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Current Insecticide Resistance Status in *Aedes Aegypti* Mosquito Populations from Mono Department and its Implications for Dengue Control in South-Western Republic of Benin, West Africa

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ABSTRACT

Aedes aegypti, an important vector in the transmission of human diseases has developed resistance to commonly used classes of insecticides in populations worldwide in public health. The current study was aimed to investigate on the current insecticide resistance status in *Aedes aegypti* mosquito populations from Mono department and its implications for dengue control in South-western Republic of Benin, West Africa. Larvae and pupae of *Aedes aegypti* populations were collected from the breeding sites in Mono department from March to July 2023 during the great rainy season. WHO susceptibility tests were conducted on unfed female mosquitoes aged 2-5 days old. WHO bioassays were performed with impregnated papers of dichlorodiphenyltrichloroethane (DDT) (4%), permethrin (0.75%), deltamethrin (0.05%), fenitrothion (1%) and bendiocarb (0.1%). The results showed *Aedes aegypti* mosquito populations were resistant to DDT, permethrin and deltamethrin insecticides but susceptible to fenitrothion and bendiocarb. There is cross-resistance to both pyrethroid and organochlorine insecticides. However, the physical barrier of Long Lasting Insecticidal Nets (LLINs) which are frequently distributed free by Beninese National Malaria Control Program throughout the entire country to increase coverage of LLINs is still important despite the insecticide resistance observed.

KEYWORDS: *Aedes aegypti*, resistance, insecticide, Republic of Benin.

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INTRODUCTION

Mosquitoes of medical importance, namely *Aedes* (Ae.) *aegypti* and *Ae. albopictus* transmit dengue, chikungunya, yellow fever, and zika infection, which kill millions of humans every year (Smith *et al*., 2016). In the absence of effective vaccines and specific antiviral drugs, the strategies for preventing and controlling dengue rely solely on vector control intervention, primarily with the use of insecticides (WHO, 2022). However, the excessive and long-term use of insecticides has led to resistance in mosquitoes.

Evidence of resistance towards all four classes of insecticides, *i.e.*, carbamates, organochlorines, organophosphates and pyrethroids have been reported in the population of the *Aedes* mosquitoes (Ahbirami *et al*., 2020; Elia-Amira *et al*., 2018; Elia-Amira *et al*., 2019).

Chemical control remains one of the primary means for combating mosquito populations worldwide (Marcombe *et al.*, 2012). There are two major mechanisms that contribute to insecticide resistance in mosquitoes: target site and metabolic resistance. Minor mechanisms of resistance include physical barriers, such as altered cuticle thickness preventing insecticide penetration, and behavioral resistance, such as altered mosquito behavior in the presence of insecticides (WHO, 1998a, Zalucki and Furlong, 2017). Target site resistance is the failure of an insecticide to bind its target site due to alterations in the structure or inaccessibility of the target site. The primary protein associated with target site insensitivity to pyrethroids is the voltage-sensitive sodium channel (Vssc) protein, which is responsible for the initiation and propagation of action potentials in the nervous system (Du *et al*., 2013). Changes to the acetylcholinesterase enzyme, which is responsible for breaking down remnants of the neurotransmitter acetylcholine in the synaptic cleft, is associated with target site insensitivity to carbamates and organophosphates (Fukuto, 1990). Alterations to the Vssc, known more commonly as knockdown resistance (*kdr*), and the acetylcholinesterase enzyme, commonly known as *Ace-1*, prevent insecticide binding; therefore, mosquitoes may survive despite exposure to an insecticide. Metabolic insecticide resistance is associated with the mutation or elevated expression of specific enzymes that lead to the rapid detoxification or sequestration of insecticides (Hemingway *et al*., 2004). Metabolic resistance involves three enzyme families: cytochrome P450 monooxygenases (P450), glutathione-S-transferases (GST), and carboxy/cholinesterases (CCE) (Strode *et al*., 2008). Previous studies have indicated that the elevated activity of α-esterase and β-esterase may confer resistance to organophosphates, while the elevated activity of mixed-function oxidases (MFOs) usually confer resistance to pyrethroids (Brogdon, 1989).

Very few researches were published on insecticide resistance status in *Aedes aegypti* mosquito populations from Mono department. Therefore, there is a need to carry out new researches for this purpose.

