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Original Article

Importance of Insecticide Resistance Monitoring and its Implications for Malaria Vector Control in South-Western Republic of Benin, West Africa

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Abstract-*Malaria is a public health concern and insecticide resistance monitoring is necessary in this disease control. The current study was aimed to compare the susceptibility to cyfluthrin in female adult Anopheles gambiae sensu lato from Lokossa district with their F1 progeny susceptibility in malaria vector control in South-Western Republic of Benin, West Africa. Larvae and pupae of Anopheles gambiae s.l. populations were collected in their breeding sites in Mono department from March to July 2024 during the great rainy season and reared up for obtaining F1 progeny. Female adult An. gambiae s.l. mosquitoes were also collected from window traps put on windows of rooms in Lokossa district surveyed using small electronic rackets. Female adult An. gambiae species collected from window traps were morphologically identified using morphological keys and then transferred into mosquito cages for WHO bioassays performed with impregnated papers of cyfluthrin (0.15%). WHO bioassays were also performed with F1 progeny. The physiological age of female adult An. gambiae s.l. collected from window traps was determined through dissection using Detinova method. The results showed that female adult Anopheles gambiae s.l. from Lokossa district collected from window traps were more susceptible to cyfluthrin than their F1 progeny. Changes in mosquito physiology occur with senescence. The resistance is a hereditary and dynamic phenomenon. Some Anopheles gambiae blood fed mosquitoes in the mosquito fauna collected in window traps put on windows of locations surveyed in Lokossa district were found. The monitoring of insecticide resistance in malaria vectors needs some biotechnologies or tools.*

Keywords *- Benin, Malaria Vector Control, Public health, Electronic racket, Resistance Monitoring.*

1. Introduction

Globally in 2024, there were an estimated 282 million malaria cases in 80 malaria endemic countries, an increase of about 9 million cases (3%) compared with 2023 [1].

Between 2000 and 2015, although the trend in case numbers fluctuated, there was a slight decrease overall of about 3.8%, from 239 million to 230 million cases, across the 108 countries that were malaria endemic in 2000. Since 2015, malaria cases have increased by 22.6%. Of the regions that showed an increase , most of this increase was observed in the WHO African Region (88%) and the WHO Eastern Mediterranean Region (12%) [1].

Globally in 2024, there were an estimated 610 000 malaria deaths, an increase of 12 000 compared with 2023. Between 2015 and 2024, deaths increased by 5.5%, with more than one-third of the increase occurring between 2023 and 2024. The mortality rate remained unchanged in 2024 compared with 2023, at 13.8 per 100 000 population at risk [1].

In Benin, vector control is highly prioritised in the national strategic plan for malaria control. It has been marked by frequent large scale free distribution of Long Lasting Insecticidal Nets (LLINs). In addition, the indoor residual spraying is complementary control tool in some pilot sites. The efficacy of these control methods would largely depend on the susceptibility of the vectors to the insecticides being used.

The ongoing spread of insecticide-resistant genes, such as the well-characterized *kdr* mutations [2-3] in populations of the major African malaria vectors, *An. gambiae*, can seriously jeopardize the efficacy of vector control programmes. The monitoring of insecticide resistance in malaria vectors is of prime importance especially where control programmes are planned or already running, in order to assess potential selection effects of insecticidal compounds on vector populations, and to take appropriate measures such as switching to other classes of compounds. For this goal, the presence and frequency of the *kdr* mutations constitute a valuable and useful resistance marker for two main reasons.

Pyrethroids are the only group of insecticides currently recommended for net treatment. The determination of insecticide susceptibility status of the target vectors will help monitor the insecticidal efficacy and possible development of resistance at early stages. So, the early detection of insecticide resistance development is the most important aspect that guides vector control programmes. The emergence of resistance in populations of *An. gambiae* to common classes of insecticides used in public health has been reported in Benin [4,5,6].

Beninese National Malaria Control Programme has frequently implemented large-scale and free distribution of Long-Lasting Insecticidal Nets (LLINs) throughout the entire country to increase coverage of LLINs. In addition, these LLINs are often impregnated with pyrethroids. So, it is crucial that information on the current status of *An. gambiae s.l.* Resistance to pyrethroids is being investigated. This will properly inform control programs of the most suitable insecticides to use and facilitate the design of appropriate resistance management strategies.

