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Geographic distribution of deltamethrin resistance in malaria vectors from 2010 to 2020 in Republic of Benin, West Africa

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Abstract

The current study aims to establish the cartography of deltamethrin resistance in malaria vectors from 2010 to 2020 in Republic of Benin. *An. gambiae s.l.* populations were collected from March to July during the great rainy season in Ouémé in 2010, Littoral in 2012, Atlantic in 2012, Couffo in 2020 and Collines in 2012. *An. gambiae s.l.* populations were also collected from May to October during the rainy season in Alibori in 2012 and in 2015. Susceptibility tests were done following WHO protocol on unfed females' mosquitoes aged 2-5 days old reared from larval and pupal collections with papers impregnated with deltamethrin (0.05%). The results showed that the populations of *Anopheles gambiae s.l.* from Adjara, Dangbo and Misséréte were resistant to deltamethrin in 2010 whereas the populations of *Anopheles gambiae s.l.* from Kandi, Malanville, Cotonou, Allada and Dassa-Zoumè were resistant to this product in 2012. The populations of *Anopheles gambiae s.l.* from Parakou were resistant to deltamethrin in 2015 whereas the populations of *Anopheles gambiae s.l.* from Dogbo were resistant to the same product in 2020. The establishment of mapping or geographic distribution of deltamethrin resistance in malaria vectors from 2010 to 2020 in Republic of Benin is useful as this will properly inform control programs of the most suitable insecticides to use and facilitate the design of appropriate future resistance management strategies.

Keywords: Mapping; Deltamethrin Resistance; Malaria Vectors; Benin

1. Introduction

Malaria continues to be a major public health problem in the tropical world. It is the principal cause of morbidity and mortality in all sub-Saharan countries accounting for 10% - 30% of all hospital admissions, responsible for 15% - 25% of all deaths in children under the age of 5 years and for a substantial number of miscarriages and underweight births [1]. According to a WHO report released in 2014, an estimated number of 437000 African children died before their fifth birthday due to malaria. The report stated that there were about 198 million cases of malaria in 2013 and an estimated 584000 deaths with most victims being children [2]. It is estimated that over a million deaths are caused annually by malaria (mainly by *P. falciparum* malaria) across the globe [3].

Controlling mosquitoes and the diseases they transmit had been a major concern globally. The different strategies formulated at different times have been insufficient in many parts of the world to eradicate mosquitoes. Cutting off or breaking the link between mosquito vectors and human hosts consequently disrupts the life cycle of the malaria

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parasite. Intervention methods in that direction involve the use of insecticides, larvicides, topical repellents, treated bed nets, indoor residual spraying among others, to intercept the vector-host interactions or contact [1].

High prevalence of vector resistance to pyrethroids has been reported in a great number of cities across sub-Saharan Africa [4-5]. Broadly two major mechanisms are responsible for the majority of insecticide resistance cases: reduced target site sensitivity due to mutations at the DNA level [6-8] and metabolic detoxification through increased enzyme activities [9-11]. Enzymes involved in insecticide metabolism are members of three large detoxification multigene families: the glutathione S transferases (GSTs), cytochrome P450s, and esterases. Reduced target site resistance is specific to insecticides with similar mode of action whereas, metabolic resistance has a broad spectrum and is nonspecific.

Very few researches were published on the geographic distribution of deltamethrin resistance in malaria vectors from 2010 to 2020 in Republic of Benin. Therefore, there is a need to carry out new researches for this purpose.

The goal of this study was to cartography the deltamethrin resistance in malaria vectors on ten years in Republic of Benin, West Africa.

