Chapter

Insecticide Resistance in Vectors of Medically Important Parasitic Infections

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Abstract

Insecticide resistance is a major threat to vector control programmes as insecticides still remain the most effective method to control the vector-borne diseases. For effective management of insecticide resistance, a knowledge of the insecticides used along with their mode of action is a prerequisite to optimize their use. Nowadays, different detection methods, *viz.,* phenotypic, genotypic and proteomic assays are used for assessment of insecticide resistance in vectors. An understanding of the phenotypic and genotypic variations present in the vectors help in implementation of these techniques to evaluate the usefulness of insecticides in an area and to determine the efficacy of an ongoing vector control programmes. The understanding of different factors involved in emergence of insecticide resistance and the alternative solutions to control this problem by the use of rotational, mixture of insecticides and use of piperonyl butoxide to increase the efficacy of indoor residual spray and insecticide treated bed nets are some of the steps taken to tackle the problem of insecticide resistance in vectors.

Keywords: insecticides, resistance, vectors, parasitic infections, bioassays

1. Introduction

Many fatal disease-causing pathogens are transmitted to humans by insects which belong to the phylum "Arthropoda". These insects are known as vectors when they harbor the causative organisms in them and transmit it to other humans and animals. Most of the vectors have blood-sucking mouth parts and can transmit pathogens like parasites, viruses and bacteria. The vector-borne diseases are one of the significant causes of morbidity and mortality, particularly in the endemic regions of the tropical and subtropical nations [1], and affect more than 80% of world population. Numerous parasitic infections such as malaria, babesiosis, trypanosomiasis, leishmaniasis and filariasis which affect vast human populations are transmitted by these vectors. For most of these diseases, there is still no effective vaccine is available and a significant strategy to prevent and control these diseases is the control of their vectors by using different methods [2].

Among the various methods used for the control of vector-borne diseases, the most effective and common method is the use of insecticides. All the vector control programmes depend upon the use of insecticides in the form of larvicides, adulticides and insecticide treated nets [3]. It is not easy to say when insecticides

were first used for vector control, but at least since 1000 BC people have been using natural chemicals, i.e., inorganic sulfur against the pest insects [4]. The first chemical insecticide synthesized for the control of medically important vector mosquito, which transmits malaria, was DDT in 1874. DDT was continuously use for the control of different pest insects until the first half of the 20th century, when due to development of resistance it was replaced by other insecticides such as organophosphates and carbamates [5]. The main hindrance to achieve success in vector control programmes is the development of insecticide resistance due to their overuse. Such resistance has a direct effect on the vector in terms of its longevity, infectiousness and on the management of disease [6].

2. Insects as vectors of parasitic infections

Most of the harmful parasitic infections are transmitted to humans by insects which have blood feeding behavior [7]. The parasitic infections such as malaria, lymphatic and non-lymphatic filariasis, leishmaniasis, sleeping sickness or the Human African Trypanosomiasis (HAT), and Chagas' disease or the American Trypanosomiasis, are a great burden to human health and life, especially in the poorest countries [8]. Malaria is a human parasitic disease with a very high burden. It is now especially important due to the widespread drug-resistant malaria and is at a risk of reemergence in many places worldwide. Malaria is transmitted by the bite of the mosquito species belonging to genus *Anopheles*, and filariasis is transmitted by the bite of *Culex* mosquito (**Table 1**). The parasitic zoonotic disease leishmaniasis is transmitted via the bite of phlebotomine sandflies in the Old and New World, tsetse fly is the vector of HAT, and triatomine kissing bugs are the vectors of Chagas' disease [8].

The vast expansion of these vector populations has become a growing concern and their control by different classes of insecticides is the most common method for their control, as insecticides suppress the insect populations by targeting insect metabolism in specific ways.

Insect	Disease	Insecticide use for their control
Mosquito	Malaria, filariasis	DDT, malathion, pyrethrum, deltamethrin, cyfluthrin,
Sandfly	Leishmaniasis	DDT, alpha cypermethrin, deltamethrin, deltamethrin + PBO
Tsetse fly	Human African Trypanosomiasis (HAT)	DDT, deltamethrin, HCB, dieldrin
Triatomine *bugs*	Chagas' disease/American trypanosomiasis	Pyrethroid, deltamethrin, fluralaner or afoxolaner

DDT: dichlorodiphenyltrichloroethane; HCB: hexachlorobenzene; PBO: piperonyl butoxide.

