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The changes in mosquito vector behaviour and the emerging resistance to insecticides will challenge the decline of malaria

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Abstract

The preventive measures against malaria recommended by the WHO include anti-vector procedures such as indoor residual spraying, the use of long-lasting insecticide-treated bed-nets, and the destruction of larval breeding sites. The presence of insecticide-treated materials inside the mosquito habitat has consequences for the vector's population, reducing density, survival, contact with humans, and feeding frequency. However, the effectiveness of these tools is being challenged by the emergence of insecticide resistance. The evolution of resistance to insecticides in *Anopheles* threatens to thwart the goal of decreasing malaria transmission, in an arms race between malaria control programmes and the vector populations. Multiple mechanisms of resistance to insecticides have been observed in *Anopheles* populations, including target site mutation (knockdown resistance), increased metabolic detoxification, and remarkable behavioural adaptation. These disturbing observations all show the capacity of *Anopheles* to adapt to and circumvent strategies aimed at reducing malaria transmission. Thus, by using nets to protect ourselves, are we providing *Anopheles* with the entire arsenal needed to hit much harder?

Keywords: Adaptation, *Anopheles*, insecticide resistance, Kdr, malaria
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Introduction

Malaria is one of the most serious vector-borne diseases, and affects millions of people, mainly in Africa. More than 90% of deaths resulting from malaria occur in children aged 1–5 years [1]. In the absence of a sufficiently efficient vaccine, the diagnosis and treatment of clinical cases, intermittent preventive treatment of targeted populations and vector control are the only tools available to combat malaria. Recent progress in reducing malaria morbidity and mortality in Africa is founded upon expanded coverage of long-lasting insecticide-treated bed-nets (LLINs), indoor residual spraying (IRS), and combination drug therapy [2,3]. Most researchers agree that vector control has a central role in achieving the ambitious goal of malaria elimination [2,4]. The 21st century has witnessed a pronounced increase in the use of insecticides for malaria control. Several major donors have invested heavily in the distribution of LLINs and IRS activities [5–7] after the recent call by the Bill & Melinda Gates Foundation and the President's Malaria Initiative for the international community to support a campaign to eradicate malaria [5,8].

Historically, the first global strategy for malaria control was adopted in 1955 at the start of the now notorious Global Malaria Eradication Program. This strategy called for the widespread and rapid application of dichlorodiphenyltrichloroethane (DDT) to interrupt the transmission of the disease in countries around the world, except for countries in sub-Saharan Africa, regardless of geography and epidemiology [6]. This approach succeeded in some countries, but failed to interrupt transmission completely in many other countries; malaria resurged to previous or even higher levels as eradication programmes crumbled, and the strategy was abandoned [9].

Anopheles vectors of malaria are constantly evolving. Of the 460 described species of *Anopheles*, only approximately 60 are able to ensure malaria transmission. In Africa, malaria is mainly transmitted by mosquitoes of the *Anopheles gambiae* complex, *Anopheles funestus* group, *Anopheles nili* complex, and *Anopheles moucheti* complex. *Anopheles* mosquitoes are probably the most efficient malaria vectors, and a large diversity of ecosystems in Africa are favourable for their presence. However, malaria transmission is highly variable throughout Africa, and it is necessary to have the best knowledge possible regarding the transmission pattern and targets—the anopheline vectors—before using any vector control measures. Many detailed studies have been conducted on the importance of mosquito resistance (resistance by modifying the target of insecticides, metabolic resistance, and behavioural adaptation) to malaria control [10,11]. The genetic complexity and ecosystem diversity of the *Anopheles* species, coupled with behavioural resistance, constitute a serious obstacle to the implementation of effective and sustainable vector control programmes with LLINs and IRS. These tools are now being challenged by the emergence of insecticide resistance, which has arisen as an adaptation in mosquitoes in response to such control measures and poses a serious threat in the fight against malaria. Indeed, some species have developed physiological and/or metabolic resistance to insecticides [12], whereas others have adopted new behaviours (e.g. new time or place of bite or new host) to avoid [13] exposure to insecticides and ensure their survival and reproduction.

In this review, we will focus on the adaptive processes of malaria vectors (i.e. behavioural changes and decreased sensitivity to insecticides) that can hamper the efficacy of vector control interventions.