2. Material and methods

2.1. Study area

The study area is located in Republic of Benin (West Africa) and includes six departments, Ouémé, Alibori, Littoral, Atlantic, Collines and Couffo. In Ouémé department located in the South-eastern, the study was carried out in Adjara, Dangbo and Misseérété districts under Indoor Residual Spraying (IRS) during our survey. In Alibori department located in the northern, the study was carried out in Kandi district, a cotton growing area, in Malanville district, a rice growing area located near the Niger River and in Parakou district, a vegetable growing area. In Littoral department, the study was carried out in Cotonou district which is an urban area, the economic capital of Benin. In Atlantic department, the study was carried out in Allada district which is a cereal growing area. In Collines department, the study was carried out in Dassa-Zoumè district which is yam growing area and in Couffo department, the study was carried out in Dogbo district which is cereal growing area. The choice of the study sites took into account the economic activities of populations, their usual protection practices against mosquito bites,and peasant practices to control farming pests. We took these factors into account to establish the cartography of deltamethrin resistance in malaria vectors from 2010 to 2020 in Republic of Benin. The southern Benin is characterized by a sub-equatorial type of 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. The central part of Benin is characterized by a sudano guinean climate with two rainy seasons (April to July and September to November) with the annual mean rainfall of 1,000 mm. The northern Benin is characterized by a sudanian climate with only one rainy season per year (May to October) and one dry season (November to April). The temperature ranged from 22 to 33°C with the annual mean rainfall of 1,300 mm.

2.2. Mosquito sampling

An. gambiae s.l. populations were collected from March to July during the great rainy season in Ouémé in 2010, Littoral in 2012, Atlantic in 2012, Couffo in 2020 and Collines in 2012. *An. gambiae s.l.* populations were also collected from May to October during the rainy season in Alibori in 2012 and in 2015. Larvae and pupae were collected in these districts within both padding and village using the dipping method on several breeding sites (brick pits, pools, marshes, streams, ditches, pits dug for plastering traditional huts, puddles of water, water pockets caused by the gutters). Then, they were kept in separated labeled bottles related to each district surveyed. Otherwise, larvae collected from multiple breeding sites were pooled together and then re-distributed evenly in development trays containing tap water. Larvae were provided access to powdered TetraFin® fish food, and were reared to adults under insectary conditions of 25 +/- 2°C and 70 to 80% relative humidity at Center for Entomological Research of Cotonou (CREC) and at Laboratory of Applied Entomology and Vector Control of the Department of Sciences and Agricultural Techniques located in Dogbo district in south-western Benin. *An. gambiae s.l.* Kisumu, a reference susceptible strain was used as a control for the bioassay tests.

Susceptibility tests were done following WHO protocol on unfed females mosquitoes aged 2-5 days old reared from larval and pupal collections. All susceptibility tests were conducted in the Laboratory of CREC and in Laboratory of Applied Entomology and Vector Control (LAEVC) at 25+/-2°C and 70 to 80% relative humidity.

2.3. 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 [12]. In this study, the insecticide tested was deltamethrin (0.05%). The choice of deltamethrin was justified by its frequent use on LLINs (Permanet 2.0) which were used by National Malaria Control Programme (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 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 [12].

2.4. Statistical analysis

The resistance status of the used mosquito sample was determined according to the WHO criteria [13-14] as follows:

- Mortality rates between 98%-100% indicate full susceptibility
- Mortality rates between 90%-97% require further investigation or 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 the test-tubes because the mortality rates in all controls was always less than 5% [15].

3. Results

The analysis of Table 1 showed that the populations of *Anopheles gambiae s.l.* from Adjara, Dangbo and Missérété were resistant to deltamethrin in 2010 whereas the populations of *Anopheles gambiae s.l.* from Kandi, Malanville, Cotonou, Allada and Dassa-Zoumè were resistant to this product in 2012. The populations of *Anopheles gambiae s.l.* from Parakou were resistant to deltamethrin in 2015 whereas the populations of *Anopheles gambiae s.l.* from Dogbo were resistant to the same product in 2020.

