UON SORT IT- March 2024 Supplement
INSECTICIDE RESISTANCE STATUS IN *ANOPHELES GAMBIAE* SENSU LATO (S.L.) IN FOUR MALARIAL EPIDEMIOLOGICAL ZONES IN KENYA, 2023
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# INSECTICIDE RESISTANCE STATUS IN *ANOPHELES GAMBIAE* SENSU LATO (S.L.) IN FOUR MALARIAL EPIDEMIOLOGICAL ZONES IN KENYA, 2023

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## ABSTRACT

***Objective*: This research aimed at determining the status of insecticide resistance in the *Anopheles gambiae* s.l malaria vectors to different classes of insecticides in the four malarial epidemiological zones in Kenya.**
***Design*: A retrospective descriptive cross-sectional study of routinely collected insecticide resistance monitoring programme data.**
***Setting*: The study considered five counties within four malarial epidemiological zones: Kwale (Coast endemic), Kirinyaga (Low transmission zone), Nandi (Highland endemic prone zone), Baringo (Seasonal transmission zone), Marsabit(seasonal transmission zone).**
***Methods*: Three to five days old adult mosquitoes that emerged in the insectary from larvae collected from breeding sites in Kirinyaga, Baringo, Kwale, Marsabit and Nandi counties were tested for susceptibility to pyrethroids, organophosphates, organochlorines and piperonyl butoxide (PBO)-pyrethriod synergist as per WHO insecticide resistance bioassay procedure. Identification of all bioassayed mosquitoes was done using appropriate morphological keys.**
**Results: Confirmed resistance in *Anopheles gambiae sl.* to pyrethroid deltamethrin was observed in Kirinyaga, Kwale and Marsabit counties. Further, resistance to the pyrethroids permethrin was observed in Kwale and to lambdacyhalothrin in Kirinyaga. The findings on susceptibility to the test insecticide were as follows;**

**permethrin in Kirinayaga, deltamethrin in Nandi Hills and lambdacyhalothrin in Kwale. Additionally, susceptibility of *Anopheles gambiae sl.* to organochlorines, organophosphates, and PBO pyrethroid synergist was observed in Kirinyaga, Baringo and Kwale respectively.**

***Conclusion*: The data from the present study showed confirmed insecticide resistance majorly to the pyrethroids across the five epidemiological zones but there was susceptibility to organochlorines, organophosphates, and synegistpyrethroid classes across all the zones. Continued insecticide resistance monitoring and management in areas with observed resistance is key in ensuring the effectiveness of insecticide-based vector control interventions in place.**

**Keywords: Insecticides resistance, *Anopheles gambiae s.l, An. Arabiensis, An. gambiae s.s.*, pyrethroids, deltamethrin, organophosphates, organochlorine, Piperonyl-Butoxide synergist), Chlorfenapyr, Clothianidin**

## INTRODUCTION

Malaria caused by Plasmodium spp parasites transmitted by the female Anopheles vector has remained a serious public concern. Globally in 2021, 241 million cases with 619,000 deaths were reported while in 2020, cases increased to 241 million from 227 million cases reported in 2019(1). Globally malaria deaths in 2020 were 627,000 with 77% of those deaths occurring in children below 5 years of age (2). In Kenya, Malaria is a serious threat to public health, with three-quarters of the country's population estimated to be at risk(3) The prevalence of malaria in Kenya is at 6%, with the lake endemic region at 19%, the Coastal endemic zone at 5%, and thelow-risk region remaining below 1% (4).