Table 1.
Major group of insects causing human diseases and insecticides used for their control [7, 8].

3. Types of insecticides and their modes of action

There are many classes of insecticides with varying modes of action. The insecticides used in different vector control programmes are generally classified into four classes on the basis of their chemistry, toxicological action, or their mode

of penetration, *viz.,* organochlorines, organophosphates, carbamates, synthetic pyrethroids insect growth regulators and bacterial larvicides (**Figure 1**).

3.1 Organochlorines

These are chlorinated hydrocarbons which represent diverse group of compounds with carbon, hydrogen and chlorine in their structure. They comprise of three subgroups, namely, dichlorodiphenylethanes (dichlorodiphenyltrichloroethane [DDT], dicofol, methoxychlor, and perthane), chlorinated cyclodienes (aldrin, endrin, dieldrin, chlordane, endosulfan, and heptachlor), and hexachlorocyclohexanes (benzene hexachloride [BHC], chlordane, lindane, mirex, and toxaphene) [9]. The only organochlorine compound being used in residual spraying is DDT with 82% of organochlorines compounds being used in Southeast Asian region, mainly India, for vector control. DDT causes the alteration of the sodium and potassium ion transport across axonal membranes; this results in increased negative after-potential and prolonged action potentials, which consequently leads to repeated firing and occurrence of sequential action potentials, thus causing spasms and death of the insect [10]. DDT was first used during World War II for the control of mosquitoes and was extensively used in the period from 1940s to 1960s, and was banned in 1972 by the Environmental Protection Agency (U.S.A.).

3.2 Organophosphates

These are a group of synthetic compounds produced by the reaction of alcohols and phosphoric acid. These inhibit the enzyme acetylcholinesterase (AChE), which is responsible for the degradation of acetylcholine. The organophosphate binds to the enzyme, causing it to undergo a conformational change at its binding site to acetylcholine [11]. Their application is mainly by three methods: residual spraying, space spraying and to a lesser extent as larvicides. The organophosphates are extensively used in Southeast Asia, followed by the Americas and the Western Pacific.

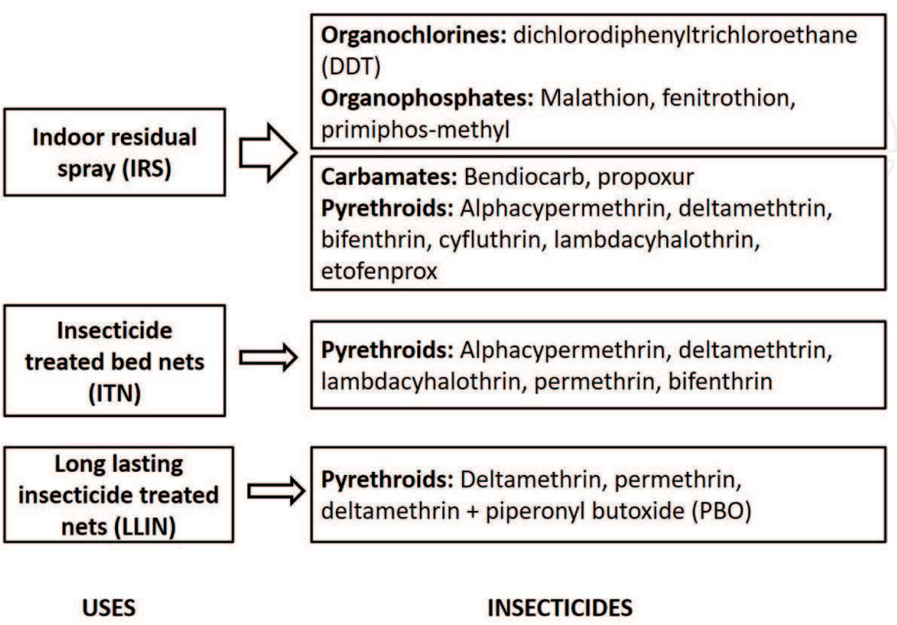

Figure 1.
Insecticides used for indoor residual spray (IRS), insecticide treated nets (ITN) and long-lasting insecticide treated nets (LLIN) [9, 11–14].

3.3 Carbamates

These are esters of carbamic acid and structurally and mechanistically similar to organophosphate (OP) insecticides. These insecticides work by inhibiting AChE and are commonly used to control agricultural pests. Compared with the other classes of insecticides, the use of carbamates is limited, and mainly used for residual spraying in the African Region [12].