Mechanisms Allowing a Decrease in Sensitivity to Insecticides

The different resistance mechanisms that enable insects to withstand insecticides can be grouped into three categories.

Resistance by modifying the target of insecticides

The main targets of insecticides are receptors or enzymes of the nervous system: acetylcholinesterase (AChE), the voltage-dependent sodium channel (CNaVdp), and the receptor of γ-aminobutyric acid. Mutation of these targets is a very effective resistance mechanism, inducing cross-resistance to all insecticides acting on the same target. The target of organophosphates and carbamates is AChE. Organochlorines of the cyclodiene group act on γ-aminobutyric acid receptors. Pyrethroids and DDT act on CNaVdp. A point mutation replacing leucine with phenylalanine at the sixth segment of domain II of the CNaVdp gene (knockdown resistance (Kdr) mutation Leu→Phe) and a point mutation replacing leucine with a serine at the same position (Kdr mutation Leu→Ser) have been described in West Africa [14,15] and East Africa [16], respectively. These two point mutations (L1014F and L1014S) are associated with Kdr to DDT and pyrethroids in *A. gambiae* s.l., whereas the same point mutations have been reported to be rare in *A. funestus*.

Metabolic resistance

Metabolic resistance is the most common resistance mechanism in insects. It relies on the enzyme systems that allow insects to ensure the natural detoxification of all foreign elements, not only insecticides. Three main categories of enzymes are involved in this function: esterases, cytochrome P450 monooxygenases, and glutathione-*S*-transferases [10]. In Mozambique, a high level of pyrethroid resistance has been observed for *A. funestus* [15]. WHO susceptibility assays indicated that *A. funestus* has very high resistance to pyrethroids, even though no DDT resistance was observed, suggesting that Kdr was not involved. Both biochemical assays and quantitative PCR implicated the upregulation of P450 genes in pyrethroid resistance, with glutathione-*S*-transferases playing a secondary role. Resistance to a carbamate, bendiocarb, was also noted and attributed to mutated AChE and the action of esterase. Several mechanisms may contribute together to the resistance to insecticides in the same insect population.

However, some authors refer to these two types of resistance (metabolic resistance and resistance by modifying the target of insecticides) as physiological resistance [10,17].

Behavioural resistance

Since the identification of behavioural mechanisms in resistance to insecticides in 1956 [13], little research has been conducted in this area. The experimental studies investigating such resistance are not easy to design, because changes in behaviour that occur in the field are not necessarily observable or quantifiable in laboratories. Several types of behavioural resistance have been described in insects [18]; some are associated with the mobility of the insect [13,19], and others are associated with its immobility [20]. Behavioural resistance refers to any modification to mosquito behaviour that facilitates the avoidance or circumvention of insecticides. Both mechanisms allow insects to avoid contact with the toxic product or limit the duration of this contact. The lack of information about behavioural resistance partly results from the difficulty of using relatively simple exposure assays and monitoring field populations in investigating behavioural resistance as compared with metabolic resistance and resistance by modifying the target of insecticides [10]. Behavioural resistance seems to be becoming increasingly more complex, suggesting that the adaptation processes are very sophisticated.

Anopheles and Adaptation

Adaptation has been defined as the functional adjustment of organisms to environmental conditions [21,22]. In evolutionary biology, there is a fundamental concept: organisms facing stressful situations can either [23,24] adapt or disappear. This concept seems to hold for *Anopheles*, especially after the massive deployment of nets and insecticide spraying as components of the international efforts during the last decade to eliminate malaria.

