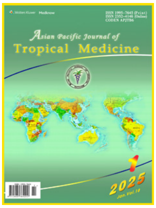

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Knockdown resistance associated organochlorine resistance in mosquito-borne diseases (*Anopheles culicifacies*): A systematic review

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ABSTRACT

Objective: To investigate the prevalence, mechanisms, and trends of knockdown resistance (kdr) in *Anopheles (An.) culicifacies* and its impact on the efficacy of organochlorine and other insecticides.

Methods: A systematic review was conducted based on PRISMA guidelines, extracting data from biooan.org, Embase, ProQuest, PubMed, Scopus, and Web of Science without a time limit until the end of 2022. Articles meeting the inclusion criteria were assessed using the STROBE checklist. Data on kdr mutations, insecticide resistance, and effectiveness were analyzed across eight selected studies from various regions.

Results: The review revealed widespread kdr-mediated resistance in *An. culicifacies*, primarily against dichloro-diphenyl-trichloroethane (DDT), persisting even decades after discontinued use. Key kdr mutations, including *L1014F* and *L1014S*, were identified. Resistance to deltamethrin was less stable, with increased sensitivity observed after short-term discontinuation. The findings underscore the vector's sustained resistance to organochlorine insecticides and relative sensitivity to pyrethroids.

Conclusions: Stable kdr resistance in *An. culicifacies* to organochlorine insecticides highlights the need for periodic susceptibility assessments and strategic rotation or combination of insecticides to combat malaria effectively and prevent the development of resistance.

KEYWORDS: Knockdown resistance (kdr); Organochlorine; Mosquito-borne diseases; *Anopheles culicifacies*; Systematic review

1. Introduction

Malaria is a major public health problem in many parts of the world. This disease has a complex epidemiology due to several vectors, different reservoirs, rainfall, global warming, vast lands, forests, hills, and various places of growth and rest[1,2]. One of

Summary

Question: What is the prevalence and impact of knockdown resistance (kdr) in *Anopheles* culicifacies on the efficacy of organochlorine and other insecticides?

Findings: This systematic review analyzed eight studies on kdr resistance in *An. culicifacies*. It found widespread and stable resistance to organochlorine insecticides such as dichloro-diphenyl-trichloroethane across multiple regions over three decades. Resistance trends were linked to kdr mutations, notably L1014F and L1014S.

Meaning: Effective malaria vector control requires periodic assessment of insecticide susceptibility in *An. culicifacies* and the rotation or combination of insecticides to prevent resistance development and ensure long-term efficacy.

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the common areas of this disease in the world is the Southeast Asian region of the World Health Organization, and the Indian subcontinent is one of the most widespread areas infected with this disease[2]. *Plasmodium vivax* and *Plasmodium falciparum* are the main parasites and *Anopheles (An.) culicifacies*, *An. fluviatilis*, *An. stephensi*, *An. minimus*, *An. dirus*, and *An. sundaicus* are the main vectors of malaria[3]. Among the vectors, *An. culicifacies* is the most common vector and has played a major role in the epidemic and severe transmission of malaria in this region. Cytogenetic studies have classified *An. culicifacies* into five subspecies: A, B, C, D, and E. These subspecies differ in terms of vector capacity, biology, and response to malaria control interventions.

Despite numerous studies in the world in the field of sibling species in *An. culicifacies*, it is still difficult to have a comprehensive view of the biological and ecological characteristics. Due to the overlap of morphological features of close species, it makes their identification and classification difficult. In the studies conducted in the field of resistance to dichloro-diphenyl-trichloroethane (DDT), type A has been more sensitive than type B. Also, the comparison between species B and C showed that species C had higher resistance and higher survival rate[4,5]. In another study in areas where organochlorine poisons are used to fight against *An. culicifacies*, the ratio of blood feeding from humans and sporozoite rates in E species was higher than other species, which indicates that the resistance in this species was higher than other siblings[6].

