
Insecticide Resistance in East Africa – History, Distribution and Drawbacks on Malaria Vectors and Disease Control

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Additional information is available at the end of the chapter

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Abstract

Malaria is a major contributor to the global disease burden and a significant impediment to socio-economic development in resource-poor countries. In contrast to improved trends of malaria morbidity and mortality in some parts of the world, malaria has remained a life threatening disease in many other regions including East Africa because of factors such as weak health systems, growing drug and insecticide resistance, ecological change, climate anomalies, socio-economic factors and changes in land use patterns. Ongoing malaria vector control strategies rely mainly on the use of indoor residual spraying (IRS) and insecticide treated nets (ITNs) which are the primary intervention strategies to reduce malaria burden. The current success in reducing malaria related morbidity and mortality has led to the optimism that elimination of the disease as a public health problem may be a realistic objective. Efforts during the last decades enabled access to ITNs in sub-Saharan Africa protecting millions of people at risk of malaria. The number of countries that employed IRS as a vector control strategy increased almost by two fold and the percentage of households owing at least one ITN in sub-Saharan Africa is estimated to increase from time to time. Currently, all ITNs are treated with pyrethroids while IRS depends on pyrethroids, DDT and recently on carbamates. Despite IRS and ITNs are known in reducing malaria incidence, insecticide resistance in malaria vectors threatens the success of malaria control program. Resistance to insecticides has occurred in most arthropod vectors with different mechanisms. If the current trends of increased insecticide resistance continue, it may jeopardise the efficacy of current vector control tools. Given the limited choice of available insecticides, i.e., only 12 insecticides belonging to 4 classes of insecticides (organochlorines, organophosphates, pyrethroids and carbamates), resistance to these insecticides has become a limiting factor for current efforts to sustain control. Currently, no other insecticide class with similar efficacy has been approved by WHOPES. The development of insecticide resistance in malaria vectors has been attributed to the prolonged use of insecticides for IRS and high coverage of ITNs/LLINs. The recent use of pyrethroids for indoor residual spraying is likely to have enhanced the selection pressure for insecticide resistance alleles among East African vector populations. Moreover, mosquitoes breeding in agricultural habitats are exposed to sub lethal

doses of pesticides used in agriculture. Since currently recommended insecticides for IRS or ITNs were developed with similar active ingredients of pesticides used for agricultural pest control, their extensive and widespread use to boost agricultural productivity is believed to foster insecticide resistance in mosquito populations. There is strong evidence on the emergence of resistance to DDT and pyrethroids in the major malaria vectors in East Africa however, current information on resistance status of the malaria vectors in different areas of the sub-region is scarce. Genes conferring resistance to malaria vectors, including *kdr*, super *kdr* and acetylcholinesterase mutations and metabolic resistance are not mapped. The frequency and spatial distribution of East and West African *kdr* mutations and their association with the phenotypic resistance in East Africa is less understood. The bioassay results after WHO diagnostic tests in different East African malaria vector populations against insecticides used in public health is not well documented. In conclusion, planning and implementing insecticide resistance monitoring and management strategy should be part of the vector control program either for pre-emptive action without waiting for the development of resistance or to slowdown the spread of resistance in malaria vectors in the sub-region.

Keywords: malaria vectors, insecticide resistance, resistant management, vector control, East Africa

1. Introduction

East Africa is a region encompassing six countries which include Kenya, Uganda, Ethiopia, Tanzania, Rwanda and Burundi, and all these countries are prone to malaria transmission with known efficient vectors. The main malaria vectors in the region are *Anopheles gambiae* s.s, *An. arabiensis* and *An. funestus* [1–4]. These vectors breed in different habitats ranging from temporary rain pools to permanent water bodies [5–8]. Vector species distribution in East Africa are governed by several factors which include anthropogenic activities [4, 5, 7], such as development projects [9–12]. Also, climate, particularly temperature and rainfall, has been regarded as the function of habitats for vector abundance and distribution between low- and high-altitude areas [13, 14]. Human migration and movement from high land to low land have facilitated the distribution of parasites [15]. Topography has influenced the abundance and distribution of vector in all areas [16–19]. Thus, the abundance and distribution of efficient vectors have led to the wide use of control tools and intensive interventions across the sub-region. The main tools used for the control of malaria vectors are long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) [20]. The pyrethroids are the only insecticides which have been used for treating LLINs while organochlorides, organophosphates, carbamates and pyrethroids are used for IRS [1, 21]. Currently, organochlorides (especially DDT) are banned in most of the East African countries for IRS use due to resistance developed by the major malaria vectors and environmental concern [20]. The development of resistance is influenced by many factors [22]. These include genetic factors including the number and frequency of resistance alleles in the insect population, fitness cost and relative dominance of the characters; biological factors including the insect life history parameters, the fitness of the heterozygous and

homozygous resistant phenotypes and initial population size; reproductive factors including the rate of increase and fluctuations in population size; and operational factors including application methods of the insecticide and properties of an insecticide in use, previous selection with other insecticides, proportion of population exposed to selective doses, dosage of insecticide taken up by exposed insects and the life stage of the mosquito selected [22, 23].

