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Dynamics of insecticide resistance in *Anopheles gambiae* sensu lato populations from Bohicon in Sudano-guinean area of Benin, West Africa

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Abstract

The current study was aimed to investigate the dynamics of insecticide resistance in *Anopheles gambiae* sensu lato populations from Bohicon in Sudano-guinean area of Benin, West Africa. Larvae and pupae of *Anopheles gambiae s.l.* populations were collected from the breeding sites in Zou department in 2015 and 2020. WHO susceptibility tests were conducted on unfed female mosquitoes aged 2-5 days old. WHO bioassays were performed with impregnated papers of permethrin (0.75%) and dichlorodiphenyltrichloroethane (DDT) (4%). *An. gambiae* mosquitoes were identified to species using PCR techniques in 2015. Molecular assays were also carried out to identify *kdr* mutations in individual mosquitoes. *An. Gambiae s.l.* populations from Bohicon were resistant to permethrin and DDT in 2015 and still remained resistant to these products in 2020. There is cross-resistance to both insecticides. PCR revealed 100% of mosquitoes tested were *Anopheles gambiae s.s*. The *L1014F kdr* mutation was found in *An. Gambiae s.s.* Bohicon at high allelic frequency.

Keywords: Dynamics; Resistance; Insecticides; Malaria; Benin

1. Introduction

Malaria remains one of the most important infectious diseases worldwide with an estimated 228 million cases and 405,000 deaths occurring in 2018 [1].

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

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and a thorough resistance monitoring programme [2]. The control of vector borne diseases uses different methods depending on physiological, behavioural and ecological features of the vector.

The intense use of DDT in agricultural settings and during the WHO malaria eradication programme in the 1950s and 1960s were suspected to be the main factors selecting for pyrethroids and DDT resistance in *An. Gambiae* populations [3]. Pyrethroids are the only option for net treatment due to their relative safety for humans at low dosage, excito-repellent properties, rapid rate of knock-down and killing effects [4]. However widespread reports of pyrethroid resistance in *An. Gambiae* in West and East Africa [5-6] and its cross-resistance with DDT are major challenges to its adoption for vector control purposes. Resistance to the insecticide DDT in the mosquito vectors of malaria has severely hampered efforts to control this disease and has contributed to the increase in prevalence of malaria cases. Over 90% of the 300-500 million annual cases of malaria occur in Africa, where the major vector is *Anopheles gambiae s.l*. [7].

In Benin as across Africa, malaria control relies heavily on vector control through the use of insecticide-treated nets (ITN) and indoor residual spraying (IRS). In West Africa, the main mechanism involved in pyrethroid-resistance in *Anopheles gambiae* is caused by target site insensitivity through a knockdown resistance (*kdr*)-like mutation caused by a single point mutation (Leu-Phe) in the para-sodium channel gene [6]. Malaria vector resistance to insecticides in Benin is conferred by two main mechanisms: (1) alterations at site of action in the sodium channel, viz the *kdr* mutations and (2) an increase of detoxification and/or metabolism through high levels of multi-function oxidases (MFOs), non-specific esterases (NSEs) [8-11].

Although it was shown by Ranson *et al.* [12], that a leucine phenylalanine substitution at position 1014 of the voltage gated sodium channel is associated with resistance to permethrin and DDT in many insect species, including *Anopheles gambiae* s.l from West Africa, another study has shown that target site mutation (*kdr*) was not responsible for DDT and permethrin resistance in *An. Arabiensis* populations, a major malaria vector in Nigeria. This study has likely suggested the involvement of metabolic resistance mechanisms in this resistance [13].

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 report the assessment of the susceptibility status, insecticide resistance levels in *Anopheles gambiae* s.l. to permethrin and DDT to evaluate the presence and extent of the distribution of the *kdr* mutation within and among these *An. Gambiae* s.l. populations in the Sudano-guinean area of Benin.

2. Material and methods

2.1. Study area

The study was carried out in the location of Bohicon selected for mosquito collection on the basis of variation in agricultural production, use of insecticides and/or ecological settings. The locality of Bohicon is located in the Sudano-guinean area of the country, where the farmers used significant amounts of pyrethroids and organophosphates for cotton protection or to control agricultural pests. Bohicon is characterized by a sudano-guinean climate with an average rainfall of 1,000 mm per year.