Table 1 Status of deltamethrin resistance in *Anopheles gambiae sensu lato* from 2010 to 2020

Populations	Years	Resistance status
Adjara	2010	R
Dangbo	2010	R
Missérété	2010	R
Kandi	2012	R
Malanville	2012	R
Cotonou	2012	R
Allada	2012	R
Dassa-Zoumè	2012	R
Parakou	2015	R
Dogbo	2020	R

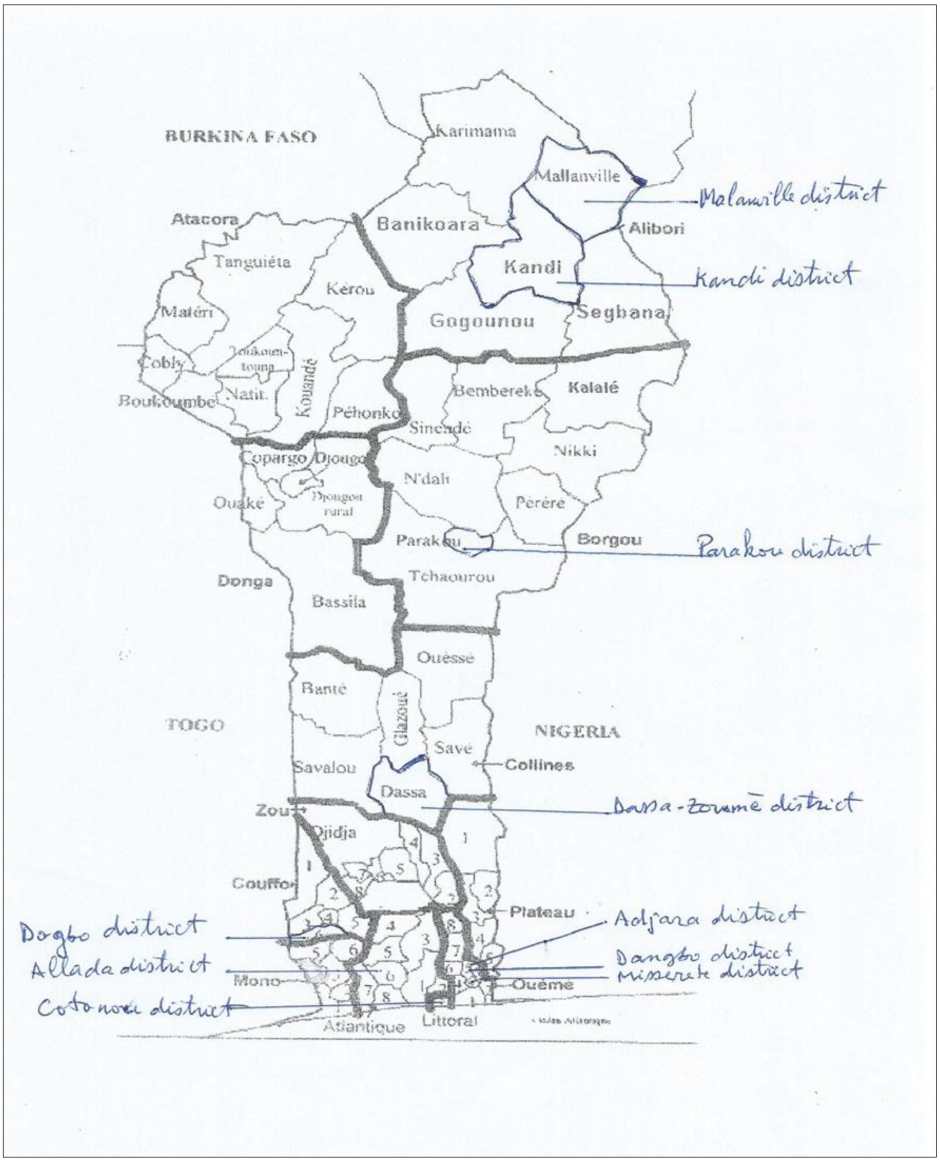

Figure 1 Map of Republic of Benin showing the districts where deltamethrin resistance were detected from 2010 to 2020

4. Discussion

Mosquitoes can be managed using an integrated approach that relies mostly on prevention, using biological and chemical controls when necessary. The key strategy is to eliminate all potential breeding sites; even one ounce of standing water can support a population of larvae.