An. culicifacies is widely distributed in arid and semi-arid regions of Bangladesh, Afghanistan, South China, Cambodia, Eritrea, Iran, India, Laos, Nepal, Myanmar, Sri Lanka, and Pakistan[4,7]. The most effective way to fight and control malaria is to use insecticides to kill the disease vector. By eliminating the vector of the disease, the transmission chain of malaria can be broken, and the spread of the disease can be prevented. In the past decades, various insecticides such as organochlorides, organophosphorus, carbamates, and pyrethroids have been used to fight malaria vectors, including *An. culicifacies*[8]. Pyrethroids are a large group of insecticidal compounds with relatively low toxicity to mammals and short environmental persistence. Although DDT has relatively higher toxicity, its use in human activity environments is obsolete[9]. The mechanism of DDT and pyrethroids' effect on sodium channels are voltage-dependent[10]. By changing the gating kinetics on sodium channels, these neurotoxins lead to the long-term opening of sodium channels and eventually lead to paralysis and death of the insect[11,12]. But in the past decades, due to the frequent and incorrect use of insecticides along with the use of agricultural pesticides and the existence of different species and different performance of *Anopheles* mosquitoes, selection pressure has been maintained among the mosquito population, which inevitably leads to the development of resistance to insecticides in many countries[13]. One of the most important mechanisms of pyrethroid resistance in insects is knockdown resistance (kdr), which is caused by a decrease in the sensitivity of the target site. This resistance is usually caused by a point mutation (*L1014F/S/H*) in the *IIS6* section of the voltage-gated sodium channel. Sodium channel mutations play an important role in the insensitivity of the sodium channel to insecticides[11,14]. In addition, resistance to pyrethroids affects the behavior of the carrier and their life characteristics such as fitness, egg production, fertility, growth and development of life stages and survival. In the studies conducted in resistant strains of malaria, the ratio of egg laying in females, fertility, the time interval between egg laying and the emergence of adult strains and the ratio of survival in adulthood were higher compared to sensitive species. Based on this, the presence of resistance in carriers by affecting their physical fitness provides them with suitable conditions for reproduction and survival[15,16].

The main strategy for controlling malaria in endemic areas is indoor residual spraying (IRS) based on the vector's susceptibility status and the insecticide's effective duration. Currently, to control *An. culicifacies*, spraying is done using malathion, deltamethrin, cyfluthrin, lambdacea halothrin, alfaci permethrin, or bifenthrin. Identifying the genetics, behavioral characteristics, and sensitivity of *An. culicifacies* and its different species are essential for the optimal fight against them[7]. Based on this, the present study investigated the effectiveness of organochlorine insecticides and other insecticides used to fight against *An. culicifacies* and the sensitivity ratio, the presence of knockdown resistance, and its trend in the world through a systematic review method.

2. Materials and methods

This research was conducted as a systematic review method in knockdown resistance in *An. culicifacies* in the world based on PRISMA guidelines (guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses)[17]. Based on this, by searching the scientific databases biooan.org, Embase, ProQuest, PubMed (MEDLINE), Scopus, and Web of Science without a time limit until the end of 2022, all related articles using the keywords *An. culicifacies*, *An. culicifacies*, DDT, deltamethrin, malathion, pyrethroids, organochlorides, insecticide resistance, insecticide-resistance, knockdown resistance (kdr) mutation, kdr mutation, malaria, *Anopheles* malaria vectors, kdr-like mutations. Voltage-gated sodium channels were extracted. Searching for keywords using OR and AND operators was done singularly and combined with the title, the abstract, and the full text of the articles. The study protocol was registered on the International Prospective Register of Systematic Reviews (PROSPERO) under CRD42021231605.

2.1. Inclusion and exclusion criteria

The English-language articles about *An. culicifacies* investigated the existence of kdr to organochlorine insecticides and were of good quality were included in the study. The articles related to other *Anopheles*, whose resistance was not investigated and were of low quality, were excluded from the study.

2.2. Quality assessment

The quality assessment of the articles was done based on 22 parts of the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist, which examined compliance with the principles of writing and implementation in the title, implementation method, findings, limitations, and conclusions. Each part of this checklist is given a score based on importance; the maximum possible score is 33. In this study, a score of 20 to 26 was considered average quality, and a score greater than 26 was considered high quality[18].