3.4 Pyrethroids

These compounds are organic, and similar to the naturally occurring pyrethrins produced by the flowers of pyrethrums. On the basis of their biological response, they are divided into two groups – Type I and Type II pyrethroids. Pyrethroids are used in all the four major methods of application: about 70%for residual spraying, 25% for space spraying, and the remainder for treatment of nets and larvicidal purposes. In terms of the weight of active ingredient, pyrethroids are not the most used insecticides, but in terms of spray coverage they are by far the most used insecticides. The large-scale usage of pyrethroid insecticides for vector control is worrisome because it exerts a high selection pressure for the development of resistance in vector populations. The genes conferring resistance against pyrethroids have been spreading in vector populations, particularly in the populations of malaria and dengue vectors [13, 14]. This is particularly concerning because the use of long-lasting insecticidal nets (LLINs), a major tool in malaria control, depend solely on the action of pyrethroids. It is critical that the susceptibility of malaria vectors to pyrethroids is preserved. Therefore, it has been recommended to not use pyrethroids for indoor residual spraying where there is a high coverage of its use with treated nets [13].

3.5 Insect growth regulators (IGRs)

These are diverse group of chemical compounds, the use of which dates back to 1980s [15]. These compounds are effective against the larval stages of insects. They are divided into juvenile hormone analogs and chitin synthesis inhibitors. They mimic insect hormones, such as juvenile hormone and ecdysone, and interfere with the normal growth and development of the insect.

3.6 Bacterial larvicides

These larvicides are based on the bacteria of the species *Bacillus sphaericus* (Bs), and *Bacillus thuringiensis* serovar *israelensis* (Bti), which are entomopathogenic. In early 1960s the first strain of *Bs* with its larvicidal activity was discovered but in 1976 the Bti subspecies was discovered which was highly toxic to larvae of many species of mosquitoes. By mid-1980s, the use of bacterial larvicides started in different vector control programmes.

4. Insecticide resistance in disease vectors

The insecticide act in many different ways, to which insects have also developed different mechanisms by which they develop resistance against the toxic effects of these insecticides. Broadly, there are three different mechanisms of development of resistance, i.e., metabolic resistance, target site and resistance to penetration of

the insecticide. Correspondingly, different biological, biochemical and molecular methods have been developed to detect these mechanisms in different vectors.

Status of insecticide resistance in vectors of parasitic diseases

4.1 Malaria

Malaria remains one of the deadliest vector-borne disease. Repeated exposure of the malaria vectors to insecticides over several decades has resulted in resistance to many of them. The insecticide resistance in vectors of malaria was first reported in 1950s for DDT [3]. Till date, the vectors of malaria are known to have developed resistance to the four major insecticide classes, *viz.,* pyrethroids, organochlorines, carbamates and organophosphate. Sub-Saharan Africa contributes to 90% and Southeast Asia contributes 7% of the malaria cases reported worldwide, whereas in the Latin Americas, malaria cases have significantly declined, the major contribution of cases now being from the Amazon region [16]. From Africa, all the four major species of *Anopheles,* which are the vector for malaria, have developed resistance to pyrethroids, except in the south-western Africa. In Southeast Asia, resistance to PY has been reported for *An. minimus*, *An. vagus* and *An. sinensis* in China, Thailand and Vietnam [17]. In India, *An. stephensi*, the prime urban vector and *An. culicifacies*, the rural vector of malaria, have developed resistance to PY, DDT and OP in the states of Goa, Tamil Nadu, Orrisa and Chhattisgarh, whereas only one population in western Columbia in the Amazon region has developed resistance to only PY and DDT and not OPs [18]. The resistance to DDT has also been reported in Thailand and Vietnam [19], and in India, the resistance has been reported from Gujarat and Rajasthan [20]. The major vector of malaria in Orrisa, *An. fluviatlis* (S form), and in the Amazon region, *An. darlingi,* are reported to be susceptible to the all classes of insecticides [21]. In addition, the resistance to bendiocarb, a CA which is commonly used in IRS, has also been reported across Africa. The resistance to OP has been limited to only west and east Africa. Multiple resistance to insecticides has been reported from different parts of all these regions [22].

