

## The impact of insecticides management linked with resistance expression in *Anopheles* spp. populations

O impacto do manejo de inseticidas relacionado com a expressão de resistência em populações de *Anopheles* spp.

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**Abstract** *The resistance of some species of Anopheles to chemical insecticides is spreading quickly throughout the world and has hindered the actions of prevention and control of malaria. The main mechanism responsible for resistance in these insects appears to be the target site known as knock-down resistance (kdr), which causes mutations in the sodium channel. Even so, many countries have made significant progress in the prevention of malaria, focusing largely on vector control through long-lasting insecticide nets (LLINs), indoor residual spraying and (IRS) of insecticides. The objective of this review is to contribute with information on the more applied insecticides for the control of the main vectors of malaria, its effects, and the different mechanisms of resistance. Currently it is necessary to look for others alternatives, e.g. biological control and products derived from plants and fungi, by using other organisms as a possible regulator of the populations of malaria vectors in critical outbreaks.*

**Key words** *Malaria, Anopheles, Knock-down resistance (kdr), Pesticides, Public Health.*

**Resumo** *A resistência de algumas espécies de Anopheles a inseticidas químicos está se espalhando rapidamente por todo o mundo e tem dificultado as ações de prevenção e controle da malária. O principal mecanismo responsável pela resistência nestes insetos parece ser o sítio-alvo conhecido como resistência knock-down resistance (kdr), que causa mutações no canal de sódio. Mesmo assim, muitos países fizeram progressos significativos na prevenção da malária, concentrando-se em grande parte no controle do vetor através redes inseticidas de longa duração (RILD), e de pulverização residual interna (PRI) de inseticidas. O objetivo desta revisão é contribuir com informações sobre os inseticidas mais aplicados para o controle dos principais vetores da malária, seus efeitos, e os diferentes mecanismos de resistências. Atualmente é necessário olhar para outras alternativas, como por exemplo, controle biológico e produtos derivados de plantas e fungos, pela utilização de outros organismos como um possível regulador de populações de vetores da malária em surtos críticos.*

**Palavras-chave** *Malária, Anopheles, Knock-down resistance (kdr), Pesticidas. Saúde Pública*

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## Background

According to the World Health Organization (WHO), Malaria kills over a million people per year and other 3.2 billion people are living in areas at risk. Over 80% of deaths occur in Africa, where approximately 66% of this population is at risk area<sup>1</sup>. In the Americas, 14% of the population is at risk of transmission. Since the year 1990, the number of malaria cases reported in South and Central America and the Caribbean remain relatively low, however there is a risk of malaria transmission in nine countries that are part of the Amazon region and in eight countries in Central America and the Caribbean<sup>1</sup>.

Malaria is a disease caused by *Plasmodium* species which are transmitted by several species of *Anopheles* mosquitoes. There are approximately 400 species of *Anopheles* world-wide and of these about 50 species are exclusive vectors of human malaria<sup>2,3</sup>. The most important vectors in Sub-Saharan Africa and the most efficient malaria vectors world-wide belong to the *Anopheles gambiae* Complex. In this complex, *A. gambiae* and *Anopheles arabiensis* are the most important vectors of *Plasmodium falciparum* (the most severe malarial pathogen) in Africa. At level of Americas, *Anopheles darlingi* is one of the most important malaria vectors<sup>4</sup>, occurring mainly in Amazon region and showed high degree of variability in behavioral traits and capable of maintaining a relatively high transmission of malaria even when found in low densities<sup>5,6</sup>. This makes it difficult to predict the impact of ongoing changes in the environment on the mosquito populations. Due to their anthropophilic behavior and physiological feasibilities, they possess a higher vector capacity than other closely related sibling species with no or low vector capacity. Falavigna-Guilherme et al.<sup>4</sup> carry out the reported autochthonous cases of malaria after construction of the Itaipu Hydroelectric in Foz do Iguacu. The impact of dams and irrigated schemes *Anopheles* populations has been elucidated by Sanchez-Ribas et al.<sup>7</sup> revealing the proliferation of *Anopheles* species in anthropogenic environments.