Insecticide resistance is not new in insect vectors, and it is a genetically inherited characteristic which increases in the populations of vectors as a result of increased resistance selection pressure and also a trait capable of rapid spread. Malaria vector control in East Africa relies principally on the use of insecticides that can be applied either as an indoor residual deposit or can be used to treat mosquito nets and curtains. However, the long-term vector control program based on prolonged and frequent insecticide application faced the problem of resistance. Vector control subjects mosquito populations to selection and survival of the fittest. Evolution of insecticide resistance in an insect population arises when there is an increase in the frequency of one or more resistance genes in the population following exposure to insecticides. Attempts to kill the tolerant individuals lead to ever increasing doses and eventually resistant pest populations. This is an inevitable limitation in the use of any new or old class of insecticides. Malaria control initiatives introduced DDT during the Second World War from 1945 to 1948 to eradicate malaria since that time DDT showed to be an effective malaria vector control, but resistance has emerged throughout endemic countries including East Africa.

2. Malaria prevention and control strategies in East Africa

In East Africa, several intervention strategies are set to reduce morbidity and mortality from malaria. Effective measures of malaria control have been achieved mainly through the use and high coverage of IRS and scaling up of LLINs in Tanzania [24, 25], Kenya [26], Uganda [27], Ethiopia [28] and Rwanda [29]. Community involvement has been another strategy in malaria prevention in different parts of East Africa [10, 27, 28].

In the recent past, house modification through window screen and blocking of eaves has been practiced in the prevention of malaria vectors in Tanzania [30, 31], Kenya [32] Ethiopia [33] and Uganda [34]. More innovative vector control strategies including control of resistant vector populations in the sub-region are use of entomopathogenic fungi [35, 36], larval source management [7] and vector trapping [37–39]. Plant-based derivatives have also been used in vector control in Uganda [40], Tanzania [41–43], Kenya [44, 45] and Ethiopia [46–48].

3. History, distribution and current status of insecticide resistance in East Africa

Insecticides (chemicals) which have been used for the control of vector-borne diseases and crop protection is believed to enhance the evolution of resistance in insects [49]. The intensive

nili, *An. paludis*, *An. pharoensis* and *An. coustani* [20]. From peer-reviewed sources, other anopheline species documented included: *An. christyi*, *An. demeilloni*, *An. gibbinsi*, *An. harperi*, *An. implexus*, *An. maculipalpis*, *An. marshalli*, *An. pretoriensis*, *An. rufipes*, *An. squamosus*, *An. swahilicus*, *An. theileri*, *An. wilsoni* and *An. ziemanni*, none of which are considered as important or primary vectors in Africa [51]. The malaria vector distribution in the country is not uniform due to variation in climatic factors, particularly temperature and rainfall.

In Kenya, the first reported case of resistance was in the context of insecticide-treated net use in Western Kenya where reduced knockdown rates have been observed [1]. Complete susceptibility of populations of *An. arabiensis* to DDT, fenitrothion, bendiocarb, lambda-cyhalothrin and permethrin was documented from Mwea rice irrigation scheme, Central Kenya [52]. Widespread resistance against pyrethroids and DDT was observed across western Kenya [53]. *An. gambiae* s.l. showed different levels of resistance to deltamethrin, lambda-cyhalothrin and bendiocarb Kilifi, Malindi and Taveta districts in coastal Kenya. Pyrethroid resistance has been reported in *An. gambiae* s.s and *An. arabiensis* from four districts of Western Kenya. Stump and others also found significant differences in *kdr* gene frequency between the large-scale insecticides treated nets [54].

Kamau and Vulule reported that *An. gambiae* s.l. and *An. funestus* from western, coastal and central Kenya were susceptible to DDT, fenitrothion, bendiocarb, lambda-cyhalothrin and permethrin [52]. The same study also showed the presence of Leucine-Serine (East African) *kdr* mutation in *An. gambiae* s.s. of western Kenya, but the leucine-phenylalanine (West African) mutation was absent in this mosquito population. Though the East African *kdr* mutation was detected from west Kenyan populations of *An. gambiae*, it has never occurred at homozygous state. The frequency of the L1014S *kdr* allele doubled in the ITN test village and its nearest neighbor from 1987 to 2001, but not outside of this area. This suggests that ITN use has further selected for the *kdr* mutation in the population.