The aim of this study was to investigate on the current insecticide resistance status in *Aedes aegypti* mosquito populations from Mono department and its implications for dengue control in South-western Republic of Benin, West Africa.

MATERIALS AND METHODS

Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Mono. Mono department is located in the south-western Benin and the study was carried out more precisely in the six districts of this department (Figure 1). The choice of the study site took into account the economic activities of populations, their usual protection practices against mosquito bites, the Long-Lasting Insecticidal Nets, Permanets and OlysetNets distribution frequently by National Malaria Control Program in these localities and peasant practices to control farming pests. These factors have a direct impact on the development of insecticide resistance in the local mosquito vectors. We took these factors into account to investigate on the current insecticide resistance status in *Aedes aegypti* mosquito populations from Mono department and its implications for dengue control in South-western Republic of Benin. Mono 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.1 Map of Republic of Benin showing the six districts surveyed in Mono department

Mosquito sampling

Aedes aegypti populations were collected from March to July 2023 during the great rainy season in Mono department selected in the south-western 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 each district surveyed. Otherwise, larvae collected from multiple breeding sites were pooled together related to each district 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 Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) of the Department of Sciences and Agricultural Techniques located in Dogbo district at Normal High School of Technical Teaching (ENSET) of Lokossa. *Anopheles gambiae* sensu lato Kisumu, a reference susceptible strain was used as a control for the bioassay tests.

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, 1998b). In this study, the insecticides tested were dichlorodiphenyltrichloroethane (DDT) (4%), permethrin (0.75%), deltamethrin (0.05%), fenitrothion (1%) and bendiocarb (0.1%). We used permethrin and deltamethrin, insecticides of the same family as pyrethroids which are used on Long-Lasting Insecticidal Nets, OlysetNets and Permanets distributed frequently by National Malaria Control Program. We used DDT to assess cross resistance with pyrethroids. Bendiocarb is the insecticide used for indoor residual spraying in the northern part of Benin. We used fenitrothion, an organophosphate to assess cross resistance with carbamate.

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 (WHO, 1998b).

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 LaRPET at 25+/-2°C and 70 to 80% relative humidity.

Statistical analysis and data interpretation

The resistance status of mosquito samples was determined according to the WHO criteria (WHO, 2017) 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.

RESULTS

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

The resistance ratios (RR50) of the wild populations of *Aedes aegypti* from Mono with regard to *Anopheles gambiae s.l.* Kisumu susceptible reference strain were 2.497, 1.999 and 1.589 respectively for DDT, permethrin and deltamethrin. These resistance ratios (RR50) were all higher than 1 (Table 1).

The same remark was made with the resistance ratios (RR95). They were 1.714, 1.652 and 1.328 respectively for DDT, permethrin and deltamethrin. These results showed that the populations of *Aedes aegypti* from Mono 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	23.987	-	38. 678	-
	Permethrin	31.393	-	39.254	-
	Deltamethrin	44.688	-	49.347	-
	Fenitrothion	Nd	-	Nd	-
	Bendiocarb	Nd	-	Nd	-
Mono	DDT	59.896	2.497	66.298	1.714
	Permethrin	62.783	1.999	64.857	1.652
	Deltamethrin	71.011	1.589	65.576	1.328

	Fenitrothion	Nd	-	Nd	-
	Bendiocarb	Nd	-	Nd	-

Nd = No determined
- = Knock down (Kd) is not characteristic of Organophosphates and Carbamates

Mortality of *Aedes aegypti* populations from Mono department after one hour exposure to WHO impregnated papers with dichlorodiphenyltrichloroethane (DDT) (4%), permethrin (0.75%), deltamethrin (0.05%), fenitrothion (1%) and bendiocarb (0.1%).

Kisumu strain (control) confirmed its susceptibility status as a reference strain. The 24 hours mortality recording showed that female *Anopheles gambiae* Kisumu which were exposed to WHO papers impregnated with dichlorodiphenyltrichloroethane (DDT) (4%), permethrin (0.75%), deltamethrin (0.05%), fenitrothion (1%) and bendiocarb (0.1%) 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 the field collected female *Aedes aegypti* populations from Mono department, they were resistant to dichlorodiphenyltrichloroethane (DDT) (4%), permethrin (0.75%) and deltamethrin (0.05%) with the mortality rates of 47%, 65% and 71% respectively. But, these female *Aedes aegypti* populations were susceptible to fenitrothion (1%) and bendiocarb (0.1%) with the mortality rates of 98% and 100% respectively (Table 2).