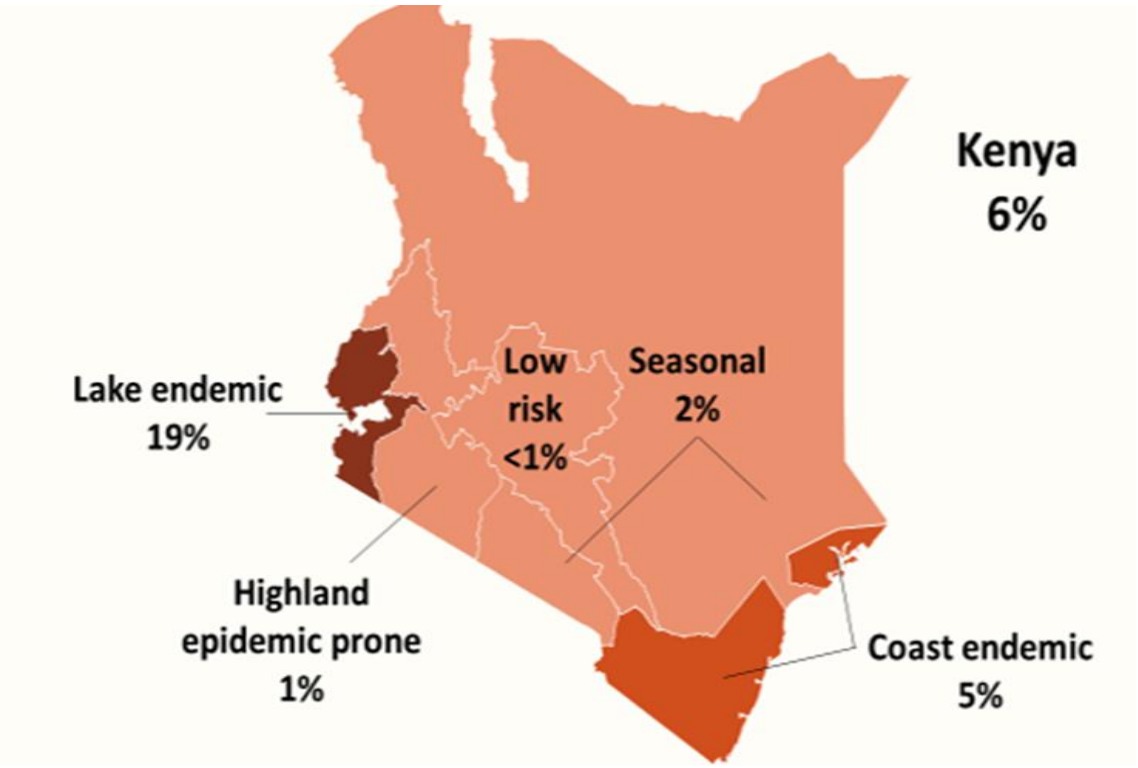



*Figure 1: Malaria epidemiological zones*

Vector-control interventions that include universal coverage with Long Lasting Insecticide Nets (LLINs) and Inscticide Residual Spraying (IRS) coupled with case management and Intermittent preventive treatment for pregnant women in endemic areas have brought about fruitful gains in Malaria prevention and treatment. This has worked towards achieving the vision of a malaria-free Kenya which is the aim of the malaria strategy (3). Kenyan malaria strategy aims at having 100% of people dwelling in malaria-endemic zones using the necessary interventions to prevent malaria by 2023.

Mosquito Vector control interventions that commonly utilize insecticides have brought about fruitful gains in the control of malaria. The emerging insecticide resistance in Anopheles mosquitoes poses the danger of losing these gains. Insecticide resistance to the commonly applied insecticide classes; organochlorides, pyrethroids, organophosphates, and carbamates, is currently on the rise across all significant malaria vectors according to WHO Global Report on Insecticide Resistance in Malaria Vectors 2010–2016. Since 2010, 68 countries have raised alarm on emerging insecticide resistance to at least one class of commonly used insecticides. In Kenya, resistance to pyrethroids, carbamates, organophosphates, and organochlorines is widespread (3). Resistance to pyrethroids has been detected in two species of the Anopheles gambiae complex (An. gambiae (s.s.) and An. Arabiensis) and in An. funestus (s.s.) while resistance to carbamates was limited to An. gambiae (s.s.) and An. arabiensis. Resistance to the organochlorine was reported in An. gambiae (s.s.) and An. funestus (s.s.) while resistance to organophosphates was reported in An. gambiae (s.l.) (3).