2.2. Mosquito sampling

An. Gambiae s.l. populations were collected from April to June 2015 and 2020 during the first rainy season in Bohicon district selected in the Sudano-guinean area of the country. Larvae and pupae were collected in this district 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 Bohicon location surveyed. Otherwise, larvae collected from multiple breeding sites were pooled together related to Bohicon location surveyed 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 of Entomological Researches of Cotonou (CREC) located in Akpakpa, in Cotonou district in 2015 and in insectary of the Laboratory of Applied Entomology and Vector Control of the Department of Sciences and Agricultural Techniques located in Dogbo district in south-western Benin in 2020. *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 2015 in the CREC laboratory at 25+/-2°C and 70 to 80% relative humidity whereas in 2020, susceptibility tests were conducted in Laboratory of Applied Entomology and Vector Control (LAEVC).

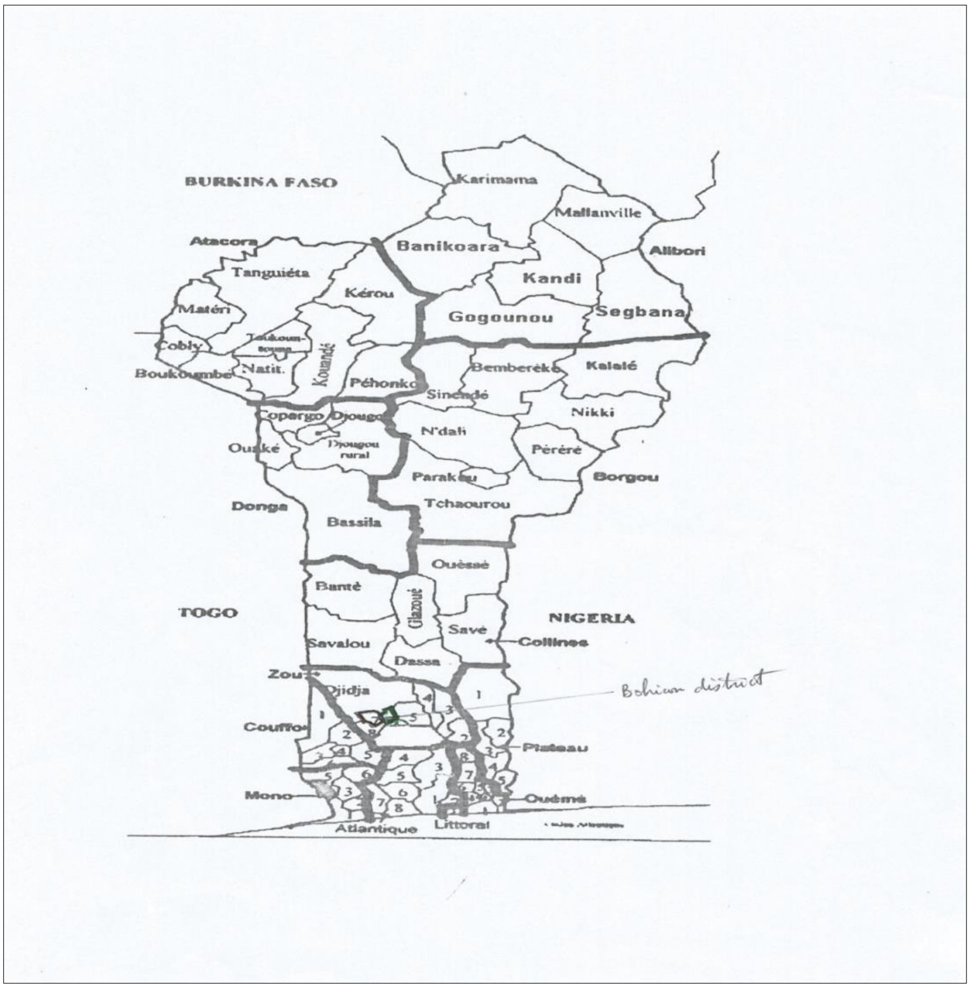

Figure 1 Map of Republic of Benin showing Bohicon district surveyed

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 [14]. In this study, the insecticide tested was permethrin (0.75%). The choice of permethrin was justified by its recent use on LLINs which were used by NMCP for implementation of large-scale and free distribution through the entire country to increase coverage. We used DDT to assess cross-resistance to both insecticides.