3.2. Uganda

The main malaria vectors in Uganda are *An. gambiae* and *An. funestus*, with *An. arabiensis* involved in local transmission. Recent study also showed that *An. funestus* and *An. gambiae* are the widely distributed vectors in Uganda. Other less dominant anophelines which were implicated in malaria transmission in the country include: *An. coustani*, *An. listeri*, *An. marshalli* and *An. kingi* [55, 56].

There is widespread insecticide resistance in the main malaria vectors, *An. gambiae*, *An. funestus* and *An. arabiensis*. In Uganda, resistance to pyrethroid insecticides has been reported in the three main malaria vectors, *An. gambiae*, *An. arabiensis* [55, 57, 58] and *An. funestus* [59]. A reduced susceptibility by *An. gambiae* s.l. to three pyrethroid insecticides, deltamethrin, cyfluthrin and cypermethrin, has been observed [60]. *An. gambiae* s.l. was DDT- and pyrethroid-resistant in central and eastern Uganda [58]. There are currently no reports of organophosphate resistance, but resistance to carbamates including propoxur has been documented. Maweje and co-workers observed high pyrethroid resistance in *An. gambiae* and *An. Arabiensis*, but both species were fully susceptible to bendiocarb and fenitrothion from eastern Uganda [55]. Resistance to DDT and deltamethrin has also been reported in populations of *An. funestus* and

An. gambiae s.l. from southwestern Uganda [56]. *An. funestus* in Tororo, eastern Uganda, was resistant to pyrethroids, permethrin and deltamethrin. Suspected DDT resistance was also observed in *An. funestus*. However, this population was completely susceptible to bendiocarb (carbamate), malathion (organophosphate) and dieldrin. Recently, widespread resistance against pyrethroids and DDT was observed across Uganda [53, 61]. Mutations which confer resistance to DDT and pyrethroids, West African (L1014F) and East African (L1014S) mutations, have been reported from the Ugandan *An. gambiae*. Increased esterase activity was also detected in pyrethroid- and DDT-resistant *An. gambiae* populations. The presence of the East African *kdr* mutation (L1014S) is shown for the first time in *An. arabiensis* from Uganda [62]. The resistance in this species was due to both target site (*kdr*) and metabolic mechanisms and there was also cross-resistance between DDT and pyrethroids. Resistance to pyrethroids is present, and apparently increasing, in *An. arabiensis* from Jinja, eastern Uganda [55], but it is not mediated by known 'knockdown resistance' target-site mechanisms (L1014F and L1014S) in the voltage-gated sodium channel, which are extremely rare in this species in this area [55]. In the absence of a known target-site mechanism, metabolic mechanisms are strongly implicated in the resistance phenotype. However, knockdown resistance mutation conferring pyrethroid/DDT resistance has also been suggested to occur in other axons of the sodium channel gene in *An. gambiae*. Biochemical assays suggest that resistance in this population is mediated by metabolic resistance with elevated level of GSTs, P450s and pNPA. The low frequency of L1014S and L1014F mutations and complete restoration of susceptibility to permethrin and deltamethrin by the two species after synergist assay using PBO indicate involvement of other mechanisms such as P450s in the same study. Populations of *An. gambiae* s.l. from eastern Uganda tested for the presence of knockdown resistance (*kdr*) and altered acetylcholinesterase (*ace-1R*) alleles showed the presence of *kdr* L1014S allele, while *ace-1R* and *kdr* L1014F alleles were absent [57]. All populations from the same area remain highly susceptible to carbamate, organophosphate and dieldrin insecticides. Metabolic resistance through elevated expression of cytochrome P450s has been implicated in these mosquito populations.

3.3. Ethiopia

Forty-two anopheline species have been recorded in Ethiopia [63]. There are only four anopheline mosquito species reported as malaria vectors. *Anopheles arabiensis* is the primary vector of malaria and it is widely distributed throughout the country [64], while *An. funestus*, *An. pharoensis* and *An. nili* are secondary vectors with localized distribution [65]. *An. arabiensis* belongs to the *An. gambiae* complex of sibling species. Only two member species of the *An. gambiae* complex, *An. arabiensis* and *An. Amharicus* (formerly known as *An. quadriannulatus* B), are reported to exist in Ethiopia. *An. quadriannulatus* species B had been described as a new species from southwestern Ethiopia [66]. This species was reported to be zoophilic and exophilic and is assumed to have no role in malaria transmission in Ethiopia [67]. *Anopheles arabiensis* is responsible for most of malaria infections in Ethiopia. Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) are pillars in malaria prevention and control strategy in Ethiopia. For over five decades, the main vector control strategy by the national malaria control program has been indoor residual spraying (IRS), using DDT with a limited