Anopheles vectors are known to have remarkable abilities to adapt that enable their survival in widely varying environmental conditions [25]. Although the use of insecticides reduces mosquito density, it has led to the selection of resistant strains [26–28]. Behavioural modifications have also been reported in mosquitoes exposed to insecticides, such as a shift from endophilic (i.e. resting in houses after blood meals) to exophilic (i.e. resting outdoors after blood meals) behaviour and changes in the time of feeding [29–32]. LLINs remain effective for reducing the burden of malaria, but the long-term effects of insecticides on vector populations and malaria transmission remain to be evaluated. A study conducted in Kenya by Githeko *et al.* in 1996 showed a dramatic change in the behaviour of *A. gambiae* s.s. after the implementation of LLIN use. This species became more exophilic after the introduction of the mosquito nets, whereas it was previously exclusively endophilic. This study also showed that, despite the species' new exophilic behaviour, *A. gambiae* s.s. remains highly anthropophilic, increasing the risk of malaria transmission. In 2009, Lefèvre *et al.* [33] studied *A. gambiae* populations living in a rice-growing area in Burkina Faso. Whereas this species was highly anthropophilic before the widespread use of nets, which lowered the availability and accessibility of human hosts, the authors observed a trophic deviation of this species to cattle. In the Kilombero Valley of Tanzania [29], before the use of LLINs, *A. gambiae* s.l. and *A. funestus* had a strong tendency to bite hosts indoors late at night. In 2009, after covering of only 47% of the population with nets, no significant difference was observed in the proportion of *A. gambiae* caught indoors with constant biting activity. Studies conducted in Senegal [34] and Benin [32] showed that *A. gambiae*, which usually bites hosts in the second half of the night (i.e. after 12 a.m.), began to bite hosts a little earlier (well before 10 p.m.), at a time when most people are not yet under mosquito nets. Despite the deployment of nets, malaria transmission has continued, because of the adaptation of this species.

Concerning *A. funestus*, the most commonly observed phenomena after the introduction of LLINs have been a strong tendency to be exophilic (in Tanzania) and a trophic deviation to cattle [29]. The widespread implementation of LLIN use has led to the disappearance of *A. funestus* from some areas. For example, in Dielmo village, the density of *A. funestus* fluctuated markedly from 1990 to 2007 [35,36], leading to the total suppression of malaria transmission by *A. funestus* [32] shortly after the implementation of LLIN use. In other regions, this species has established an entirely new strategy. A study conducted in Benin by Moiroux *et al.* [37] in 2012 showed that this species, which was once aggressive at night, became aggressive in the daytime, as >26% of mosquitoes were caught between 6 a.m. and 9 a.m. in broad daylight. In addition, instead of attacking victims at night when they are sleeping, the species now prefers to wait until the early morning hours, when people leave home to go to work or eat breakfast. Another study, conducted by Corbel *et al.* in 2012 [32], just 1 year after the large-scale introduction of impregnated mosquito nets, showed that *A. funestus*, which used to plague houses, began to bite hosts more frequently in outside dwellings. The exophagy rate rose from 45% before the intervention to >70% after the intervention.

Malaria Control and *Anopheles* Resistance to Insecticides: an Arms Race

DDT played a key role in the successful control, and in some places the elimination, of malaria [38] during and after the first global campaign to eradicate malaria in the 1950s and 1960s, especially in areas where the climate was temperate and transmission was unstable. However, this initial success has not been sustained, owing to, among other things, the emergence of resistance to this insecticide among mosquito populations. The present vector control strategies against malaria are based on the massive deployment of LLINs and IRS [2,3]. Currently, pyrethroids are the only insecticides that are approved for treating bed-nets. Pyrethroids are preferable for bed-net impregnation because of their high effectiveness, with a strong excito-repellent effect on mosquitoes, and low mammalian toxicity as compared to organochlorine, carbamate and organophosphate compounds. The insecticides that are currently used for IRS belong to the same pyrethroid family or to a small number of other more toxic and often more expensive (particularly when considering their persistence) insecticide families, i.e. carbamates and organophosphates [18].

The long-term efficacy of LLINs in reducing malaria morbidity has recently been questioned in West Africa. The first evidence of low long-term LLIN efficacy was identified in a rural area of Senegal, where there was evidence of a rebound in malaria morbidity coinciding with the emergence of Kdr [34]. Moreover, in Benin, universal coverage with LLINs and/or IRS has shown no beneficial effect on morbidity in comparison with targeted LLIN use [32]. Of all types of resistance, perhaps the most significant for *A. gambiae* populations is resistance by modifying the target of insecticides, especially Kdr. This mutation is most concerning, because it is transmitted from generation to generation.

The emergence and rapid spread of pyrethroid resistance in *Anopheles* populations may be a threat to the sustained effectiveness of malaria vector control activities across Africa. However, in a true arms race, *Anopheles* mosquitoes continue to develop increasingly more resistance to insecticides. Unfortunately, Kdr to pyrethroids and DDT, as first reported in *A. gambiae* s.s. populations in the Ivory Coast [15], has been observed to be spreading, mainly in West Africa. The spread could be attributed to the intensive use of DDT and pyrethroids for protection of crops, particularly cotton, and public health purposes. Indeed, the use of pyrethroids in pesticides for agriculture and net treatment has been recognized as a factor that is responsible for the selection of resistant mosquitoes in sub-Saharan Africa [26,27,39].

