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Cartography of permethrin resistance in malaria vectors from 2008 to 2020 in the Republic of Benin, West Africa

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Abstract

The use of chemical insecticides causes important damage to the environment and human health and there is a need to monitor it.The current study aimsto establishthe cartography of permethrin resistance in malaria vectors from 2008 to 2020 in the Republic of Benin. Larvae of *An. gambiae s.l.* were collected from March to July during the great rainy season in Ouémé in 2008 and in 2012, Littoral in 2008, Mono in 2014 and in 2018, Couffo in 2020, Zou in 2008 and in 2012 and Collines in 2013. Larvae of *An. gambiae s.l.* were also collected from May to October during the rainy season in Alibori in 2012. Susceptibility tests were done following WHO protocol on unfed female adult mosquitoes aged 2-5 days old reared from larval and pupal collections with papers impregnated with permethrin (0.75%). The results showed that the populations of *Anopheles gambiae s.l.* from Akron, Suru-léré, Bamè, Ladji and Houéyiho were resistant to permethrin in 2008, whereas the populations of *Anopheles gambiae s.l.* from Agbalilamè, Parakou and Bohicon were resistant to permethrin in 2012. The populations of *Anopheles gambiae s.l.* from Savè were resistant to permethrin in 2013. The populations of *Anopheles gambiae s.l.* from Grand-Popo, Comè and Lokossa were resistant to permethrin in 2014, whereas the populations of *Anopheles gambiae s.l.* from Houéyogbé and Bopa were resistant to permethrin in 2018. The populations of *Anopheles gambiae s.l.* from Dogbo were resistant to permethrin in 2020.

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Introduction

Malaria disease remains a major public health problem, most notably in sub-Saharan Africa, despite all progress recorded in reducing malaria morbidity and mortality (WHO, 2020).

In Benin, malaria vector control relies mainly on the mass distribution of LLINS and on IRS operations. Although LLINS and IRS have been shown to be effective, they have performed below expectations in some settings including several locations in the country (N'Guessan *et al.*, 2007; Kelly-Hope *et al.*, 2008; Ranson *et al.*, 2011).

After many years of prolonged use of pyrethroid insecticides to control agricultural pests and disease vectors, malaria vectors with increasing levels of pyrethroid resistance have emerged and this has impacted the ability of LLINs to control these mosquito populations (Hemingway *et al.*, 2016; WHO, 2018). Insecticide resistance has now been reported in malariavectors against the four main classes of public health insecticides used in the control of these malaria vectors. Different insecticide resistance mechanisms havebeen reported, including metabolic resistance, target-sitemutations, and behavioral changes, as well as cuticular thickening (Hemingway *et al.*, 2000; WHO, 2012).

Controlling mosquitoes and the diseases they transmit has 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 parasite. Intervention methods that directly involve the use of insecticides, larvicides, topical repellents, treated bed nets, and indoor residual spraying, among others, to intercept the vector-host interactions or contact (Effiom *et al.*, 2012).

Very few researches were published on the cartography of permethrin resistance in malaria vectors from 2008 to 2020 in the Republic of Benin. Therefore, there is a need to carry out new research for this purpose.

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

Materials and methods

Study area

The study area is located in the Republic of Benin (West Africa) and includes seven departments: Ouémé, Littoral, Alibori, Zou, Collines, Mono and Couffo. Ouémé,Littoral, Mono, Couffo and Zou are departments located in Southern Benin, whereas Collines is a department located in the central part of the country. Alibori is a department located in Northern Benin. In the Ouémé department, the study was carried out more precisely in the Sèmè district and in the Akron location in the Porto-Novo district. In the Littoral department, the study was carried out more precisely in Suru-léré, Ladji and Houéyiho locations in the Cotonou district. In the Mono department, the study was carried out more precisely in Grand Popo, Comè, Lokossa, Houéyogbé and Bopa districts. In the Couffo department, the study was carried out more precisely in the Dogbo district, whereas in the Zou department, the study was carried out more precisely in Bamè location in Zagnanando district and in Bohicon district. In the Collines department, the study was carried out more precisely in the Savè district. In the Alibori department, the study was carried out more precisely in the Parakou district. 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 permethrin resistance in malaria vectors from 2008 to 2020 in the Republic of Benin. 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.