The goal of the current study is to compare the susceptibility to cyfluthrin in female adult *Anopheles gambiae* sensu lato from Lokossa district with their F1 progeny susceptibility and its implications for malaria vector control in South-western Republic of Benin, West Africa.

2. Review of Literature

Malaria remains one of the leading public health problems worldwide, particularly in sub-Saharan Africa, where it accounts for high levels of morbidity and mortality. Vector resistance to pyrethroids has been reported in many African countries, including West Africa (Ivory Coast, Burkina Faso, Senegal) [7-11], central Africa (Cameroon) [12], East Africa (Kenya) [13] and Southern Africa (South Africa) [14]. In Benin, the first cases of resistance of *Anopheles gambiae* s.l. to pyrethroids, the main insecticides used for net impregnation, were reported in 1999 [15]. Since then, several studies have demonstrated the expansion of this resistance nationwide, involving molecular mechanisms such as *kdr* mutations (L1014F, N1575Y) and ace-1 (G119S), as well as the overexpression of detoxification enzymes [16,17].

3. Materials and Methods

3.1. Study Area

The study area is located in the Republic of Benin (West Africa) and includes the department of Mono. Mono department is located in the south-western Benin, and the study was carried out more precisely in Lokossa district (Figure.1). The southern borders of this district are Athiémé and Houéyogbé districts. The northern border is the Dogbo district. The eastern border is the Bopa district, and the western border is the Togo Republic. The Lokossa district covered 260 km^2. The choice of the study site took into account the economic activities of populations, their usual protection practices against mosquito bites, and peasant practices to control farming pests. These factors have an impact on resistance development in the local vector mosquitoes. We took them into account to compare the susceptibility to cyfluthrin in female adult *Anopheles gambiae* s.l. from Lokossa district with their F1 progeny susceptibility in South-Western Benin. Mono has a climate with four seasons, two rainy seasons (March to July and August to November) and two dry seasons (November to March and July to August). The temperature ranges from 25 to 30°C, with the annual mean rainfall between 900 and 1100 mm.

Fig. 1 Map of Republic of Benin showing Lokossa district surveyed

3.2. Mosquito Sampling

Anopheles gambiae sensu lato mosquitoes populations were collected from March to July 2024 during the great rainy season in the Lokossa district. Larvae and pupae were collected from their breeding sites using the dipping method and kept in labeled bottles. The samples were reared to adults in the insectary of the Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) of the Department of Sciences and Agricultural Techniques located in Dogbo district.

Anopheles gambiae s.l. mosquitoes were also collected with some small electronic rackets from March to July 2024 during the great rainy season in window traps put on windows of four rooms in each of locations surveyed in Lokossa district such as Agamè, Houin, Koudo, Ouèdèmè. Between 6.00 a.m. to 7.00 a.m., small electronic rackets were used to collect mosquitoes from these window traps. They were then put in some plastic cups covered with a small cutting of untreated net on which was put cotton wool moistened with a 10% honey solution.

3.3. Obtaining of F1 Progeny

After larvae and pupae were collected in locations of Lokossa district, they were reared up to adult emergence at insectary. Male and female adult mosquitoes aged 5-7 days old were used in the reproduction. After the female mosquitoes had been mated and given a rabbit's blood meal, an ovipositor was put in a mosquito cage containing these females.

After the eggs were laid by these females, they were placed in some containers that contained water. Larvae of the first stage were fed with yeast. They were then reared up to F1 progeny emergence for bioassay tests.

3.4. Mosquito species identification

After female Anophelines were collected from window traps, they were identified to species based on morphological characters using identification keys [18] and then transferred into mosquito cages for bioassays tests.

3.5. Testing Insecticide Susceptibility

The principle of the WHO bioassay is to expose insects to a given dose of insecticide for a given time to assess susceptibility or resistance. The standard WHO discriminating dosages are twice the experimentally derived 100% lethal concentration (LC100 value) of a reference susceptible strain [19].