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 [14].

2.4. PCR detection of species and the *kdr* mutation

At the end of WHO bioassays in 2015, a polymerase chain reaction test for species identification [15] was performed to identify the members of *An. Gambiae* complex collected from Bohicon district. PCR for the detection of the *kdr* Leu-phe mutation was carried out on alive *An. Gambiae* mosquitoes as described by Martinez-Torres *et al.* [16].

2.5. Statistical analysis

The resistance status of mosquito samples was determined according to the WHO criteria [17] as follows:

- Mortality rates between 98%-100% indicate full susceptibility
- Mortality rates between 90%-97% require further investigation
- 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% [18]. The correlation between the results of insecticide susceptibility and molecular results (*kdr* frequency) was also assessed for the district surveyed.

3. Results

3.1. Evolution of *Anopheles gambiae* s.l. populations resistance to permethrin and DDT in Bohicon district from 2015 to 2020

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 permethrin (0.75%) and DDT (4%) 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 Bohicon, they were resistant to permethrin and DDT with the mortality rates of 73% and 16% respectively in 2015. These *Anopheles gambiae* s.l. populations still remained resistant to these same products in 2020 with the mortality rates of 67% and 07% respectively (Table 1).

Table 1 Mortality of *An. Gambiae s.l.* populations from Bohicon district after one hour exposure to WHO impregnated papers with permethrin (0.75%) and DDT (4%) in 2015 and 2020.

Populations	Years	Insecticides	Number tested	% Mortality	Resistance status
Kisumu (Control)	2015	Permethrin	100	100	S
	2020	Permethrin	100	100	S
	2015	DDT	100	100	S
	2020	DDT	100	100	S
Bohicon	2015	Permethrin	100	73	R
	2020	Permethrin	100	67	R
	2015	DDT	100	16	R
	2020	DDT	100	07	R

3.2. Mosquito species identification

PCR revealed that 100% of mosquitoes tested were *Anopheles gambiae* s.s. (Table 2).

3.3. Detection of the *Kdr* mutation

The results of molecular tests performed on *Anopheles gambiae* populations from Bohicon district revealed very high frequency of Kdr mutation. This allelic frequency of L1014F kdr mutation was 90% (Table 2).

Table 2 *Kdr* frequency in surviving *An. Gambiae* populations from Bohicon district 24 h post-exposure to WHO impregnated papers with permethrin and DDT in 2015

			Kdr mutation			
Location	Number tested	Species Ag	RR	RS	SS	F(Kdr)
Bohicon	25	25	20	5	0	0.90

Ag: *An. Gambiae* s.s.

4. Discussion

Female *Anopheles gambiae s.l.* populations from Bohicon were resistant to permethrin in 2015 and still remained resistant to the same product in 2020. So, *Anopheles gambiae s.l.* natural populations have developed resistance to permethrin in the Sudano-guinean area of Benin. This resistance to permethrin may be due to knock down effect which characterize pyrethroids as there is a correlation between resistance level to pyrethroids and knock-down time [19]. Knock down effect is a characteristic of pyrethroids. It happens immediately after the insects are exposed to pyrethroids [20]. Therefore, if the time need for insects to be knocked down increases, it indicates that the insects may be resistant to the insecticide [21]. When insects are exposed to pyrethroids, they fall down but will not die immediately. For susceptible insects, they will eventually die. But for resistant insects, after they are knocked down for a while, they will recover and soon be able to fly again after the pyrethroids entering their bodies are detoxified by their metabolism [21]. Aïzoun *et al.* [11] have also already reported permethrin resistance in *Anopheles gambiae s.l.* populations from the location of Agbalilamè in the Sèmè district of Ouémé department in the southern Benin. It is worth mentioning that the locality of Agbalilame is crossed by the Nokoue Lake streams, which sweep and converge several environmental pollutants and pesticide residues from the neighbouring peri-urban cities and farms to the coastal locality of Agbalilame. It is also possible that several ranges of xenobiotics present in these water bodies around Agbalilame might have also contributed to the selection of this resistance in *Anopheles gambiae.*

Female *Anopheles gambiae s.l.* populations from Bohicon were resistant to DDT in 2015 and still remained resistant to the same product in 2020. The Knock down effect is not only a characteristic of pyrethroids. It is also a characteristic of DDT. Resistance to DDT was widespread in the early 1970s because of its intensive use in public health and agriculture [22] and emerged after about 11 years of application [23]. Although DDT has been used in limited quantities for disease vector control during the past 3 decades, there have been recent reports of resistance in malaria vectors from African countries [24-26].

