Chapter 7

Insecticide Resistance in Malaria Vectors: An Update at a Global Scale

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Abstract

Malaria remains the deadliest vector-borne disease in the world. With nearly half of the world's population at risk, 216 million people suffered from malaria in 2016, with over 400,000 deaths, mainly in sub-Saharan Africa. Important global efforts have been made to eliminate malaria leading to significant reduction in malaria cases and mortality in Africa by 42% and 66%, respectively. Early diagnosis, improved drug therapies and better health infrastructure are key components, but this extraordinary success is mainly due the use of long-lasting insecticidal nets (LLINs) and indoor residual sprayings (IRS) of insecticide. Unfortunately, the emergence and spread of resistance in mosquito populations against insecticides is jeopardising the effectiveness of the most efficient malaria control interventions. To help establish suitable resistance management strategies, it is vital to better understand the distribution of resistance, its mechanisms and impact on effectiveness of control interventions and malaria transmission. In this chapter, we present the current status of insecticide resistance worldwide in main malaria vectors as well as its impact on malaria transmission, and discuss the molecular mechanisms and future perspectives.

Keywords: malaria, mosquito, insecticide resistance, pyrethroids, bed nets, metabolic resistance, cytochrome P450, knockdown resistance

1. Introduction

In 1993, Steven Spielberg produced 'Jurassic Park', one of the most internationally acclaimed movies at that time. This science-fiction story is based on the cloning of dinosaurs using its DNA from mosquitoes that had been preserved in amber. Although the idea is brilliant, the technical

limitations to get entire genome of dinosaurs from ancient DNA make it impossible [1]. However, the movie is right on one fact that mosquitoes existed at the same time as dinosaurs probably biting them as other animals before evolving to become human biters [2]. But only few mosquitoes have specialised in biting humans (anthropophily), although those that succeeded have caused devastating consequences to mankind. From all diseases that mosquitoes can transmit, malaria has been and still is the one with the greatest health and socioeconomic impact, from the ancient Egypt to present time [3]. For example, malaria has been suggested as one of the causes of the death of the great Tutankhamun [4], one of the Egypt's famous pharaoh. Malaria remains the deadliest vector-borne disease in the world. With nearly half of the world's population at risk, 216 million people suffered from malaria in 2016, with over 400,000 deaths, mainly in sub-Saharan Africa [5]. Recent global efforts have been made to control and eliminate malaria leading to significant reduction in malaria cases and mortality in Africa by 42% and 66%, respectively. Early diagnosis, improved drug therapies and better health infrastructure are key components, but this success is mainly due the use of insecticide-treated nets (ITNs), long-lasting insecticidal nets (LLINs) and indoor residual sprayings (IRS) of insecticide [6]. Unfortunately, the emergence and spread of resistance in mosquito populations against insecticides is jeopardising the effectiveness of the most efficient malaria control interventions [7]. Insecticide resistance is spreading globally. Currently, of 73 countries with ongoing malaria transmission that provided data, 60 countries reported resistance to at least one class of insecticides, while 50 reported resistance to two or more insecticide classes [5]. In this chapter, we present the current status of insecticide resistance worldwide in main malaria vectors, as well as its impact on the epidemiology, and discuss the molecular mechanisms and future perspectives.

2. Insecticide resistance in malaria vectors

The term insecticide resistance is defined as the ability of an insect to withstand the effects of an insecticide by becoming resistant to its toxic effects by means of natural selection and mutations [8]. Repeated exposure to insecticides selects individuals possessing biochemical machineries that can detoxify the insecticides more rapidly or are less sensitive to it [9]. These individual survivors could then pass the resistance mechanism to the successive generations resulting in pest populations that are more resistant.

2.1. Development of the insecticide resistance

Resistance has been observed in more than 500 insect species worldwide [10], including malaria mosquitoes. Mosquitoes are typical R-strategists (animals that reproduce fast and produce a large number of offspring), and can adapt fast to environmental changes. As a consequence of this and the widespread use of insecticides in agriculture and public health, resistance has arisen relatively rapidly in malaria vectors. Insecticide-resistant phenotypes are favoured where mosquitoes are exposed to sub-lethal doses of the insecticide. Under these conditions, resistant individuals have a better chance to survive and reproduce; this means

selection pressure towards resistant populations. Such conditions can result from vector control through insecticide decay (on treated walls or nets) or bad spraying technique. Insecticide resistance was first reported in malaria vectors in the 1950s [11], and resistance to dichlorodiphenyltrichloroethane (DDT) and pyrethroids is now widespread [12]. Resistance is predicted to impair malaria control efforts but evidences from field studies remain limited and potentially conflicting [7]. To date, malaria vectors have developed resistance to the main chemical classes used in public health, i.e., pyrethroids (PYs), organochlorines (OCs), carbamates (CAs) and organophosphates (OPs). Although public health use of insecticide has an impact on the development of resistance in mosquitoes, one key source of resistance in malaria vectors remains the massive use of insecticides for control of agricultural pests [13]. Other chemicals and factors aside from insecticides may create a selective environment, which favours build-up of resistant populations [14].

2.2. Monitoring of insecticide resistance

Surveillance to monitor the emergence and spread of resistance is an essential step in insecticide resistance management (IRM) providing baseline data for programme planning and choice of insecticide [15, 16]. Effective resistance monitoring can improve the efficacy of vector control and may also delay or prevent the onset and spread of resistance. Insecticide resistance is commonly assessed by exposing mosquitoes to a diagnostic dose using standard protocols published by WHO [17]. However, if resistance alleles are partially or fully recessive, like *kdr* [18], bioassays will only detect resistance when alleles have already reached a frequency high enough for resistant homozygotes to occur. Detection of resistance at the molecular level is more sensitive and can provide early warning of target-site and metabolic resistance.

2.3. Worldwide pattern of insecticide resistance

The worldwide distribution of the dominant malaria vectors is represented in **Figure 1**.