The primary resistance mechanisms can be grouped into metabolic resistance and target site resistance. Metabolic resistance arises when changes in a mosquito's enzyme cause

rapid detoxification of an insecticide than normal. The enzymes include; esterases, monooxygenases, and glutathione S-transferases. Target-site resistance occurs when there is a mutation in the sodium channel receptor that an insecticide is intended to bind. This makes the insecticide no longer able to bind on the intended target site of the receptor making the insect either unaffected or less affected by the insecticide. The type of resistance conferred is referred to as "knockdown resistance" (mediated by the kdr genes).

In Kenya, mechanisms of insecticide resistance among malaria vectors reported include the kdr mutations (L 1014S and L 1014F) and elevated enzyme activity in carboxylesterase, glutathione S-transferases (GST), and monooxygenases. Kdr mutations L 1014S and L 1014F were detected in An. gambiae (s.s.) and An. arabiensis populations. Elevated activity of monooxygenases has been detected in both An. arabiensis and An. gambiae (s.s.) populations while the elevated activity of carboxylesterase and GST has been detected only in An. arabiensis populations.(3,4)

WHO has given recommendations for mitigations in the control of insecticide resistance (5). This includes;

New generations of LLINs, which can be a combination of Piperonyl butoxide (PBO) and a pyrethroid or a pyrethroid with a neonicotinoid as an insecticide resistance strategy.

Mosaic spraying in which one compound is used in one geographic area and a different compound belonging to a different class in neighboring areas.

Mixtures in which two or more compounds Rotations of insecticides using two or preferably more insecticides that have different modes of action;

Combination of interventions in which two or more insecticides of different classes are used in a house. Currently, there of different insecticide classes are mixed into one compound.

To maintain the gains from the main adult vector control interventions using LLINs and IRS, it is important to know the distribution of the dominant vector species in the different geographical regions, alongside a comprehensive understanding of the status of resistance to insecticides by the vector species in those regions. These will guide the application of vector control interventions and prevent further development of resistance. Indoor residual spraying and insecticide-treated nets have so far worked well in the control of malaria but these interventions will not be efficient in the control of malaria if insecticide resistance in the malaria vector species is not monitored and addressed. This research aimed to determine the status of malaria vector species resistance to selected classes of insecticides in the four malarial epidemiological zones in Kenya.

*Specific Objectives*

To determine the status of insecticide resistance in *Anopheles gambiae s.l.* to different classes of insecticides in four Malarial Epidemiological zones in Kenya.

## MATERIALS AND METHODS

*Study design*

A retrospective descriptive cross-sectional study of routinely collected data. Data used was generated from the exposure of 3 to 5-day-old adult mosquitoes reared from larvae collected from various study sites to different types of insecticide to determine resistance.

*Study setting*

Kenya has four main malaria epidemiological zones with diversity in risk determined largely

by altitude, rainfall patterns, and temperature, as well as the prevalence of malaria. Insecticide resistance in malaria vectors in these epidemiological zones threatens the effectiveness of the main vector control tools in use; standard long-lasting insecticidal nets and Indoor residual spraying. Insecticide resistance monitoring is key in response to the increasing resistance in malaria vectors to various classes of insecticides. In the face of increasing insecticide resistance, Insecticide resistance monitoring and management plans have become a requirement to guide the selection and implementation of vector control interventions. In Kenya, there is an existing gap in the knowledge on the spread of insecticide resistance in the predominant malaria vectors to different classes of insecticides across epidemiological zones. Additionally, there is a gap in systematic surveillance monitoring of insecticide resistance where vector control interventions are being rolled out. This study considered five counties within four malarial epidemiological zones; Kwale in Coast endemic, Kirinyaga in the low transmission, Nandi in highland endemic prone, Baringo in seasonal transmission and Marsabit in seasonal transmission.

