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Scientific Research

Pyrethroid Insecticide Resistance *kdr* Gene in the House Fly, *Musca domestica* (Diptera: Muscidae), in the United Arab Emirates

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Abstract

The house fly, *Musca domestica* L., is a very important insect because of its role as a vector of several human, livestock, and poultry diseases. Different groups of insecticides, including the pyrethroids, are used to control house flies. The objective of the current study was to conduct a survey of the *kdr* insecticide resistance gene in the house fly population in the United Arab Emirates (UAE). Flies were collected from five locations (Abu Dhabi, Dubai, Fujairah, Ajman, and Ras Al-Khaymah). Genomic DNA was extracted and polymerase chain reaction (PCR) amplification of specific allele (PASA) was performed. The maximum percentage (70%) of homozygous genotype (*kdr*/*kdr*) occurred at Ajman, followed by Dubai (59.5%), and the minimum percentage occurred at Ras Al-Khaymah (14.3%). Resistant insects of the genotype *kdr*/*kdr* were detected in all of the tested house fly populations. Ajman and Fujairah house fly populations were in Hardy-Weinberg equilibrium. The resistance *kdr* allele was found at a high frequency (0.54 - 0.8) at all locations except at Ras Al-Khaymah (0.21). Together, this study demonstrated that the pyrethroid insecticide resistance *kdr* allele was found in UAE house fly populations, strongly suggesting that a countrywide pyrethroid insecticide resistance management program needs to be implemented.

Keywords

House Fly, Pyrethroid, *kdr*, Resistance, UAE

1. Introduction

The house fly, *Musca domestica* L. (Diptera: Muscidae), is a very important insect that can have negative impacts on human, livestock, and poultry because of its role as a disease vector. It has been reported to be a poten-

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tial vector of metazoan parasites found in pig pens [1]. House flies also can be mechanical vectors of *Vibrio cholera* (biotype El Tor), thus increasing its dissemination [2]. In addition, a total of 497 fungal isolates of 15 genera were identified from the external surfaces of both female and male house flies, which showed that the house fly can be a vector for fungal spores [3]. House flies are also effective vectors of *Escherichia coli* O157:H7 among cattle, as well as an effective vector from cattle to humans [4] [5].

Pyrethroid insecticides are used in the chemical control of house flies. The voltage-gated sodium channel (*Vssc*) is the main target of these insecticides, but target site insensitivity conferred by mutations in the *Vssc* has been a major mechanism of resistance to pyrethroids [6] [7]. At amino acid residue 1014, a mutation of leucine to phenylalanine (L1014F), which is known as the *kdr* mutation in the *para*-type sodium channel gene, has been consistently associated with knockdown resistance in house flies [8] [9]. Resistant flies have alleles such as *CYP*6*D*1 and *Vssc*1 that conferred resistance to permethrin and other pyrethroids [10].

In the United Arab Emirates (UAE), house flies can be disease vectors and can pose a risk to human and animal health. Although they are routinely controlled by chemical insecticides, there have been no reports on the prevalence of insecticide resistance, especially at the molecular level (detection of resistance alleles). Therefore, the objective of the current study was to conduct a survey on the presence of the *kdr* pyrethroid insecticide resistance gene in house fly populations at five locations in the UAE.

2. Materials and Methods

2.1. House Flies

A total of 122 adult house flies were collected from five field populations (Abu Dhabi, Dubai, Ras Al-Khaymah, Ajman, and Fujairah) in the UAE in 2013.

2.2. DNA Extraction

Genomic DNA was extracted from individual house flies and stored at −80˚C. Extractions were performed using an automated Maxwell 16 DNA extraction machine equipped with a tissue DNA purification kit (Promega, USA) using a procedure according to the manufacturer's protocol.

2.3. Presence of the *kdr* Resistance Gene in the House Fly Population Using PCR Amplification of Specific Alleles (PASA)

The PASA protocol described by Huang *et al*. [11] was followed. Briefly, it is an assay based on Williamson *et al*. [8] which genotypes each house fly for the presence of the L1014F replacement in the sodium channel's S6 transmembrane segment of domain II. Two outer allele-specific primers, *kdr*1, 5'-AAGGATCGCTTCAAGG-3' and *kdr*4, 5'-TTCACCCAGTTCTTAAAACGAG-3', were used. Also, two inner allele-specific primers, *kdr*2, 5'-TCGTGATCGGCAATT-3' and *kdr*3, 5'-GTCAACTTACCACAAG-3', were used. PASA was performed in thin walled microcentrifuge tubes using 25 µL reaction-volumes. The following are the optimized PCR conditions: 2 µL of genomic DNA (90 ng/µL) extracted from a single house fly, 10 pmol of each primer, 12.5 µL Taq PCR Master Mix (Qiagen, Germany), and 8.5 µL nuclease free water. Amplification was initiated by 95˚C for 2 minutes, followed by 40 cycles, 94˚C for 45 seconds, 54˚C for 30 seconds, 72˚C for 90 seconds, and a final extension step at 72˚C for 10 minutes. A control fragment of 480 bp was the result of amplification by using *kdr*1 and *kdr*4 primers. Amplification of the susceptible allele by *kdr*1 and *kdr*3 primers produced a 200 bp fragment. Instead of the 200 bp fragment, a 280 bp fragment was amplified by *kdr*2 and *kdr*4 primers from the *kdr*-type allele. Every PCR included a negative control (no-template DNA) to make sure there was no contamination. PCR amplifications were carried out in a Swift Max Pro thermocycler (ESCO, Singapore). The PCR amplified fragments were resolved by electrophoresis using 1.5% agarose gels that were stained with ethidium bromide and photographed under UV light using a UVDI gel documentation system (Major Science, Taiwan). Frequencies of the resistance *kdr* allele were tested for Hardy-Weinberg equilibrium for each house fly population at each location by using a chi-square X^2 test at $P = 0.05$ for one degree of freedom where $X^2 = 3.84$ [12].