Anopheles gambiae s.l natural populations have developed high resistance to both DDT and permethrin in the Sudano-guinean area surveyed in the current study. This cross-resistance was also observed in different ecological settings in the country. According to Vulule *et al.* [5] and Chandre *et al.* [6], the pyrethroid resistance in *An. Gambiae* in West and East Africa and its cross-resistance with DDT are major challenges to its adoption for vector control purposes.

The *kdr* frequency in *Anopheles gambiae* populations from Bohicon recorded in this study was higher. So, there is a correlation between the results of insecticide susceptibility and molecular results (*kdr* frequency) in the current study.

Female *An. gambiae s.l.* populations from the Sudano-guinean area of Benin were resistant to permethrin and DDT with high allelic frequency of *L1014F kdr* mutation. There is cross-resistance to both insecticides.

5. Conclusion

With the rapid spread of pyrethroid resistance in the malaria vectors from the main ecological settings and the various resistance mechanisms involved, the geographic distribution of vector susceptibility to pyrethroids is critically needed as it will provide baseline information for vector control. In order to guide future malaria vector control interventions in Benin, the presence though at high frequency of the West African *kdr* mutation in *Anopheles gambiae* populations from the main ecological settings needs to be carefully monitored in the country.

Compliance with ethical standards

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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.

References

[1] WHO. World malaria report. Geneva: World Health Organization. 2019; 232 pages (ISBN: 978-92-4-156572-1).

[2] Aïzoun N, Ossè R, Azondekon R, Alia R, Oussou O, Gnanguenon V, Aïkpon R, Padonou GG, Akogbéto M. Comparison of the standard WHO susceptibility tests and the CDC bottle bioassay for the determination of insecticide susceptibility in malaria vectors and their correlation with biochemical and molecular biology assays in Benin, West Africa. Parasit. Vector. 2013a; 6:147.

[3] Akogbeto MC, Djouaka R, Noukpo H. L'utilisation des insecticides en agriculture au Bénin. Bull. Soc. Pathol. Exot. 2005; 98: 400-405.

[4] Zaim M, Aitio A, Nakashima N. Safety of pyrethroid-treated mosquito nets. Med. Vet. Entomol. 2000; 14:1-5.

[5] Vulule JM, Beach RF, Atieli FK, Mount DL, Roberts JM and Mwangu RW. Reduced Susceptibility of Anopheles gambiae to permethrin associated with the use of permethrin impregnated bednets and curtains in Kenya. Med. Vet. Entomol. 1994; 8:71-5.

[6] Chandre F, Darriet F, Manga L, Akogbeto M, Faye O, Mouchet J, Guillet P. Status of pyrethroid resistance in Anopheles gambiae sensu lato. Bull. World Health Organ. 1999; 77(3):230-234.

[7] Ranson H, Jensen B, Wang X, Prapanthadara L, Hemingway J and Collins HF. Genetic mapping of two loci affecting DDT resistance in the malaria vector Anopheles gambiae Insect Mol. Biol. 2000a; 9(5): 499-507.

[8] Corbel V, N'Guessan R, Brengues C, Chandre F, Djogbenou L, Martin T, Akogbeto M, Hougard JM, Rowland M. Multiple insecticide resistance mechanisms in Anopheles gambiae and Culex quinquefasciatus from Benin, West Africa. Acta Trop. 2007; 101: 207–16.

[9] Djogbénou L, Pasteur N, Akogbéto M, Weill M, Chandre F. Insecticide resistance in the Anopheles gambiae complex in Benin: a nationwide survey. Med. Vet. Entomol. 2009; 69: 160-164.