application of malathion as an alternate insecticide. However, DDT use for IRS was replaced in favor of deltamethrin in 2009, and after 2 years of use deltamethrin was also replaced with bendiocarb in 2011 due to the reduced susceptibility of the principal vector to the mentioned insecticides. Insecticide susceptibility tests carried out in different parts of the country have shown different levels of resistance by the principal vector to insecticides in use for IRS and/or to treat LLINs. Insecticide resistance by *An. arabiensis* to DDT was reported during the early 1990s [64, 68]. Balkew and others reported resistance by *An. arabiensis* to permethrin and DDT [69]. Another study by Yewhalaw and his colleagues from southwestern Ethiopia indicated that *An. arabiensis* developed resistance to DDT, permethrin, deltamethrin and malathion. In contrast, *An. arabiensis* was susceptible to bendiocarb and Propoxur [70] and primiphos methyl (PMI/USID unpublished data). Abate and Haddis also reported high level of DDT and pyrethroid resistance in populations of *An. gambiae* s.l, presumably *An. arabiensis* from different parts of the country [71]. Another recent report by Massebo and others showed that populations of *An. arabiensis* from southwest Ethiopia developed resistance against lambda-cyhalothrin, alpha-cypermethrin, cyfluthrin, deltamethrin and DDT [33]. Moreover, high knockdown resistance mutation (West African *kdr*) was detected in populations of *An. arabiensis* from northwestern, central and southwestern Ethiopia [70, 72]. Bottle bioassay studies using synergists also revealed possible involvement of metabolic resistance in addition to *kdr* mutations in these populations of *An. Arabiensis*, which could further complicate the current malaria vector control program in the country [73]. The development of resistance by malaria vectors against insecticides used for public health could potentially jeopardize the malaria vector control strategy in Ethiopia, and hence it is imperative to monitor the level and distribution of insecticide resistance to develop new effective vector control tool and/or plan sound insecticide resistance management (IRM) strategy in the country.

3.4. Tanzania

The principal vectors of malaria in Tanzania are mosquitoes of the *An. gambiae* s.s, *An. arabiensis* and *An. funestus*. Other vectors which have limited role in malaria transmission include: *A. merus*, *A. nili*, *A. paludis*, *A. pharoensis*, *An. coustani*, *An. lesoni*, *An. parensis*, *An. merus*, *An. marshallii* and *An. rivulorum* [74, 75]. Recent entomological data indicate that *An. funestus* is prevalent on the mainland as well, particularly in the Kagera Region. Moreover, in coastal areas of north-eastern Tanzania and Zanzibar, high coverage of ITNs and IRS has resulted in a shift in the malaria vector population from *An. gambiae* to *An. arabiensis*. Resistance to pyrethroids by *An. gambiae* s.s. and *An. arabiensis* has been reported from several districts of the mainland of Tanzania [76–78]. Okumu and his colleague reported that *An. arabiensis* from southeastern Tanzania showed 100% susceptibility to DDT but 95.8% to deltamethrin, 90.2% to lambda cyhalothrin and 95.2% to permethrin [79]. In Zanzibar, *An. arabiensis* was resistance to pyrethroids (lambda-cyhalothrin, deltamethrin and permethrin), but was susceptible to carbamates (bendiocarb) and organochlorides (DDT). Moreover, in a similar study, resistance was documented in *An. gambiae* s.s to the same pyrethroid insecticides but was susceptible to bendiocarb, DDT and malathion [80]. In Pemba, resistance was detected in sites monitored for lambda-cyhalothrin, permethrin, deltamethrin and DDT, but no resistance was detected for bendiocarb and primiphos-methyl CS. Similarly in Unguja, lambda-cyhalothrin resistance

was detected in four of the five sites tested and permethrin resistance in one of the two sites tested. However, insecticide resistance was not detected for bendiocarb, pirimiphos-methyl CS and DDT. *Anopheles gambiae* s.s showed reduced susceptibility to the carbamate insecticide, bendiocarb [81]. *An. arabiensis* collected from Lower Moshi showed complete susceptibility to pirimiphos-methyl and malathion, but reduced susceptibility to permethrin [82, 83]. In northwestern Tanzania, there was cross-resistance between pyrethroids and DDT. In Zanzibar, resistance is not homogeneously expressed across islands, and pyrethroid resistance is stronger in Pemba than Unguja.

