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SHORT COMMUNICATION

Molecular evidence of knockdown resistance (*kdr*) mutations in the head lice (*Pediculus humanus capitis*) collected from disadvantaged children in Klang Valley, Malaysia

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ABSTRACT

Pediculosis capitis, caused by infestation of the human head louse *Pediculus humanus capitis* is endemic all over the world, and Malaysia is no exception. Permethrin is recommended as one of the first-line treatments for pediculosis capitis. However, after decades of intensive and continuous use, numerous treatment failures and recurrent cases have been reported globally due to established evidence of permethrin resistance. The fact that permethrin-based products are still widely used and available over the counter prompted this study to investigate the genotypic basis of permethrin resistance and to identify *kdr* alleles in head lice collected from seven children shelters located across Klang Valley, Malaysia. The PCR-RFLP employed in this study successfully demonstrated the *kdr* T917I mutation in head lice resulting in two genotypes: 31 (49.21%) homozygous susceptible (SS) and 32 (50.79%) heterozygous resistant (RS). These findings provide baseline data on permethrin resistance in Malaysia, which has not been previously investigated.

Keywords: Head lice; insecticide resistance; permethrin; *kdr*; Malaysia.

INTRODUCTION

In Malaysia, pediculosis capitis is one of the most reported ectoparasitic infestations among primary school children and children in welfare institutions. Permethrin is a synthetic pyrethroid used as a first line treatment to treat pediculosis capitis. Typically used as 1% cream on the scalp and hair, this topical pediculicide acts as a neurotoxin by interfering with sodium transport in the head lice leading to depolarization of neuromembranes and respiratory paralysis and eventually resulting in the death of the head lice (Ko & Elston, 2004). Generally, permethrin has the advantage of being both pediculicidal and ovicidal, with minimal toxicity, making it the pediculicide of choice (Leung *et al*., 2022). However, after decades of intensive and continuous use of permethrin, many treatment failures and recurrent cases due to established evidence of permethrin resistance have been reported worldwide.

The knockdown resistance (*kdr*) due to three-point mutation of amino acid substitutions on the M815I, T917I, and L920F of the α-subunit of voltage sensitive sodium channel (*VSSC*) gene is a major attribute of resistant lice. M815I and L920F mutations reduce susceptibility to permethrin whereas T917I mutation, whenever present alone or combination with L920F mutation, is key in developing permethrin resistance and commonly used as a molecular biomarker to detect permethrin resistance in head lice (Brownell *et al*., 2020).

Numerous epidemiological studies have been conducted to assess the prevalence of pediculosis capitis in Malaysian children since 1981, with a continuous significant increasing trend. Therefore, a crucial understanding of permethrin resistance is necessary for strategizing effective treatment and alternative therapeutic measures for a better prevention and control of pediculosis capitis especially among underprivileged children of Malaysia. This study aims to investigate the phenotypic and genotypic basis of permethrin resistance status in the head lice amongst disadvantaged children from Klang Valley, which has not been investigated before.

MATERIALS AND METHODS

This study was approved for the protocol involving human subjects by the UMMC Medical Research Ethics Committee (MEC ID NO: 2024813-14045). Informed assents were obtained from the participants and their legal guardians provided informed consent for this study.

Head lice sampling was conducted in Klang Valley, Malaysia from October 2024 to January 2025. Seven welfare homes sheltering orphans, neglected children and refugees were randomly selected to represent the appropriate host population. The sample size was determined using the Slovin's formula, $n = N / (1 + Ne^2)$, assuming an estimated population of approximately 300 children residing in the selected institutions, a margin of error of 5%, and a confidence level of 95%, yielding a minimum of 171 individuals to be screened. A total of 172 children were examined for the presence of head lice and pediculosis capitis is confirmed when living adults, nymphs or viable nits were detected. Head lice were combed from the hair and

transferred into a microcentrifuge tube containing 70% ethanol for preservation. All specimens were brought back to the Arthropod Laboratory, Department of Parasitology, Universiti Malaya for further processing.

From each infested child, one adult louse was randomly selected, and a total of 63 head lice DNA were extracted using the PrimeWay Genomic II DNA Extraction Kit (Apical Scientific, Malaysia). To screen the T917I mutation by using PCR-RFLP, the α-subunit of voltage sensitive sodium channel (VSSC) gene were amplified according to Durand *et al*. (2007). 10ul of the resulting 332-bp PCR products were digested with SspI enzyme (Thermo Fisher Scientific, USA) according to manufacturer's recommendations. The digested products were separated on 3% agarose gel electrophoresis at 80 V for 50 min.

