Original Article

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

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ABSTRACT

Introduction: *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.

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

Materials & Methods: Larvae and pupae of *Aedes aegypti* populations were collected from the breeding sites in Couffo 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%).

Results: 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.

Conclusion: The physical barrier of Long Lasting Insecticidal Nets (LLINs) which are regularly 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.

INTRODUCTION

Aedes aegypti (Linnaeus), also known as the yellow fever mosquito, is the primary vector of chikungunya, dengue, yellow fever, and Zika viruses[1,2]. These arboviruses cause significant morbidity and mortality and incur billions of dollars in healthcare costs each year[3]. Half of the world's population live in dengue endemic areas with 50-100 million infections estimated annually worldwide[4]. *Aedes aegypti* and the viruses that they transmit have expanded into new geographic territories with the increase in global movement of people and goods. Zika and chikungunya viruses have spread throughout the Americas, while yellow fever virus was recently reported in China and resurged in Central Africa[5-10].

Insecticides utilization is considered as the most efficient tools in vector control programs. Insecticide applications can thereby vary from aerosol-space spraying, coils, lotions, clothes, or curtains embedded with certain active insecticide compounds and mass fogging to usage of larvicides in breeding waters. Consequences of national policy breakdown by usage of massive insecticide-based controls with the same active compounds might result in insecticide resistance development. As such, *Ae. aegypti* resistance development to commonly used insecticides has been reported from different countries worldwide such as Colombia[11], Brazil[12], Grand Cayman[13], Thailand[14], India[15], Malaysia[16], Mexico[17], and China[18]. Consistently to these findings, it is previously reported on urban *Ae. aegypti* resistance development in the cities Denpasar[19] and Jakarta[20] and evidencing increased resistance to commonly used insecticides in Indonesia.

Pyrethroids are synthetic analogs of pyrethrin. Similar to DDT, type I pyrethroids (permethrin, tetramethrin, allethrin, and phenothrin) interfere with the function of VGSCs to prolong neurotransmission and paralyze mosquitoes [21-23]. Type-II pyrethroids that contain an α-cyano group (cyfluthrin, cyhalothrin, deltamethrin, and cypermethrin) induce choreoathetosis–salivation syndrome by modulating GABA receptors[24]. GABA binds $GABA_A$ receptors on ligand-gated chloride ion channels, which control the chloride (Cl^-) influx that maintains the membrane potential of neurons. The $GABA_A$ receptor is the target of type-II pyrethroids. When the receptor is blocked by type-II pyrethroids, Cl^- influx and inhibitory functions are prevented, resulting in convulsions and death[25].

Carbamate insecticides are derivatives of carbamic acid. Carbamate insecticides, such as carbaryl, carbofuran, propoxur, and aldicarb, exhibit effects similar to OPs by inhibiting AChE activity [26]. Unlike OPs, carbamates can be rapidly metabolized by mosquitoes[27]. The major challenge

of utilizing chemical control is the emergence of insecticide resistance in the targeted populations.

Very few researches were published on insecticide resistance status in *Aedes aegypti* mosquito populations from Couffo 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 Couffo 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 Couffo. Couffo 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 Couffo department *and its implications for* dengue *control* 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.

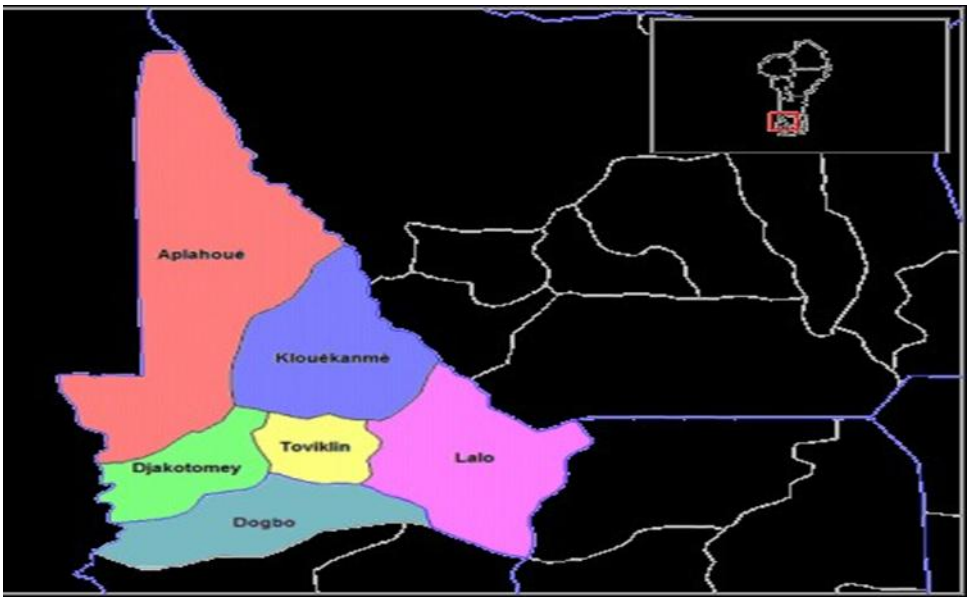

Figure 1: Map of Republic of Benin showing the six districts surveyed in Couffo department

Mosquito sampling

Aedes aegypti populations were collected from March to July 2023 during the great rainy season in Couffo 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 separate 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[28]. 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[28].