West African leucine phenylalanine *kdr* mutation was detected in two heterozygous individuals field-collected *An. arabiensis* from Tanzania [84]. A study also showed that a low frequency of permethrin resistance mediated by mixed function oxidases and esterases are present in *An. arabiensis* from Lower Moshi. The permethrin resistance is probably caused by the agricultural use of insecticides, especially in the rice fields, as permethrin-treated nets were not widely used in Lower Moshi [76]. The *kdr*-eastern variant was present in homozygous form in 97% of *An. gambiae* s.s but was absent in *An. arabiensis*. Synergist assays with PBO showed to restore susceptibility to pyrethroids, indicating that the resistance is in part due to an oxidase enzyme mechanism. Knockdown resistance mutation (target site insensitivity) was also detected in Pemba [84, 85].

3.5. Burundi

The primary vector of malaria in Burundi is *Anopheles gambiae* s.s, while secondary vectors *An. funestus*, *An. arabiensis* and *An. nili*. The most predominant members of vector species complex in the highlands of Burundi are *An. gambiae* s.s and *An. funestus* s.s [86, 87]. Insecticide susceptibility study in Karusi for *An. gambiae* s.l. showed reduced mortality to permethrin, DDT and deltamethrin. There was complete susceptibility of *An. funestus* to DDT and pyrethroids. A high frequency of East African *kdr* allele was detected in *An. gambiae* s.l., leading to cross resistance between DDT and permethrin in mosquito population. As there is little information on the frequency and distribution of insecticide resistance and the status of the susceptibility level of malaria vectors to insecticides used for vector control in the country, there is an urgent need for a nationwide and systematic evaluation of vector susceptibility level to current WHOPEs-approved insecticides for malaria vector control, to inform ongoing interventions and control program.

3.6. Rwanda

Earlier entomological studies indicate that *Anopheles gambiae* s.l. and *An. funestus* are the main vectors responsible for malaria transmission in Rwanda. *An. arabiensis* is also a locally important vector of malaria. The main malaria foci are in the east and southeast areas where the altitude is generally below 1,500 m and surrounded by marshy plains.

Insecticide susceptibility studies conducted in 2012 in several sites indicated signs of resistance to DDT in some areas, possible emergence of resistance to some pyrethroid compounds, and complete susceptibility to bendiocarb and fenitrothion. A similar insecticide susceptibility

study conducted by the national malaria control program in the same year showed established resistance to pyrethroids in Mimuri, a sentinel site in the country. A high frequency of the *kdr* gene in *An. gambiae* s.l. has been attributed to explain the new established resistance to pyrethroids in one district. A countrywide resistance monitoring also showed resistance to pyrethroids, DDT and bendiocarb and higher resistance was reported from eastern province, southern province and Kigali city. A continuous monitoring of resistance and resistance mechanisms is required in order to guide program for the best strategies to prevent the development and spread of resistance in the country.

4. Possible causes of insecticide resistance

Emergence of resistance in disease vectors in particular mosquitoes have been associated with different factors and sources. One of the factors is the intensive use of some classes of insecticides such as pyrethroids both in public health and in agriculture, which led to its reduced efficacy of insecticides [88–90]. Agricultural use of pesticides plays a role on the development of resistance and cross-resistance in malaria vectors has been implicated in literature. Resistance of *An. arabiensis* to pyrethroids in Tanzania [76] and Ethiopia [72] was attributed to use of insecticides in agriculture and livestock.

Insecticide resistance selection pressure in malaria vectors in East Africa region has also been attributed to wide coverage of LLINs and/or IRS [91] and use of agricultural pesticides [92]. As the most commonly used pesticides in agriculture and IRS are pyrethroids, organophosphate and organochlorides and for the treatments of LLINs are pyrethroids, cross-resistance is common between pyrethroids and DDT [85, 93].

5. Frequency and mechanisms of resistance in malaria vectors in East Africa

There is variation in the frequency of resistance in malaria vectors and the mechanisms conferring resistance in different sites of different countries in East Africa (Table 1). The frequency and mechanism of resistance in insects depend on the degree of selection pressure and the mode of action of the insecticide, respectively. Insecticides target the nervous system of an insect. Organophosphate insecticides are cholinesterase inhibitors. Cyclodienes insecticides affect the chloride channel by inhibiting the gamma amino butyric acid (GABA) receptor. Pyrethroids and DDT act on the sodium channel preventing those channels from closing, resulting in continual nerve impulse transmission which eventually leads to the death of an insect [94].

