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ORIGINAL RESEARCH

Mapping Knock-Down Resistance Mutations in Head-Louse Populations in Jazan, Saudi Arabia

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Introduction: Pediculosis *capitis* remains a persistent public-health concern. Although pyrethroids are still the first-line treatment, their effectiveness is threatened by knock-down resistance (kdr), which arises from point mutations in the voltage-sensitive sodium channel (VSSC). This study aimed to assess the prevalence of kdr mutations in head lice populations across different regions of Jazan, Saudi Arabia.
Methods: In this study, 59 head-louse specimens from multiple regions of Jazan were analyzed by PCR amplification and Sanger sequencing of a 908-bp fragment of the VSSC α-subunit gene.
Results: The T917I mutation showed the highest allele frequency (0.38) and was concentrated in Jazan's southern and eastern border areas.
Discussion: While overall kdr prevalence in Jazan is low, targeted surveillance is warranted in these regions, where resistance appears to be emerging.
Keywords: Pediculosis *capitis*, knock-down resistance, voltage-sensitive sodium channel, T917I, Jazan

Introduction

Pediculus humanus capitis is an obligate blood-sucking ectoparasite that infects human scalp.[1] The infection may be asymptomatic, or it may be severe, leading to excessive itching and scratching of the skin.[2]

Previous and recent records indicate a rising increase in the prevalence of *Pediculus* infestation with time. In 2021, Baghdadi et al reported 87.86% *Pediculus* infestation in the Eastern Region, Saudi Arabia, amongst 750 participants.[3] In a study on 438 participants in Makkah, Western Region, Saudi Arabia, Assaedi et al found a high infestation degree of head lice (64.2%).[4] Another study conducted in 2017 in Jeddah, Western Region, Saudi Arabia, showed *Pediculus* infestation of 31% amongst 337 female secondary school students.[5] In 2016, a prevalence of 45.45% was recorded in Albaha, Southern Region, Saudi Arabia, amongst 672 primary schoolgirls.[6]

However, in earlier studies, head louse infestation was less common. The prevalence of pediculosis was between 5 and 13% amongst study participants in various areas: 5.2% in Al-khobar, Eastern Region, Saudi Arabia;[7] 9.6% in Abha, Southern Region, Saudi Arabia;[8] 12% in Jeddah, Western Region, Saudi Arabia,[9] and 13.3% in Jazan, Southern Region, Saudi Arabia.[10] This increase in *Pediculus* infestation needs to be taken into consideration in the treatment of head lice, as it is a sign of resistance.

Several studies have shown that the resistance of head lice to insecticide might be related to pyrethroid-associated knockdown mutation (*kdr*).[11,12] This is especially relevant to Jazan due to growing local concerns about treatment resistance. This is due to the fact that pyrethroid is the most commonly used insecticide for combating lice.[13] A study in Jeddah showed that the frequency of pyrethroid-resistant alleles was 62.2%, with all alleles being homozygous in T917I.[14] Another study in Riyadh showed higher frequency (83%) of homo- and heterozygous resistant alleles in T917I, when compared to 75% homozygous resistant alleles in L920H, in addition to novel double mutations in V966F and F967L with fequencies of 90 and 85%, respectively.[15] In view of the high frequency of resistance, more studies are needed to identify the *kdr* mutations in other regions of Saudi Arabia.

The aim of study was to determine the frequency of resistance-linked mutation associated with pyrethroid treatment in Jazan.

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Materials and Methods

The steps of the methodology are shown in the flowchart in Figure 1.