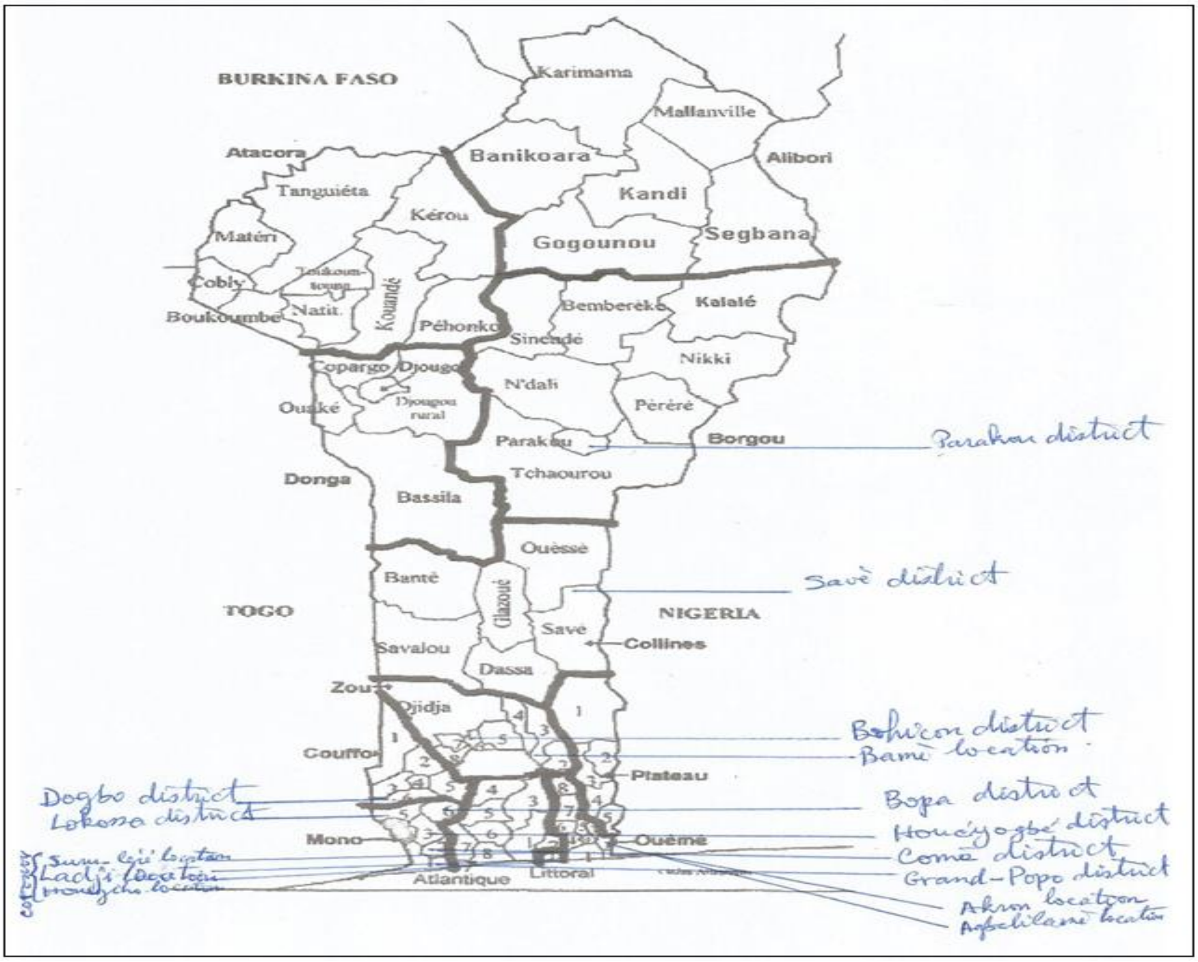

Fig.1. Map of the Republic of Benin showing locations where permethrin resistance was detected from 2008 to 2020.

The central part of Benin is characterized by a sudano guinean climate with two rainy seasons (April to July and September to November) with an annual mean rainfall of 1,000 mm. 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 an annual mean rainfall of 1,300 mm.