In Kenya, the main vector species of malaria include members of the *Anopheles gambiae* complex (*An. gambiae s.s*, *An. arabiensis*, and *An. merus*) and *Anopheles funestus* complex (6).

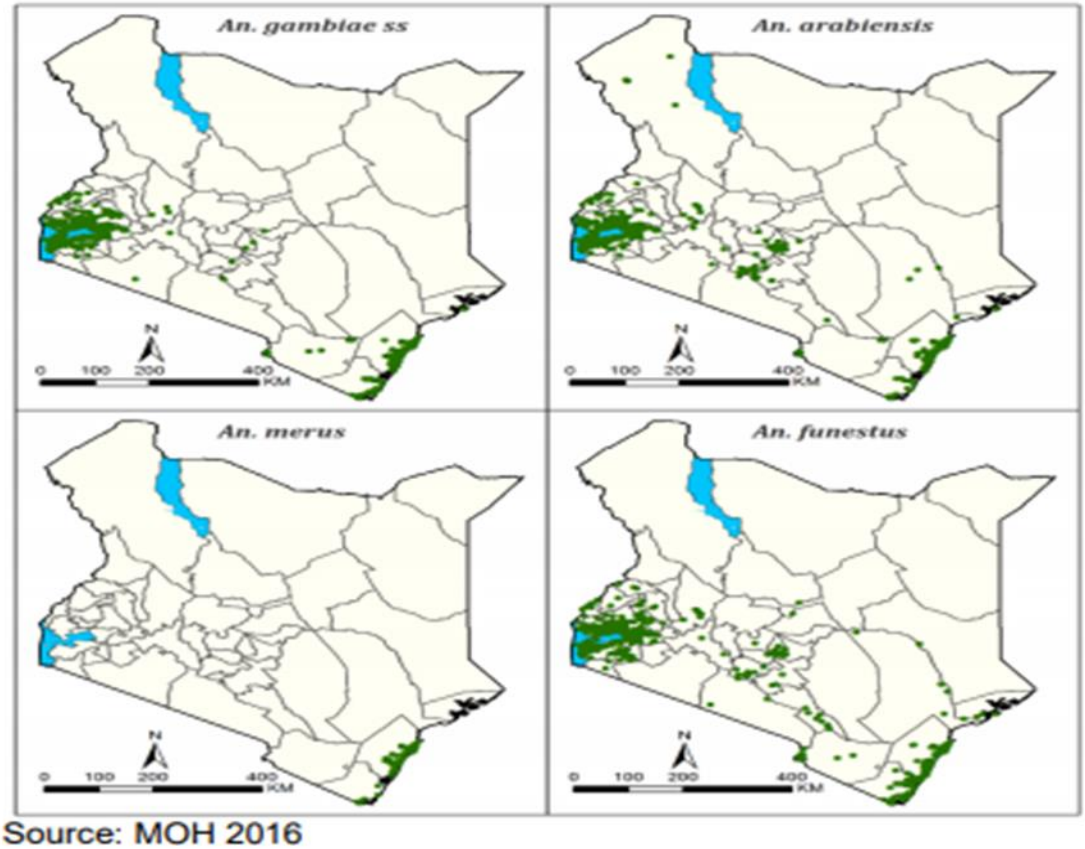



*Figure 2: Major Malaria Vectors in Kenya*

Vector distribution varies from region to region influenced by temperature, humidity, and rainfall. Because of the medical importance of mosquitoes, regular understanding and recording of the diversity of mosquitoes in different epidemiological zones is of importance. This forms the baseline to study vector species bionomics as well as correlations with the abiotic factors of the environment and to make a strategy for the control of mosquito-borne diseases. Knowledge of vector bionomics which includes their ecology and behavior alongside their susceptibility status to the commonly used insecticide is extremely important to guide the implementation of vector control tools and their effective distribution. Thorough familiarity with vector bionomics also plays a significant role in the rolling out of integrated vector management strategies. This is because the behavioral tendencies of the various malaria vector species inform the choice of tools of vector control to be employed.