Several studies have shown that a direct relationship exists between the rapid increase in the frequency of Kdr and the widespread use of LLINs. Accordingly, there could be a direct relationship between the increase in Kdr and the rebound of malaria observed over the past 2 years in endemic areas [32,34]. A study conducted in Dielmo (Senegal) showed that the dramatic increase in Kdr frequency was directly related to a massive deployment of LLINs [34,40]. The Kdr frequency increased from >5% (a level that had not varied for years) to >47% just 2 years after the introduction of LLINs. This increase in Kdr frequency was associated with a rebound of malaria. Near Dielmo, although some gardening and rice cultivation activities are performed, the use of pesticides was limited, and did not change during the study period. Insecticide-treated net use was implemented in Dielmo in July 2008 as part of the malaria study. The authors speculate that the implementation of insecticide-treated net use may have contributed to the selection of pyrethroid-resistant populations of *A. gambiae*. Therefore, agricultural practices probably had a limited role in the emergence of resistance to insecticides in this area. Interestingly, in 2012, Ndiath *et al.* [41] studied the distribution of Kdr in the Senegal River region (20 villages of the region of Podor). The authors observed a high level of Kdr in Guédé Chantier, the only village where LLINs were used by the majority of the population (approximately 65% of the inhabitants). The authors speculated that the use of pyrethroid-treated nets has contributed to the selection of a resistant mosquito population, as in other areas [27,39]. Similar observations have been made in Benin [32] and Niger [27]. In Kenya, lower susceptibility of *A. gambiae* to permethrin was found in villages where permethrin-impregnated nets had been used for 1 year than in villages without nets. The mechanism involved was postulated to include Kdr together with metabolic resistance [39].

Because of the high anthropophily of malaria vectors, the widespread use of LLINs and IRS in an increasing number of countries, and the use of a small number of related insecticides, some malaria vectors are now exposed to a level of insecticide pressure that has never been reached before. *Anopheles* vectors have no choice but to adapt, as *A. gambiae* s.l. has disappeared in an increasing number of areas, and *A. funestus* has begun to disappear in some places. In the arms race launched between the malaria control programmes and the vector populations, the arthropods seem to have an advantage over the developers of insecticides and insecticide-based vector control measures.

Conclusion

Insecticide resistance is a phenomenon that is growing at an alarming rate. There is no doubt that vector control is being challenged by two phenomena that are both disturbing and troubling: biological resistance to insecticides, and behavioural adaptation to insecticide-based vector control interventions. If the current trends continue, these phenomena may compromise the effectiveness of malaria control and elimination, as they did at the time of the previous eradication campaign [5]. The previous knowledge of the mechanisms of resistance and the knowledge acquired recently through molecular biology may provide opportunities for resistance management. There is, however, little evidence that the presently recommended resistance management strategies are effective against malaria vectors. In fact, the need for the implementation of truly effective strategies for delaying the spread of insecticide resistance is so urgent, and the time needed for the development of new insecticides is so great, that it is doubtful whether the approaches that have been used up to now, which are mainly based on the use of insecticides or a combination of insecticides, will be able to sustain the efficacy of malaria vector control interventions for the next decade. From this perspective, the present model of insecticide-based vector control that is promoted by chemical companies has not proved to be effective enough in countering the adaptation of the vectors. Therefore, the development of new vector control interventions that are not based on the use of insecticides should be considered.

In the meantime, other additional malaria control measures are needed. In areas of highly seasonal transmission with a high burden of disease in young children, such as in south Senegal, a new strategy, called seasonal malaria chemoprevention [42], recommended by the WHO, can be used to reduce the incidence of malaria. Similar strategies have proved to be effective in Burkina Faso [43] and in Mali [44] for children who were already using LLINs. In Senegal, the Ministry of Health plans to implement seasonal malaria chemoprevention in five regions in the south of the country, starting in 2013.

Transparency Declaration

The authors declare that they have no conflicts of interest.

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