4.2 Lymphatic filariasis

The prominent vector of lymphatic filariasis is *Culex quinquefasciatus*. The resistance against synthetic insecticides, i.e., DDT and malathion was reported from filariasis endemic states of India, Uttar Pradesh, Bihar and Kerala [23]. In a study carried in different localities of Brazil, the resistance to DDT, pyrethroids and carbamates have been recorded [24]. This mosquito was found resistant to malathion, permethrin in Kuala Lumpur and to pyrethroid, deltamethrin, permethrin in Zambia, only to pyrethroids in Zanzibar, and highly resistance to permethrin in Central Java [25, 26].

4.3 Leishmaniasis

It is the second most prominent parasitic infection after malaria in terms of fatalities caused by it globally. The phlebotomine sandflies are the prominent vectors of leishmaniasis, mainly found in tropics and subtropics [27]. For its vector control, the chemical interventions used are IRS and ITNs. Since 1944, DDT-based IRS was mainly used for the control of sandfly, but after 1970s, due to its toxic effect and reports on DDT resistance in sandfly from the disease endemic regions in India resulted in the use of alternative insecticide, i.e., pyrethroids [28, 29]. The deltamethrin, lambda-cyhalothrin and alpha-cypermethrin resulted in more

than 70% of reduction in both *Lutzomyia* spp. and *Phlebotomus argentipes* sandflies [30]. Insecticide-treated durable wall lining (DWL) is being used as an alternative type of indoor residual intervention to increase the residual effect of insecticides used in IRS [31] and it drastically reduced the abundance of *P. argentipes* in south Asian countries [32]. Presently, IRS spraying and use of ITNs are the most common methods implemented in vector control programmes for the control of sandfly.

4.4 Human African Trypanosomiasis (HAT)

HAT is transmitted by the bite of the tsetse fly, i.e., *Glossina* spp., across most of the 38 countries of the sub-Saharan Africa [33]. In 1945, DDT and BHC were the only synthetic insecticides used for their control, but later on pyrethroids like deltamethrin were used. In Ethiopia, deltamethrin impregnated nets were used in 1990 for the control of *Glossina pallidipes* [34], but later on resistance was reported to deltamethrin [35].

4.5 Chagas' disease/American trypanosomiasis

The Chagas' disease, transmitted by triatomine bugs, is a major disease affecting millions of people in the Latin America countries. In 1950s, DDT was used to control the triatomine density [36], following which HCB was considered to be more effective, and later, dieldrin was also used for its control. In 1999, resistance in triatomine bugs was not a serious problem except for some reports where *Rhodnius prolixus* showed resistant to pyrethroids in Venezuela, and *Triatoma infestans* in Brazil [37, 38]. Some reports of deltamethrin resistance in *T. infestans* are also there from Argentina [37]. In a study carried out in Bolvia also, *T. infestans* populations were found resistant to deltamethrin [39]. The expression of resistance to pyrethroids during the early phase of embryonic development in *T. infestans* has also been reported [40].

5. Targets and techniques for detection of insecticide resistance:

5.1 Metabolic resistance

It is the most common mechanism of development of resistance to insecticides. In this type of resistance, either the enzymes which detoxify the insecticide are over expressed or there is an altered affinity of the enzyme for the compound used, mainly caused by substitution of amino acids, mainly in the three major enzyme families (cyrochrome P450 monooxygenase, glutathione S-transferase [GSTs] and esterase) which are involved in metabolism of the insecticide compound [41].

In mosquito species, the vectors of malaria and lymphatic filariasis, several CYP450 genes has been documented to be involved in resistance to pyrethroids, *viz.,* $CYP6P_3$ and $CYP6M_2$, CYP6P9a, $CYP6Z_3$ [42], while $CYP6Z_1$ is attributed for conferring resistance to both carbamates and pyrethroids [43]. $CYP9M_{10}$ and $CYP6AA_7$ confer resistance to the insecticide permethrin in *Culex* species [44, 45]. The GSTs confer resistance to DDT in mosquitoes, i.e., *Anopheles*, and GSTe2 GSTe3, GSTe4 have been reported to be involved in pyrethroid resistance in *An. funestus* in Uganda and Kenya [46]. Several P450s and GST genes were reported to be overexpressed in a deltamethrin-resistant *An. sinensis* in China and Southeast Asian countries [47]. In a recent study carried out by Yan et al., several genes have been identified which confer permethrin resistance to *Culex pipiens quinquefasciatus,* particularly, 2 CCEs, 6 GSTs, and 7 P450s gene were highly expressed [49].

