

Molecular identification of allelic genotypes of pyrethroid-insecticide resistance in housefly, Iraq

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Abstract

According to global-wide presence of insecticides resistance to pyrethroids, the current study identified the purpose to detect the allelic genotypes regarding this issue in house flies in Iraq. From the governorate of Al-Qadisiyah, Iraq, 60 morphologically and molecularly recognized house flies were caught from 6 different regions. Using a technique called polymerase chain reaction (PCR) amplification of specific allele (PASA), PCR was employed to reveal the presence of allelic-genetic variations in the *para*-type sodium channel (*para*) gene to recognize knockdown resistance (*kdr*) mutation from the homozygous-wild type of complete susceptibility (*sus/sus*) to the mutated-homozygous type of complete resistance (*kdr/kdr*) or to the mutated-heterozygous type (*kdr/sus*). Here, these genotypes were targeted using specific primers to identify these genetic variations. The results have declared the presence of the *sus/sus* at 100%-frequency rate in all flies, and none of the other genotypes were detected (0%) in all flies. This valued piece of result indicates the reality of resistance persistence due to lack of insecticide-spraying programs in the governorate. This study provides high-quality information about the current status of insecticide resistance in house flies in Iraq about supporting the fact of genetic-base development of such resistance via frequent use of insecticides.

Keywords: Insecticide resistance, *Kdr*, *Musca domestica*.

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التحديد الجزيئي للأليل المورث لمقاومة الحشرات للمبيدات الحشرية من نوع Pyrethroid في ذبابة المنزل، العراق

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الخلاصة

استناداً للوجود العالمي للمبيدات الحشرية المقاومة للبيرثرويد، حددت الدراسة الحالية الغرض من الكشف عن الأنماط الجينية الأليلية فيما يتعلق بهذا الموضوع المسألة في فصائل الذباب المنزلي في العراق. تم الإمساك بستون فصيلة معترف بها مورفولوجياً وجزيئياً من ستة مناطق مختلفة من محافظة القادسية، العراق. باستخدام تقنية تفاعل البلمرة المتسلسل لتضخيم البلات خاصة (PASA)، تم استخدام تفاعل البوليميريز المتسلسل للكشف عن وجود اختلافات أليلية وراثية في جين قناة الصوديوم-النوع المجاور (*para*) لتمييز المقاومة الناتجة عن طفرة في بعض الأحماض الأمينية لهذا الجين (*kdr*) من النوع البري المتجانس كامل الحساسية *sus/sus* إلى النوع الطافر المتجانس كامل المقاومة *kdr/kdr* أو النوع الطافر المختلف *kdr/sus*. تم استهداف هذه الأنواع الوراثية وذلك باستخدام بواقي متخصصة لتحديد هذه الاختلافات الجينية. أظهرت النتائج وجود النوع الوراثي *sus/sus* بمعدل تردد ١٠٠٪ في جميع الذباب، ولم يتم الكشف عن أي من الأنواع الوراثية الأخرى (٠٪). تشير هذه النتيجة القيمة إلى حقيقة استمرار المقاومة بسبب نقص برامج رش المبيدات الحشرية في محافظة القادسية. كما توفر هذه الدراسة معلومات عالية الجودة حول الوضع الحالي لمقاومة المبيدات الحشرية في الذباب المنزلي في العراق لدعم حقيقة تطوير مثل هكذا مقاومة مستندة على الوراثة عن طريق الاستخدام المتكرر للمبيدات الحشرية.

Introduction

Because insecticides help in reducing the effect of vector-borne diseases, therefore, insecticide-resistance is a dangerous problem that affects environment and its components especially humans, plants, and animals. This problem costs countries around the world about billions of dollars due to the use of not-working chemicals to eradicate insects. This economic-destroying issue could also add dangerous threats to humans, plants, and animals via the persistence and escalating ability of these insects to transmit and store several infectious agents. This ability might induce developing new set of infectious agents that would not be controlled easily (1-4). House flies, *Musca domestica*, are wide-spread vector that affects human, plants, and animals, and could transfer many fly-borne diseases such as *Vibrio cholera*, hundreds of fungi, parasites and *Escherichia coli* O157:H7 (5-9). The problem of resistance by house flies against pyrethroid-based insecticides is increasingly present in the world. The voltage-gated sodium channel (*Vssc*) plays an important role as a primary target for pyrethroid effect in house flies; however, this target is exposed to certain mutation to insert phenylalanine rather than the original leucine at the residue 1014. This phenylalanine-based replacement is recognized as the *kdr*-gene mutation (10-13). This issue of allele-genotype mutation has been studied in areas of the world but not in Iraq. For this purpose, this study was performed to recognize the knockdown resistance (*kdr*) mutation from the homozygous-wild type of complete susceptibility (*sus/sus*) to the mutated-homozygous type of complete resistance (*kdr/kdr*) or to the mutated-heterozygous type (*kdr/sus*) of the *para* gene in house flies in Iraq.