Apparently, pathogen/parasite and vector (mosquito) either adapted to each other during the course of evolution, resulting in coexistence, or the pathogen/parasite was repelled<sup>8</sup>. *Anopheles gambiae* has been considered the most anthropophilic species, followed by *A. arabiensis*, *Anopheles melas*, and *Anopheles merus*. However, host preference likely varies considerably within species owing to population structure and environ-

mental conditions, and reports of host preference also vary depending upon the methods used to measure this poorly defined behavior<sup>9-11</sup>.

In an effort to malaria transmission reduction, some countries have implemented indoor residual spraying (IRS) and universal distribution of insecticide-treated nets ITNs in combination in years<sup>12,13</sup>. Many countries have made significant progress in preventing malaria by focusing largely on vector control through long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) of insecticides and use of DDT has been compromised, not only because of resistance, but also because of its environmental effects<sup>14,15</sup>. In areas with insecticide-susceptible mosquito populations, the insecticide on LLINs mitigates the loss of personal protection if the net becomes holed.

The reduction in disease burden of malaria in recent years has in large part been attributed to the massive scale up of the two main vector control interventions, LLINs and IRS, particularly in Africa south of the Sahara. A number of countries have deployed the two interventions in combination in an attempt to further reduce transmission<sup>16</sup>.

However, both IRS and LLINs face the development of physiological resistance (against insecticide) and 'behavioural resistance' in mosquitoes, which can reduce effectiveness of these interventions and possibly reverse the gains made in reducing malaria morbidity<sup>17</sup>, but there are currently not enough data to determine the impact of resistance on the effectiveness of such combinations<sup>16</sup>. Maybe, IRS has some utility in areas with low resistance as part of an overall resistance management strategy aimed at preserving the effectiveness of pyrethroids<sup>16</sup>. Although such resistance may be inevitable with successful control programmes, new strategies need to be developed to mitigate development and spread of insecticide resistance and to preserve the efficacy of currently available insecticides and the effectiveness of malaria control interventions.

The aim of this review is to elucidate the *Anopheles* spp. literature about the mainly vector species of malaria disease, what insecticides are applied, their effects and the different mechanisms resistances arising in these species.

### Approach about *Anopheles* in agricultural fields

Agricultural practices have significant influence on mosquito species diversity and abun-

dance and that certain habitat characteristics favor production of malaria vectors. For example, the creation of fishponds basins and the development of market-gardening activities, which also serves as breeding sites, can increase drastically proliferation of *Anopheles* species, especially *A. gambiae*<sup>18</sup>. These factors should be considered when implementing larval control strategies which should be targeted based on habitat productivity and water management<sup>19</sup>. There is evidence for direct relationship between irrigation development and increased malaria transmission<sup>20,21</sup>. Rice fields have proved to be particularly well suited as larval sites for *A. gambiae* s.l.. Jarju et al.<sup>22</sup> showed that the treating rice fields close to the landward edge of the floodplains with larvicides would help to reduce transmission levels because demonstrates the human-made changes are exploited by anopheline mosquitoes resulting in increased malaria transmission.

### Insecticide resistance

DDT (dichlorodiphenyltrichloroethane) and pyrethroids share the same mode of action on the insect nervous system, targeting the neuronal voltage-gated sodium ion channels. Molecular characterizations have revealed that various mutations in the S1-S6 transmembrane segments of domain II of the sodium ion channel gene give rise to resistance to these insecticides<sup>23,24</sup>. Santolamazza et al.<sup>25</sup> reported the distribution of knock-down resistance mutations in *A. gambiae* molecular forms, and suggest that there is an impact of human activities on *kdr* distribution in some samples. The different distribution of the two *kdr* mutations between sympatric M and S populations could also reflect different ecological/behavioural traits between M and S-forms that might promote different exposure to insecticide selective pressures. For example, the M-form may be more adapted to urbanized, man-influenced ecological settings, whereas the S-form tends to prevail in rural settings where a use of insecticides for agricultural purposes is expected to be greater<sup>26,27</sup>.