The detection of the enzymatic activity in biochemical assays is the most commonly used method to assess development of resistance to insecticides in insects. In several studies done to detect pyrethroid resistance, increased P450 monooxygenases and esterase activity have been recorded in the resistant strains of *Anopheles*, *Culex*, and triatomine bugs [40]. For sandfly, metabolic resistance is not adequately studied and only some studies of insecticide resistance related to bioassays on *P. argentipes* and *P. papatasi* populations from India and on *Lutzomyia* populations in South America have been reported [29].

5.2 Target site resistance

Here, the target site of the action of the insecticide may be modified genetically. As a result, the binding or interaction of the insecticide at its site of action is thereby prevented which decreases the efficacy of the insecticide.

Mechanism of resistance	Molecular determinants	Known point mutations	Type of vector
Metabolic resistance	Glutathione S-transferase gene (GSTe2)	L119F-GSTe2 mutation	*An. funestus* [46, 50]
	Cytochrome P450 monooxygenases	CYP6M2, CYP6P3 CYP4G16, CYP4G17 CPAP3-E and CPLCX1 *CYP9K1, CYP6M7, CYP4H18, CYP4H17, CYP4C36,* CYP6Z1, CYP6M2 and CYP6P3 in bendiocarb resistance Overexpression of Esterase A and Esterase B genes CYP9M10 (cyp450); CYP6AA7 overexpressed in pyrethroid resistance; $CYP_{5122}A1$	*An. gambiae*; *An. funestus, Cx. quinquefasciatus* [45, 48, 49]
Target site resistance	Voltage gated sodium channel gene (*VGSC*) gene (*kdr* mutations)	S6 segment of domain II- L1014F/S; N1575Y L925I mutation L1014F and L925I in the second domain of the sodium channel gene protein *VGSC*-1014F and *VGSC*-1014S, *ace-1* (G119S) and *rdl*-A296S or *rdl*-A296G	*An. funestus*; *An. sinensis*; *An. gambiae*; *An. culicifacies*); *An. vagus*; *Phlebotomus argentipes;* triatomne bugs [40, 43, 51–54]
	Acetylcholinesterase gene	G119S; N485I	*An. gambiae*; *An. funestus*; *An. coluzzii; Phlebotomus papatasi* [55, 56]
Penetration resistance		CPLCG3 gene	*An. gambiae*; *Cx. pipiens pallens* [18, 57]
	Aminobutyric acid	Alanine to serine at position 302 or 296	*An. gambiae*; *An. funestus* [58]

Table 2.
Genetic determinants and point mutations associated with insecticide resistance in various insect vectors [18, 40, 43, 45, 46, 48–58].

Various target-site mutations in the voltage-gated sodium channel (*VGSC*) gene were recorded in response to pyrethroids in different species of mosquitoes, *viz*., *Anopheles gambiae* complex, *An. funestus* and *An. culicifacies*, *Phlebotomus argentipes* and triatomine bugs (**Table 2**). The most common resistance associated mutation reported in the AChE gene is G119S in species of *Anopheles* and *Phlebotomus*.

6. Methods of detection of insecticide resistance

The development of resistance to insecticides in insects is a complex phenomenon which depends on many direct and indirect factors. The direct factors include the natural variations in the genetic, biochemical, physiological, ecological and behavior of insects, while the indirect factors are the operational factors such as categories of insecticides used, the timing and method used for their application [59]. There are different bioassays which exploit some of these factors and help in detecting insecticide resistance, i.e., by phenotypic, genotypic and proteomic analysis.

The laboratory bioassays are useful in detecting susceptibility, tolerance and resistance in vectors against insecticides [60]. The phenotypes of vectors are utilized in detecting the insecticide resistance in methods such as by bottle bioassays. In this, a range of concentrations of the insecticide is used to study the target site resistance followed by knocking certain genes in the insect population [61]. The WHO cone tests, wireball assays, tube tests [62] and the Centers for Disease Control and Prevention (CDC) bottle bioassays take long exposure times, therefore, an alternative method – mosquito contamination device (MCD) bottle bioassay – has been developed for the control of malarial vectors in resource poor settings [63]. In vector control programmes, it is recommended that bottle bioassays should be routinely use in laboratory to measure the phenotypic resistance of a vector against a particular insecticide so as to determine whether it is still effective [64].