Materials and methods

Sampling

From the governorate of Al-Qadisiyah, Iraq, 60 morphologically and molecularly recognized house flies were caught from 6 different regions. Samples were moved to a laboratory using clean and dry containers.

DNA extraction

For the purpose of extracting genomic DNA from flies, gSYAN® DNA extraction Kit (Bioneer Company, South Korea) was utilized to perform this process, and the insect protocol of this kit was followed. The quality and quantity of the isolated DNA were measured using NanoDrop spectrophotometer and then were followed by storing the DNA at -20 °C to later complete the next step of PASA technique.

PCR amplification of specific allele (PASA)

The allelic-*kdr* mutations in the *para* gene were used to detect the genotypes of pyrethroid resistance in house flies, and the protocol for such analyses was adopted from (14). Two-specific primers of the outer allele, *kdr1*: AAGGATCGCTTCAAGG and *kdr4*: TTCACCCAGTTCTTAAAACGAG, were used to generate a 480bp-piece amplification as a house fly-recognition control. The specific primers for the inner allele, *kdr2*: TCGTGATCGGCAATT and *kdr4*: TTCACCCAGTTCTTAAAACGAG, were utilized to recognize the *kdr*-based amplification of a 280-bp piece. A specific set of primers for the inner allele, *kdr1*: AAGGATCGCTTCAAGG and *kdr3*: GTCAACTTACCACAAG, were employed to enhance the *sus*-based amplification of a 200 bp-sized piece. These sets of primers were purchased from Bioneer Company, South Korea.

Preparation of PCR mastermix

Recruiting the AccuPower® PCR PreMix kit (Bioneer Company, South Korea), PCR mastermix was produced following the company information that was accompanied with the kit. DNA polymerase 1U, dNTPs 250 µM, Tris-HCl (pH 9.0) 10 mM, KCl 30 mM, MgCl₂ 1.5 mM, stabilizer, and tracking dye were previously added in the tubes of the kit by the company. The information provided with the kit was followed to perform the mastermix preparation. Adding 2 µL of DNA and 1 µl 10 pmole from each primer to the above-mentioned tubes was performed, and then the total volume was brought up to 20µl. A brief episode of mixing by vortexing was done. The thermocycler conditions were initial denaturation temperature of 94 °C for 5 min, 35 cycles of the denaturation 94°C for 30 s, annealing 54°C for 30 s, and extension 72°C for 1m, and final extension at 72°C for 5m. Electrophoresis that recruited a 1%-agarose gel treated with ethidium bromide was performed to then be visualized under a UV illuminator.

Statistical analyses

The statistics was performed using fraction of total analysis. The results were plotted using GraphPad Prism 7.04 (GraphPad Software Inc., USA).

Results

According to the PASA results, the study identified the presence of the *sus/sus* at 100%-frequency rate in all flies, and none of the other genotypes were detected (0%) in all flies. These results could be shown and noticed in figure 1 and 2. Figure 1 reveals that the control fragment was amplified at 480 bp in all the samples. Moreover, it shows that the genotype of wild type, *sus/sus*, has been amplified

in all the fly samples at 200 bp. However, none of the genotypes regarding *kdr/kdr* or *kdr/sus* was amplified in any sample. Figure 2 shows the percentages of these genotypes in the sampled flies.