Vector resistance to pyrethroids has been reported in African continent<sup>28-33</sup> and China<sup>34</sup>. Many studies conducted in Africa have reported the presence of the *kdr* gene, associated with the "knock-down resistance" mechanism, in *A. gambiae* complex species<sup>35-39</sup>. The development and spread of malaria vector resistance to insecticides has been attributed to the intensive use of insecticides in agriculture<sup>40,41</sup>. To date, the resistance of

*Anopheles* species to the four families of insecticides available for public health use (organochlorines, organophosphates, carbamates, and pyrethroids) is a genuine concern throughout Africa<sup>18,42-44</sup>. In *Anopheles gambiae* sensu stricto, there are two point mutations at the voltage-gated sodium channel gene confer knockdown resistance (*kdr*) to DDT and pyrethroids insecticides.

Martinez-Torres et al.<sup>45</sup> identified a Leucine-Phenylalanine substitution at position *L1014F* of the gene encoding the S6 transmembrane segment of domain II of the sodium channel, in laboratory strains derived from field resistant samples of Burkina Faso and Ivory Coast. A second mutation, a Leucine-Serine substitution at the same codon (*L1014S*), has been identified in a colony derived from specimens from Kenya<sup>30</sup>. Kabula et al.<sup>46</sup> observed the co-occurrence of *L1014S* and *L1014F* mutations associated to pyrethroids resistance and maybe this phenomenon is spreading in vector malaria populations. Field surveys revealed a widespread distribution of the *1014F* allele in West Africa<sup>28,47</sup>. In addition, significant differences were found in the frequency of this allele between two molecular forms, denoted M and S, that are considered units of incipient speciation within *A. gambiae*<sup>26</sup>. These forms are characterized by sequence differences in transcribed and non-transcribed spacers of the ribosomal DNA. The S-form is the most widespread throughout Sub-Saharan Africa while the M-form is mostly confined to the West part of Africa, from Senegal to Angola. In general, the *1014F* allele is common in the S-form but rare in the M-form, even when populations of both forms occur in sympatry<sup>26,48</sup>. In the few M-form populations where it has been found, sequencing analysis of the upstream intron-1 of the *kdr* locus showed that the *1014F* allele apparently occurred through introgression with the S-form<sup>49</sup>. Recent surveys have reported the co-occurrence of both *1014F* and *1014S* alleles in localities of Gabon, Cameroon and Uganda<sup>37,50</sup>.

The distribution and frequency of these mutations poses serious questions about the sustainability of insecticide-based vector control programs. This is particularly evident when one considers that pyrethroids are the only insecticides recommended by the World Health Organization for insecticide-treated materials and that DDT is being re-introduced for malaria control in several countries<sup>51</sup>. The pyrethroids produced a more immediate irritant effect than DDT<sup>52</sup>. Knowledge of the way *kdr* resistance is evolving in *A. gambiae* is therefore of great epidemiological impor-

tance. Lol et al.<sup>53</sup> showed that molecular evidence suggests the presence of *kdr*-type resistance in field-collected *Anopheles albimanus* associated to mutations on the voltage-gated sodium channel (VGSC) gene. VGSC can be in many cases the responsible to resistance of pyrethroids.

Xu et al.<sup>54</sup> discuss the diagnostic of insecticide resistance and showed how the sample collection and preparation methods affect insecticide susceptibility bioassay. Field-collected female adults consistently exhibited the highest monooxygenase and Glutathione-S-transferases activities, responsible by mechanism is metabolic detoxification of pyrethroids. Awolola et al.<sup>55</sup> tested PermaNet® 3.0, known as insecticide synergist-combination long-lasting insecticidal net used to metabolic resistance when combined with *kdr*. The PermaNet® 3.0 significantly reduced *Anopheles gambiae* densities per house and confirmed increased efficacy of PN 3.0 compared to the pyrethroid-only LLIN.

Pinto et al.<sup>56</sup> genotyped the *kdr* locus and sequenced the upstream intron-1 in samples of *A. gambiae* S-form, so their studies proved that selection through insecticide pressure will favor *kdr* alleles and remove wild-type alleles. Therefore, the frequency of haplotypes carrying the wild-type allele will be lower than expected under neutrality, and so frequency need not reflect age.

