia. *Scientific Reports* 11(1): 5180.