In this study, the insecticide tested was cyfluthrin (0.15%). The choice of cyfluthrin was justified by the recent use of pyrethroids on LLINs which were used by NMCP for implementation of large-scale and free distribution through the entire country to increase coverage.

An aspirator was used to introduce 20 to 25 unfed female mosquitoes aged 2-5 days into five WHO holding tubes (four tests and one control) that contained untreated papers. They were then gently blown into the exposure tubes containing the insecticide-impregnated papers. After one hour of exposure, mosquitoes were transferred back into holding tubes and provided with cotton wool moistened with a 10% honey solution.

The number of mosquitoes "knocked down" at 60 minutes and mortalities at 24 hours were recorded following the WHO protocol [19].

Anopheles gambiae Kisumu, a reference susceptible strain, was used as a control for the bioassay tests. We used Kisumu more precisely to confirm the quality of WHO impregnated papers of cyfluthrin.

All susceptibility tests were done following WHO protocol in Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) of Department of Sciences and Agricultural Techniques located in Dogbo district at 25+/-2°C and 70 to 80% relative humidity.

3.6. Dissection of Anopheles Gambiae Mosquitoes

The physiological age of a female adult. *gambiae* from window traps was determined through dissection using the Detinova method [20].

3.7. Statistical Analysis

The resistance status of mosquito samples was determined according to the WHO criteria [21] as follows:

- Mortality rates between 98%-100% indicate full susceptibility
- Mortality rates between 90%-97% indicate possible resistance
- Mortality rates < 90%, the population is considered resistant to the tested insecticides.

Analysis using Fisher's exact test and test of proportion was performed on the data sets gathered from the locations surveyed in Lokossa district to compare mortality rates obtained with F1 progeny to those of their parent female adult *Anopheles gambiae s.l.* mosquitoes.

4. Results and Discussion

4.1. Susceptibility Status to Cyfluthrin in Anopheles Gambiae s.l. Populations from Lokossa District

Kisumu strain (control) confirmed its susceptibility status as a reference strain. The 24 hours mortality recording shows that female *Anopheles gambiae* Kisumu which were exposed to WHO papers impregnated with cyfluthrin (0.15%) were fully susceptible to this product.

They were dead, and none of them could fly after a 24-hour mortality recording required by the WHO (Table 1 and Table 2).

Regarding F1 progeny, *An. gambiae s.l.* mosquitoes from Agamè, Houin, Koudo, Ouèdèmè in Lokossa district, they were resistant to cyfluthrin with the mortality rates of 57%, 33%, 41% and 29% respectively (Table 1).

Table 1. Mortality of F1 progeny *An. gambiae s.l.* mosquitoes from Lokossa district after one hour exposure to WHO impregnated papers with cyfluthrin (0.15%)

Locations	Number tested	% Mortality	Resistance status
Kisumu (Control)	100	100	S
Agamè	100	57	R
Houin	100	33	R
Koudo	100	41	R
Ouèdèmè	100	29	R

Regarding field-collected female adult *Anopheles gambiae s.l.* populations from Agamè, Houin, Koudo, Ouèdèmè in Lokossa district, they were also resistant to cyfluthrin with the mortality rates of 71%, 55%, 62% and 47 % (Table 2). But, these mortality rates were higher than those recorded with their F1 progeny ($p<0.05$).

Table 2. Mortality of female adult *Anopheles gambiae* s.l. mosquitoes from Lokossa district after one hour exposure to WHO impregnated papers with cyfluthrin (0.15%)

Locations	Number tested	% Mortality	Resistance status
Kisumu (Control)	100	100	S
Agamè	100	71	R
Houin	100	55	R
Koudo	100	62	R
Ouèdèmè	100	47	R

4.2. Dissection of Anopheles Gambiae Mosquitoes

The results obtained regarding the physiological age of female adult *An. gambiae* determined through dissection using Detinova method showed that almost all surviving *An. gambiae* mosquitoes from bioassays dissected were nullipares. The number of pare mosquitoes in the different locations surveyed were very few and ranged from Zero (0) to eight (8) mosquitoes (Table 3).

