

Current insecticide resistance status in malaria vector populations from Dogbo district in South-western Republic of Benin, West Africa

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Abstract

This study was aimed to investigate the current insecticide resistance status in malaria vector mosquitoes from Dogbo district in South-western Benin, West Africa. Larvae and pupae of *Anopheles gambiae s.l.* populations were collected from the breeding sites from March to July 2020 during the first rainy season in Couffo department using the dipping method. 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%), deltamethrin (0.05%), cyfluthrin (0.15%), lambda-cyhalothrin (0.05%) and dichlorodiphenyltrichloroethane (DDT) (4%). *An. gambiae s.l.* populations from Dogbo district were resistant to permethrin, deltamethrin, cyfluthrin, lambda-cyhalothrin and DDT. There is cross-resistance to both pyrethroid and DDT insecticides. However, the physical barrier of Long Lasting Insecticidal Nets (LLINs) which are regularly distributed free by Beninese National Malaria Control Programme throughout the entire country to increase coverage of LLINs is still important despite the insecticide resistance observed.

Key words: *Anopheles gambiae*; Resistance; Pyrethroids; DDT; Republic of Benin

1. Introduction

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 [1]. 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 [2]. However, widespread reports of pyrethroid resistance in *An. gambiae* in West and East Africa [3-4] 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.* [5].

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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 [4]. 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) [6-10].

Although it was shown by Ranson *et al.*[11], 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 [12].

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, deltamethrin, cyfluthrin, lambda-cyhalothrin and DDT.

2. Material and methods

2.1. Study area



Figure 1 Map of Republic of Benin showing Dogbo district surveyed

The study area is located in Republic of Benin (West Africa) and includes the department of Couffo. Couffo department is located in the south-western Benin and the study was carried out more precisely in Dogbo district (Fig.1). The

southern borders of this district are Lokossa and Bopa districts. The northern border is Djakotomey district. The eastern border is Lalo district and the western border of Dogbo district is Togo republic. Dogbo district covered 475 km² and belongs to geographic region of ADJA. 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. We took these factors into account to study the current insecticide resistance status in *Anopheles gambiae sensu lato* populations from Dogbo district in South-western Republic of Benin. 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.

2.2. Mosquito sampling

An. gambiae s.l. populations were collected from March to July 2020 during the first rainy season in Dogbo district. 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 Dogbo 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 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 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 (WHO, 1998). In this study, the insecticides tested were permethrin (0.75%), deltamethrin (0.05%), cyfluthrin (0.15%), lambda-cyhalothrin (0.05%) and dichlorodiphenyltrichloroethane (DDT) (4%). The choice of permethrin and deltamethrin was justified by their recent use on LLINs (OlysetNets for permethrin and Permanet 2.0 for deltamethrin) which were used by National Malaria Control Programme (NMCP) for implementation of large-scale and free distribution through the entire country to increase coverage. We used cyfluthrin and lambda-cyhalothrin to assess cross-resistance between pyrethroid insecticides. DDT was tested because of its intensive use in the past as well as to assess cross-resistance to organochlorine and pyrethroid 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 [13].

2.4. Statistical analysis

The resistance status of the used mosquito sample was determined according to the WHO criteria [14] 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 the test-tubes because the mortality rates in all controls was always less than 5% [15]. Analysis using Fisher’s exact test and test of proportion was performed on the data sets gathered from the Dogbo district surveyed to compare each of tested insecticides and to assess the resistance status.

3. Results

3.1. Determination of Knock down time (Kdt) and resistance ratio (RR)

The resistance ratios (RR50) of the wild populations of *Anopheles gambiae s.l.* from Dogbo with regard to *Anopheles gambiae s.l.* Kisumu susceptible reference strain were 1.853, 2.220, 2.483, 2.600 and 3.484 respectively for DDT, permethrin, deltamethrin, cyfluthrin and lambdacyhalothrin. These resistance ratios (RR50) were all higher than 1 (Table 1).

The same remark was made with the resistance ratios (RR95). They were 1.677, 1.876, 2.027, 2.089 and 2.410 respectively for DDT, permethrin, deltamethrin, cyfluthrin and lambdacyhalothrin. These results showed that the populations of *Anopheles gambiae s.l.* from Dogbo were resistant to these products (Table 1).