To confirm the reliability of RFLP result, randomly selected samples from two genotypes (SS and RS) were then subjected to cycle sequencing performed by Apical Scientific Sdn. Bhd. (Selangor, Malaysia) using both forward and reverse primers. The VSSC gene partial sequences obtained were aligned and compared with the published wild-type sequence (Genbank Accession No.: AY191156) using BioEdit Sequence Alignment Editor Software, Version 7.7.1 (Hall, 1999).

The frequency of resistance alleles was calculated by dividing the total number of resistance alleles by the total number of alleles at the locus. Genotype frequencies were assessed for the Hardy-Weinberg expectations using a chi-square (χ^2) goodness of fit (Black & Krafsur, 1985). Wright's inbreeding coefficient (F_{is}) was used to test the departure from Hardy-Weinberg equilibrium (HWE) particularly heterozygous deficiency or excess using the formula:

$$F_{is} = 1 - (H_{obs} / \mathbf{H}_{exp});$$

where Hobs is the number of heterozygous observed genotype and Hexp is number of heterozygous expected genotype.

RESULTS

To detect the *kdr* T917I mutation associated with pyrethroid resistance, RFLP analysis was carried out using the SspI restriction enzyme, which recognizes the sequence AAT|ATT. In the RR genotype (homozygous mutant), the enzyme fully digests the DNA, producing two fragments of 261 bp and 71 bp. In the RS genotype (heterozygous), partial digestion occurs, resulting in three fragments: 332 bp, 261 bp, and 71 bp. In contrast, the SS genotype (homozygous wild type) lacks the restriction site, so the DNA remains uncut, showing a single band of 332 bp (Figure 1).

Of the 63 head lice, the SS and RS genotypes were detected in 31 (49.21%) and 32 (50.79%) head lice, respectively. Overall, the frequency of the *kdr* T917I mutation was 0.25. The distribution of *kdr* genotypes frequencies were calculated from only five shelters (Table 1). The remaining two shelters with only one genotype (RR)

