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(RESEARCH ARTICLE)

Establishment of the correlation between resistance level to deltamethrin and physiological age of *An. gambiae sensu lato* populations from Dogbo district in South-Western Benin, West Africa

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Abstract

The current study was aimed to establish the correlation between the resistance level to deltamethrin and physiological age of *An. gambiae sensu lato* populations from Dogbo district in South-Western Benin, West Africa. Female *An. gambiae s.l.* mosquitoes were collected from window traps put on windows of rooms in Dogbo district surveyed. *An. gambiae s.l.* mosquitoes were collected from March to July and August to November 2020 during the rainy season in the locations of Ayomi, Dévé, Honton, Lokogohoué, Madjrè and Totchangni. Female *An. gambiae* species were morphologically identified using morphological keys and then transferred into mosquito cages for WHO bioassays performed with impregnated papers of deltamethrin (0.05%). The physiological age of adult female *An. gambiae* was determined through dissection using Detinova method. The results showed that more *An. gambiae* mosquitoes were old, more they were susceptible to deltamethrin. Otherwise, the young *An. gambiae* mosquitoes were more resistant to deltamethrin than the old. There is a correlation between resistance level to deltamethrin and physiological age of *An. gambiae sensu lato* populations from Dogbo district in South-Western Benin.

Keywords: Physiological age; Resistance; Malaria vectors; Deltamethrin; Benin

1. Introduction

World Health Organization (WHO) recommends a multi-pronged strategy to control and eliminate malaria, which includes vector control interventions, preventive therapies, diagnostic testing, treatment with quality-assured artemisinin based combination therapies (ACTs), and strong malaria surveillance. Effective malaria control and elimination requires strong and well-funded National Malaria Control Programmes (NMCPs), tailored national and regional strategies, extensive applied and operational research, and a close collaboration among partners in the global malaria and development community.

Achieving effective scale-up of malaria interventions also requires significant human resources at national, district and community levels, and the regular training of malaria programme staff [1].

Even in areas where resistance has been identified, LLINs continue to provide some level of protection by acting as a physical barrier against disease vectors. Countries should therefore continue to promote the goal of universal LLIN

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coverage. In areas with high levels of LLIN coverage in which pyrethroid resistance is identified, WHO recommends the deployment of focal IRS with a non-pyrethroid insecticide. The presence of a non-pyrethroid on wall surfaces reduces the probability that pyrethroid resistance will spread [1].

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. More recently, the emergence of resistance in populations of *An. gambiae* to common classes of insecticides used in public health has been reported in Benin [2-17]. The ongoing spread of insecticide-resistant genes, such as the well-characterized *kdr* mutations [18, 19] in populations of the major African malaria vectors, *An. gambiae*, can seriously jeopardize the efficacy of vector control programmes [8]. Metabolic resistance or biochemical mechanisms that involve the detoxifying enzymes was also involved in resistance *of An. gambiae s.l.* populations from Benin [5, 6]; [9-11].

Beninese National Malaria Control Programme has recently implemented large-scale and free distribution of long-lasting insecticidal nets (LLINs) throughout the entire country to increase coverage of LLINs. It is crucial that information on current status of *An. gambiae s.l.* resistance to pyrethroid being investigated. This will properly inform control programs of the most suitable insecticides to use and facilitate the design of appropriate resistance management strategies. In this study, we establish the correlation between resistance level to deltamethrin and physiological age of *An. gambiae sensu lato* populations from Dogbo district in South-Western Benin, West Africa.

2. Material and methods

2.1. Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Couffo. Couffo department is located in the south-westernBenin and the study was carried out more precisely in Dogbo district. 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 establish the correlation between resistance level to deltamethrin and physiological age of *An. gambiae* sensu lato populations from Dogbo district. Couffo 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.