In addition to the traditional bioassays for determining resistance to insecticides, currently, various molecular markers and techniques to detect target site mutations have been developed. These genetic mutations can be identified by various PCR based methods such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), microsatellites and single nucleotide polymorphisms (SNP). To observe changes at the RNA level, molecular techniques such as real-time polymerase chain reaction (RT-PCR), differential display reverse transcription PCR, northern blot and microarrays may be used. For protein estimation enzyme assays, various techniques such as enzyme linked enzyme-linked immunosorbent assay (ELISA), western blot, sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) may be used [65, 66]. In addition to this, to study the mechanism of resistance at the molecular level through proteomics, the identification of resistance-related proteins with their expression profiling can provide knowledge of the activity of proteins related to insecticide resistance in insects [67].

6.1 Detection of insecticide resistance in malaria vectors

6.1.1 Phenotypic assays

The WHO and CDC bioassays are the most common assays carried out to detect the insecticide resistance in malarial vectors till now. The WHO bioassays have been carried out in various studies, such as in detection of *Anopheles culicifacies* resistance to DDT, malathion and deltamethrin in India (60), *An. stephensi* in eastern

Ethiopia [68], and *An. arabiensis* susceptibility to bendiocarb, lambda-cyhalothrin and deltamethrin in Yemen [69]. The CDC bottle bioassay had been carried out to detect metabolic resistance as well as biochemical resistance against permethrin in *An. arabiensis* in Tanzania [70], and to quantify the resistance of insecticides of deltamethrin, lambda-cyhalothrin, alpha-cypermethrin, permethrin and DDT in *An. darlingi, An. nuneztovari* and *An. albimanus* in Colombia [71]. Another bioassay used for detecting the phenotypic resistance in the malarial vectors is MCD bottle bioassay. These bioassays are helpful and significant as they detected the effect on the behavior of *An. stephensi* and *An. gambiae* after the exposure to insecticides [63].

6.1.2 Genetic assays

To detect target site mutations, due to resistance to insecticides such as pyrethroids, carbamates and organophosphates in different *Anopheles* species, PCR assay is most commonly used [72]. It helps in detecting kdr mutations in different species of *Anopheles* due to overuse of pyrethroids/DDT [73, 74]. This assay also detected the resistance in vector population at the metabolic level which involves genes such as cytochrome P450 genes, carboxylesterases and glutathione S-transferases in *Anopheles coluzzii*. Profiling of gene expression was carried out by multiplexing followed by qRT-PCR, the findings from these studies proved it as another effective method for detection of insecticide resistance in malarial vectors. Moreover, study had been carried for the polymorphic genes: P450 genes CYP6Z1, CYP6Z3 and CYP6M7 against pyrethroid resistance in the malaria vector *An. funestus.* The analysis reported the changes in amino acids by QTL, which showed the contribution of these polymorphic genes in the insecticide resistance [75]. Thus, the characterizations and genetic profiling methods can improve the understanding regarding the target site mutations and metabolic resistance in malarial vectors.

6.1.3 Proteomics assays

The major driving factors which contribute to the malaria transmission are the age, method of blood feeding and way of infection spread. These factors could be promising target for detecting the insecticide resistance through proteomics. In *Anopheles* mosquitoes, with the help of artificial neural networks (ANNs) and MALDI-TOF/MS, the effect of insecticide resistance on these factors were described [76]. Proteomics detected the contribution of age as one of significant factor to insecticide resistance by using matrix-assisted laser desorption ionization tandem time-of-flight mass spectrometry or capillary high-pressure liquid chromatography with linear ion-trap (LTQ)-Orbitrap XL hybrid mass spectrometer which was further quantified by Western Blot leading to detection of protein biomarkers [77]. Proteomics study also detected metabolic resistance and target sited mutations by NCBInr/Protein BLAST and MS/MS-FTMS in *An. gambiae* in Burkina Faso [78]. Multiple resistance, which is emerging as the big issue in insecticide resistance management has also been detected with the help of proteomics like 2D electrophoresis and MALDI TOF in *An. stephensi* [79]. Thus, proteomics analysis can be a promising tool to tackle various obstacles in insecticide resistance management.