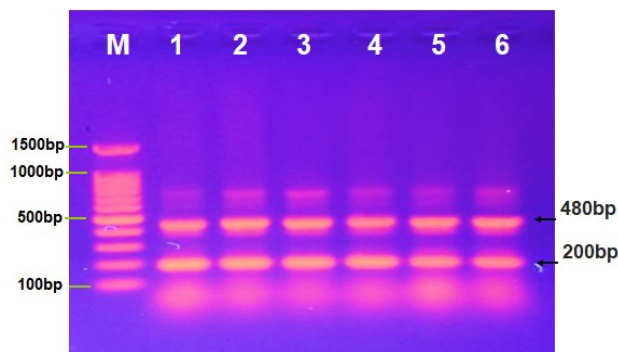


Figure 1: Image of the electrophoresis of the *kdr*-based genotypes for 6 sampled local locations for the houseflies. Lane M is the ladder, 1500-100bp. Lanes 1 to 6 are the sampled locations for the houseflies, and they show the amplification of the homozygous-wild type of the *para* gene (*sus/sus*) at 200 bp using PASA technique.

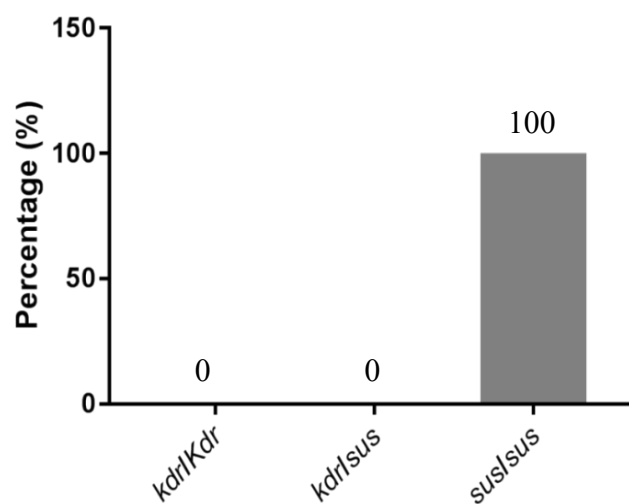


Figure 2: Shows the percentages of the allelic variations of the *kdr* and the susceptibility to pyrethroid in the sampled flies.

Table 1: Shows the percentages of *kdr/kdr*, *kdr/sus*, and *sus/sus* in some Countries of the world

| Researcher | n | <i>kdr/kdr</i> | | <i>kdr/sus</i> | | <i>sus/sus</i> | | Country | Reference |
|--------------------|-----|----------------|------|----------------|------|----------------|------|-----------------|-----------|
| | | n | % | n | % | n | % | | |
| Our Study | 60 | 0 | 0 | 0 | 0 | 60 | 100 | Iraq | Our |
| WHO | 20 | 0 | 0 | 0 | 0 | 20 | 100 | - | 15 |
| Huang <i>et al</i> | 743 | 338 | 45.5 | 267 | 36 | 138 | 23.9 | China | 15 |
| Al-Deeb | 122 | 56 | 45.9 | 24 | 19.7 | 48 | 39.3 | United Emirates | 14 |

n= number of tested flies.

Discussion

The current study results showed that all the house flies caught in the regions of the governorate carried the homozygous-wild genotype, *sus/sus*, and didn't carry any of the genotypes regarding *kdr/kdr* or *kdr/sus*. This means that our houseflies have no insecticide resistance based on the disappearance of the mutations, *kdr/kdr* or *kdr/sus*. If there is no exposure to insecticides, there is no development of resistance to these chemicals by house flies, and this fact supports our results. Table 1 identifies some studied regions in the world with different ratios of tested allelic variations of the *para* gene regarding the presence of the *kdr* or the *sus* genotypes (14,15) in which (15) agree with our result of no presence of the *kdr*-based allelic variations in all of their samples. However, Authors from China (15) indicate that there were 45.5%, 36%, and 23.9% of the *kdr/kdr*, *kdr/sus*, and *sus/sus* respectively. In addition, a researcher from United Emirates (14) detected that ratios of *kdr/kdr*,

kdr/sus, and *sus/sus* were 45.9%, 19.7%, and 39.3 respectively. The presence of the *kdr/kdr* and *kdr/sus* genotypes was explained via the frequent use of ineffective insecticides in these countries mentioned in the literature cited above (14,15). Because Iraq has no mass insecticide-using programs by responsible officials or by the local people in the governorate, house flies collected in our study had 0% presence of the *kdr/kdr* and/or *kdr/sus*. This study provides high-quality information about the current status of insecticide resistance in houseflies in Iraq but more globally about supporting the fact of genetic-base development of such resistance via frequent use of insecticides.