Through these studies the *kdr* mutations are not homogeneously distributed in the two molecular forms of *A. gambiae* (termed M and S), which are considered as incipient species. In fact in early studies, the absence of the *L1014F* allele in the M-form was considered one of the major pieces of evidence for a severe restriction of gene flow between the two forms.

Ilboudo-Sanogo et al.<sup>57</sup> reported malaria vector resistance to pyrethroids and DDT in five localities exposed to high insecticide pressure in Burkina Faso and assessed by PCR the frequency of the *kdr* gene allele, associated with the “knock-down resistance” mechanism in *A. gambiae* and *A. funestus*. Thus, *A. gambiae* indicates a decrease in vector susceptibility to DDT in four localities included rural, suburban, and urban areas. The problem of physiological resistance against insecticides is more acute for LLINs than for IRS, as LLINs rely solely on pyrethroids, whereas IRS can be done with several classes of insecticides. There is evidence of increasing levels of pyrethroid resistance<sup>58</sup> and corresponding decreases in the effectiveness of malaria control programmes that rely on pyrethroid-based interventions<sup>59</sup>.

Since, Briët et al.<sup>60</sup> assessed the use of pyre-

throids, Deltamethrin and Permethrin in LLINs, despite resistance being an important factor in reducing their effectiveness and showed that they are likely to be cost effective against malaria even in areas with strong pyrethroid resistance<sup>31</sup>. The insecticide susceptible mosquitoes can become extremely adapted your feeding behavior after LLINs implementation and occur the increase in frequency of *kdr L1014F* genotype. Despite this, through the observations, *Plasmodium falciparum* prevalence and gametocyte rate in villagers decreased dramatically after LLINs deployment<sup>61</sup>. These authors support the view that it is important to pursue the use of LLINs in compliance with the WHO recommendations<sup>62</sup>.

In *A. darlingi*, Long-term DDT use resulted in a changed susceptibility of *A. darlingi* populations over time<sup>63</sup>. Indoor Residual Spraying (IRS) and Insecticide Treated Nets (ITNs) reduced intra-domiciliary vector densities<sup>64</sup>. ITNs for instance proved very successful against *A. darlingi* in southern Venezuela, where a reduction of 56% of malaria cases was recorded in local indigenous populations after the introduction of lambda-dacyhalotrin- treated hammock nets<sup>65</sup>. There is strong evidence that *A. darlingi* may be insecticide resistant to deltamethrin, due to the low mortality in bioassays<sup>66</sup>. *Anopheles arabiensis* is a member of the *Anopheles gambiae* Giles complex and the third most important malaria vector mosquito in Africa<sup>67</sup>. In recent reports, it was shown that the *kdr* Leu → Phe mutation was present in a single specimen of *A. arabiensis* from Burkina Faso<sup>68</sup> and two specimens from Tanzania<sup>58</sup>. The Leu Phe mutation has been found in two specimens from Uganda<sup>50</sup> and one specimen from Kenya<sup>69</sup>. All the above specimens were heterozygous for the *kdr* alleles and none of the reports correlate the *kdr* alleles with resistance phenotypes. A study with colonies of *A. arabiensis* from Sudan was done by Matambo et al.<sup>70</sup> with the aim of investigation the importance of the *kdr* mutation and potential enzyme-related resistance mechanisms in a DDT-resistant and other insecticides classes throughout of populations' generations. Exposures of F-16 generation adults to other insecticides showed a high level of resistance to all four classes of insecticides used for malaria vector control, whereas the base colony exhibited a high level of resistance to dieldrin only. All specimens from F-16 exposure DDT- strain, regardless of insecticide susceptibility phenotype, were homozygous (RR) and showed no correlation between the resistant phenotype as ascertained by bioassay and the presence of the *kdr* mutation.

In Sudan, Abdalla et al.<sup>71</sup> also verify that in *A. arabiensis* the *L1014F* allele was significantly associated with resistance to pyrethroids and DDT.