3. Results

The 200 bp susceptible allelic fragment was amplified by *kdr*1 and *kdr*3 primers and the 280 bp *kdr* allelic

fragment was amplified by *kdr*2 and *kdr*4 primers (**Figure 1**). The percentages of the three genotypes (*kdr*/*kdr*, *kdr*/*sus*, and *sus*/*sus*) varied among the five tested fly populations (**Table 1**). The maximum number of flies (70%) with the *kdr*/*kdr* genotype occurred at Ajman, followed by Dubai (59.5%), while the minimum number occurred at Ras Al-Khaymah (14.3%) (**Figure 2**). Moreover, insects with the *kdr*/*kdr* genotype were detected in all tested populations (**Figure 3**). Two of the house fly populations, namely Ajman and Fujairah, were in Hardy-

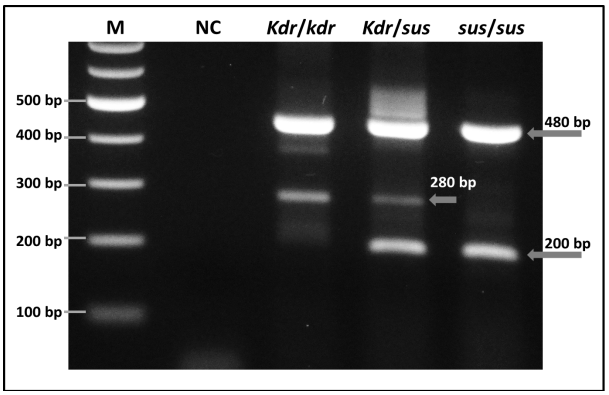

Figure 1. PCR products from individual house flies after separation on a 1.5% agarose gel. The 200 bp susceptible allelic fragment was amplified by *kdr*1 and *kdr*3 primers. The 280 bp kdr allelic fragment was amplified by *kdr*2 and *kdr*4 primers. A control fragment (480 bp) was amplified by *kdr*1 and *kdr*4 primers. M = Marker 100-bp (Promega, USA); NC = Negative control.

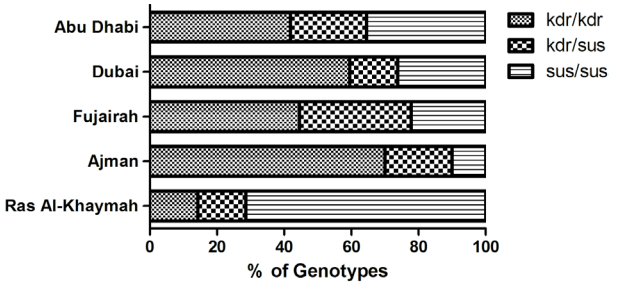

Figure 2. Genotype percentages in house fly populations at different UAE locations.

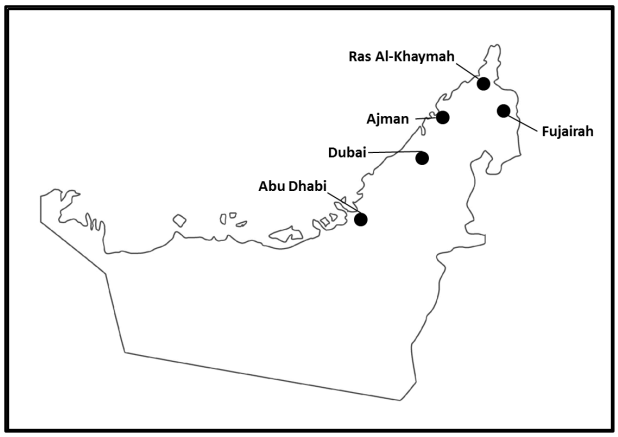

Figure 3. UAE map showing the location of house fly populations containing the resistance *kdr*/*kdr* genotype.

Table 1. House fly genotypes and pyrethroid knockdown resistance *kdr* allelic frequency at different UAE locations.