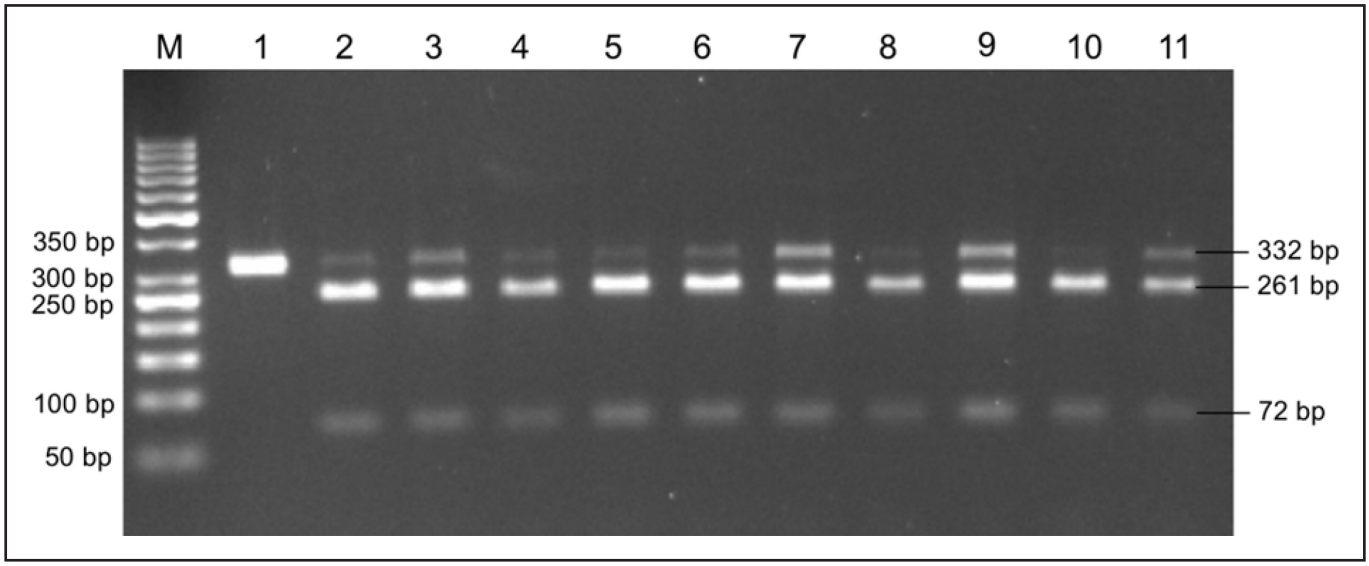

Figure 1. 3% agarose gel electrophoresis demonstrates the RFLP patterns of *kdr* T917I genotypes. Lane 1 is a representative of the homozygous susceptible or wild type (SS). Lanes 2-11 are representing heterozygous genotypes (RS) presented with three bands. Lane M; 50-bp DNA ladder.

Table 1. Frequency of *kdr* T917I genotypes detected in this study

Sampling site	No. of head lice	*kdr* genotypes				HW (χ^2)	F_{is}
		RR (%)	RS (%)	SS (%)	Frequency of mutation		
United Arakan Institute	2	0 (0.00)	1 (50.00)	1 (50.00)	0.25	0.22	-0.33[b]
HRIPHI Refugee School	2	0 (0.00)	0 (0.00)	2 (100.00)	0.00	–	–
RACTAR Orphanage	7	0 (0.00)	3 (42.86)	4 (57.14)	0.21	0.52	-0.27[b]
Pure Life Society	11	0 (0.00)	10 (90.91)	1 (9.09)	0.45	7.64[a]	-0.83[b]
Baitul Ehsan	23	0 (0.00)	5 (21.74)	18 (78.26)	0.11	0.34	-0.12[b]
Akademi Tahfiz Al-Hidayah	3	0 (0.00)	0 (0.00)	3 (100.00)	0.00	–	–
Yayasan Sunbeams Home	15	0 (0.00)	13 (86.67)	2 (13.33)	0.43	8.77[a]	-0.76[b]
Total	63	0 (0.00)	32 (50.79)	31 (49.21)	0.25	7.30[a]	-0.34[b]

[a]Not in Hardy-Weinberg equilibrium ($P < 0.05$; χ^2=3.84).
[b]$F_{is} > 0$ indicates deficit of heterozygotes (homozygous excess), while $F_{is} < 0$ indicates excess of heterozygotes (homozygous deficiency).

did not meet the assumptions required for HWE. Statistical analysis shows two shelters deviated from HWE, whereas HWE hold in three shelters. Overall, the chi-square of 7.30 shows a significant deviation from HWE. However, most of this deviation is due to the absence of RR genotype. In addition, the *Fis* < 0 from all five shelters indicates excess of heterozygotes (homozygous deficiency). The distribution of *kdr* T917I genotypes is summarised in Figure 2. Sequencing results of RS representative samples revealed non-synonymous mutations in both codons 917 and 920, resulting in C→T substitution, leading to Thr (ACA)→Ile (ATA) mutation and Leu (CTT)→Phe (TTT) mutation, respectively (Figure 3). SS head lice showed no substitution in both codons. Table 1 summarizes the frequency of *kdr* T917I genotypes in head lice collected from seven children shelters in Klang Valley.