Target site insensitivity is the most frequently reported mechanism conferring resistance to several insecticides used for vector control by altering the target site of the insecticides. The mode of action of each insecticide on insects is site-specific. For instance, the mode of action of organophosphate and carbamate insecticides is mainly by inhibition of the enzyme acetyl-

Country	Insecticide	Mosquito species	Mechanism	Reference(s)
Kenya	DDT & pyrethroids	<i>An. gambiae</i> , <i>An. arabiensis</i>	<i>Kdr</i> (L1014S, L1014F)	[21, 52, 54, 104, 105, 116, 130–132]
	Pyrethroids	<i>An. gambiae</i> , <i>An. arabiensis</i> , <i>An. funestus</i>	Cytochrome P450s monooxygenases, esterases	[53, 61, 130, 132]
Uganda	DDT & pyrethroids	<i>An. gambiae</i> , <i>An. arabiensis</i>	<i>Kdr</i> (L1014S, L1014F)	[55, 57, 58, 62, 114, 133]
	DDT & pyrethroids	<i>An. gambiae</i> , <i>An. arabiensis</i> , <i>An. funestus</i>	cytochrome P450s, GSTs, pNPA	[53, 55, 59, 91]
Ethiopia	DDT & Pyrethroids	<i>An. arabiensis</i>	<i>kdr</i> (L1014F)	[67, 70, 72, 134]
	Pyrethroids	<i>An. arabiensis</i>	Cytochrome P450s monooxygenases	[73]
Tanzania	DDT & pyrethroids	<i>An. gambiae</i> , <i>An. arabiensis</i>	<i>Kdr</i> (L1014F, L1014S), <i>rdl</i>	[76, 84, 92]
	DDT & Pyrethroids	<i>An. arabiensis</i> , <i>Cx. quinquefasciatus</i>	Mixed function oxidases, b-esterases, P450s, cuticle proteins, GABA, sulfotransferase	[76, 92, 135, 136]
Burundi	DDT & pyrethroids	<i>An. gambiae</i> s.l.	<i>Kdr</i> (L1014S)	[133]
Rwanda	DDT & Pyrethroids	<i>An. gambiae</i> s.l.	<i>Kdr</i>	[137]

Table 1. Insecticide resistance mechanisms conferring resistance to different insecticide families in the major malaria vectors and other mosquitoes in East Africa

cholinesterase (ACHE). Insects develop resistance to these insecticides through structural modification of ACHE due to large number of point mutations that occurs in gene encoding the protein for acetyl cholinesterase (ACHE), an active target site for carbamates and organophosphates which operates in the nerve cell synapses. These mutations result in altered ACHE, which reduces the sensitivity of target site to an insecticide. Another common site insensitivity mechanism is referred as knockdown resistance (*kdr*): insects usually get paralyzed rapidly following exposure to DDT and pyrethroids, and this is expressed as 'knockdown resistance' (*kdr*). However, knockdown is absent in insects exposed to DDT and pyrethroids due to mutations in the para-gated sodium channel gene, whose protein sub-units make up the voltage-sensitive sodium channels on the nerve membranes. Voltage-gated sodium channels are the target for both pyrethroid insecticides and DDT by which insecticides alter the function of the sodium channels in nerve membranes. Knockdown resistance mutation results from a single nucleotide polymorphism in the domain II, segment 6 of the sodium channel gene. Lucine (TTA) to serine (TTT) and leucine (TTT) to phenylalanine (TCA) amino acid substitutions at this position result in West and East African *kdr* mutations, respectively, which confer resistance to DDT and/or pyrethroids in the East African malaria vectors *An. gambiae* s.s and *An. arabiensis* [95, 96]. Another mutation of methionine to threonine, known as the super-*kdr* mutation, occurs between segment 4 and segment 5 of domain II of the sodium channel gene

and results in a much higher resistance than *kdr*. The super-*kdr* mutation is mostly occurring together with the *kdr* mutation. The *kdr* resistance mechanism produces cross-resistance between DDT and pyrethroids and it is a genetically recessive mechanism.

Metabolic resistance is another important mechanism conferring resistance to insect vectors which is associated with the production of increased quantities of families of enzymes involved in insecticide metabolism. It resulted from structural change in the enzyme molecule that enhances its ability to detoxify or bind the insecticides which alter the affinity of the enzyme to insecticides. In the latter case, this mechanism enhances insecticide tolerance status in insects. Some of the common enzymes involved in detoxifying or sequestering insecticides in insects are monooxygenases which include the cytochrome P450 enzymes. These large groups of enzymes confer resistance mainly to pyrethroids and carbamates and to a lesser extent to organochlorines and organophosphates. Another extremely important group of enzymes which confer resistance to organophosphate, carbamates and to some extent pyrethroid insecticides are esterases. Elevated level of esterases results in sequestration and metabolism of the target insecticides. Elevated glutathione S-transferases (GSTs) also play a role in the detoxification and excretion of organophosphates and DDT in insects. Cross-resistance between DDT and organophosphates is often caused by GSTs.