Location	N	*kdr*/*kdr*	*kdr*/*sus*	*sus*/*sus*	*kdr* frequency	H.W. X^2
Abu Dhabi	31	13	7	11	0.54	9.26
Dubai	42	25	6	11	0.67	19.34
Ras Al-Khaymah	21	3	3	15	0.21	6.96
Ajman	10	7	2	1	0.8	1.41*
Fujairah	18	8	6	4	0.61	1.61*

N = Number of tested flies; H.W. = Hardy-Weinberg equilibrium. *Signifies the population is in H.W. $P = 0.05$; chi-square $X^2 = 3.84$.

Weinberg equilibrium based on the chi-square X^2 test (1.41 and 1.61, respectively) (**Table 1**). The resistance allele *kdr* was found at a high frequency (0.54 - 0.8) at all locations, except at Ras Al-Khaymah (0.21) (**Table 1**).

4. Discussion

Genotyping each house fly for the presence of the resistance *kdr* allele using PASA revealed that this allele was present in the five tested populations. This finding was confirmed by the amplification of a 280-bp *kdr*/*kdr* homozygous allelic fragment. Other flies were heterozygous (*kdr*/*sus*) as indicated by the amplification of two allelic fragments (280 bp for *kdr* and 200 bp for *sus*) or homozygous (*sus*/*sus*). These findings were in agreement with the results of Huang *et al*. [11]. Because flies with the *kdr*/*kdr* genotype are resistant to pyrethroid insecticides, the results suggested that resistance levels could increase with time in fly populations of the UAE, which could lead to control failure.

The screened house fly populations in the current study showed variabilty in the precentages of the three genotypes (*kdr*/*kdr*, *kdr*/*sus*, and *sus*/*sus*), indicating different levels of pyrethroid resistance. Similarly, Huang *et al*. [11] reported variabiliy in genotypes among house fly populations. Rinkevich *et al*. [10] reported that combinations of pyrethroid insecticide resistance alleles of *Vssc*1 and *CYP*6*D*1 were highly variable between the states from which the house flies were collected. Resistant insects of the genotype *kdr*/*kdr* were detected at Abu Dhabi, Dubai, Fujairah, Ajman, and Ras Al-Khaymah. However, only two of the examined house fly populations, namely Ajman and Fujairah, were in Hardy-Weinberg equilibrium based on the chi-square X^2 test in which the observed numbers of the *kdr* homo- and heterozygotes were compared with the expected numbers calculated from the *kdr* frequency. Deviation from Hardy-Weinberg equilibrium could indicate different levels of selection caused by the use of pyrethroid insecticides on house fly populations. The results of the current study are consistent with other studies, which indicated that insect populations are not always in Hardy-Weinberg equilibrium [11]. Toloza *et al*. [13], in a study of the pyrethroid resistance allelic frequency in head lice (*Pediculus humanus capitis*), reported that tests for the Hardy-Weinberg equilibrium for each location showed that genotype frequencies differed significantly from expectations in four of the six studied sites.

The resistance *kdr* allele was found at a high frequency (0.54 - 0.8) at all locations except at Ras Al-Khaymah (0.21). Similarly, Rinkevich *et al*. [10] reported that the resistance allele *CYP*6*D*1*v*1 was found at a high frequency (0.63 - 0.91) in house fly populations at all sites. In the present study, the maximum frequency (0.8) of the *kdr* allele was found at Ajman. Thus, most of the fly population (70%) at that location was the resistant homozygous *kdr*/*kdr* genotype. Nonetheless, it should be mentioned that the tested number of flies in Ajman was small (n = 10) and may not reflect a precise representation of the *kdr* allelic frequency in the fly population. However, the minimum number (14.3%) of flies with *kdr*/*kdr* genotype was found at Ras Al-Khaymah. This finding indicated that the Ras Al-Khaymah house fly popualation was still dominated by susceptible flies and consequently could be managed by using pyrethroid insecticides.

Genomic DNA was extracted relatively from small numbers of individual house flies in the current study because of limited time and funding. Testing of larger numbers of flies is recommended in future studies, because larger fly numbers should provide more accurate estimations of the *kdr* allelic frequency and pyrethroid resistance levels. However, the current study provided sufficient evidence that the *kdr* allele was present in the tested house fly populations in the UAE. Furthermore, the current study is the first report on the *kdr* pyrethroid resistance allele in the UAE. Thus, it serves as a foundation for future studies on insecticide resistance. Once an insect population shows resistance to an insecticide, control programs using this insecticide are no longer effective. Accordingly, the pyrethroid insecticide list used for the control of house flies in the UAE should be reevaluated in order to reduce the frequency of the *kdr* resistance allele in the fly population. In addition, a country-

wide operational implementation of a global plan for insecticide resistance management is also warranted [14].

5. Conclusion

This study demonstrates that the pyrethroid insecticide resistance *kdr* allele is found in UAE house fly populations. Future studies should include pyerethroid insecticide bioassays on house flies before performing screening for the *kdr* allele. This will examine the relationship between the *kdr* mutation and the toxicity of the pyrethroid insecticides and determine whether a countrywide pyrethroid insecticide resistance management program needs to be implemented.

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