2.3.1. Sub-Saharan Africa

Malaria morbidity in sub-Saharan Africa represents 90% of the total cases reported worldwide [5, 19]. Many vectors play an important role in malaria transmission across Africa, notably the four major malaria vector species, i.e., *Anopheles gambiae* (including *An. gambiae sensu stricto* (s.s.) and *An. coluzzii*), *An. arabiensis* and *An. funestus* s.s. [20]. In the past decade, PY resistance in these major malaria vectors has spread across the continent being prevalent in west, central, east and southern Africa [12]. As far as we know, south-western Africa (Namibia and Botswana) remains the only region where PY-resistant *Anopheles* populations have not yet been reported (**Figure 2A**; data source: irmapper.com, 2017). The PY resistance is a great concern because PYs are the main insecticide class recommended for LLINs impregnation [21]. Resistance to LLIN exposure increases mosquito survival, which may lead to rising malaria incidence and fatality in Africa [22]. However, insecticide resistance of malaria vectors is not limited to PYs only but also exists to the other three classes of insecticides used in public health, such as CAs, OCs and, to a lesser extent, OPs [12]. However, some differences have been observed in the distribution of resistance among regions across the continent. For

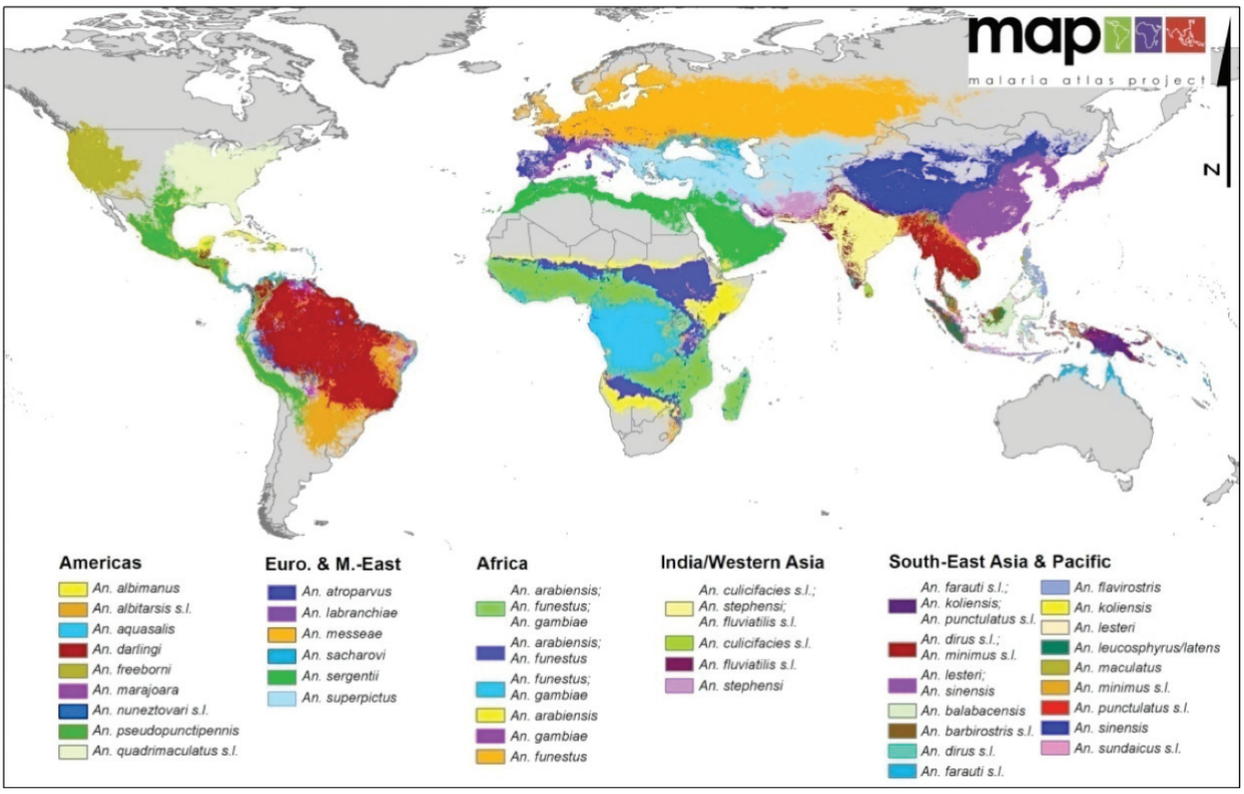

Figure 1. Global distribution of the dominant vector species of malaria [137].

example, resistance to DDT, the most common OC used in IRS, has been reported in *An. gambiae* and *An. funestus* in western, central and eastern Africa [23–28], whereas it is practically absent in southern Africa (**Figure 2B**), with the exception of an *An. funestus* s.s. population in southern Malawi [29]. DDT resistance has been also reported in *An. arabiensis* in southern Africa, specifically in Madagascar, Mozambique and South Africa [30–32]. In addition, resistance to CA, especially bendiocarb, which is commonly used in IRS, has been reported across Africa (data source: irmapper.com, 2017), although the regions with widespread CA resistance are focused in west and southern Africa (**Figure 2C**). So far, resistance to OP is less prevalent, limited to few reports in West and East Africa for *An. gambiae* s.s. and *An. arabiensis*, respectively (**Figure 2D**) [33, 34], whereas *An. funestus* s.s. populations remain fully susceptible across the continent.

2.3.2. Southeast Asia and Western Pacific Region

After Africa, Southeast Asia is the area with a higher incidence of malaria, with 7% of the cases reported [5]. A good number of vectors (belonging to complexes or groups of species that are difficult to distinguish) are involved in transmission, presenting an extraordinary biodiversity, heterogeneity in distribution, linked with a high variety in host feeding and ecological habitat preferences, as well as high differences in vector competence [35–37]. Currently in Southeast Asia, PY resistance has been detected in *An. epiroticus* in Vietnam [38], *An. minimus* in Thailand and Vietnam [35, 38], *An. sinensis* in China and Vietnam [39, 40] and *An. vagus* in Cambodia and Vietnam [38]. Similarly, DDT resistance has been detected in *An. minimus* in Cambodia