Figure 1a Map of Republic of Benin showing Dogbo District

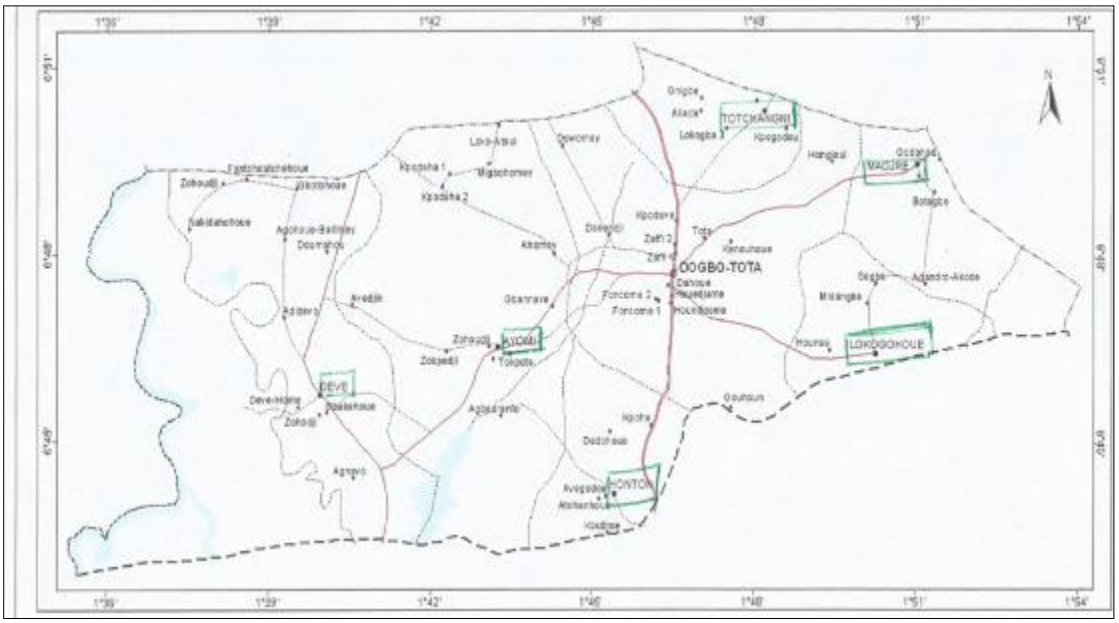

Figure 1b Map of Dogbo District showing the study area

2.2. Mosquito sampling

Anopheles gambiae s.l. mosquitoes were collected in 2020 in window traps put on windows of four rooms in each of locations surveyed in Dogbo district such as Ayomi, Dévé, Honton, Lokogohoué, Madjrè and Totchangni. Between 6.00 a.m. to 7.00 a.m., aspirators were used to collecting mosquitoes from these window traps. They were then put in some plastic cups covered with small cutting untreated net on which was put cotton wool moistened with a 10% honey solution.

2.3. Mosquito species identification

Female Anophelines were identified to species based on morphological characters using identification keys [20] and then transferred into mosquito cages for bioassays tests.

2.4. 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 [21]. In this study, the insecticide tested was deltamethrin (0.05%). The choice of deltamethrin 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 3–5 days into six WHO holding tubes (four tests and two controls) that contained untreated papers. They were then gently blown into the exposure tubes containing the insecticide impregnated papers. After one-hour 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 every five minutes and mortalities at 24 hours were recorded following the WHO protocol [22].

An. 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 deltamethrin.

All susceptibility tests were done following WHO protocol in Laboratory of Applied Entomology and Vector Control (LAEVC) of the Department of Sciences and Agricultural Techniques located in Dogbo district at 27°C +/- 2°C and 75% +/- 10% relative humidity.

2.5. Dissection of *Anopheles gambiae* mosquitoes

The physiological age of adult female *An. gambiae* was determined through dissection using Detinova method [23].

2.6. Statistical analysis

The resistance status of mosquito samples was determined according to the WHO criteria [22] 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.

Abbott s formula was not used in this study for the correction of mortality rates in test tubes because the mortality rates in control tube were less than 5% [24].

3. Results

3.1. Susceptibility status to deltamethrin in *Anopheles gambiaes.l.* populations from Dogbo 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 deltamethrin (0.05%) were fully susceptible to this product. They were dead and none of them could fly after 24 h mortality recording required by WHO (Table 1).

Regarding field collected female *Anopheles gambiae s.l.* populations from Ayomi, Dévé, Honton, Lokogohoué, Madjrè and Totchangni in Dogbo district, they were resistant to deltamethrin with the mortality rates of 43%, 31%, 28%, 53%, 18% and 16% respectively (Table 1).

Table 1 Mortality of *An. gambiae s.l.* populations from Dogbo district after one hour exposure to WHO impregnated papers with deltamethrin (0.05%)

Locations	Number tested	% Mortality	Resistance status
Kisumu (Control)	100	100	S
Ayomi	100	43	R
Dévé	100	31	R
Honton	100	28	R
Lokogohoué	100	53	R
Madjrè	100	18	R
Totchangni	100	16	R

3.2. 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 alive *An. gambiae* mosquitoes from bioassays dissected were nullipares. The number of pare mosquitoes in the different locations surveyed were very few and ranged from 01 to 04 mosquitoes (Table 2).