Table 1 Determination of Knock down times 50 (Kdt50), Knock down times 95 (Kdt95), resistance ratios 50 (RR50) and resistance ratios 95 (RR95)

Populations	Insecticides	Kdt50 (min)	RR50	Kdt95 (min)	RR95
Kisumu (Control)	DDT	43.313	-	54.024	-
	Permethrin	30.245	-	41.093	-
	Deltamethrin	24.689	-	35.103	-
	Cyfluthrin	22.790	-	33.246	-
	Labdacyhalothrin	15.289	-	26.142	-
Dogbo	DDT	80.282	1.853	90.635	1.677
	Permethrin	67.156	2.220	77.123	1.876
	Deltamethrin	61.310	2.483	71.179	2.027
	Cyfluthrin	59.263	2.600	69.456	2.089
	Labdacyhalothrin	53.278	3.484	63.012	2.410

3.2. Mortality of *An. gambiae s.l.* populations from Dogbo district after one hour exposure to WHO impregnated papers with permethrin (0.75%), deltamethrin (0.05%), cyfluthrin (0.15%), lambdacyhalothrin (0.05%) and dichlorodiphenyltrichloroethane (DDT) (4%)

Table 2 Determination of resistance status in *Anopheles gambiae s.l.* populations from Dogbo to permethrin, deltamethrin, cyfluthrin, lambdacyhalothrin and DDT

Populations	Insecticides	Number tested	% Mortality	Resistance status
Kisumu (Control)	DDT	100	100	S
	Permethrin	100	100	S
	Deltamethrin	100	100	S
	Cyfluthrin	100	100	S
	Labdacyhalothrin	100	100	S
Dogbo	DDT	100	4	R
	Permethrin	100	34	R
	Deltamethrin	100	77	R
	Cyfluthrin	100	71	R
	Labdacyhalothrin	100	84	R

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%),

deltamethrin (0.05%), cyfluthrin (0.15%), lambdacyhalothrin (0.05%) and dichlorodiphenyltrichloroethane (DDT) (4%) were fully susceptible to these products. They were dead and none of them could fly after 24 hours mortality recording required by WHO (Table 2).

Regarding field collected female *Anopheles gambiae s.l.* populations from Dogbo district, they were resistant to permethrin (0.75%), deltamethrin (0.05%), cyfluthrin (0.15%), lambdacyhalothrin (0.05%) and dichlorodiphenyltrichloroethane (DDT) (4%) with the mortality rates of 34%, 77%, 71%, 84% and 4% respectively (Table 2).

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 [9]. The control of vector borne diseases uses different methods depending on physiological, behavioural and ecological features of the vector.

Female *Anopheles gambiae s.l.* populations from Dogbo have developed resistance to DDT, permethrin, deltamethrin, cyfluthrin and lambdacyhalothrin. The resistance to permethrin, deltamethrin, cyfluthrin and lambdacyhalothrin may be due to knock down effect which characterizes pyrethroids as there is a correlation between resistance level to pyrethroids and knock-down time [16-17]. Knock down effect is a characteristic of pyrethroids and it happens immediately after the insects are exposed to pyrethroids [18]. Therefore, if the time need for insects to be knocked down increases, it indicates that the insects may be resistant to the insecticide [19]. 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 [19]. Aïzoun *et al.* [10] 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 south eastern 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*. Deltamethrin, cyfluthrin and lambdacyhalothrin resistance was also reported in the country [20-22].

Female *Anopheles gambiae s.l.* populations from Dogbo were also resistant to DDT. The Knock down effect is not only a characteristic of pyrethroids. It is also a characteristic of DDT [17]. Resistance to DDT was widespread in the early 1970s because of its intensive use in public health and agriculture [23] and emerged after about 11 years of application [24]. 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 [25-26, 11, 27].

There is cross-resistance to both pyrethroid and organochlorine insecticides in the current study. This cross-resistance was also observed by other authors. In fact, according to Vulule *et al.* [3] and Chandre *et al.* [4], 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.

5. Conclusion

There is cross-resistance to both pyrethroid and organochlorine insecticides. However, the physical barrier of Long-Lasting Insecticidal Nets (LLINs) which are regularly distributed free by Beninese National Malaria Control Programme throughout the entire country to increase coverage of LLINs is still important despite the insecticide resistance observed. Only the torn nets cannot protect people from malaria vector bites.

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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