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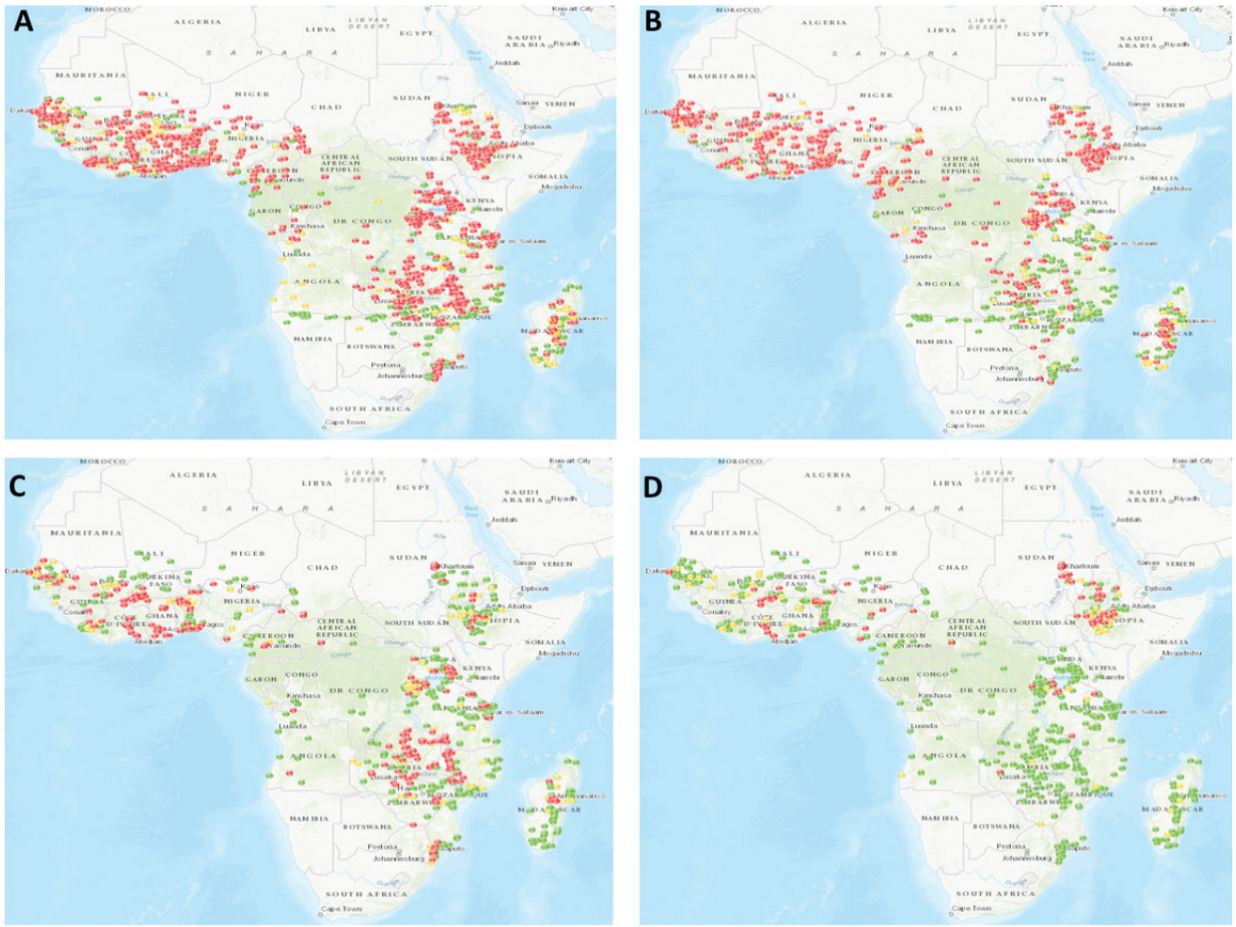

Figure 2. Distribution of resistance to all four classes of insecticides in the major malaria vectors belonging to *An. gambiae* complex and *An. funestus* group in Africa from 1985 to 2017. The green dots represent full susceptibility, orange is for suspected resistance and red for confirmed resistance. (A) Widespread resistance to pyrethroids. (B) Widespread resistance to DDT (organochlorines) although susceptibility is observed in southern Africa in *An. funestus* populations. (C) Profile of resistance to carbamate with significant areas of resistance in west and southern Africa. (D) Broad susceptibility to organophosphates across the continent but with pockets of resistance in West Africa and Ethiopia in *An. gambiae* s.l.

and Laos [40], *An. dirus* and *An. minimus* in Thailand [35] and possibly *An. epiroticus* in Vietnam [38]. Alarming, high level of multiple resistance to all classes of insecticides used in public health has been reported recently in *An. sinensis* in malaria endemic areas of China, including permethrin, deltamethrin, bendiocarb, DDT, malathion and fenitrothion, among others [41–43]. In South Asia, represented mainly by India, *An. baimaii* and *An. minimus* are also present but geographically restricted to East and Northeast regions and are fully susceptible to all classes of insecticides [44, 45]. *An. stephensi*, *An. culicifacies* species E and *An. fluviatilis* species S are the other predominant vectors responsible for malaria transmission in mainland India [36]. *An. stephensi*, prime urban vector in India, has shown resistance to PY, DDT and OPs in Goa State [46]. In addition, resistance to DDT was also detected in *An. stephensi* populations from Gujarat and Rajasthan [47]. PY-resistant populations of *An. culicifacies* s.l., present mainly in rural areas, have been reported in almost all the regions [48, 49]. In the last few years, resistance of *An. culicifacies* s.l. is increasingly being spread in many States such as Chhattisgarh, Odisha and Tamil Nadu. Also, high resistance to DDT and

OP has been reported in most districts of Odisha, a State with high prevalence of malaria, as well as in other regions with lower endemicity. *An. fluviatilis* S, the other major malaria vector in Odisha, remains fully susceptible to all insecticides [49].