Louse Samples

Fifty-nine samples of adult lice were collected by dry combing from 4 to 6 years old female kindergarten children in different schools in Jazan regions, using a metal comb, in line with relevant guidelines and regulations. The number of samples was determined based on the need to represent different subregions within Jazan, and the availability of samples during surveillance period. This sample size was considered sufficient for initial detection of kdr mutations in Jazan, based on a previous study that used 45 samples to investigate kdr mutations in different regions of Jeddah.[14]

Each sample was placed in a sterile microcentrifuge tube, transported to the laboratory in an ice box and stored at −20°C for further analysis. All samples were examined under a magnifying lens to separate the larger, well-characterized adult lice from nymphs. Eggs were also excluded from the analysis. This study was conducted in accordance with the

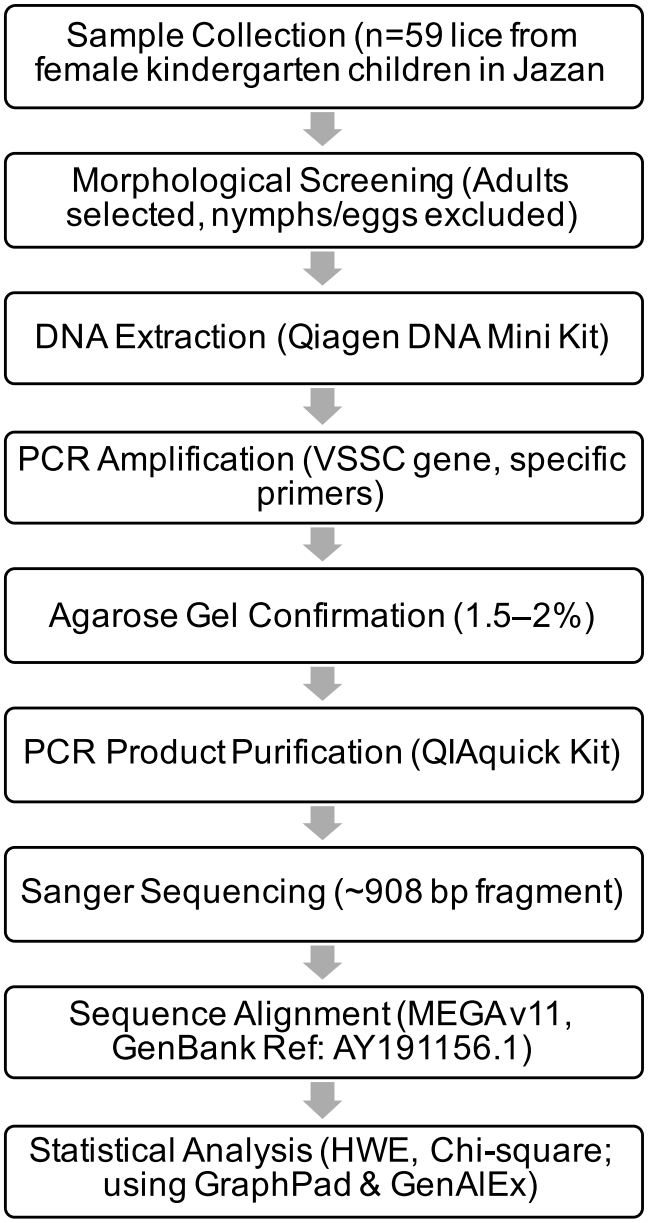

Figure 1 Flowchart of the workflow from sample collection to statistical analysis.

ethical principles outlined in the Declaration of Helsinki. The study was approved by the Biomedical Ethics Committee of King Abdulaziz University Faculty of Medicine (approval reference no.: 130-2). Informed consent for this study was obtained from the legal guardians of the participants.

DNA Extraction and Conventional PCR

After excluding nymphs and eggs, the adult louse samples were homogenized, and DNA was extracted using Qiagen DNA Mini Kit (Cat. No. 51304) in accordance with the kit manufacturer's protocol. The VSSC α-subunit gene PCR was done as described earlier using Trans 2X EasyTaq PCR SuperMix (+Dye) Cat. no. AS111-AJ-11.[16] The primers were selected based on a previous study: 5′-ATTTTGCGTTTGGGACTGCTGTT; 3′ (sense) and 5′CCATCTGGGAAGTTCTTTATCCA (antisense).[17] The PCR conditions were initial denaturation for 5 min at 94 °C and 35 cycles of denaturation for 45 sec at 94 °C, annealing for 45 sec at 55 °C, extension at 72 °C for 1 min, and final primer extension for 7 min at 72 °C.