*Study population*

The project uses the recommended F0 mosquitoes that emerged in the insectary from field-collected larvae to carry out insecticide bioassays.

*Study Variables*

The objective of this project was to determine the insecticide resistance status in Anopheles mosquitoes to different classes of insecticides from routinely collected insecticide resistance monitoring data by DNMP. Resistance was determined by percentage mortality after exposure to the different insecticides and interpreted using WHO 2016 guidelines.

*Data collection procedures*

This study will review and analyze insecticide resistance data obtained from the Division of National Malaria Program (DNMP) Entomology.

Sample collection and Bioassay procedures followed

Larvae collection was carried out in various breeding sites in Kirinyaga, Baringo, Kwale, Marsabit and Nandi. Rearing of the mosquito larvae was done between 28°C -31°C with a humidity of 80%-85% in the insectary using tetramin® fish food as the larval diet. Pupae that emerged from the larvae were maintained in pupae cups within adult cages in the insectary and reared to adults. The adult mosquitoes were maintained in adult chambers in the insectary at temperatures and humidity of between 25°C - 27°C and 84% - 87% using 10% sugar solution as diet. 3 to 5 days old adults (F0 mosquitoes) were tested for susceptibility to pyrethroids (permethrin 0.05%, deltamethrin 0.05%, and lambdacyhalothrine 0.05%), Organophosphates (Malathion 5%, Fenitrothion 1%), Organochlorine (Dieldrin 0.4% and 4%) and Synergist + Pyrethroid PBO + Permethrin 0.75%) as per WHO insecticide resistance bioassay procedure- Annex 1

*Morphological Identification*

All bioassayed mosquitoes were morphologically identified as *An. gambiae* s.l. (9)

*Analysis and statistics*

Data was entered into Microsoft Excel sheets for analysis to determine the degree of resistance. In cases where there was mortality of above 5% in the control test, correction on mortality was done. Phenotypic resistance frequency was interpreted as per the WHO guideline of 2016: Mortality between 98–100% was considered susceptible while mosquitoes with mortality <98% but ≥ 90% were considered possibly resistant, and those with mortality <90% were considered confirmed resistant.

*Ethics consideration*

Scientific and ethical clearance was obtained from the Maseno University Scientific and Ethics Review Committee (approval number MUSERC/01234/23). Permission to use the assessment data was sought from NMCP. Personal identification information was omitted from the data to ensure patient confidentiality.

## RESULTS

A total of 1,244 *An. gambiae* s.l. mosquitoes were included in bioassay testing for different classes of insecticides (Pyrethroids and (Synergist + Pyrethroid), Organophosphates, and Organochlorines) to determine insecticide resistance status (Table 1)