Table 3. Determination of physiological ages with surviving Anopheles gambiae mosquitoes from WHO bioassays

Locations	Number Tested	Physiological ages	
		Pare	Nullipare
Agamè	68	8	60
Houin	39	1	38
Koudo	47	2	45
Ouèdèmè	29	1	28

4.3. Identification of Anopheles Gambiae-fed Mosquitoes

Some *Anopheles gambiae* fed mosquitoes in the mosquito fauna collected in window traps put on windows of four rooms in each of locations surveyed in Lokossa district were found. The figure 2 showed the blood of killed *Anopheles gambiae* fed mosquitoes collected from window traps in the study area.

Fig. 2 The blood of killed *Anopheles gambiae* fed mosquitoes collected from window traps

The advantages and disadvantages of using *Anopheles gambiae* F1 progeny were shown in table 4.

Table 4. Advantages and disadvantages of using F1 progeny

Vector sample	Advantages	Disadvantages
F1 progeny	Age of vectors can be kept constant between tests, allowing results from different times and places to be compared.	Requires better entomological facilities, which limits where the tests can be carried out.
	In areas with low mosquito density, can be used even if it is not possible to catch sufficient numbers of adult wild female mosquitoes.	Environmental conditions will differ from those within the insectary.
		Since many eggs may be derived from just a few adult females, the number of genomes sampled from the wild population is likely to be less than the number of insects tested.

In the current study, female adult *An. gambiae s.l.* mosquitoes collected from window traps put on windows of rooms in Lokossa district surveyed were those from emergence of pupae of *Anopheles gambiae s.l.* populations collected from the breeding sites using the dipping method. So, even if in the current study, the F1 progeny were obtained from mosquitoes collected from the breeding sites using the dipping method and reared up to adult emergence, they (F1 progeny) also have the female adult *An. gambiae s.l.* mosquitoes collected from window traps put on windows of rooms as parent.

The F1 progeny *An. gambiae s.l.* mosquitoes from Agamè, Houin, Koudo, Ouèdèmè in Lokossa district were resistant to cyfluthrin. In similar way, field collected female adult *Anopheles gambiae s.l.* populations from window traps in the same locations in Lokossa district were also resistant to cyfluthrin. The resistance of *Anopheles gambiae s.l.* populations from Lokossa district to cyfluthrin may be explained by increased use of various insecticidal products (including pyrethroids) for crop protection. These results corroborated with those obtained which have shown that after pesticide treatments in agricultural settings, residues of insecticides get into mosquito breeding sites. These residues have lethal effects on larvae of some populations of mosquito whereas they exert a selective pressure on other populations, leading to a gradual tolerance of insecticide concentrations and to the emergence of resistant populations [22]. In addition, a research carried out in Côte d'Ivoire has studied the pyrethroid resistance in *Anopheles gambiae* s.l. from Côte d'Ivoire [23]. Among the pyrethroids tested, there was beta-cyfluthrin. In their study, eleven insecticide powder formulations belonging to six insecticide classes (pyrethroid, carbamate, organophosphate, neonicotinoid, entomopathogenic fungus and boric acid) were initially screened for residual activity over 4 weeks against pyrethroid resistant *Anopheles gambiae* sensu lato (s.l.) from the M'bé valley, central Côte d'Ivoire. Tests were performed using the eave tube assay that simulates the behavioural interaction between mosquitoes and insecticide-treated inserts. With the best performing insecticide, persistence was monitored over 12 months and the actual contact time lethal to mosquitoes was explored, using a range of transient exposure time (5s, 30s, 1min up to 2 min) in the tube assays in laboratory. The mortality data were calibrated against overnight release-recapture data from enclosure around experimental huts incorporating treated inserts at the M'bé site. The natural recruitment rate of mosquitoes to the tube without insecticide treatment was assessed using fluorescent dust particles. Their results showed that although most insecticides assayed during the initial screening induced significant mortality (45–100%) of pyrethroid resistant *An. gambiae* during the first 2 weeks, only 10% beta-cyfluthrin retained high residual efficacy, killing 100% of *An. gambiae* during the first month and > 80% over 8 subsequent months. Transient exposure for 5 s of mosquitoes to 10% beta-cyfluthrin produced 56% mortality, with an increase to 98% when contact time was extended to 2 min. In the experimental hut enclosures, mortality of *An. gambiae* with 10% beta-cyfluthrin treated inserts was 55% compared to similar rate (44%) of mosquitoes that contacted the inserts treated with fluorescent dusts. This suggests that all host-seeking female mosquitoes that contacted beta-cyfluthrin treated inserts during host-seeking were killed. These authors concluded that the eave tube technology is a novel malaria control approach which combines house proofing and targeted control of anopheline mosquitoes using insecticide treated inserts. Beta-cyfluthrin showed great promise for providing prolonged control of pyrethroid resistant *An. gambiae* and has potential to be deployed year-round in areas where malaria parasites are transmitted by highly pyrethroid resistant *An. gambiae* across sub-Saharan Africa.