2.3. Extracting the data

Data extraction was done independently by two researchers trained in review, quality assessment, and evaluation of articles. Based on this, the titles and abstracts of the articles that met the inclusion criteria for the systematic review were first examined. If the articles were completely related, their full text was studied, quality assessment was done, and the required information was extracted. However, if two researchers rejected the articles, the reason was mentioned, and in case of disagreement between them, the article was judged by a third person. Data extraction was done using a checklist that included the characteristics of the first author, place and time of the article, sample size, type of insecticide, and mutation that occurred.

2.4. Selection of studies

14 536 Related articles were found in the initial search. Then, to organize the studies, the sources were entered into the Endnote software. The repeated sources and 4 382 duplicates were removed. After that, the full text of the articles was obtained and their titles and abstracts were evaluated. 9 927 Articles that were not relevant for the systematic review were removed. Finally, the full text of 227 related articles and 219 articles conducted in the field of other species of *Anopheles*, insecticides, or other types of resistance were removed. A total of 8 related articles were included in the systematic review process (Figure 1).

3. Results

Eight studies conducted between 2007 and 2020 in the field of kdr resistance in *An. culicifacies* vector (malaria vector) were included in the systematic review[8,9,13,19–23]. Among the conducted studies, five studies were conducted in India, one in Iran, one in Afghanistan, and one in Sri Lanka. The specifications of the reviewed articles are presented in Table 1.

An. culicifacies is one of the main vectors of malaria, especially in Southeast Asia. During the past decades, the fight against this vector to control malaria in this region has been carried out and continues with numerous insecticides, especially organochlorine. However, the resistance of this vector to some of these insecticides has made it difficult to fight against. The most important type of resistance against these insecticides is kdr, which we will continue to investigate its trend and sensitivity ratio against insecticides in *An. culicifacies*.

Dinparast Djadid and colleagues investigated the presence of kdr resistance in *An. culicifacies* against pyrethroids in Iran (Sistan and Baluchistan province). In the preliminary investigation, the sensitivity of *An. culicifacies* against the insecticides DDT (4%), dieldrin (0.4%), malathion (5%), permethrin (0.25%), lambadacyhalothrin (0.1%), and deltamethrin (2.5%) were 95%, 97%, 99%, 99.5%, 100%, and 100%, respectively. In terms of kdr resistance, the analysis of exon Ⅰ and Ⅱ showed that a transversal type point mutation had occurred at position 29 of exon Ⅰ of strain *67GF*, which resulted in the substitution of Lue/His amino acid upstream of the common kdr mutation identified in other species of insects. However, except for the above case, all the altered bases in the samples of *An. culicifacies* were similar[9].

In another study conducted by Dykes *et al*. in India, two kdr mutations, including *L1014F* and *L1014S*, were evaluated in *An. culicifacies* using PCR-based methods. Based on the findings, the *L1014F* mutation was observed in all regions of India (except the north), and the *L1014S* mutation was observed in the samples of central and eastern India. Deviation from Hardy-Weinberg equilibrium was not observed in both kdr mutant alleles (in heterozygous conditions). Both deltamethrin resistance mutations and only the *L1014S* mutation showed resistance to DDT[19].

Singh and colleagues evaluated the presence of kdr resistance to DDT and pyrethroids in India. The results of DNA sequencing of *An. culicifacies* samples showed three amino acid substitutions in the *IIS6* membrane segments of VGSC (Voltage-gated Na^+ channels), each due to a single-point mutation. Two non-synonymous substitution mutations were observed in the *L1014* residue, which resulted in Leu (TTA)-to-Phe (TTT) or -Ser (TCA) amino acid substitutions and were due to the *3042A*>T transition or the *3041T*>C transition, respectively. Also, a new mutation was created in the *V1010* residue