Due to the rapidity with which high levels of resistance to DDT it is suggested that this insecticide should be avoided for vector control interventions and underlines the need for rotational use of the other classes of insecticides to manage the situation. Pyrethroids are the only group of insecticides approved by WHO Pesticide Evaluation Scheme (WHOPES) for LLINs<sup>13</sup>.

In otherwise, Okumu et al.<sup>72</sup> no detected *kdr* gene mutation in wild *A. arabiensis* populations tested LLINs and IRS compounds, however the observed tolerance to pyrethroids necessitates prudence against possibility of physiological resistance arising and spreading rapidly across the area and reported the lose insecticidal efficacy with time.

Similar studies were conducted by Protopopoff et al.<sup>73</sup> showing opposing results reporting high levels of resistance in *A. gambiae* populations to pyrethroids and DDT. *Anopheles gambiae* s.s developed phenotypic resistance to these insecticides and *kdr* frequency has in the *A. gambiae* s.s population but was absent in *A. Arabiensiensis*. Due to selection of pyrethroids resistance is probably that *A. gambiae* s.s persists at high frequency in north-western Tanzania.

DDT and pyrethroids resistance are at least partially attributed to the reductions in insecticide efficacy caused by point mutations in their common target site, the voltage-gated sodium channel (VGSC), and perhaps also some sharing of metabolic resistance mechanisms<sup>45</sup>. Organophosphates and carbamates inhibit Acetylcholinesterase (AChE), an important enzyme in the central nervous system. Inhibition of AChE leads to accumulation of acetylcholine in nerve junctions (or synapses), which prevents the interruption of electrical impulse propagation<sup>74</sup>. Essandoh et al.<sup>75</sup> revealed that absence of *L119S* allele strongly predicted susceptibility on carbamate and organophosphate resistance in *Anopheles gambiae* s.s. and *Anopheles coluzzii*. However, Matambo et al.<sup>70</sup> reported the inhibitory effect of the carbamate insecticide propoxur on acetylcholinesterase activity and did not show correlation with the resistant phenotype. Hemingway et al.<sup>76</sup> recorded that larvae and adults of some samples revealed broad-spectrum organophosphate and carbamate resistance in *Anopheles nigerrimus* but not in *Anopheles culicifacies*. The authors suggested that the lack of resistance in the sample *A. culicifacies* probably reflects the lower levels of

selection pressure of this species because of its restricted breeding sites in agricultural water.

A resistance case of multiple insecticide was reported for Qin et al.<sup>77</sup> where *Anopheles sinensis* populations showed high monooxygenase and carboxylesterase activities and resistant to DDT and deltamethrin. *Anopheles vagus* was susceptible to deltamethrin but resistant to DDT and malathion with high monooxygenase, glutathione S-transferase and carboxylesterase activities. Low *kdr* mutation (*L1014F* allele) and high *Ace-1* frequency was detected in *A. sinensis*, but no *kdr* and *Ace-1* mutation was detected in *A. vagus* populations. Aizoun et al.<sup>42</sup> used carbamates and organophosphate with the attempt to develop strategies that will be alternatives against pyrethroids-resistant malaria vectors in the field. These mutations were observed in *A. gambiae* populations that can be confers resistance to organophosphates and carbamates compounds. Chang et al.<sup>78</sup> verified the effect of rotational use of insecticides with different modes of action in *A. sinensis* populations, the results showed multiple resistances to chemical insecticides, mainly long-term rotational use of various insecticides has to evolved a high insecticide resistance, being metabolic detoxification was the dominant mechanism of resistance. Nkya et al.<sup>79</sup> detected elevate frequency in *L1014Skdr* mutation in *A. gambiae* s.s. in the urban area and in the same area showed a moderate DDT resistance in larvae and adults in populations. The link between agriculture and pyrethroids resistance was confirmed by a significant correlation between deltamethrin resistance levels and agriculture intensity across all populations. The authors discussed that both agriculture and urban areas are likely favoring the emergence of resistance to insecticides. In agriculture areas, the mainly factor is the massive usage of pesticides and in urban areas the uncontrolled indoor spraying with insecticides may strongly select for *kdr* mutations and metabolic resistance mechanisms with a potential role of pollutants in favoring the selection of particular detoxification enzymes.