The PCR products were loaded on 1.5–2% Agarose gel and visualized using Qiagen DNA ladder (GelPilot 100 bp Plus ladder, Cat. No. 239045).

DNA Sequencing

PCR products were purified using a PCR purification kit (QIAquick PCR Purification Kit, Qiagen) following the manufacturer's instructions. Purified amplicons were assessed for quality using a nanodrop spectrophotometer before proceeding to sequencing. Sanger sequencing of 908 bp gDNA fragment of the VSSC a-subunit gene was done in order to identify the resistant and susceptible genes in the samples.[17] Negative controls were included in each run for quality control. Molecular Evolutionary Genetics Analysis version 11 was used for aligning and visualizing sample sequence, with reference to GenBank ID: AY191156.1.

Statistical Analysis

The percentage of each genotype (RR, RS, and SS) was calculated for each sample. In addition, Hardy–Weinberg expectations with Wright's inbreeding coefficient were used to calculate the frequencies of expected genotype. Then, chi square test was applied to compare Hardy–Weinberg expectations with the observed counts. Analysis was done using GraphPad Prism version 9 and GenAlEx version 6.5.

Results

Head lice samples were taken from female nursery children (4–6 years) in Northern (n=15), Southern (n=15), Eastern (n=15) and Western (n=14) regions of Jazan in order to study the likelihood of head louse resistance to pyrethrin in this location.

Sanger sequencing was done for all samples, and bioinformatic analysis was applied to determine the presence and heterogenicity of mutations in VSSC gene. The results showed that V966F and F967L were absent in all tested samples, an indication of wild-type alleles.

The other three loci: T917I, L920H and M815I showed the absence of heterozygotes (RS=0). High Fis indicated perfect heterozygotes deficit (Table 1).

Sampling was done from the north, south, east and west of Jazan. The distribution of T917I, L920H and M815I mutations is illustrated in Table 2:

The results showed that northern Jazan had wild type VSSC gene (χ^2 = 0), as it lacked mutations. However, the southern area of Jazan showed high frequencies of the three mutations T917I, L920H and M815I (0.7, 0.6 and 0.6, respectively) and (χ^2 = 1, 1.01 and1.0, respectively), with a positive Fis which indicated a heterozygote deficit. The eastern area of Jazan showed high frequency (0.7) of T917I (χ^2 = 1) and low frequency (0.3) of L920H (χ^2 = 1). In the western area of Jazan, only one mutation was detected in T917I at low frequency of 0.2 (χ^2 = 1). Thus, the population deviated significantly from HW equilibrium.

Discussion

In this study, pyrethroid-resistant mutation was determined in Jazan, Saudi Arabia. Three point mutations were detected in T917I, L920H, and M815I.

Table 1 Genotype Distribution of Kdr Mutations in Jazan (n=59)

Mutation	Genotype			Frequency of Mutated Allele	HW	χ^2	F_{is}	Interpretation
	SS	RS	RR					
T917I (ACA>ATA)	0.62	0	0.38	0.380	0.384	1.000	1.000	SS above HW by ≈ 0.24
L920H (CTT>CAT)	0.77	0	0.23	0.230	0.593	1.000	1.000	SS above HW by ≈ 0.18
M815I (ATG>ATT)	0.85	0	0.15	0.150	0.722	1.000	1.000	SS above HW by ≈ 0.13
V966F (GTT>TTT)	1	0	0	0.000	1.000	0.000	–(undefined)	SS match HW
F967L (TTT>TTA)	1	0	0	0.000	1.000	0.000	–(undefined)	SS match HW

Table 2 Allele Variability in Different Locations Inside Jazan (n=15 in the Northern, Southern and Eastern Regions, While N = 14 in the Western Region)