2.3.3. Eastern Mediterranean

Malarial morbidity in this region accounts for only 2% of reported cases world over [5]. Afghanistan, Pakistan, Sudan, South Sudan and Yemen account for the majority of the malaria cases. In Afghanistan, a 2016 study done in five different locations reported that *An. stephensi* is the main vector, followed by *An. culicifacies* s.l. and *An. superpictus*, and other marginal species such as *An. subpictus*, *An. splendidus* and *An. nigerrimus* [50]. Different populations of three most abundant vectors, *An. culicifacies* s.l., *An. superpictus* and *An. subpictus*, showed resistance to the PY class II, deltamethrin. However, only populations of *An. culicifacies* s.l. and *An. superpictus* showed resistance to the PY class I permethrin and the OC insecticide DDT, while *An. subpictus* remained susceptible to both insecticides. Furthermore, *An. stephensi* showed resistance to the OP insecticide malathion, whereas *An. culicifacies* s.l. and *An. superpictus* were susceptible to this insecticide. Finally, these three species remained susceptible to the CA insecticide bendiocarb. Similarly, in Pakistan, a neighbouring country of Afghanistan, *An. stephensi* and *An. subpictus* are the main malaria vectors [51]. Populations of *An. subpictus* and *An. stephensi* showed resistance to the PY insecticide class I permethrin, the PYs class II deltamethrin and lambda-cyhalothrin and the OC insecticide DDT, while susceptible to the OP insecticide malathion, with the only exception of the populations of southern districts of the Punjab, resistant to malathion [52–54]. In Sudan, *An. arabiensis* is the major malaria vector reported from all parts of the country, coexisting sympatrically with *An. gambiae* s.s. and *An. funestus* [55]. *An. arabiensis* populations in Sudan are resistant to all the insecticides used in public health: PYs [56–60], CAs [58], OCs [57, 59–61] and OP [57, 58]. Limited data, however, are available in South Sudan, where resistance to the PY deltamethrin has been reported in *An. arabiensis* in two localities, Juba Payam and Northern Bari Payam [62].

2.3.4. Latin America

Malaria cases have declined considerably in this region in the past two decades, with many of the countries going into pre-elimination phase [63]. However, with 562,000 cases reported during 2015–2016, malaria is still a high burden, especially in countries in the Amazonia region such as Brazil, Colombia, Peru and most recently Venezuela [5], that showed an alarming increase over 76% of the reported cases (from 136,402 to 240,613) between 2015 and 2016, displaying an unprecedented 365% increase in malaria cases between 2000 and 2015 [5]. This country now encompasses Brazil as the larger contributor to the malaria burden in the Americas. *An. darlingi* is the primary malaria vector in the Amazonia region [64]. Fortunately, *An. darlingi* has shown susceptibility to all the insecticides across most of its distribution range, with exception of one population in western Colombia, which showed resistance to PY and DDT, but susceptibility to OPs [65–67]. However, studies to track the insecticide resistance and the available data are scanty. Thus, we cannot discard that resistance to insecticides in *An. darlingi*, as well as other malaria vectors, does not exist but rather could be more widespread in the Amazon region [63]. Similarly, the insecticide resistance of the secondary malaria vectors, often zoophilic but occasionally anthropophilic, is likely induced by the insecticide selection

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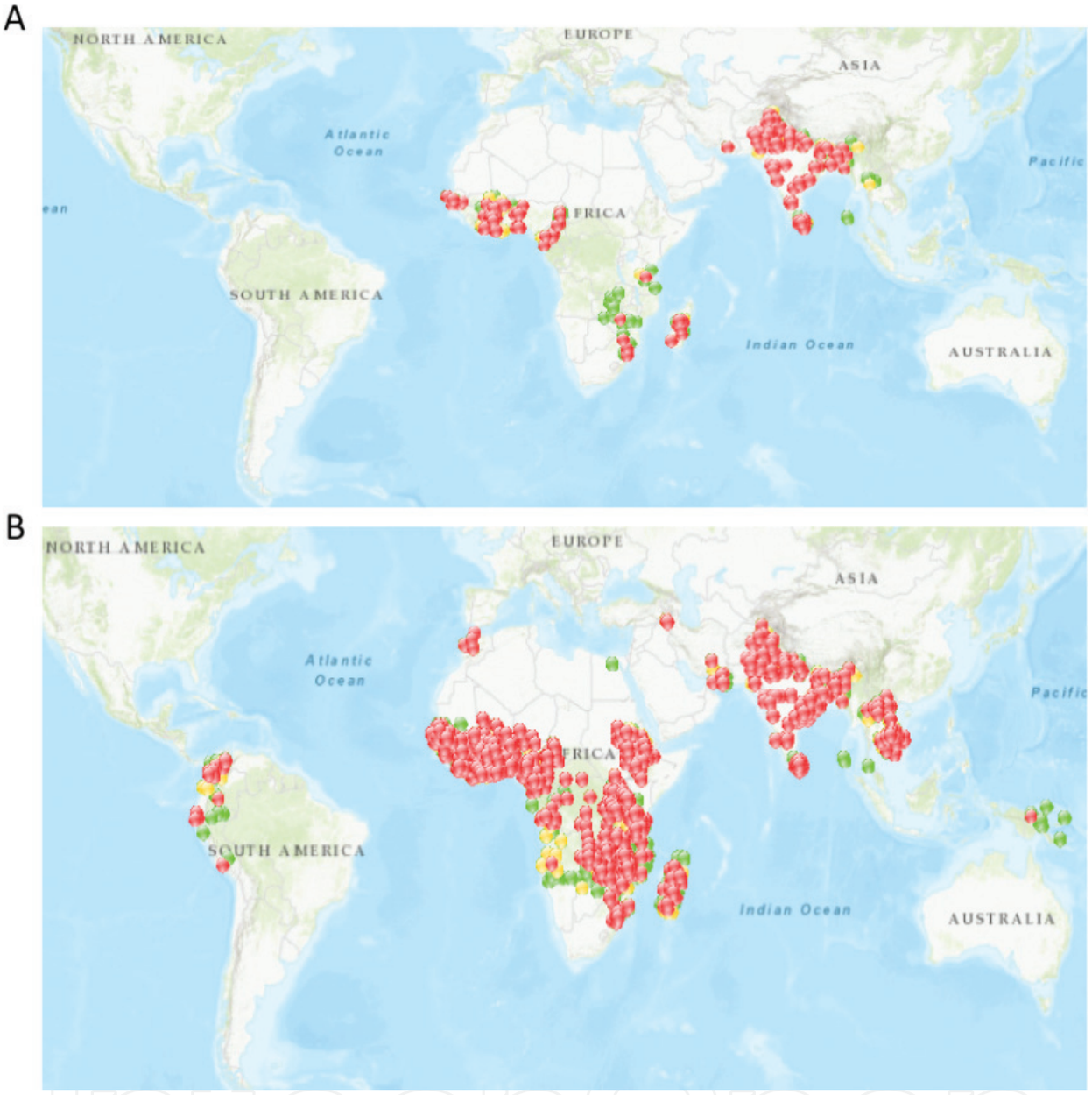