Behavioral resistance in mosquito vectors tends to change their behavior due to long-term exposure to insecticide-treated surfaces such as walls and LLINs. This behavior has been found to be associated with avoidance of exposure to lethal doses of insecticides due to reduced contact with the insecticide [97, 98]. The behavior is known to increase the longevity of insects in an environment where there is insecticide application through IRS, LLINs or both for vector control. Insects show limited tendency to enter sprayed houses or in houses with LLINs. For example, the evaluation of LLINs or IRS compounds in East African experimental huts have shown avoidance behavior by *An. gambiae*, *An. arabiensis* and *An. funestus* [98]. This also results in irritancy and excito-repellency, which keeps the mosquitoes away from different treated surfaces before contact with the host [99–102]. Shifting of vector species composition (from *An. gambiae* to *An. arabiensis*) due to implementation of LLINs or IRS has also been observed in Tanzania and Kenya [61, 103, 104]. In Africa, there is high proportion of *An. gambiae* and *An. funestus* in areas with high coverage of LLINs [105]. The host-seeking behavior of vectors have changed from endophagic to exophagic due to intensive LLINs coverage [106].

Moreover, mutation in the GABA-gated chloride channel, which leads to dieldrin resistance other than DDT, has been described in different species of mosquitoes. The role of cuticular resistance mechanism is not yet known in the phenotypic resistance in the East African malaria vectors.

6. Impact and Implications of insecticide resistance on the efficacy of LLINs, IRS and malaria transmission

The increased bed nets ownership and its utilization have significantly reduced malaria-related cases and mortality in Kenya [107, 108], Tanzania [109, 110], Uganda [111] and Ethiopia [112]. However, the high coverage of IRS and scaling up of LLINs is believed to induce the

development of resistance in vector species to various classes of insecticides. These have been documented in Kenya [21], Tanzania [79, 113], Uganda [114] and Ethiopia [70, 73, 115].

After the successful reduction of the malaria vector and disease transmission, the increased resistance among potential vector populations has been witnessed across East Africa [1, 21, 116]. The current status of pyrethroid resistance in malaria vectors and an increase in malaria incidence shows the compromised vector control system due to insecticide resistance, which calls the need for the development of new tools for malaria control. Insecticide resistance has shown to compromise the effectiveness of malaria control efforts in Kenya and other West African countries. The use of non-pyrethroid insecticides for IRS is a potential option as the ITN are mainly pyrethroid-based. It has been observed that pyrethroid resistance mosquitoes are entering and surviving exposure to LLINs, which may quantify the indoor transmission resurgence in areas with high level of pyrethroid resistance.

7. Prospects of prevention on development and spread of IR in malaria vectors in East Africa

In vector control, constant use of the same insecticide induces resistance selection pressure in small vector population which subsequently spread to the large population. The spread of resistance depends on the frequency of the resistance genes within the vector population. In operational programs, the coverage of LLINs and IRS are most critical to be considered in the prevention of the development and spread of the insecticide resistance. Further, insecticide-resistant monitoring plan and management strategy should be developed and implemented to delay the development or spread of resistance.

8. Insecticide monitoring and insecticide resistance management option

8.1. Mixture

In resistance management strategies, four tools (rotation, mosaic, mixture and combination) have been suggested [117] either to slow down resistance or reduce the rate of insecticide selection pressure. In control programs, simultaneous use of two or more insecticide compounds with different modes of action within a single product or formulation is preferred to manage resistance in insects. Two mixed insecticides with different modes of action can lead to reduced chance of double resistance by killing an insect which is resistant to one of the insecticide compounds [71, 82]. The use of mixture of insecticides relies on the assumption that the number of insects carrying a resistant allele at both loci is rare if the frequency of resistant allele at two loci is low [23, 118]. This approach may have a reduced efficacy if resistance in insects is at detected level to one of the mixed insecticide compounds. The major aim of insecticide mixture is to overcome the resistance selection pressure rather than maintaining the high susceptibility status of the insect population. The mixture should be up to the standard

application concentration ratio of two insecticide compounds for effective control. Mixture of insecticides has usually high cost implication which may not be affordable in community protection against malaria. The mixture of insecticide compounds has practically shown to be effective when applied in small scale [82].