T917I	SS	RS	RR	Frequency of mutated allele	HW	χ^2	F_{is}	Interpretation
North	1	0	0	0.00	1.00	0.00	N/A	Matches HW
South	0.3	0	0.7	0.70	0.09	1.00	1.00	SS above (~3.3 ×)
East	0.3	0	0.7	0.70	0.09	1.00	1.00	SS above (~3.3 ×)
West	0.8	0	0.2	0.20	0.64	1.00	1.00	SS above (~1.2 ×)
L920H	**SS**	**RS**	**RR**	**Frequency of mutated allele**	**HW**	χ^2	F_{is}	**Interpretation**
North	1	0	0	0.00	1.00	0.00	N/A	Matches HW
South	0.3	0	0.6	0.60	0.09	1.01	1.00	SS above (~3.3 ×)
East	0.7	0	0.3	0.30	0.49	1.00	1.00	SS above (~1.4 ×)
West	1	0	0	0.00	1.00	0.00	N/A	Matches HW
M815I	**SS**	**RS**	**RR**	**Frequency of mutated allele**	**HW**	χ^2	F_{is}	**Interpretation**
North	1	0	0	0.00	1.00	0.00	N/A	Matches HW
South	0.3	0	0.6	0.60	0.09	1.01	1.00	SS above (~3.3 ×)
East	1	0	0	0.00	1.00	0.00	N/A	Matches HW
West	1	0	0	0.00	1.00	0.00	N/A	Matches HW

Abbreviations: SS, homozygous susceptible; RS, Heterozygous resistant; RR, homozygous resistant; HW, Hardy–Weinberg; χ^2, chi square test; F_{is}, Inbreeding coefficient.

The most important mutation was T917I which was associated with threonine-to-isoleucine substitution in 917, and it was due to the substitution of cytosine with thymine. The L920H mutation was correlated with the exchange of leucine with histidine in 920, corresponding to the substitution of CTT with CAT. The M815I mutation was associated with substitution of methionine with isoleucine in 815 due to replacement of ATG with ATT. In 966, V966F mutation was related to substitution of GTT with TTT leading to amino acid change from valine to phenylalanine. In addition, a mutation led to the replacement of phenylalanine with leucine in 967 (TTT to TTA).

These mutations were found in low frequencies. The highest frequencies of these mutations were observed in the southern and eastern regions of Jazan. This may be attributed to the emergence of novel local mutations that cause this heterogenicity.[18]

The L920H is a point mutation which was detected previously in Riyadh, Saudi Arabia.[15] Similarly, this mutation was detected in Jazan at a lower total frequency of 0.230, when compared to a frequency of 0.73 in Riyadh. However, the novel double mutations identified in Riyadh (V966F and F967L) were not detected in this study.

Mutation in M815I is one of the three mutations (T929I, L932F and M815I) that may form a haplotype, and it is commonly found together with the other mutations as an indication of pyrethroid resistance in the tested samples.[19] This mutation was detected at a low frequency of 0.150, mainly in the Southern region. In previous studies, M815I mutation was seen at higher frequencies of 0.98 in the United States[20] and 1 in Turkey.[21] This disparity may be explained by the variation in the frequency and protocol of pyrethroid use. The uncontrolled usage of pyrethroid-based treatment could be the reason for the elevated incidence in the United States and Turkey.[22] In addition, the boarder research area and greater social and behavioral variation in head lice control procedures in these countries may also affect the frequency of this mutation. In the other hand, the lower frequency of mutation in Jazan could be attributed to a more restricted use, a decreased reliance on chemical pediculicides, or an earlier phase of resistant allele spread. Furthermore, the distribution of resistant mutations may also be influenced by variations in public health practices and population movement, in addition to environmental factors.

The T917I mutation is a strong indication of *kdr* mutation, even if found alone. Previous studies in Saudi Arabia revealed a higher frequency of T917I than that seen in the present study (0.38). In Riyadh and Jeddah, Saudi Arabia, the frequencies of this mutation were 0.83 and 0.62, respectively. This may be attributed to the smaller size and lower heterogenicity of populations in Jazan, when compared to those in Jeddah and Riyadh, Saudi Arabia.

Conclusion

Jazan has a low overall frequency of kdr mutations than other areas in Saudi Arabia. The most frequent mutations were found in the southern regions, suggesting a development of regional resistance. These findings highlight the need to control the spread of pyrethroid resistance through continuous surveillance and the development of novel strategies using up-to-date technology.

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Disclosure

The author reports no conflicts of interest in this work.

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