[10] Djègbé I, Boussari O, Sidick A, Martin T, Ranson H, Chandre F, Akogbéto M and Corbel V. Dynamics of insecticide resistance in malaria vectors in Benin: first evidence of the presence of L1014S kdr mutation in Anopheles gambiae from West Africa. Malar J. 2011; 10: 261.

[11] Aïzoun N, Aïkpon R, Padonou GG, Oussou O, Oké-Agbo F, Gnanguenon V, Ossè R, Akogbéto M. Mixed function oxidases and esterases associated with permethrin, deltamethrin and bendiocarb resistance in Anopheles gambiae s.l. in the south-north transect Benin, West Africa. Parasit. Vector. 2013b; 6: 223.

[12] Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan Anopheles gambiae associated with resistance to DDT and pyrethroids. Insect Mol. Biol. 2000b; 9(5): 491-497.

[13] Oduola OA, Olojede BJ, Ashiegbu OC, Adeogun OA, Otubanjo AO and Awolola ST. High level of DDT resistance in the malaria mosquito: Anopheles gambiae from rural, semi-urban and urban communities in Nigeria. J Rural Trop. Public Health. 2010; 9: 114-120.

[14] WHO. Report of the WHO Informal Consultation. Tests procedures for insecticide resistance monitoring in malaria vectors, bioefficacy and persistence of insecticides on treated surfaces. Geneva: World Health Organization: Parasitic Diseases and Vector Control (PVC)/Communicable Disease Control, Prevention and Eradication (CPE); 1998; 48 pages (WHO/CDS/CPC/MAL/98.12).

[15] Scott JA, Brogdon WG, Collins FH. Identification of single specimens of the Anopheles gambiae complex by the polymerase chain reaction. Am. J. Trop. Med. Hyg. 1993; 49: 520-529.

[16] Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Berge JB, Devonshire AL, Guillet P, Pasteur N, Pauron D. Molecular characterization of pyrethroid knockdown resistance (kdr) in major malaria vector An. gambiae s.s. Insect Mol. Bio. 1998; 7: 179-184.

[17] WHO. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes Second edition. Geneva: World Health Organization. 2013; 48 pages (ISBN 978 92 4 151157 5).

[18] Abbott WS. A method of computing the effectiveness of an insecticide. J. Am. Mosq. Control Assoc. 1987; 3(2):302-303.

[19] Akogbeto M, Yakoubou S. Résistance des vecteurs du paludisme vis-à-vis des pyréthrinoïdes utilisés pour l'imprégnation des moustiquaires au Bénin, Afrique de l Ouest. Bull. Soc. Pathol. Exot. 1999; 92:123-130.

[20] Coats JR. Insecticide Mode of Action. Academic Press London; 1982.

[21] Cochran DG. Effects of three synergists on Pyrethroid Resistance in the German Cockroach (Dictyoptera: Blatellidae). J. Econ. Entomol. 1994; 87(4): 879-884.

[22] Metcalf RL. A century of DDT. J. Agric. Food Chem. 1973; 21: 511–519.

[23] Magesa SM, Wilkes TJ, Mnzava AE, Njunwa KJ, Myamba J, Kivuyo MD, Hill N, Lines JD, Curtis CF. Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. Part 2. Effects on the malaria vector population. Acta Trop. 1991; 49: 97–108.

[24] Coetzee M, Van WP, Booman M, Koekemoer LL, Hunt RH. Insecticide resistance in malaria vector mosquitoes in a gold mining town in Ghana and implications for malaria control. Bull. Soc. Pathol. Exot. 2006; 99: 400–403.

[25] Hargreaves K, Hunt RH, Brooke BD, Mthembu J, Weeto MM, Awolola TS, Coetzee M. Anopheles arabiensis and An. quadiannulatus resistance to DDT in South Africa. Med. Vet. Entomol. 2003; 17:417-422.

[26] Tia E, Akogbeto M, Koffi A, Toure M, Adja AM, Moussa K, Yao T, Carnevale P, Chandre E. Pyrethroid and DTT resistance of Anopheles gambiae s.s. (Diptera: Culicidae) in five agricultural ecosystems from Cote-d'Ivoire [in French]. Bull. Soc. Pathol. Exot. 2006; 99:278–282.