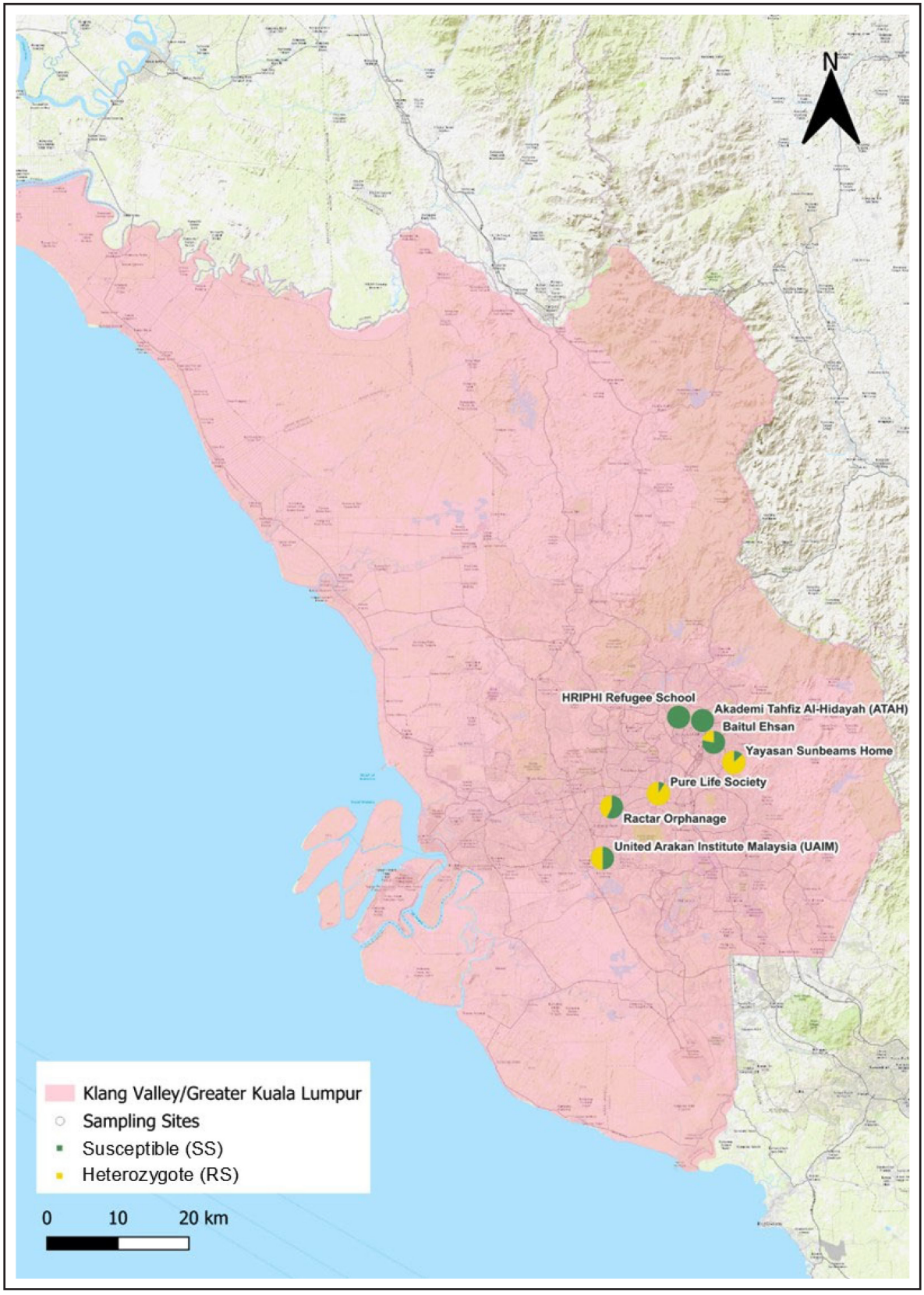

Figure 2. Distribution of *kdr* T917I genotypes of head lice collected from children shelters in the Klang Valley.