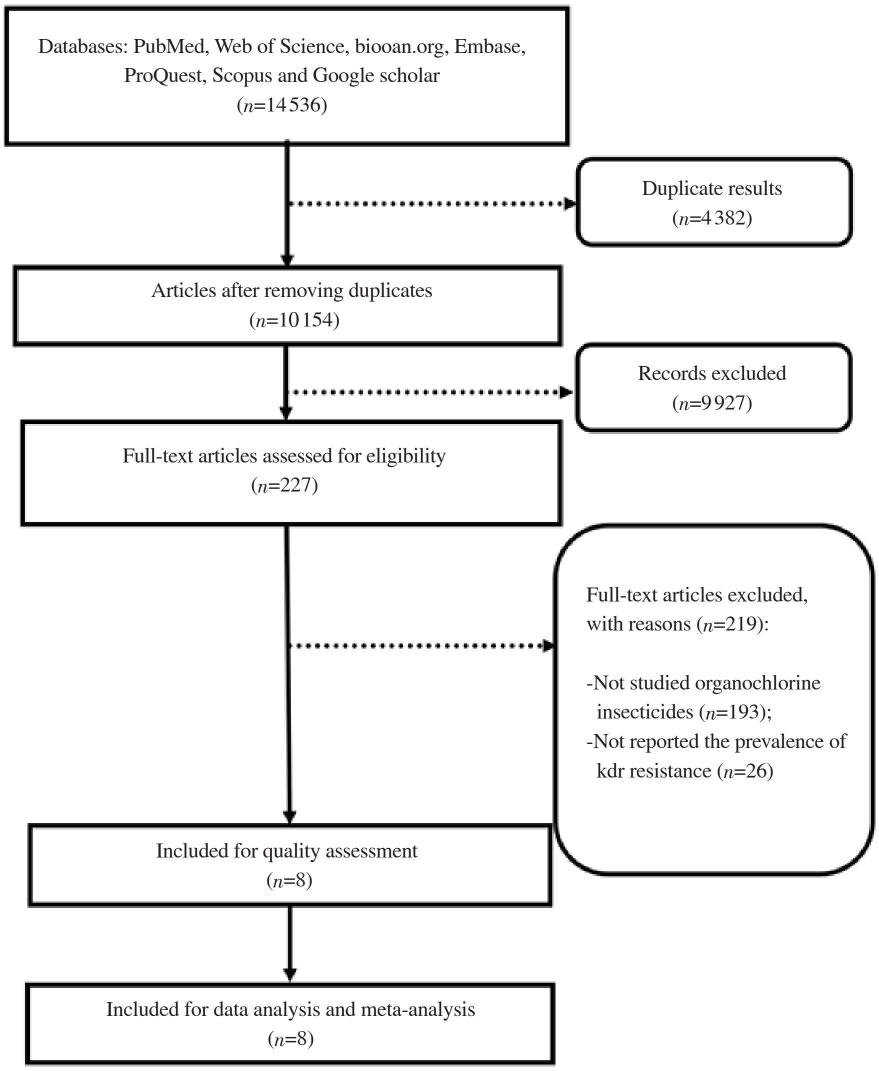

Figure 1. The PRISMA flow diagram.

that led to Val (GTG)-to-Leu (TTG or CTG) substitution caused by *3028G*>T or *3028G*>C transitions. A third and new substitution, Val (GTG)-to-Leu (TTG or CTG), was also identified in the *V1010* residue caused by one of the two transitions -*3028G*>T or *3028G*>C[22].

Surendran *et al*. investigated resistance in *An. culicifacies* in Sri Lanka. Based on the findings, the adults of the *An. culicifacies* species were resistant to DDT, probably resistant to deltamethrin, and sensitive to malathion. DNA sequencing revealed an *L1014F* (TTA to TTC) mutation in the transmembrane part of the voltage-gated sodium channel protein *IIS6* (this mutation was previously observed in India)[13].

Sahu *et al.* investigated the phenotypic resistance in *An. culicifacies* in the state of Odisha in the central east of India. Based on this, to evaluate resistance, bioassays were performed before and after the mass distribution of long-term insecticide on female mosquitoes with DDT, deltamethrin, and malathion, according to the standard guidelines of the World Health Organization (WHO). According to the findings, 82% resistance to DDT, 26.7% to malathion, and 19.1% to deltamethrin were reported[20].

Singh *et al.* investigated *L1014* kdr mutation in *An. culicifacies sensu lato* in Gujarat state, India. According to the findings, among the total of 186 samples that were genotyped using the AS-PCR method, 167 samples were identified as homozygous sensitive, one sample was homozygous resistant, and 18 samples were identified as heterozygous resistant. Therefore, Phe mutation (TTT) in *L1014* residue was found in 19 samples, which included 18 heterozygous samples and one homozygous sample[23].