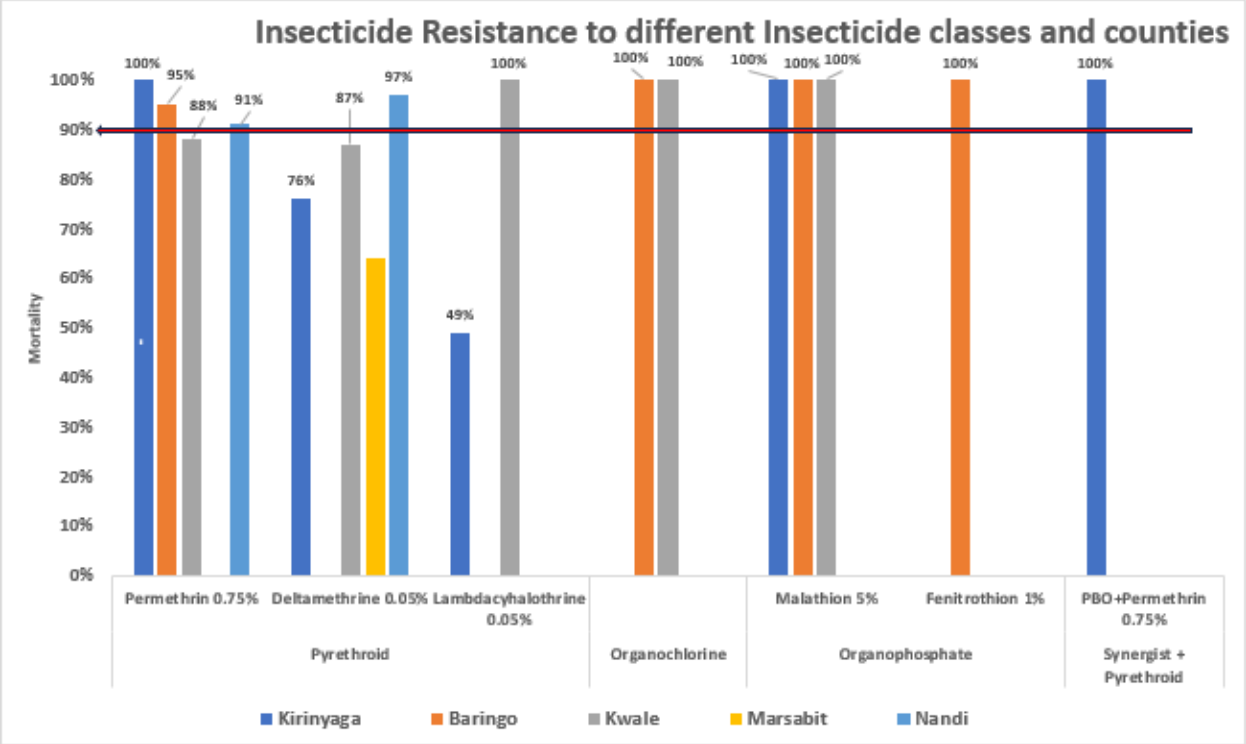
**Table 1**

*Insecticide Resistance status in Anopheles gambiae sl per county to different classes of Insecticides*

| County | Insecticide | Insecticide class | Number Exposed | %KD | %Mortality at 24 hrs | WHO Insecticide resistance interpretation |
|---|---|---|---|---|---|---|
| Kirinyaga | Permethrin 0.75% | Pyrethroid | 30 | 90 | 100 | Susceptible |
| Kirinyaga | Deltamethrine 0.05% | Pyrethroid | 50 | 100 | 76 | Resistant |
| Kirinyaga | Lambdacyhalothrine 0.05% | Pyrethroid | 75 | 80 | 49 | Resistant |
| Kirinyaga | PBO+Permethrin 0.75% | Synergist + Pyrethroid | 20 | 100 | 100 | Susceptible |
| Kirinyaga | Malathion 5% | Organophosphate | 80 | 100 | 100 | Susceptible |
| Baringo | Permethrin 0.75% | Pyrethroid | 21 | 33% | 95 | Possible resistance |
| Baringo | Dieldrin 4% | Organochlorine | 19 | 58% | 100 | Susceptible |
| Baringo | Malathion 5% | Organophosphate | 10 | 70% | 100 | Susceptible |
| Kwale | Permethrin 0.75% | Pyrethroid | 100 | 96% | 88 | Resistant |
| Kwale | Fenitrothion 1% | Organophosphate | 100 | 29% | 100 | Susceptible |
| Kwale | Deltamethrin 0.05% | Pyrethroid | 100 | 100% | 87 | Resistant |
| Kwale | Lambdacyhalothrine 0.05% | Pyrethroid | 100 | 100% | 100 | Susceptible |
| Kwale | Dieldrin 0.4% | Organochlorine | 200 | 85% | 100 | Susceptible |
| Kwale | Malathion 5% | Organophosphate | 100 | 100% | 100 | Susceptible |

| | | | | | | |
|---|---|---|---|---|---|---|
| Marsabit | Deltamethrin 0.05% | Pyrethroid | 59 | 92% | 64 | Resistant |
| Nandi | Deltamethrin 0.05% | Pyrethroid | 100 | 98% | 97 | Possible resistance |
| Nandi | Permethrin 0.75% | Pyrethroid | 80 | 95% | 91 | Possible resistance |