Figure 3. Worldwide view of the escalation of insecticide resistance in malaria vectors: (A) resistance profile in malaria vectors between 1985 and 2000 with limited resistance reported in West Africa and Southeast Asia; (B) significant increase of resistance in African and in other regions from 2000 to 2017.

pressure from agriculture activities. Field populations of *An. albimanus* have been reported resistant to PY in Colombia, Panama and Peru [67–69]. *An. albimanus* population in the north-western coast of Peru have shown cross-resistance to all classes of insecticides used in public health for being resistant to PYs, CAs, OCs and OPs [70]. Nevertheless, this is not the only case of cross-resistance reported for *An. albimanus*. Another population in southern Mexico has shown low resistance to PY and OP, linked with high resistance to DDT [71]. Similarly, a population of *An. nuneztovari*, secondary malaria vector distributed mainly in Colombia and Venezuela, showed cross-resistance to DDT and OPs in one specific location of Colombia, close to the border with Venezuela [72]. Other malaria vectors, such as *An. benarrochi* and *An.*

pseudopunctipennis, have shown susceptibility to PY across most range of their distribution with only two exceptions: one *An. benarrochi* population at the border between Peru and Brazil resistant to permethrin, and one *An. pseudopunctipennis* population in the northwest Peru resistant to permethrin, cypermethrin, deltamethrin and lambda-cyhalothrin [36], the latter population also showed cross-resistance to OP (malathion).

In conclusion, resistance to insecticide is steadily spreading worldwide in most vectors as shown by the comparison of resistance profile between 1985 and 2000 (**Figure 3A**) and 1985 to 2017 (**Figure 3B**) from IR mapper (http://www.irmapper.com/). This represents a serious challenge to malaria control, which relies heavily on insecticide-based tools.

3. Insecticide resistance mechanisms

A proportion of insect populations can tolerate doses of insecticides which have been proved lethal to the majority of the individuals in a normal population of the same species through various mechanisms such as: (i) insecticide can be broken down or detoxified much faster in the resistant mosquitoes than in the susceptible ones, hence quickly eliminated from their body (metabolic resistance); (ii) the target of the insecticide can be genetically altered to prevent the insecticide from binding thereby reducing the insecticide effect (target-site resistance); or (iii) resistant mosquitoes may absorb the toxin slower than susceptible insects (penetration resistance). An illustration of these mechanisms is represented in **Figure 4**.

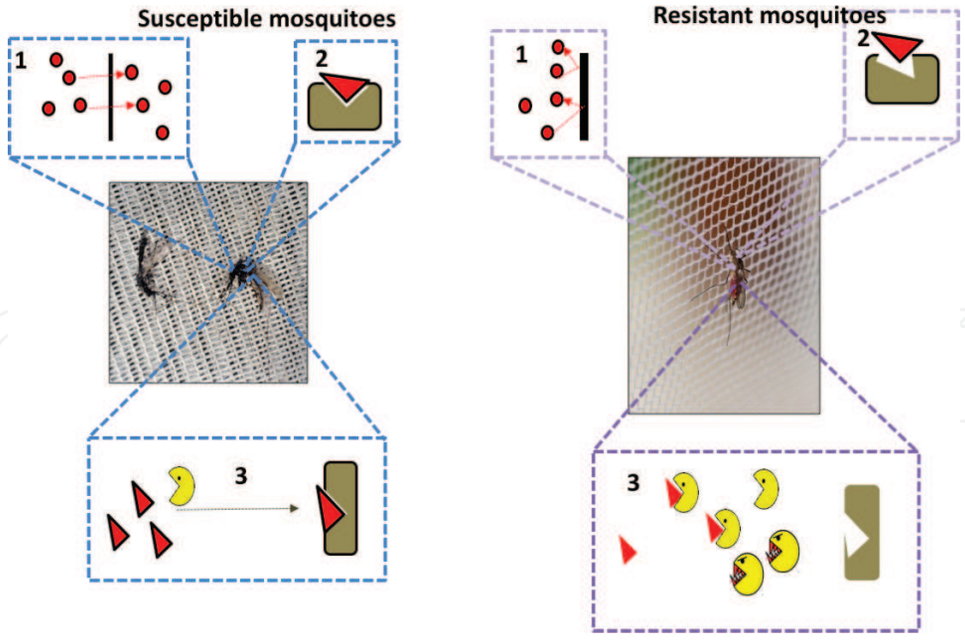

Figure 4. Illustration of the physiological insecticide resistance mechanisms in mosquitoes. (1) Reduced penetration: physiological changes to the cuticle of the mosquitoes prevent the absorption or penetration of insecticide. (2) Target-site resistance: insecticides have a target site within the mosquito. This site can become modified so that the insecticide no longer binds to it. (3) Metabolic resistance: enhanced enzyme systems break down insecticides before they can have a toxic effect on the mosquito.

3.1. Methods used to study resistance mechanisms

Insecticide resistance monitoring is essential to understand the actual threat and how resistance is spreading among malaria vectors [7]. Once resistance has reached very high levels (fixed in the population), most insecticide resistance management strategies, which are based to restore susceptibility, would not work. Thus, regular monitoring is crucial. Three detection methods (**Table 1**) can be used to monitor insecticide resistance, each method providing different information. Bioassays are the most popular way to monitor resistance where mosquitoes are exposed fixed doses of insecticides for a fixed time and the percentage mortality is recorded 24 h post-exposure [73]. Even though they are simple to perform, bioassays have several disadvantages such as requiring a large number of mosquitoes, affected by variations in humidity, temperature and time of the day [74]. Some authors argue that bioassays should be supplemented with DNA markers or even partially replaced by these DNA markers [75]. It should be noted that DNA markers are usually specific to certain mechanisms hence the need to perform them is to avoid unknown mechanisms going undetected. Until now, no assay has been developed that is suitable to monitor cuticular or behavioural resistance.