Mosquito sampling

An. gambiae s.l. populations were collected from March to July during the great rainy season in Ouémé in 2008 and in 2012, Littoral in 2008, Mono in 2014 and in 2018, Couffo in 2020, Zou in 2008 and in 2012and Collines in 2013. *An. gambiae s.l.* populations were also collected from May to October during the rainy season in Alibori in 2012. 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 separate 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 the Center for Entomological Research of Cotonou (CREC) and at the 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 female adult mosquitoes aged 2-5 days old reared from larval and pupal collections.

All susceptibility tests were conducted in the Laboratory of CREC and in the Laboratory of Applied Entomology and Vector Control (LAEVC) at 25+/-2°C and 70 to 80% relative humidity.

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 (WHO, 1998). In this study, the insecticide tested was permethrin (0.75%). The choice of permethrin was justified by its frequent use on LLINs (Olyset Nets) which were used by the National Malaria Control Programme (NMCP) for implementation of large-scale and free distribution throughout the entire country to increase coverage. An aspirator was used to introduce 20 to 25 unfed female adult 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 was recorded following the WHO protocol (WHO, 1998).

The resistance status of the used mosquito sample was determined according to the WHO criteria (WHO, 2013; WHO, 2017) 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 were always less than 5%(Abbott, 1987).

Results and discussion

The analysis of Table 1 showed that the populations of *Anopheles gambiae s.l.* from Akron, Suru-léré, Bamè, Ladji and Houéyiho were resistant to permethrin in 2008, whereas the populations of *Anopheles gambiae s.l.* from Agbalilamè,Parakou and Bohicon were resistant to permethrin in 2012. The populations of *Anopheles gambiae s.l.* from Savè were resistant to permethrin in 2013. The populations of *Anopheles gambiae s.l.* from Grand -Popo, Comè and Lokossa were resistant to permethrin in 2014, whereas the populations of *Anopheles gambiae s.l.* from Houéyogbé and Bopa were resistant to permethrin in 2018. The populations of *Anopheles gambiae s.l.* from Dogbo were resistant to permethrin in 2020.

The populations of *Anopheles gambiae s.l.* from Akron, Suru-léré and Bamè were resistant to permethrin in 2008.Permethrin is one of the pyrethroids used to impregnate mosquito nets (Olysets) which are regularly distributed free to populations throughout Benin country by the National Malaria Control Programme (NMCP).

Permethrin resistance in *Anopheles gambiae s.l.* from Suru-léré in the Cotonou district might be explained by the increased use of household insecticide and availability of xenobiotics for larval breeding sites in the urban area. They were one of the possible factors selected for pyrethroid resistance in *Anopheles gambiaes.l.* in the urban area. The underlying mechanism of resistance patterns observed in populations of *Anopheles gambiae s.l.* from Suru-léré was explored through a synergist assay. The synergist assay with PBO, an inhibitor of cytochrome P450 mono-oxygenases, indicated that this enzyme family plays a little role in the permethrin resistance observed in Suru-léré. Indeed the mortality rate to permethrin slightly varied when mosquitoes were exposed to PBO. The use of synergist PBO to overcome permethrin resistance in *Anopheles gambiaes.l.* from Suru-léré showed that this synergist had partially inhibited mono-oxygenase activity and, therefore, slightly improved permethrin effectiveness in these*Anopheles gambiaes.l.* populations (Aïzoun *et al.*, 2014a).

Permethrin resistance in *Anopheles gambiae s.l.* from Akron might be explained by a vegetable growing area located in Porto-Novo district where insecticide products are used to control farming pests. According to Djouaka *et al.* (2008), the permethrin resistance in *Anopheles gambiae s.l.* from Akron might be explained by the implication of metabolic mechanism (cytochrome P450 genes). In addition, this study also reported on multiple resistance mechanisms in populations of *Anopheles gambiae s.l.* from Akron which emphasizes several additional potential resistance mechanisms which need further investigation.

Table 1. Status of permethrin resistance in *Anopheles gambiae sensu lato* from 2008 to 2020.