Resistance to pyrethroids, deltamethrin 0.05% was observed in Kirinyaga, Kwale and Marsabit counties while resistance to Lambdacyhalothrine 0.05% was only observed in Kirinyaga. Resistance to Permethrin 0.75% a pyrethroid was observed in Kwale (Figure 3). Possible resistance to Permethrin 0.75% and Deltamethrin 0.05% was observed in Nandi and to Permethrin 0.75% in Baringo. Susceptibility was observed: in Kirinyaga to dieldrin an organochlorine, in Baringo to malathion and fenitrothion of which both are organophosphate and in Kwale to PBO + permethrin, a synergist + Permethrin (Figure 3)




*Mortality: ≥ 98% Susceptible, 90-97% Possible resistance, < 90% Confirmed resistance*

*Figure 3: Insecticide resistance status to different insecticides and counties in the year 2023, Kenya*

## DISCUSSION

This study generated data on the status of insecticide resistance to different classes of insecticides in *An. gambiae s.l* sampled from various sites across the five malarial epidemiological zones. Confirmed resistance in *Anopheles gambiae sl.* to the pyrethroid deltamethrin was observed in Kirinyaga, Kwale and Marsabit counties which are within the low transmission, coast endemic and seasonal transmission epidemiological zones respectively. Further resistance was observed to pyrethroids: permethrin in Kwale, and

lambdacyhalothrine in Kirinyaga but there was observed susceptibility to permethrin in Kirinayaga, to deltamethrin in Nandi in the highland epidemic-prone zone, and to lambdacyhalothrin in Kwale. Possible resistance to Permethrin and Deltamethrin was observed in Nandi and to Permethrin in Baringo.

Susceptibility in *Anopheles gambiae sl.* was observed to insecticide classes organochlorine (dieldrin), organophosphate (malathion, fenitrothion), and synergist + pyrethroid (PBO + permethrin) in Kirinyaga, Baringo and Kwale.

The data showed mainly confirmed insecticide resistance and partly possible resistance majorly to the pyrethroid class of insecticides widespread across the five epidemiological zones but there is susceptibility to organochlorine, organophosphate, and synergist + pyrethroid classes across all the zones. In Kirinyaga where the predominant Anopheles *gambiae sl* is *Anopheles arabiensis*, resistance was observed to pyrethroid, deltamethrin and lambdacyhalothrin but susceptibility remained to permethrin. The resistance could be attributed to the use of pesticides in agriculture which could have contributed to the selection of the pyrethroid resistance in the malaria vectors. The use of insecticides to control agricultural pests has been cited as one of the contributing factors to the emergence of insecticide resistance in the malaria vector *An. gambiae s.l.* (8) (10). Furthermore, Insecticide resistance to deltamethrin and permethrin is likely associated with the widespread use of mosquito control interventions in the areas showing resistance to the pyrethroid class (11) Mosquitoes were susceptible to synergist + pyrethroid (PBO + permethrin) which is now being used as an alternative in addressing the problem of monooxygenase-mediated insecticide resistance to pyrethroids.

*Policy implications:*

Data generated is essential in guiding policies for insecticide-based malaria vector control interventions. WHO 2012 Global Plan for Insecticide Resistance Management (GPIRM) guidelines direct that where resistance is confirmed, remedial action is recommended for the management of insecticide resistance. This will ensure the effectiveness of insecticide-based malaria vector control tools and preserve the gains in malaria control.