3.2. Target-site resistance

One of the mechanisms mosquito becomes resistant is by altering the target site of the insecticide thereby preventing it from binding effectively hence the insecticide has little or no effect on the insect. Most insecticide targets are found within the nervous system and mutations in these target sites (mainly receptors) lead to reduced sensitivity. For example, PYs and DDT act on the voltage-gated sodium channels (VGSCs) and mutation in the amino sequence of this gene results in reduced sensitivity of the channels preventing PYs and DDT from binding [76]. Insects with this mutation can withstand prolong exposure to insecticide without being knocked down, hence the name "knockdown resistance" (*kdr*) [77]. The replacement of the *leucine* residue for a *phenylalanine* or a *serine* at position 1014 in the VGSC is one of the most common amino acid substitution associated with PY resistance in malaria vector [77]. Also an *alanine* to *serine* substitution at position 302 (or 296) of the γ-amino-butyric acid (GABA) receptor is found in the dieldrin-resistant (*rdl*) insect species including *An. gambiae* [78] and *An. funestus* [79]. Similarly, mutations in the gene coding for the neurotransmitter acetylcholinesterase (*ace-1*), the target site of OPs and CAs, have been found [80], which reduces the inhibition effect of the insecticide on the enzyme [81, 82]. Substitution of *glycine* to *serine* at position 119 has been reported in *An. albimanus* and *An. gambiae*, and this mutation confers resistance to OPs and CAs [83]. Duplication of the *ace-1* gene has been reported in the *An. gambiae* and *An. coluzzii* [84]. However, in species such as *An. funestus*, other mutations were detected in *ace-1* including the N485I shown to be associated with bendiocarb resistance in southern African populations [85].

3.3. Metabolic resistance

Metabolic resistance is the most common and challenging of all insecticide resistance mechanisms. Mosquitoes have enzyme systems that protect them from xenobiotic compounds and

Susceptibility bioassay tests	Biochemical assays	Molecular assays
Description	Description	Description
Vectors are exposed to fixed insecticide concentrations, and the level of vector mortality is subsequently recorded. The results are expressed as the percentage of vectors knocked down, alive or dead. Susceptibility testing requires samples of at least 100 live mosquitoes per testing site. These susceptibility tests are generally used for routine monitoring, as they can be applied in the field. They provide standardised data that are relatively easily interpreted. Either WHO paper bioassays or CDC bottle bioassays can be used. The results obtained with the two methods are not comparable. In order to observe longitudinal or temporal patterns in resistance, countries and academic institutions in all regions must therefore use the same method consistently over time.	Biochemical assays detect the presence of a particular resistance mechanism or an increase in enzyme activity. They require fresh mosquitoes, but much fewer than for bioassays. Unlike bioassays, biochemical assays can identify some specific resistance mechanisms and indicate an increase in metabolic enzyme activity. Biochemical assays are normally used in conjunction with synergist and molecular assays.	Molecular tests are used on the actual gene, allowing detailed and direct analysis of resistance genes. Testing can be done with straightforward polymerase chain reaction techniques (30) with DNA or in more elaborate microarray tests with RNA. More advanced molecular methods can provide complex genetic information including whether the mutation is unique or has spread. These are the most accurate tests for measuring resistance frequency in vector populations. Molecular tests must, however, be correlated with susceptibility testing.
Limitations	Limitations	Limitations
Susceptibility tests identify the existence of resistance once it is at a detectable level but do not establish the resistance mechanism involved. They may also not identify resistance if the frequency is too low. Several countries have reported shortages in the supply of testing materials and have switched between the WHO and CDC tests, making results difficult to compare. In some cases, they have limited their testing.	The method is more difficult to use in the field as it requires sophisticated equipment, and interpretation of the results requires strong technical skills. Further, the correlation between chemical reactions in these tests and increased ability to metabolise insecticides is not yet well defined.	The method requires sophisticated equipment and entomological capacity. It can be used to detect target site resistance and a few identified metabolic mechanisms. Therefore, susceptibility tests should be used to complement molecular results, as the absence of identifiable genotypic resistance does not necessarily mean that resistance does not exist.

Table 1. Different methods for monitoring insecticide resistance in mosquito vectors and their limitations.

some of these enzyme systems can break down insecticide before it can reach its site of action. In metabolic resistance, enzymes that detoxify the insecticide can be overexpressed or alter the affinity of the enzyme for the insecticide through amino acid substitutions [86]. Overexpression of insecticide resistance genes is the most frequent mechanism in resistant mosquitoes. This increased expression of insecticide resistance genes can be due to *cis*- or *trans*-acting elements in the promoter or gene amplification [87, 88]. This overexpression results in the high level of enzyme production in the resistant mosquitoes that enables them to break down the insecticide at a faster rate before it reaches the target site. Cytochrome P450 monooxygenases,

glutathione S-transferases (GSTs) and esterases are the three major enzyme families that are involved in breaking down of insecticides.

3.3.1. Cytochrome P450 monooxygenases

Of the six families of P450s, genes belonging to the CYP4, CYP6 and CYP9 have been observed in resistant mosquitoes with increased transcriptional level [89], with the majority of those implicated in resistance belonging to the CYP6 family. For a P450 to be involved in resistance, it does not only have to be overexpressed but also must be able to metabolise/sequester the insecticide to which the insect is resistant and also be better metaboliser than those for the susceptible strain [90]. In *An. gambiae*, CYP6P3 and CYP6M2 have been shown to metabolise type I and type II PYs [91], and CYP6M2 can metabolise DDT [92]. In *An. funestus*, the duplicated P450 CYP6P9a and CYP6P9b and CYP6M7 have been shown to metabolise PYs [93, 94], whereas CYP6Z1 confers cross-resistance to both pyrethroids and carbamates [85]. Furthermore, allelic variation of P450 genes, such as CYP6P9a/b, has been shown to drive pyrethroid resistance in field populations of *An. funestus* [95] with signature of selective sweep associated with scale-up of bed nets [96].