In the study of Ahmad *et al*, which investigated kdr resistance in culicifacies in Afghanistan, the findings showed resistance to all four classes of insecticides: DDT (4%), malathion (5%), permethrin (0.75%) and deltamethrin (0.05%). It was found in the provinces located in the east and south of the Hindu Kush Mountain range. Thus, the death ratio of *An. culicifacies* against DDT was 81%, malathion (95%), permethrin (89%), and deltamethrin (64%). Genotype analysis to identify kdr mutations showed the existence of resistant alleles *L1014S* and *L1014F*[21].

In the study of Raghavendra *et al.* in India, they investigated

Table 1. Characteristics of the articles included in the systematic review.

Author	Year of study	Place of study	Sample size	Mosquito (adult/ larvae /pupae)	Methods (molecular/insecticide/ biochemical)	Type of molecular method	Type of insecticide	Type of allele mutation	Quality assessment
Dinparast Djadid *et al*.[9]	2007	Iran	1452	Adult	Molecular/ Insecticide	PCR/coloning/ Sequencing	DDT, malathion, permethrin, lambadacyhalothrin, and deltamethrin	Position 29 of exon 1 (CCT > CAT)-Lue/ His	High
Dykes *et al*.[19]	2015	India	9	Adult	Molecular/ Insecticide	PCR/Genotyping (ARMS)/ Sequencing	DDT and deltamethrin	*L1014F* and *L1014S*	Moderate
Singh *et al*.[22]	2010	India	20	Adult	Molecular	Sequencing/ PIRA-PCR/RFLP/ genotyping	DDT and pyrethroids	*L1014* and *V1010*	Moderate
Surendran *et al*.[13]	2020	Sri Lanka	376	Adult	Molecular/ Insecticide	Sequencing	DDT, malathion, and deltamethrin	*L1014F*	High
Sahu *et al*.[20]	2019	India	1500	NA	Insecticide	NA	DDT, malathion, and deltamethrin	-	High
Singh *et al*.[23]	2009	India	186	NA	Molecular/ Insecticide	Sequencing/PCR/ genotyping	pyrethroid and DDT	*L1014F*	High
Ahmad *et al*.[21]	2016	Afghanistan	2880	Larvae and pupae	Molecular/ Insecticide	genotyping	DDT, malathion, permethrin and deltamethrin	*L1014S* and *L1014F*	High
Raghavendra *et al*.[8]	2010	India	2332	Adult	Molecular	PCR	DDT, malathion, and deltamethrin	-	High

DDT: dichloro-diphenyl-trichloroethane.

and compared the resistance ratio of *An. culicifacies* against DDT, malathion, and deltamethrin insecticides between 1985 and 2006. According to the findings, resistance to DDT insecticide was still present after 30 years, and malathion resistance after 9 years after not using IRS (indoor residual spray). However, resistance to deltamethrin showed a significant decrease within 2 to 3 years after discontinuation of IRS. So the mortality of *An. culicifacies* caused by DDT in 1985 [15 years after complete elimination (DDT-IRS)] was 9%, and in 2006 [more than 30 years after complete elimination (DDT-IRS)], it was estimated to be 20%. In terms of malathion, the death rate between 1985 and 1992 was less than 44%, and this rate was less than 68% in 2005 and 2006, although the death rate increased between the mentioned years. But the present review showed that even 8-9 years after malathion was removed from the IRS, resistance remains. *An. culicifacies* was completely (100%) sensitive to deltamethrin in 1985 and 1987. In 2001, the mortality ratio of *An. culicifacies* exposed to deltamethrin was reported to be 66%. In 2005 and 2006 (3 years after the removal of deltamethrin-IRS), the mortality ratio increased to more than 98%, which shows that the increased duration of not being exposed to this insecticide has increased the sensitivity to it. It was also observed that the lethal time to kill 50% and 90% of mosquitoes exposed to DDT and malathion insecticides increased during the studied years, but decreased to deltamethrin[8].