Populations	Years	Resistance status
Akron	2008	R
Suru-léré	2008	R
Bamè	2008	R
Ladji	2008	R
Houéyiho	2008	R
Agbalilamè	2012	R
Parakou	2012	R
Bohicon	2012	R
Savè	2013	R
Grand-Popo	2014	R
Comè	2014	R
Lokossa	2014	R
Houéyogbé	2018	R
Bopa	2018	R
Dogbo	2020	R

The populations of *Anopheles gambiae s.l.* from Bamè were resistant to permethrin in 2008, even if Bamè is a rice-growing area where little quantity of insecticide is used to control farming pests (Aïzoun *et al.*, 2014a).

The populations of *Anopheles gambiae s.l.* from Ladji, a peri-urban location of Cotonou were resistant to permethrin in 2008. In the same way,the populations of *Anopheles gambiae s.l.* from Agbalilamè in Sèmè district were resistant to permethrin in 2012. It is worth mentioning that the locations of Ladjiand Agbalilamè are crossed by the Nokoué Lake streams, which sweep and converge several environmental pollutants and pesticide residues from the neighbouring peri-urban cities and farms to the coastal locations of Ladji and Agbalilamè. It is also possible that several ranges of xenobiotics present in these water bodies around Ladji and Agbalilamè might have also contributed to the selection of this resistance in *Anopheles gambiae s.l.*. In 2008, metabolic resistance conferred by detoxifying enzymes such as cytochrome P450 mono-oxygenases and esterases were found to play a role in populations of *Anopheles gambiae s.l.* from Ladji resistance to permethrin (Aïzoun *et al.*, 2014b). The underlying mechanism of the resistance pattern observed in this population was explored using a synergist assay. The synergist assay with Piperonylbutoxide (PBO), an inhibitor of cytochrome P450 monooxygenases, indicated that this enzyme family plays a role in the high permethrin resistance observed in Agbalilamè. Indeed, the mortality rate of permethrin was shown to vary significantly when mosquitoes were exposed to PBO. The use of synergist PBO to overcome permethrin resistance in *Anopheles gambiaes.l.* from Agbalilamè showed that this synergist has inhibited mono-oxygenaseactivity and therefore improved permethrin effectiveness in these*Anopheles gambiae s.l.* populations (Aïzoun *et al.*, 2013).

The populations of *Anopheles gambiae s.l.* from Houéyiho, a vegetable growing area of Cotonou, were resistant to permethrin in 2008. This could be explained by the amount quantity of insecticides used to control farming pests in this area (Aïzoun *et al.*, 2014c).

The populations of *Anopheles gambiae s.l.* from Parakou and Bohicon were resistant to permethrin in 2012. This resistance to permethrin in these populations of *Anopheles gambiae s.l.* might be due to L1014 F *kdr* mutation as its frequency was very high and was 0.74 for populations of *Anopheles gambiae s.l.* from Parakou, a vegetable growing area (Aïzoun *et al.*, 2014d).

The populations of *Anopheles gambiae s.l.* from Savè were resistant to permethrin in 2013 even if Savè is a cereal-growing area where little quantity of insecticide is used to control farming pests (Aïzoun *et al.*, 2014e).

The populations of *Anopheles gambiae s.l.* from Grand-Popo, Comè and Lokossa were resistant to permethrin in 2014, whereasthe populations of *Anopheles gambiae s.l.* from Houéyogbé and Bopa were resistant to permethrin in 2018. The mechanisms of permethrin resistance in adult populations of *Anopheles gambiae s.l.* from Mono department are not well investigated yet, same as permethrin resistance in adult populations of *Anopheles gambiae s.l.* from Dogbo district in Couffo department. However, recent studies carried out by Aïzoun (2021a-2021c) showed the implication of metabolic resistance mechanisms in pyrethroid resistance in larvae of *Anopheles gambiae s.l.* from the Mono department.

Conclusion

The use of chemical insecticides causes important damage to the environment and human health.The establishment of mapping of permethrin resistance in malaria vectors from 2008 to2020 in the 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.

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Author contribution

The authors designed the study, supervised the experiment, analyzed and interpreted the data, contributed to the mapping and drafted the manuscript.

Conflict of interest

We declare that there is no conflict of interest regarding the publication of this article.

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