3.3.2. Glutathione S-transferases

The GSTs are involved in the phase two of the detoxification of xenobiotic compounds where they conjugate the substrate with glutathione enhancing solubility thus facilitating the excretion. In insect, six classes of GSTs, i.e., delta, sigma, epsilon, omega, theta and zeta have been identified [97]. Insects resistant to major classes of insecticide show elevated levels of GSTs activities. For example, GSTs confer resistance to DDT in mosquitoes including *An. gambiae* [98], *An. dirus* [99], *Aedes aegypti* [100] and *An. funestus* [26]. A single amino acid change in GSTe2 (L119F) has been shown to confer a cross-resistance to DDT and PYs in *An. funestus* [26], whereas a similar change is also reported in *An. gambiae* (I114T) [101].

3.3.3. Esterases

CAs and OPs are the main insecticides that are metabolised or sequestered by esterase-mediated insecticide resistance. Esterase levels in the resistant mosquitoes can either be elevated like in *Culex* or non-elevated like in *Anopheles* species (*An. arabiensis, An. stephensi* and *An. culicifacies*). Esterase-mediated insecticide resistance in *Anopheles* spp. was associated with allelic variants that can metabolise the insecticide at a faster rate than those of the susceptible and shown to confer resistance to malathion [102, 103]. The role of esterases in PY resistance still needs to be investigated.

3.4. Cuticular or reduced penetration resistance

Cuticular resistance occurs when mosquitoes reduce the absorption of insecticide into their bodies by altering the structure or composition of the cuticle. A wide range of insecticides are threatened by this mechanism as for their lethal effect to occur, most insecticides must cross the cuticle in order to reach their site of action. Cuticular resistance enhances the resistance

conferred by other mechanisms. This mechanism has not been extensively studied as compared to the other mechanisms because there are very few examples. Recently, Yahouédo et al. [104] studied the role of the cuticular resistance in PY-resistant strain of *An. gambiae* called MRS, free of *kdr* mutations. They succeeded to show that lower amount of insecticide was absorbed in the MRS strain than in the susceptible strain and also that the MRS strain had a significantly thicker cuticle layers than those of the susceptible strain. *CPLCG3* gene, which codes for a structural protein contributing to the cuticle thickness, was found to be constitutively upregulated. Similar evidences of cuticular resistance were shown for *An. funestus* with proofs of cuticle thickening in PY-resistant mosquitoes [105].

4. Impact of current insecticide resistance in parasite transmission: a global warning based on reported level of resistance?

4.1. Fitness cost of resistant lab and field *Anopheles* populations

The use of insecticide selects small proportion of individuals possessing resistance genes allowing them to resist and survive the effects of the insecticide, transferring the genetic modifications conferring resistance to the progeny. This should most likely increase the proportion of resistant individuals within the population. However, mutations or genes conferring resistance are usually associated with a fitness cost and may disrupt normal physiological functions [106, 107]. For example, resistant vectors may have lower mating success [108, 109], lower fecundity and fertility, higher developmental time and lower longevity. Resistant individuals may be also more susceptible to natural predators [110] or more prone to mortality during overwintering. Most insecticide resistance management strategies rely on the fact that fitness cost may impact the spread and persistence of resistance alleles in the vector populations [7].

4.2. Impact of resistance on life traits: longevity, fecundity and mating male competitiveness

Resistance caused by overproduction of metabolic enzymes generally shows lower fitness cost than target site resistance, most probably because the primary function of the enzyme is not disrupted [111]. But to date, little is known about the effective impact of metabolic resistance on the life traits of the vector due to the absence of DNA-based molecular marker. Nevertheless, many studies demonstrated that resistant strains of arthropods often present lower fitness compared to their susceptible counterparts [112]. For example, it was shown that resistance strains may be associated with relatively slower larval development, reduced survival rates among larvae and adults, reduced fecundity in females and reduced fertility [106, 113, 114]. It was shown that target-site resistance due to *kdr* and *rdl* mutations is able to impact negatively the male mating competitiveness in the malaria vector *An. gambiae,* whereas metabolic resistance had no effect [109]. Among all the parameter elucidated above, vector longevity is an essential parameter in disease transmission because it increases the potential for infective bites to hosts. Furthermore, the effect of longevity on disease transmission is crucial for parasites like *Plasmodium* that need a minimum incubation period in the vector before being transmitted to a new host. Insecticide resistance is generally thought to increase longevity of resistant

vectors, thereby increasing infectiousness of parasites and threatening vector control. However, the development of resistance in a mosquito often comes with a price subsequently affecting the fitness of the vector [115]. As a consequence of the fitness cost of insecticide resistance on the life traits (mentioned above) of the vectors, reversion to susceptibility is expected. A good example of reversal to susceptibility occurred in *An. arabiensis* in Sudan. In this country, after antimalarial house spraying in the early 1980s, resistance to malathion was noticed. This prompted a switch of insecticide treatment to fenitrothion (OP insecticide), and susceptibility to malathion was restored in the following years [10]. However, reversal rates are variable and may be very slow, particularly when an insecticide has been used for many years. For example, the same *rdl* gene has been reported to be maintained in field populations of Sri Lanka despite the withdrawal of cyclodiene insecticides for mosquito control for more than 30 years [116]. Before implementing any resistance management strategy in the field, knowledge of the reversal rate is crucial.