Generally, based on the findings of the investigated studies, kdr resistance in *An. culicifacies* has existed for many years and is increasing. This has finally led to the obsolescence of some insecticides, such as DDT. Due to the increase in resistance, the duration of the effect of some insecticides has also increased. It should be mentioned that due to the lack of use of some insecticides for several years, the resistance of *An. culicifacies* to them has decreased. As a result, in endemic areas, before using insecticides to control this species of *Anopheles* mosquito, it is necessary first to evaluate its sensitivity to the insecticide in question, and if it is effective, use the insecticide or use a combination of insecticides to control these vectors.

4. Discussion

Collecting information about the malaria vector, the way to fight it, the presence of resistance to insecticides, and understanding the resistance mechanism is an essential component for investigating, managing, and developing entomological measures and fighting it to control this disease. In the present study, *An. culicifacies* had relatively high resistance against organochlorine insecticides, especially DDT. The main mechanism of this resistance was kdr, which was also spread in the native countries where this carrier operates. Also, persistence in resistance to DDT and malathion insecticides was still present after long-term discontinuation (about 30 years) of IRS. Various factors are effective in creating resistance in *Anopheles* mosquito, including the short life cycle of the insect, high diversity and genetic exchange, widespread and incorrect use of insecticides, and selective pressure. Studies have shown that the extensive, incomplete, and long-term use of pesticides in agricultural activities and the increase in the coverage of long-lasting insecticidal nets to control vectors have directly led to increased insecticide resistance[24,25].

Genetic stability is another factor affecting the non-return of insect sensitivity to insecticides in insects, which has led to maintaining the resistant gene and balancing it with other genetic changes. The persistence and development of insecticide resistance is an evolutionary phenomenon that insects use to maintain their survival. However, one of the approaches to return sensitivity in insects is to remove selection pressure from populations that cannot develop homozygous resistance. Mixing resistant mosquitoes with homozygous susceptible mosquitoes or heterozygous resistant mosquitoes can gradually increase the number of susceptible mosquitoes. Based on this, maintaining or returning insecticide resistance depends on the nature and stability of resistance genes, the intrinsic fitness of heterozygotes and homozygotes, and the frequency of resistance genes. As a result, sensitivity can be increased by reducing the pressure caused by insecticides in certain conditions. For example, resistance to malathion and DDT (after 30 years of non-use) has remained stable, and one of the reasons can be genetic stability. However, the resistance to deltamethrin has decreased after not using it for a short period. The short-term return of sensitivity to deltamethrin can be due to the heterozygosity of the resistance gene. Studies have shown that the return of insecticide resistance in a population is relative and depends on the genetic stability of mosquitoes' resistance[8,20,26].

An. culicifacies has multiple resistances to insecticides. As a result, due to the lack of alternative effective insecticides, the onset of resistance can be prevented or delayed by proper rotation of different groups of insecticides[27]. Among the types of insecticides to deal with *An. culicifacies*, carbamates, deltamethrin, and synthetic pyrethroids are superior to other types of insecticides, so if they are used wisely to control this vector, their useful life can be increased.

The limitations of the present study include 1) Conducting studies in different years and the different effectiveness of insecticides in these years; 2) Not reporting kdr ratio in all studies; 3) Uncertainty of the amount and duration of insecticide use in studies; 4) small sample size in some studies.

In conclusion, the findings showed that knockdown resistance in *An. culicifacies* has led to stable resistance in organochlorine insecticides. However, there is still relative sensitivity to deltamethrin and pyrethroids insecticides. Also, the resistance ratio was reduced with short-term no use of deltamethrin. Based on this, to prevent the spread of kdr resistance in *An. culicifacies*, it is recommended to use effective or combined insecticides periodically to fight against this vector to prevent the development of resistance to other insecticides and fight malaria successfully.

Conflict of interest statement

The authors declare no conflict of interest.

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Availability of data and materials

All obtained data are included in the text.

Authors' contributions

EA determined the title, wrote and registered the protocol, and submitted the article. EA, M.M and S.M extracted the files from the databases. A.Z, N.A, and EA, screening, and selection of final articles. M.M and EA, data extraction. SD wrote the article. All authors read and approved the final manuscript.

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