6.2 Detection of insecticide resistance in filarial vectors

6.2.1 Phenotypic assays

WHO bioassays are most commonly used diagnostic assay for detecting insecticide resistance in *Culex* phenotypically which is proved by extensive experimental

studies such as to detect the knockdown resistance in *Cx. quinquefasciatus* in Sri Lanka [80]; as the diagnostic assay in *Cx. pipiens* against organochlorine, pyrethroid, organophosphate and carbamate insecticides. Larval bioassay by microplate method in the *Cx. pipens* larvae has also been carried out to study the knockdown and metabolic resistance in filariasis endemic areas of Egypt [81]. In India, following the WHO diagnostic methods for phenotypic detections, susceptibility studies have been carried out against DDT and Deltamethrin in *Cx. quinquefasciatus* from northeastern India to study the target sited resistance in this vector [82]. Some other studies have also been carried out using WHO bioassays for detecting the insecticide resistance such as metabolic resistance in *Cx. pipiens pallens* in China [83]; target sited mutations of genes G119S ace-1 and L1014F in *Cx. pipiens* complex and their hybrids in Morocco [84].

6.2.2 Genotypic assays

PCR based methods such as amplification of specific gene targets by PCR followed by sequencing of the amplified product can be used to detect specific mutations associated with resistance. For example, L1014F mutation on the VGSC domain IIS6 had been reported in *Cx. quinquefasciatus* in Sri Lanka associated with resistance to insecticides [85]. Various resistance genes have also been discovered through transcriptome profile by whole-transcriptome microarrays in *Culex* species. With the help of pyrosequencing, the mutations and metabolic insecticide resistance due to deltamethrin in *Cx. quinquefasciatus* from Zanzibar was determined [86]. These assays provided evidence for insecticide resistance caused due to target site mutations and metabolic alterations at the genetic level [87].

6.2.3 Proteomics assays

A global protein profile among insecticide resistance strains and susceptible strains of *Culex* may be obtained through proteomics. The alterations at the proteomics level can be determined by quantification of certain tags using liquid chromatography/tandem mass spectrometric analysis. The protein profiles have also been detected using isobaric tag for relative and absolute quantitation (iTRAQ) data analysis method which has confirmed the susceptibility status of strains of *Culex* in studies [88]. Such studies assure that proteomics can be considered as promising tools for studying insecticide resistance in mosquito populations.

6.3 Detection of insecticide resistance in the vector of *Leishmania*: Sandfly

6.3.1 Phenotypic assays

The WHO bioassays detected the knockdown resistance type mutations and certain metabolic enzyme resistances due to exposure of DDT, malathion, deltamethrin and propoxur in *Phlebotomus argentipes* [89]. Another study detected the resistance in *Paralongicollum sergenti* and *P. papatasi* on exposure to DDT and lambdacyhalothrin in Morocco [90]. The WHO and CDC bottle bioassays have also been conducted to study the diagnostic time and dose in *P. papatasi* to study insecticide resistance for pyrethroids, organophosphates, carbamates and DDT [91].

6.3.2 Genotypic assays

The detection of molecular alterations such as the mutations in *VGSC* gene have been detected in sandflies [92]. The pyrethroid resistance mutations (kdr) were

also studied in the sandfly vector population worldwide with PCR as the major tool for detection [93]. Target site resistance of voltage gated sodium channel gene (kdr mutations) at position L1014F/S in *Phlebotomus papatasi* has also been determined through PCR [91].

6.4 Detection of insecticide resistance in the vector of Chagas' disease: Triatomine bug

6.4.1 Phenotypic assays

The WHO bioassays have been conducted in many species of Triatominae against DDT resistance [94], where behavioral responses were observed in *T. infestans* strains to deltamethrin, on the basis of which the insect populations were differentiated into susceptible and resistant [95]. The other behavioral and environmental factors such as dispersal or migration of the vector populations leading to the changes in feeding behavior, nutritional status and reproductive behavior also contribute to the alterations in the response to insecticides [96]. Therefore, observation of such changes can be utilized in detection of insecticide resistance in vector populations.

6.4.2 Genotypic assays

Target site mutations and metabolic resistance are the major pathways of insecticide resistance in insect vectors. In case of *Triatomine* bugs, studies have utilized the detection of these altered effects on certain enzymes and genes. The qRT-PCR was used to compare three triatomine species: *Triatoma dimidiata, T. infestans* and *T. pallidipennis* by studying their resistance associated metabolic and the target mutations in Latin America [97]. The transcriptome analysis of the genes encoding various proteins played a significant role in *T. infestans* to analyze the metabolism of essential compounds in the vector's body and its effect on insecticide resistance [98]. The detection of pyrethroid induced alterations in the developing eggs of *Triatomine* at the molecular level has proved to be another promising tool [99, 100].