Table 2 Determination of physiological ages with alive *An. gambiae* mosquitoes from WHO bioassays

		Physiological ages	
Locations	Numbertested	Pare	Nullipare
Ayomi	57	01	56
Dévé	69	04	65
Honton	72	03	69
Lokogohoué	47	03	44
Madjrè	82	01	81
Totchangni	84	02	82

Figure 2 Ovary dissection

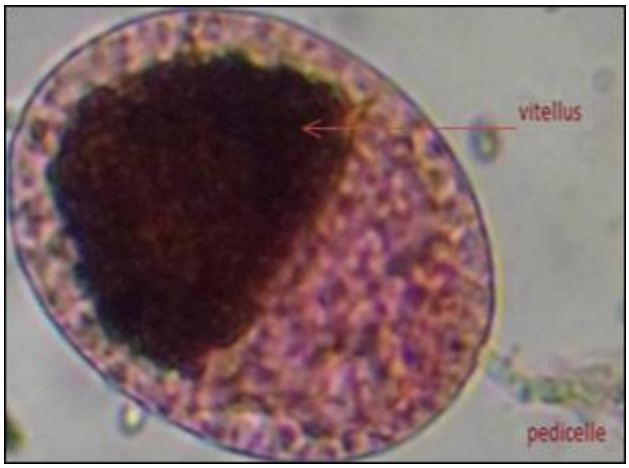

Figure 3 Nullipare ovariole

Figure 4 Unipare ovariole

4. Discussion

The management of insecticide resistance is a major issue, which must interest the different National Malaria Control Programmes. This management requires two kinds of information: sound knowledge of the mechanisms of resistance and a thorough resistance monitoring programme [5]. The control of vector borne diseases uses different methods depending on physiological, behavioural and ecological features of the vector.

Field collected female *Anopheles gambiae s.l.*populations from Dogbo district, were resistant to deltamethrin. The resistance of *Anopheles gambiae s.l.* populations from Dogbo district to deltamethrin may be explained by increased use of various insecticidal products (including pyrethroids) for crop protection. In fact, Akogbéto *et al.* [25], showed 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. According to Zaim *et al.* [26], pyrethroids have unique modes of action such as fast knockdown and excito-repellent effects.

The status of deltamethrin resistance was already studied by Aïzoun *et al.* [14], in three *Anopheles gambiae sensu lato* populations from main ecological settings in Benin. This study showed that the geographic distribution of vector susceptibility to pyrethroids is critically needed as it will provide baseline information for vector control. In addition, there was the presence at high frequency of the West African *kdr* mutation in *Anopheles gambiae* Dassa-Zoume and Allada which needs to be carefully monitored.

The results obtained regarding the physiological age of adult female *An. gambiae* determined through dissection using Detinova method showed that almost all alive *An. gambiae* mosquitoes from bioassays dissected were nullipares. The number of pare mosquitoes observed in the different locations surveyed were very few and ranged from 01 to 04 mosquitoes. Nullipare mosquitoes were younger than pares. More the mosquito was old, more it was susceptible to deltamethrin. Otherwise, the young *An. gambiae* mosquitoes were more resistant to deltamethrin than the old. A similar pattern was already observed by Aïzoun *et al.* [27], with two *Anopheles gambiae s.l.* populations from Ladji and Sèkandji in southern Benin. A similar pattern was also observed in *An. gambiae* Giles from Zanzibar by Lines and Nassor who showed that the mortality rate rose with age when mosquitoes were old [28]. Another similar pattern was also observed in *Anopheles funestus* FUMOZ-R from Southern Mozambique [29]. It has also been observed that older mosquitoes are sometimes less resistant to insecticides, especially when resistance is conferred by the presence of a detoxifying enzyme, the activity of which tends to decline with age [30].

Female *An. gambiaes.l.* populations from the Dogbo district in south-western Benin were resistant to deltamethrin.The current study clearly showed that more *An. gambiae* mosquitoes were old, more they were susceptible to deltamethrin. Otherwise, the young *An. gambiae* mosquitoes were more resistant to deltamethrin than the old. There is a correlation between resistance level to deltamethrin and physiological age of *An. gambiae* sensu lato populations from Dogbo district in South-Western Benin.

5. Conclusion

The current study clearly shows that there is a correlation between resistance level to deltamethrin and physiological age of *An. gambiae sensu lato* populations from Dogbo district in South-Western Benin.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest regarding the publication of this paper.

Statement of ethical approval

The study follows proper ethical procedures.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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