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 [29] 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 Couffo with regard to *Anopheles gambiae s.l.* Kisumu susceptible reference strain were 2.579, 1.629 and 1.898 respectively for DDT, permethrin and deltamethrin. These resistance ratios (RR50) were all higher than 1 (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	23.287	-	35. 123	-
	Permethrin	31.893	-	50.875	-
	Deltamethrin	39.678	-	43.206	-
	Fenitrothion	Nd	-	Nd	-
	Bendiocarb	Nd	-	Nd	-
Couffo	DDT	60.075	2.579	74.683	2.126
	Permethrin	51.985	1.629	73.456	1.443
	Deltamethrin	75.334	1.898	84.487	1.955
	Fenitrothion	Nd	-	Nd	-
	Bendiocarb	Nd	-	Nd	-

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

Table 2: Determination of resistance status in *Aedes aegypti* populations from Couffo 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
Couffo	DDT	100	34	R
	Permethrin	100	57	R
	Deltamethrin	100	45	R
	Fenitrothion	100	99	S
	Bendiocarb	100	100	S

S= susceptible; **R=** Resistant

The same remark was made with the resistance ratios (RR95). They were 2.126, 1.443 and 1.955 respectively for DDT, permethrin and deltamethrin. These results showed that the populations of *Aedes aegypti* from Couffo were resistant to these products (Table 1).

Mortality of *Aedes aegypti* populations from Couffo 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 Couffo department, they were resistant to dichlorodiphenyltrichloroethane (DDT) (4%), permethrin (0.75%), deltamethrin (0.05%) with the mortality rates of 34%, 57% and 45% respectively. But, these female *Aedes aegypti* populations were susceptible to fenitrothion (1%) and bendiocarb (0.1%) with the mortality rates of 99% and 100% respectively (Table 2).

DISCUSSION

Aedes aegypti represents the principal vector of many arthropod-borne diseases in tropical areas worldwide. Since mosquito control strategies are mainly based on use of insecticides, resistance development can be expected to occur in frequently exposed *Ae. aegypti* populations. Surveillance on resistance development as well as testing of insecticide susceptibility is therefore mandatory and needs further attention by national/international public health authorities.

In the current study, the field collected female *Aedes aegypti* populations from Couffo department were resistant to dichlorodiphenyltrichloroethane (DDT), permethrin and deltamethrin, but susceptible to fenitrothion and bendiocarb. Our study corroborated with that carried by Al-Amin *et al*[30] who had investigated on insecticide resistance status of *Aedes aegypti* in Bangladesh. In fact, arboviral diseases, including dengue and chikungunya, are major public health concerns in Bangladesh where there have been unprecedented levels of transmission reported in recent years. The primary approach to control these diseases is to control the vector *Aedes aegypti* using pyrethroid insecticides. Although chemical control has long been practiced, no comprehensive analysis of *Aedes aegypti* susceptibility to insecticides has been conducted to date. The aim of their study was to determine the insecticide resistance status of *Aedes aegypti* in Bangladesh and investigate the role of detoxification enzymes and altered target site sensitivity as resistance mechanisms. For that, eggs of Aedes mosquitoes were collected using ovitraps from five districts across Bangladesh and in eight neighborhoods of the capital city Dhaka, from August to November 2017. CDC bottle bioassays were conducted for permethrin, deltamethrin, malathion, and bendiocarb using 3 to 5 day old F0–F2 non-blood-fed female mosquitoes. Biochemical assays were conducted to detect metabolic resistance mechanisms, and real-time PCR was performed to determine the frequencies of the knockdown resistance (kdr) mutations Gly1016, Cys1534, and Leu410. Their results showed high levels of resistance to permethrin were detected in all *Aedes aegypti* populations, with mortality ranging from 0 to 14.8% at the diagnostic dose. Substantial resistance continued to be detected against higher (2×) doses of permethrin (5.1-44.4% mortality). Susceptibility to deltamethrin and malathion varied between populations while complete susceptibility to bendiocarb was observed in all populations. Significantly higher levels of esterase and oxidase activity were detected in most of the test populations as compared to the susceptible reference Rockefeller strain. A significant association was detected between permethrin resistance and the presence of Gly1016 and Cys1534 homozygotes. The frequency of kdr (knockdown resistance) alleles varied across the Dhaka Aedes populations. Leu410 was not detected in any of the tested populations. The detection of widespread pyrethroid resistance and multiple resistance mechanisms highlights the urgency for implementing alternate *Aedes aegypti* control strategies. In addition, implementing routine monitoring of insecticide resistance in *Aedes aegypti* in Bangladesh will lead to a greater understanding of susceptibility trends over space and time, thereby enabling the development of improved control strategies.

Another study carried out by Hamid *et al*[31] had investigated on *Aedes aegypti* resistance development towards several often-used insecticides, such as malathion, deltamethrin, permethrin, λ-cyhalothrin, bendiocarb, and cyfluthrin, in the periurban area of Banjarmasin city, Kalimantan, Indonesia. Their results clearly showed resistance development of *Aedes aegypti* populations against tested insecticides. Mortalities of *Aedes aegypti* were less than 90% with the highest resistance observed against 0.75% permethrin. Collected mosquitoes from Banjarmasin also presented high level of resistance development to 0.1% bendiocarb. Molecular analysis of voltage-gated sodium channel (*Vgsc*) gene showed significant association of V1016G gene point mutation in resistance *Aedes aegypti* phenotypes against 0.75% permethrin. However, F1534C gene point mutation did not correlate to *Aedes aegypti* insecticide resistance to 0.75% permethrin. Irrespective of periurban areas in Kalimantan considered as less densed island of Indonesia, *Aedes aegypti*-derived resistance to different routinely applied insecticides occurred. The findings of these authors evidence that *Aedes aegypti* insecticide resistance is most likely spreading into less populated areas and thus needs further surveillance in order to delay *Aedes aegypti* resistance development.

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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