The populations of *Anopheles gambiae s.l.* from Adjara, Dangbo and Missérété in Ouémé department were resistant to deltamethrin in 2010. In fact, the three districts were under Indoor Residual Spraying (IRS) in Ouémé department during our survey. In addition, Aïzoun *et al.*[16] showed that the populations of *Anopheles gambiae s.l.* from Missérété had developed more than one resistance mechanism to deltamethrin. In southern Benin, Corbel *et al.* [17] had already reported on multiple insecticide resistance mechanisms in populations of *Anopheles gambiae s.l.* from Ladji. Among these mechanisms, there were mixed function oxidase (MFO) and alpha-esterase with the presence of *Kdr* at high frequency (80%).

The populations of *Anopheles gambiae s.l.* from Kandi were resistant to deltamethrin in 2012. In fact, Kandi is a cotton growing area where various insecticidal products are used to control agricultural pests. The use of pesticides in agriculture has been pointed as one of the major factors driving insecticide resistance in malaria vectors [18]. In addition, high activity of mono-oxygenases was also detected in deltamlethrin resistance in *Anopheles gambiae s.l.* from

Kandi district [9]. This result may reflect an increased selection pressure on malaria vectors due to agricultural practices [19].

The populations of *Anopheles gambiae s.l*. from Malanville and Cotonou were resistant to deltamethrin in 2012. In the rice field area of Malanville, the increase in resistance to deltamethrin could be attributed to the increase of the L1014F *kdr* mutation [7] and detoxifying enzymes such as cytochrome P450 monooxygenases [20]. Regarding deltamethrin resistance in *Anopheles gambiae s.l*. from Cotonou district, it might be explained by increased use of household insecticide and availability of xenobiotics for larval breeding sites in this urban area.

The populations of *Anopheles gambiae s.l*. from Allada and Dassa-Zoumè were resistant to deltamethrin in 2012. Very high *kdr* allelic frequency was observed in populations of *Anopheles gambiae* s.s. from Dassa-Zoumè, yam growing area and might likely be explained by absence of metabolic-based resistance whereas Allada is a cereal growing area where no insecticidal product is generally used to control agricultural pests [21]. The populations of *Anopheles gambiae s.l*. from Parakou were resistant to deltamethrin in 2015. In fact, Parakou is a vegetable growing area where an important quantity of insecticidal products are generally used to control agricultural pests [22].

The populations of *Anopheles gambiae s.l*. from Dogbo were resistant to deltamethrin in 2020. The resistance mechanisms involved in this resistance were not explored yet [23]. However, the use of *Bacillus thuringiensis* in the management of larvicide resistance in larvae of *Anopheles gambiae sensu lato* from Dogbo district had given good results [24]. In similar ways, the effect of *Carica papaya Linn.* (Caricaceae) latex gel in the management of larvicide resistance in larvae of *Anopheles gambiae sensu lato* from Dogbo district [25], the effect of aqueous extract of lemon (*Citrus limon)* on *Anopheles gambiae sensu lato* larvae tolerance in malaria vector control in Dogbo district [26] and the effect of coconut oil on *Anopheles gambiae sensu lato* larvae tolerance in malaria vector control in Dogbo district [27] were different studies recently carried out with good results.

5. Conclusion

The establishment of mapping of deltamethrin resistance in malaria vectors from 2010 to 2020 in Republic of Benin is useful as this will properly inform control programs of the most suitable insecticides to use and facilitate the design of appropriate future resistance management strategies.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest among the authors.

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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