The mortality rates recorded with female adult *An. gambiae s.l.* mosquitoes collected from window traps put on windows of rooms in Lokossa district surveyed were higher than those obtained with their F1 progeny *An. gambiae s.l.* mosquitoes obtained after reproduction. So, more the mosquito was old, more it was susceptible to cyfluthrin. Otherwise, the young *An. gambiae* mosquitoes were more resistant to cyfluthrin than the old. These results corroborated with those obtained which have shown that changes in mosquito physiology that is not specifically associated with insecticides but that occurs with senescence such as an increase in the rate of cuticle permeability or a decrease in the rate of xenobiotic excretion, could also lead to an increase in susceptibility to insecticides [24].

The results obtained regarding the physiological age of female adult *An. gambiae* determined through dissection using Detinova method showed that almost all surviving *An. gambiae* mosquitoes from bioassays dissected were nullipares. The number of pare mosquitoes in the different locations surveyed were very few and ranged from zero to eight mosquitoes. That is good news for malaria vector control in Lokossa district.

Some *Anopheles gambiae* fed mosquitoes in the mosquito fauna collected in window traps put on windows of four rooms in each of locations surveyed in Lokossa district were found. These results corroborated with those obtained recently which have shown that the mosquitoes collected in bedrooms where mosquito nets were not well-appointed were higher than those collected in bedrooms where mosquito nets were well-appointed. Almost all people who slept in the beds where blood-fed *Anopheles gambiae* were found were sick. It is important to install mosquito nets well around and above the beds before sleeping at night[25].

As advantages of using F1 progeny, the age of vectors can be kept constant between tests, allowing results from different times and places to be compared. Also, in areas with low mosquito density, can be used even if it is not possible to catch sufficient numbers of adult wild female mosquitoes. As disadvantages of using F1 progeny, requires better entomological facilities, which limits where the tests can be carried out. In addition, environmental conditions will differ from those within the insectary. Also, since many eggs may be derived from just a few adult females, the number of genomes sampled from the wild population is likely to be less than the number of insects tested.

Novelty of the Study

Comparatively to the previous studies, the originality of the current study remains in the method used in mosquito collection. In fact, in the previous studies, the authors used mouth aspirators to collect mosquitoes in window traps put on windows of rooms. However, the current study is the first which used small electronic rackets in mosquito collection in window traps put on windows of rooms in the evaluation of malaria vectors' susceptibility to cyfluthrin.

Study Limitations

The current study needs more informations about molecular resistance data, sample sizes, site selection, quality control, and so on. For that, further studies will be carried out in the future in more locations of the country by taking these factors or parameters into account.

5. Conclusion

The monitoring of insecticide resistance is necessary to overcome malaria disease which is public health concern. This monitoring needs some biotechnologies or tools.

Recommendations

In order to guide future malaria vector control interventions in Benin, it is useful to report frequently the susceptibility status to pyrethroid or pyrethroid resistance level in *Anopheles gambiae* s.l. This will properly inform control programs of the most suitable insecticides to use and facilitate the design of appropriate resistance management strategies.

Conflicts of Interest

There are no conflicts of interest in regard to the publication of this paper.

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Author Contribution Statement

LNA and CDC conceived and designed the research. SEM, AASY and GGA conducted experiments. MHT analyzed data. LNA wrote the manuscript. All authors read and approved the manuscript.

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