6.4.3 Proteomics assays

Some insecticides interfere with the physiological functions in insects such as affecting the neuro-endocrine hormones. This property may be used to study the development of resistance to these insecticides and proposing candidate molecular targets responsible for resistance. LC/MS–MS has been utilized to validate certain post translational modifications in the neuro-endocrine factors in *Triatomine* [101]. The proteins essential for the structural and the metabolic constructs in Triatomine can prove to be good targets for detecting insecticide resistant populations. The transcriptome analysis, BLAST analysis and other sequencing platforms can pave the way for detecting the resistance effects on the basis of transcription factors, cell signaling pathways and cellular biology of the vector [98].

7. Alternative strategies to combat insecticide resistance

7.1 Rotation and mixture

This method involves use of insecticides of one or different classes with different target sites or distinguishable mechanism of actions in rotation or in sequence. The hypothetical basis of this strategy is that if the resistance to a particular insecticide

is rare, then the use of multiple insecticides may decrease the chances of resistance to the least possible. This strategy should be allowed to run for prolonged periods of time so that there is no reversal of the effect [102]. Thus, annual rotation programmes are carried out extensively with the rotation of multiple classes of insecticides with different target of actions for vector populations. Usually, this practice is carried out during the growing season in agricultural practices. The rotation and mixture method of insecticide resistance may further be modified by using insecticide mosaics and combinations. This includes use of two different insecticides in two different areas thus lowering down the probability of development of resistance against one particular insecticide [103].

7.2 Bioinsecticides

Insecticides create a huge bioburden due to their chemical nature and pose a threat to human health and environment. Hence, there is a need to replace insecticides with biologically friendlier methods, such as by using bioinsecticides such as bacterial and fungal species against insecticides. Following this approach, the production of bioinsecticide has gained momentum, but have yet not been put to common use due to their high production cost. There is also a need to more research regarding their use and reliability. Many species of *Bacillus* are used in agriculture and have showed effective results. Plant extracts such as nicotine, pyrethrum, and neem oils are also are also been increasingly used with the green revolution. Another promising biocontrol agent is *Androctonus australis* anti-insect toxin (AaIT), which targets the neurological system of the insect vectors. Thus, exploitation of certain characteristics of the naturally occurring organisms in nature may potentially lead to the development of useful biocontrol agents against vector populations.

8. Future perspectives

Insecticide resistance management is the only way to reduce the selection pressure of insecticides. Newer tools are needed to be designed to detect the resistance apart from the existing phenotypic and genotypic methods. The lack of adequate research and development of methods for resistance detection is a major obstacle in insecticide management. Advancements in these areas hold the potential to eradicate vector borne diseases in the future and must be promoted. There are many approaches of genetic and proteomics studies for resistance detection which are yet unutilized for certain vectors such as *Leishmania*, *Triatomine*, and *Glossina*, indicating that much work needs to be done in this area. The development of these techniques can pave the way to study alterations in the physiology of vectors at genetic level due to insecticide resistance. The various other factors influencing the resistance development in vectors such as seasonality, distribution of vectors in given geographical area, alterations at allelic level which may lead to modified phenotypic traits are yet to be explored. An overall understanding of the vector species, their genotypes, phenotypes and proteomes involved in insecticide resistance for various vectors is required. Such models will help in deploying various vector control strategies optimally, and will also help in innovating newer methods to combat insecticide resistance.

9. Conclusions

The key component of vector control programmes is the use of insecticides, however, a timely emergence of resistance in insects is very crucial for the success

of this component. Large number biological, environmental, and geographical factors are responsible for emergence of insecticide resistance in vectors. The two most common mechanisms by which vectors develop resistance to insecticides are metabolic and target site. In metabolic resistance, the three major enzymes involved are cytochrome P450 oxygenases, GSTs and esterases. The activity of these enzymes can be studied for different insecticides by standard biochemical tests. For target site resistance, the known or unknown mutations in *VGSC* and AChE genes can be studied by various techniques like PCR, RT-PCR and AFLP. To combat the emergence of insecticide resistance, various alternative strategies which involve the use of rotational insecticides, bioinsecticides and ITNs/LLINs are used. Yet many more strategies like extensive and regular surveillance of insecticide resistance, development of more sensitive techniques for the detection and area wise mapping of insecticide resistance of individual vectors may help to overcome the development of resistance in vectors.

Conflict of interest

None to declare.

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