*Limitations and strengths of the assessment:*

Data was collected during the dry season making it challenging to obtain larvae for rearing

Data collection was made possible through global fund allocation to enable insecticide resistance monitoring for malaria vector control tools.

## CONCLUSION

The data showed mainly confirmed insecticide resistance and partly possible resistance majorly to the pyrethroid class of insecticides widespread across the five epidemiological zones but there is susceptibility to organochlorine (dieldrin), organophosphates (malathion, fenitrothion), and synegist + pyrethroid classes across all the zones. Data generated on insecticide resistance across the five epidemiological zones shows a picture of where resistance is developing and may compromise vector control interventions. This alerts surveillance teams to where they closely need to monitor and carry out insecticide resistance management before the failure of vector control interventions. Continued insecticide resistance monitoring and management is key in ensuring the

effectiveness of the insecticide-based vector control interventions in place.

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**ANNEXES**

*Annex 1: WHO insecticide susceptibility test procedures (7).*

The investigator puts on gloves. Six sheets of clean white paper (12 × 15 cm), rolled into a cylinder shape, are inserted into six holding tubes (with the green dot), one per tube, and fastened into position against the wall of the tube with a steel spring wire clip. The slide unit is attached to the tubes at the other end.

Ideally, 120–150 active female mosquitoes are aspirated (in batches) from a mosquito cage into the six green-dotted holding tubes through the filling hole in the slide, to give six replicate samples of 20–25 mosquitoes per tube.

Once the mosquitoes have been transferred, the slide unit is closed and the holding tubes set in an upright position for 1 hour. At the end of this time, any moribund mosquitoes (i.e. those unable to fly) and dead mosquitoes are removed. a

The investigator inserts one oil-treated paper (the control) into each of two yellow-dotted tubes, ensuring that the label of the paper is visible on the outside of the tube. The paper is fastened with a copper clip and the tube closed with a screw cap.

Four exposure tubes with red dots are prepared in much the same way as the yellow-dotted tubes. Each of the four red-dotted exposure tubes is lined with a sheet of insecticide-impregnated paper such that print label is visible on the outside. Each paper is then fastened into its position against the wall with a copper spring-wire clip and the tube is closed with a screw cap.

The empty exposure tubes are attached to the vacant position on the slides and, with the slide unit open, the mosquitoes are blown gently into the exposure tubes. Once all the mosquitoes are in the exposure tubes, the slide unit is closed (usually a cotton wool plug is inserted into the hole to lock the slide) and the holding tubes are detached and set aside. The investigator now removes the gloves.

Mosquitoes are kept in the exposure tubes, which are set in a vertical position with the mesh-screen end uppermost, for a period of 1 hour (unless otherwise specified). The tubes are placed in an area of reduced lighting or covered with cardboard discs to reduce light intensity and to discourage test mosquitoes from resting on the meshscreen lid.

At the end of the 1-hour exposure period (or longer for certain compounds, as outlined in Table 3.1), the mosquitoes are transferred back to the holding tubes by reversing the procedure outlined in Step 6. The exposure tubes are detached from the slide units. A pad of cotton wool soaked in 10% sugar water is placed on the mesh-screen end of the holding tubes.

Mosquitoes are maintained in the holding tubes for 24 hours (or longer for slow-acting compounds). During this time, it is important to keep the holding tubes in a shady, sheltered place in the laboratory or in a chamber maintained at 27 °C ± 2 °C temperature and 75% ± 10% relative humidity. Temperature and humidity should be recorded during the recovery period.

At the end of the recovery period (i.e. 24 hours post-exposure or longer for slow-acting compounds), the number of dead mosquitoes is counted and recorded. An adult mosquito is considered to be alive if it is able to fly, regardless of the number of legs remaining. Any knocked down mosquitoes, whether or not they have lost legs or wings, are considered moribund and are counted as dead. A mosquito is classified as dead or knocked down if it is immobile or unable to stand or take off.