4.3. Epidemiological consequences of the insecticide resistance on malaria incidence

4.3.1. Past and current evidences

There are large number of confounding factors threatening the assessment of epidemiological consequences of the insecticide resistance on malaria incidence and data interpretation [117]. For this reason, only few studies have assessed the epidemiological impact of insecticide resistance. Impact of PY resistance on control failure was reported from the borders of Mozambique and South Africa. In 1996, the malaria control programme in KwaZulu-Natal (South Africa) switched from using DDT to deltamethrin for indoor spraying [118]. After four years of deltamethrin spraying, reported malaria cases increased approximately fourfold. *An. funestus*, previously eradicated, had reappeared and was observed emerging alive from PY-sprayed houses. Bioassays showed that this species was resistant to PYs but susceptible to DDT [119]. The decision to revert to IRS with DDT was accompanied by a decline in malaria cases by 91% [120]. On the Bioko Island on the West African coast, increased density of PY-resistant *An. gambiae* was also reported after IRS campaign with lambda-cyhalothrin, although a significant reduction in transmission index and malaria reported cases was observed [121, 122]. High frequencies of the L1014F *kdr* allele were observed in the local *An. gambiae* population. When PYs were replaced by CAs (bendiocarb), mosquito population declined [122]. Nevertheless, in an operational scale programme such as this, the possible contribution of other factors to the failure of PY IRS to control mosquito population density cannot be overlooked; thus, the direct consequence of the high *kdr* frequency is uncertain. After initiation of interventions combining IRS with PYs and ITNs in the highland provinces of Burundi in 2002, significant reduction was recorded in *Anopheles* density by 82% [123]. Consequently, transmission intensity was reduced by 90% and occurrence of malaria cases by 43% in children, despite high frequencies of the L1014S *kdr* allele in the main vector *An. gambiae* s.s. [123]. Many interventions took place in Africa in order to investigate the efficacy of ITNs for malaria prevention [124]. However, the extent to which PY resistance might affect the effectiveness of such interventions is not well elucidated. In Korhogo area, north of Côte d'Ivoire where the 1014F *kdr* allele frequency in *An. gambiae* is up to 80% [125], and malaria is endemic, lambda-cyhalothrin-treated nets had a significant impact on the entomological inoculation rate with around 55% reduction. Malaria

incidence in children <5 years of age decreased also (56% reduction of clinical attacks) compared to a control group having no nets [126]. This was the first clear-cut evidence of ITNs continuing to provide effective personal protection against malaria in an area with a high frequency of *kdr* in the vector populations. However, absence of a physical barrier in the control group might have overestimated the impact of PY-treated nets against *kdr* mosquitoes in this study. In southern Benin, a randomised controlled trial was carried out in a mesoendemic area to assess the impact of LLINs scale-up on malaria morbidity in children <5 years of age [127]. In this area, where the *kdr* frequency is around 50–60% in *An. gambiae* s.s., transmission increased during the rainy season but was not followed by a seasonal variation in parasite infection and clinical incidence. The evidence is clear that implementation of vector control tools (ITNs and/or IRS) has significantly decreased malaria incidence and parasite infection prevalence in children in endemic countries across Africa, despite moderate-to-high PY resistance observed in local malaria vectors.

5. Behavioural resistance to insecticides used in public health

As we have mentioned previously, the extraordinary success of malaria reduction in Africa is largely due the use of insecticides applied indoors through LLINs and IRS [6]. This malaria control approach takes advantage of the strong human preference, as well as the indoor feeding and resting behaviour of African malaria-transmitting mosquitoes [128]. As we have shown in this chapter, progress has been made in understanding the genetic basis of the ability of mosquitoes to survive insecticide entering the body. However, little is known about the causes of increasingly reported changes in blood-feeding behaviour developed by certain species of malaria-transmitting mosquitoes to avoid exposure to insecticides [7]. This phenomenon is known as behavioural resistance and it is defined as any modification in insect behaviour that helps to circumvent the lethal effects of insecticides. Thus, through intraspecific behavioural shifts in biting time, location and host preference, malaria-transmitting mosquitoes avoid exposure to insecticides, feeding on humans when most people are not protected [129], jeopardising the current control strategy in Africa primarily based on indoor application of insecticides [130–132]. Recent studies conducted in West and East Africa have shown that indoor application of insecticides may induce intraspecific behavioural shifts towards early biting, exophagic biting and exophilic resting behaviour in malaria-transmitting mosquitoes [130, 131, 133]. Similarly, current studies conducted in Central Africa showed a comparable shift towards exophilic resting behaviour [134]. Mathematical modelling and field evidences have proved that these shifts in blood-feeding behaviour could threaten and impact on the current control programmes [132, 135]. The mechanisms driving these shifts have not yet been elucidated, although some studies have shown that both genetic and environmental factors play a key role [135, 136].

6. Conclusion and perspectives

Insecticide resistance is undoubtedly a major challenge to the control of malaria vectors worldwide as it limits the tools available to achieve the goal of controlling and eliminating this

debilitating disease. It is therefore of the utmost importance that novel insecticides and new control tools be designed to help manage and mitigate the impact of resistance. Through the work of various partners such as Innovative Vector Control Consortium (IVCC), UNITAID and several manufacturers, the challenge of producing new insecticides and tools is beginning to be met. This is exemplified by the recent prequalification by the WHO of the new insecticide Sumishield (clothianidin, a neonicotinoid) in October 2017. This new insecticide together with the organophosphate Actellic (pirimiphos-methyl) could now allow countries to effectively design and implement suitable resistance management strategies for IRS interventions according to WHO's Global Plan for Insecticide Resistance Management (GPIRM). With other new insecticides expected to enter the market in the near future, resistance management strategies such as rotation of insecticides could become more realistic to implement. However, even with new insecticides available, the community should avoid being complacent as the mosquitoes will surely develop resistance with time if consideration is not given to how to use such new insecticides including between public health and agriculture sectors. Detection of resistance markers notably for metabolic resistance is also urgently needed to not only track the spread of resistance but to better assess its impact on control interventions or mosquito fitness and malaria transmission. The recent detection of markers such as L119F-GSTe2 in *An. funestus* shows that this is possible, but more efforts are needed focusing importantly on cytochrome P450s, the key metabolizers. It will be important to take advantage of the advances in genomics with the power of next-generation sequencing tools to detect potential resistance markers early enough to allow control programmes to track resistance when it is still at early stage when it could easily be managed. This will allow avoiding repeating the situation observed with PY resistance and ensure a continued effectiveness of current and future insecticide-based interventions.

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Conflict of interest

No conflict of interest.

Acronyms

CA	carbamate
CDC	Centers for Disease Control and Prevention
DDT	dichlorodiphenyltrichloroethane
DNA	deoxyribonucleic acid

GABA	gamma-aminobutyric acid
GPIRM	Global Plan for Insecticide Resistance Management
GST	glutathione S-transferase
IRM	insecticide resistance management
IRS	indoor residual spraying
ITN	insecticide-treated nets
IVCC	Innovative Vector Control Consortium
Kdr	knockdown resistance
LLIN	long-lasting insecticidal net
OC	organochlorines
OP	organophosphates
PY	pyrethroids
RNA	ribonucleic acid
VGSC	voltage-gated sodium channel
WHO	World Health Organization

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