Table 2. Determination of resistance status in *Aedes aegypti* populations from Mono to DDT, permethrin, deltamethrin, fenitrothion and bendiocarb.

Populations	Insecticides	Number tested	% Mortality	Resistance status
Kisumu (Control)	DDT	100	100	S
	Permethrin	100	100	S
	Deltamethrin	100	100	S
	Fenitrothion	100	100	S
	Bendiocarb	100	100	S
Mono	DDT	100	47	R
	Permethrin	100	65	R
	Deltamethrin	100	71	R
	Fenitrothion	100	98	S
	Bendiocarb	100	100	S

S= susceptible
R= Resistant

DISCUSSION

In the current study, the field collected female *Aedes aegypti* populations from Mono department were resistant to dichlorodiphenyltrichloroethane (DDT), permethrin and deltamethrin. But, these female *Aedes aegypti* populations were susceptible to fenitrothion and bendiocarb. Our results corroborated with those obtained by Wang *et al* (2023) who had examined sensitivity/resistance to chlorpyrifos, fenitrothion, malathion, deltamethrin, permethrin, and β-cyfluthrin, along with possible metabolic detoxification and target site insensitivity, in three *Aedes aegypti* mosquito strains. The resistant strain (PR) had developed high levels of resistance to all three pyrethroid insecticides compared to a susceptible population, with 6, 500-, 3200- and 17,000-fold resistance to permethrin, β-cyfluthrin, and deltamethrin, respectively. A newly emerged *Ae. aegypti* population collected from St. Augustine, Florida (AeStA) showed elevated levels of resistance to malathion (12-fold) and permethrin (25-fold). Synergists DEF (S,S,S,-tributyl phosphorotrithioate) and DEM (diethyl maleate) showed no or minor effects on insecticide resistance in both the AeStA and PR^{G20}strains, but PBO (piperonyl butoxide) completely abolished resistance to both malathion and permethrin in AeStA and partially suppressed resistance in PR. The voltage-gated sodium channel sequences were examined to explore the mechanism that only partially inhibited the suppression of resistance to PBO in PR. Two mutations, V1016G/I and F1534C substitutions, both of which are associated with the development of pyrethroid resistance, were identified in the PR^{G20} strain but not in AeStA. These results suggest that while cytochrome P450 mediated detoxification may not be solely responsible, it is the major mechanism governing the development of resistance in AeStA. Both P450 mediated detoxification and target site insensitivity through the mutations in the voltage-gated sodium channel contribute to the high levels of resistance in the PR^{G20} strain.

Another study carried out by Fonseca-González *et al* (2011) had evaluated the insecticide susceptibility status of *Aedes aegypti* (L.) in Colombia, and as part of the National Network of Insecticide Resistance Surveillance, 12 mosquito populations were assessed for resistance to pyrethroids, organophosphates and DDT. Bioassays were performed using WHO and CDC methodologies. The underlying resistance mechanisms were investigated through biochemical assays and RT-PCR. Their results showed that all mosquito populations were susceptible to malathion, deltamethrin and cyfluthrin, and highly resistant to DDT and etofenprox. Resistance to lambda-cyhalothrin, permethrin and fenitrothion ranged from moderate to high in some populations from Chocó and Putumayo states. In Antioquia state, the Santa Fe population was resistant to fenitrothion. Biochemical assays showed high levels of both cytochrome P450 monooxygenases (CYP) and non-specific esterases (NSE) in some of the fenitrothion- resistant and pyrethroid-resistant populations. All populations showed high levels of glutathione-*S*-transferase (GST) activity. *GSTe2* gene was found overexpressed in DDT-resistant populations compared with Rockefeller susceptible strain. Their results provide baseline data on insecticide resistance in Colombian *Aedes aegypti* populations, and will allow comparison of changes in susceptibility status in this vector over time.

CONCLUSION

There is cross-resistance to both pyrethroid and organochlorine insecticides**.** However, the physical barrier of Long-Lasting Insecticidal Nets (LLINs) which are frequently distributed free by Beninese National Malaria Control Program 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 vector bites.

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