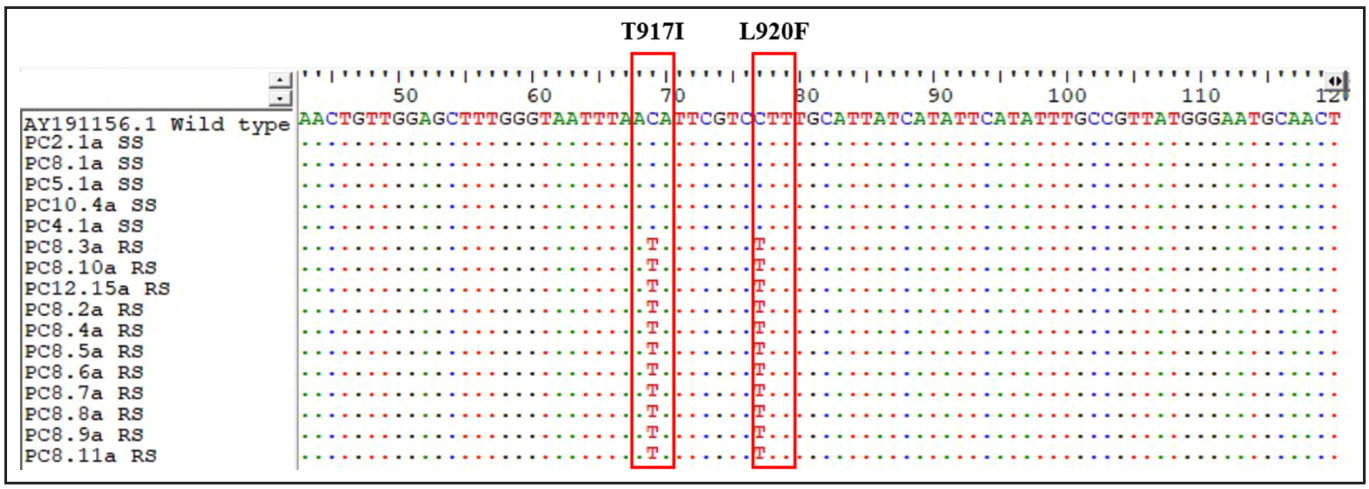

Figure 3. ClustalW alignment of *kdr* partial nucleotide sequences of SS and RS head lice collected in this study. The position of *kdr* T917I and L920F mutations are indicated by the red vertical column.

DISCUSSION

Permethrin is a neurotoxic synthetic pyrethroid that exerts its insecticidal action primarily by targeting voltage-gated sodium channels. It disrupts sodium transport across neuronal membranes in arthropods, inducing depolarization in the nervous system and ultimately leads to respiratory paralysis of affected arthropods (Nanda *et al*., 2025). However, the widespread use of permethrin has led to the emergence of resistance in various insect populations, including head lice. This resistance is often associated with mutations in the α-subunit of voltage sensitive sodium channel (*VSSC*) gene and commonly referred to as knockdown resistance (*kdr*) mutations. In this study, the VSSC gene was selected as the molecular target because it encodes the α-subunit where knockdown resistance (*kdr*) mutations occur, directly affecting the pyrethroid binding site. Hence, the VSSC gene serves as a validated molecular biomarker for detecting pyrethroid resistance in arthropods, including head lice (Durand *et al*., 2007; Brownell *et al*., 2020).

This issue is a growing concern with the worldwide prevalence has been estimated at 59% (Abbasi *et al*., 2023). Recently, the occurrence of permethrin resistance has been reported in several Asian countries including Thailand (Brownell *et al*., 2020), India (Mallick *et al*., 2023), Iran (Ghahvechi-Khaligh *et al*., 2021; Mohammadi *et al*., 2022) and Saudi Arabia (Alsaady *et al*., 2023; Alghashmari *et al*., 2025), to name a few. In Malaysia, despite a substantial number of studies on pediculosis capitis, especially on its prevalence among underprivileged children, data on the pediculicides resistance is not available.

The genotype distribution presented in Table 1 highlights a predominance of heterozygotes and an absence of homozygous resistant head lice, suggesting an incipient resistance stage within the population. Future studies should therefore focus on monitoring allele frequency shifts relative to permethrin usage patterns and assessing whether co-occurring mutations such as M815I and L920F may synergistically enhance resistance expression. Given the emergence of the *kdr*-associated resistance, reliance on permethrin alone is unsustainable. Alternative pediculicides such as benzyl benzoate lotion and dimethicone-based products have demonstrated high efficacy and safety in children (Leung *et al*., 2022). Community-based control programs should also incorporate mechanical removal of head lice (wet combing), education on personal hygiene, and treatment of close contacts to reduce infestation cycles. For resource-limited populations, integrating school-based screening, regular health education, and access to pesticide-free treatments may provide a sustainable and culturally acceptable approach to manage pediculosis capitis.

Our study has shown that PCR-RFLP is a promising technique to detect *kdr* mutations in head lice, as verified by sequencing result. However, our findings might not be sufficiently powered due to small sample size and hence might not represent the actual situation of permethrin resistance in the Klang Valley. To strengthen future analysis, larger and geographically stratified sampling across different states should be conducted to increase statistical power and capture local variations in allele frequency. Additionally, integrating phenotypic bioassays with molecular genotyping would enhance correlation between genotype and resistance phenotype. Longitudinal surveillance at the same institutions could also help to determine whether resistance alleles are increasing over time, providing a more accurate assessment of permethrin resistance dynamics. Nonetheless, this report constitutes the first molecular evidence of *kdr* T917I mutation in Malaysia, underscoring the need for ongoing surveillance and development of alternative treatment strategies in guiding public health policies especially among underprivileged children of Malaysia.

Conflict of interests

The author declares that they have no conflict of interests.

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