8.2. Mosaic

This approach is the use or application of two different classes of insecticides to control the same disease vector in the same area [119]. The mosaic approach is effective if application takes into consideration the spatial pattern. This technique helps in restoring the susceptibility status of the vector to an insecticide. It is a method for control of resistance secured to be working if properly done and monitored [120]. In some malaria endemic countries, large-scale mosaic application has shown to effectively control resistant populations of *An. albimanus* [119]. It has been observed that resistance developed fast in areas with pyrethroid alone than in areas with mosaic application along organophosphate, pyrethroids and carbamates [119]. Recently, industries have developed mosaic LLINs (PermaNet 3.0 and OlysetPlus) containing a pyrethroid insecticide and a synergist (piperonyl butoxide), an oxidase inhibitor on the fabric to increase the bio-efficacy against pyrethroid-resistant vectors [73, 114, 121]. Further research is needed in the future to use mosaic in LLINs and IRS.

8.3. Rotation

This is employing two or more insecticide compounds of different insecticide classes with different modes of action by switching the insecticide of choice each round or in alternating sequences. This approach is based on the assumption that resistance genes have a selective disadvantage in the absence of an insecticide used in operational program. If vector resistance to each insecticide is low, then the occurrence of multiple insecticide resistance is minimal or practically impossible [122]. The rotational use of insecticides plays a major role in killing resistant insects when the switch is made to a second insecticide. The defined rotation time should be as short as possible to reduce the risk of resistance development against the insecticide in use. It also slows down the evolution of the resistance [119]. For LLINs, it is difficult to implement rotation technique as only pyrethroids are used for the treatment of nets [123]. This method has higher financial implications for the implementation in vector control.

8.4. Combination of tools

In monitoring and management of resistance, the use of two or more tools or combinations of interventions simultaneously is an option in insecticide resistance management. The use of tools targeting adults such as LLINs and implementation of IRS or vice versa or combined with larviciding or larval source reduction is shown to have effects on vector control in Kenya [26, 124], Tanzania [43, 79, 113], Uganda [125] and Ethiopia [126].

Combination of tools is appreciated as it is cost-effective, prohibits mosquito feeding and causes mortality instead of reducing resistance alone. In this approach, using insecticides which share the same resistance mechanism should be avoided as resistance in malaria vectors

develops faster. Combination tools have shown to increase the protection efficiency against vectors and maintain reduced susceptibility status of the vectors for longer period.

9. Challenges in insecticide resistance management

The growing and widespread of insecticide resistance among vector species have been a major challenge in vector control and managing resistance. The resistant vectors have developed different mechanisms to tolerate the insecticides [53]. Each of the mechanism has its own target site for an insecticide [53]. The main insecticides used for the treatment of LLINs are pyrethroids, to which the major malaria vectors have shown tolerance [21]. The main challenge is that there is no other new class of insecticide to be used for LLINs and IRS [53, 61, 127]. Malaria control programs in East Africa and most sub-Saharan Africa rely heavily on donor-funded programs for LLINs distribution and IRS implementation. Insecticide resistance monitoring and management and operational research were not the primary agenda for the main donors. The control programs of East African countries have also not yet established a mechanism (s) for generating local funds to foster malaria control efforts [128]. This makes the whole effort of vector control program more challenging with the risk of malaria resurgence in some foci along the emergence and widespread of resistance in large areas of East Africa [59, 129, 130]. In general, insecticide resistance data in East Africa are patchy, and in some countries such as Burundi and Rwanda nearly non-existent. Therefore, countries need to create a national insecticide resistance data base for insecticide resistance monitoring data to understand the trend of insecticide resistance for timely decision-making and sharing of information..

10. Conclusions and future directions

Insecticides resistance against malaria vectors has spread throughout the East African countries. Some of the countries like Tanzania and Rwanda have already established a national insecticide resistance monitoring and management plan, and others are in the process of developing the plan mainly to prevent the emergence of resistance or as a response to detected resistance. However, effective implementation of the plan requires national capacities in terms of trained human power and infrastructure to undertake surveillance and monitoring of resistance to advice policy to look for alternate control options or new vector control tools or ensure that current interventions remain a choice in vector control program. Effective implementation of insecticide resistance monitoring and management also needs coordination and inter-sectorial collaboration in the respective countries.

The lack of enough funds in East African countries may delay the implementation of resistance monitoring and management strategies. This may hinder to start monitoring of resistance or responding to resistance, use of suggested insecticide of choice for vector control, change control strategy as soon as strong evidence on resistance is available. Internal sources of funding, internally driven resource mobilization and allocation of adequate resources are of

paramount importance in implementing national insecticide resistance monitoring and management strategies in the context of integrated vector management. The NMCP of each country needs also to develop the working guideline with donors and other relevant partners to implement insecticides resistance monitoring and management strategies.

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