In Côte d'Ivoire a multiple-resistance of pyrethroids were reported, organochlorides and carbamate in *A. Gambiae* and the resistance mechanisms seem to be varied<sup>41,80,81</sup>. *Ace-1* is strongly associated with organophosphate and carbamate<sup>81</sup>, but Koffi et al.<sup>80</sup> did not detected the *L1014S kdr* and *Ace-1R* mutations, Knox et al.<sup>82</sup> established an online tool for mapping insecticide resistance, susceptibility in major *Anopheles* vectors of human malaria parasites and what

resistance mechanisms data were detected. The online platform can be accessed by IR Mapper ([www.irmapper.com](http://www.irmapper.com)).

### Comments and Perspectives

In American continent, the insecticide DDT was used widely, although developed countries have made use of this insecticide for much longer time. With respect to South American countries, the “technology gap” benefited somehow because it began using DDT later, thus avoiding higher concentrations in rivers and reservoirs. The use of DDT in Brazil and Latin America was mainly performed with the aim to control of malaria vectors, being *A. darlingi* as the main target, once this species is generally considered susceptible to DDT. Though, signs of resistance were detected in Colombia and Venezuela, as well as in some areas of the Brazilian Amazon, its real role but have not been sufficiently studied. It is clear that studies of resistance of *A. darlingi* in South America have been neglected and a lot remains to be done. Such that remains a possibility of have resistant strains to the current insecticides (ex: pyrethroids). There are a wide variation in the *A. darlingi* behavior<sup>83,84</sup>. For example in the Brazilian Amazon, reports described that some *A. darlingi* populations can modify their endophilic behavior for exophilic and anthropophilic<sup>85,86</sup> to zoophilic, possibly caused by excito-repellency of these insecticides used in household spraying<sup>87</sup>.

The program managers have few options available when confronted with multiple-insecticide resistance and its level, reported in this review, combined with continual selection pressure will inevitably lead to suboptimal mosquito control. Other strategies of monitoring and samplings were utilized: odour baits contain carbon dioxide<sup>88</sup>.

We could conclude that the resistance has

spread mainly all over Africa dramatically. Use of insecticides in agriculture has been linked to resistance in malaria vectors. Although surveys of insecticide resistance are limited to a few species, the results can be alarming and can be understood that the mechanisms of resistance occurs in unstudied species of *Anopheles*. The rotational strategies applying a diversity insecticide classes, exploring their different modes of action is most usual, in order to decrease the probability of expressing resistance<sup>89</sup>. The rotational strategies showed successful in many applications in field showing to be effective if the resistance gene has an associated fitness cost<sup>90</sup>.

Other very important issue is how to apply these insecticides, these practices which often were made without adequate monitoring, leading to wrong handling of what and how much should be applied.

Currently it is necessary to look for others alternatives, e.g. biological control, by using other organisms as a possible regulator of the population of malaria vectors in critical outbreaks. Possibly the “biological products” appear to be undergoing laboratory tests or even define the protozoan *Plasmodium* as a target to these new technologies.

Products derived from plants and fungi are in constant study currently, as they offer several antagonistic activities on *Anopheles* mosquitoes<sup>91-93</sup>, through of crude extracts from the fungus and plants.

These kind of studies open the possibility for further investigations of the efficacy of larvicidal properties of natural product extracts and should be encouraged to find out the new products with mosquitocidal and larvicidal activities, supporting the idea that they are less harmful to the environment.

## Collaborations

GL da Silva, TN Pereira, NJ Ferla and OS da Silva participated in the elaboration of the manuscript at every stage. GL da Silva and TN Pereira worked in the writing and revision of this manuscript. OS da Silva and NJ Ferla contributed to the literature review.

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Artigo apresentado em 03/12/2014

Aprovado em 09/06/2015

Versão final apresentada em 11/06/2015