References

1. Ranson H, Lissenden N. Insecticide Resistance in African Anopheles Mosquitoes: A worsening situation that needs urgent action to maintain malaria control. Trends Parasitol. 2016;32(3):187-196. Doi: 10.1016/j.pt.2015.11.010.

2. Ilias A, Vassiliou VA, Vontas J, Tsagkarakou A. Molecular diagnostics for detecting pyrethroid and abamectin resistance mutations in *Tetranychus urticae*. *Pestic Biochem Physiol*. 2017;135:9-14. Doi: 10.1016/j.pestbp.2016.07.004.
3. Kasai S, Sun H, Scott JG. Diversity of knockdown resistance alleles in a single house fly population facilitates adaptation to pyrethroid insecticides. *Insect Mol Biol*. 2017;26(1):13-24. Doi: org/10.1111/imb.12267.
4. Butler D. Mosquitoes score in chemical war. *Nature*. 2011;475(7354):19. Doi: 10.1038/475019a.
5. Förster M, Klimpel S, Sievert K. The house fly (*Musca domestica*) as a potential vector of metazoan parasites caught in a pig-pen in Germany. *Vet Parasitol*. 2009;160(1-2):163-167. Doi: 10.1016/j.vetpar.2008.10.087.
6. Fotedar R. Vector potential of house flies (*Musca domestica*) in the transmission of *Vibrio cholerae* in India. *Acta Trop*. 2001;78(1):31-34. Doi:10.1016/S0001-706X(00)00162.
7. Phoku JZ, Bamard TG, Potgieter N, Dutton MF. Fungi in house fly (*Musca domestica* L.) as a disease risk indicator: A case study in South Africa. *Acta Trop*. 2014;140:158-165. Doi:10.1016/j.actatropica.2014.08.019.
8. Ahmad A, Nagaraja TG, Zurek L. Transmission of *Escherichia coli* O157:H7 to cattle by house flies. *Prev Vet Med*. 2007;80(1):74-81. Doi:10.1016/j.prevetmed.2007.01.00.
9. Sasaki T, Kobayashi M, Agui N. Epidemiological potential of excretion and regurgitation by *Musca domestica* (Diptera: Muscidae) in the dissemination of *Escherichia coli* O157: H7 to food. *J Med Entomol*. 2000;37(6):945-949. Doi:10.1603/0022-2585-37.6.945.
10. Elliott M, Janes NF, Potter C. The future of pyrethroids in insect control. *Annu Rev Entomol*. 1978;23(1):443-469. Doi:10.1146/annurev.en.23.010178.002303.
11. Dong K. Insect sodium channels and insecticide resistance. *Invert Neurosci*. 2007;7(1):17-30. Doi:10.1007/s10158-006-0036-9.
12. Williamson MS, Martinez-Torres D, Hick CA, Devonshire AL. Identification of mutations in the house fly para-type sodium channel gene associated with knockdown resistance (kdr) to pyrethroid insecticides. *Mol Gen Genet*. 1996;252(1-2):51-60. Doi.org/10.1007/BF02173204.
13. Miyazaki M, Ohyama K, Dunlap DY, Matsumura F. Cloning and sequencing of the para-type sodium channel gene from susceptible and kdr-resistant German cockroaches (*Blattella germanica*) and house fly (*Musca domestica*). *Mol Gen Genet*. 1996;252(1-2):61-68. Doi:10.1007/BF02173205.
14. Al-Deeb MA. Pyrethroid insecticide resistance kdr gene in the house fly, *Musca domestica* (Diptera: Muscidae), in the United Arab Emirates. *Agric Sci*. 2014;05(14):1522-1526. Doi:10.4236/as.2014.514163.
15. Huang J, Kristensen M, Qiao C-L, Jespersen JB. Frequency of kdr gene in house fly field populations: correlation of pyrethroid resistance and kdr frequency. *J Econ Entomol*. 2004;97(3):1036-1041. Doi:10.1603/0022-0493(2004)097[1036:FOKGIH]2.0.CO;2.