

Original Research Article

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Comparison of Two Mosquito Samples in Resistance Monitoring to Permethrin in Malaria Vectors from Mono Department in South-Western Benin, West Africa

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ABSTRACT

Pyrethroids are the only group of insecticides currently recommended for net treatment. Resistance monitoring is essential to investigate the susceptibility of wild populations of *An. gambiae s.l.* to pyrethroids. We investigated the kind of mosquito sample useful in the assessment of insecticide susceptibility tests in malaria vectors in the field conditions. Two mosquito samples were used. The first sample concerned larvae and pupae collected using the dipping method on several breeding sites and the second sample concerned female *An. gambiae s.l.* mosquitoes collected from window traps put on windows of rooms in districts surveyed. female *An. gambiae s.l.* mosquitoes were collected from March-July and August-November 2014 during the rainy season in Grand Popo, Comè, Lokossa districts selected in south-western Benin. WHO bioassays were performed with impregnated papers of permethrin 0.75% with the first sample whereas CDC bioassays were performed with stock solution of permethrin 21.5 µg per bottle with the second sample. The current study showed that although two different mosquito samples were used with two different protocols for the determination of insecticide susceptibility in malaria vectors, the susceptibility status to permethrin recorded with WHO and CDC methods were the same. Otherwise, female *An. gambiae s.l.* populations from Grand Popo, Comè, Lokossa were resistant to permethrin. However, the use of female *An. gambiae s.l.* mosquitoes collected using the dipping method on several breeding sites may be better than using samples from window traps in the assessment of insecticide susceptibility tests in malaria vectors in field conditions.

Keywords

Mosquito samples,
Window traps,
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Introduction

Malaria is a major public health problem and *Anopheles gambiae* is one of the major vectors

of this disease in sub-Saharan Africa (Gillies and Coetzee, 1987). *Anopheles gambiae* Giles (Diptera: Culicidae) is so the major malaria vector in West Africa. Resistance monitoring

is essential to investigate the susceptibility of wild populations of *An. gambiae s.l.* to pyrethroids.

Pyrethroids are the only group of insecticides currently recommended for net treatment. The determination of insecticide susceptibility status of the target vectors will help monitor the insecticidal efficacy and possible development of resistance at early stages. So, the early detection of insecticide resistance development is the most important aspect that guides vector control programmes. More recently, the emergence of resistance in populations of *An. gambiae* to common classes of insecticides used in public health has been reported in Benin (Djogbénu *et al.*, 2008; 2009; Djègbé *et al.*, 2011; Aizoun *et al.*, 2013a; 2013b; 2013c; 2014a; 2014b; 2014c; 2014d; 2014e, 2014f; 2014g; 2014h; 2014i; 2014j).

The ongoing spread of insecticide-resistant genes, such as the well-characterized *kdr* mutations (Martinez-Torres *et al.*, 1998; Ranson *et al.*, 2000) in populations of the major African malaria vectors, *An. gambiae*, can seriously jeopardize the efficacy of vector control programmes (Aizoun *et al.*, 2014a). Metabolic resistance or biochemical mechanisms that involve the detoxifying enzymes was also involved in resistance of *An. gambiae s.l.* populations from Benin (Aizoun *et al.*, 2013a; 2013b; 2014b; 2014c; 2014d).

OlysetNet distribution was made free in July, 2011 throughout the entire country including Mono department by Beninese National Malaria Control Programme to increase coverage of long-lasting insecticidal nets (LLINs). So, people living in areas targeted for the distribution of these long-lasting insecticidal nets, OlysetNet had received them. The insecticide susceptibility status is major factor determining what insecticides

should be used in the control campaigns and for that, its determination needs mosquito sample.

The aim of this study is to investigate the kind of mosquito sample useful in the assessment of insecticide susceptibility tests in malaria vectors in the field conditions.

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 Grand Popo, Comè, Lokossa districts. 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. These factors have an impact on resistance development in the local vector mosquitoes. We took them into account to compare both mosquito samples with regard to the resistance level. Mono has a climate with four seasons, two rainy seasons (March-July and August-November) and two dry seasons (November-March and July-August). The temperature ranges from 25 to 30°C with the annual mean rainfall between 900 and 1100 mm.

Mosquito sampling

The first sample concerned *An. gambiae s.l.* mosquitoes collected from March-July and August-November 2014 during the rainy season in Grand Popo, Comè, Lokossa districts selected in south-western Benin. Larvae and pupae were collected in these districts within both padding and town 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). Once, larvae and pupae collected, they were then kept in labeled bottles related to the districts surveyed. Otherwise, larvae collected from multiple breeding sites were pooled together 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 Centre de Recherche Entomologique de Cotonou (CREC) located in Akpakpa, in Cotonou district. These samples were reared up to adult emergence at the CREC insectary. *An. gambiae* Kisumu, a reference susceptible strain was used as a control for the bioassay tests. We used Kisumu more precisely to confirm the quality of treated or impregnated papers. Susceptibility tests were done following WHO protocol on unfed females mosquitoes aged 2-5 days old reared from larval and pupal collections. All WHO susceptibility tests were conducted in the CREC laboratory at 25±2°C and 70 to 80% relative humidity.

The second sample concerned *An. gambiae s.l.* mosquitoes collected in window traps put on windows of four rooms in each district surveyed. Between 6.00 a.m. to 7.00 a.m., aspirators were used to collecting mosquitoes from these window traps. They were then put in some plastic cups covered with small cutting untreated net on which was put cotton wool moistened with a 10% honey solution. Female *An. gambiae* species were morphologically identified using morphological keys (Gillies and De Meillon, 1968) and then transferred into mosquito cages. *An. gambiae* Kisumu, a reference susceptible strain was carried out from Centre de Recherche Entomologique de Cotonou (CREC) insectary to mono department and used as a control for the bioassay tests. We used Kisumu more precisely to confirm the

quality of treated Wheaton bottles. All Susceptibility tests were done following CDC protocol on unfed female *An. gambiae s.l.* mosquitoes.

Testing insecticide susceptibility

WHO Protocol

Females *An. gambiae s.l.* aged 2 to 5 days old were exposed to WHO diagnostic dosage of permethrin 0.75% according to the WHO protocol (WHO, 2013). Thus, an aspirator was used to introduce 20 to 25 unfed female mosquitoes 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 10, 20, 30, 40, 50, 60 minutes and mortalities at 24 hours were recorded following the WHO protocol (WHO, 2013). The choice of permethrin was justified by OlysetNet distribution made free in July, 2011 throughout the entire country by Beninese National Malaria Control Programme to increase coverage of long-lasting insecticidal nets (LLINs).

CDC protocol

The principle of CDC bottle bioassay is to determine the time it takes an insecticide to penetrate an arthropod, traverse its intervening tissues, get to the target site, and act on that site relative to a susceptible control. Anything that prevents or delays the compound from achieving its objective of killing the arthropods contributes to resistance. Diagnostic dose that was applied in the current study was the dose recommended by CDC (Brogdon and Chan, 2010). These doses were

checked on the *An. gambiae* Kisumu susceptible reference strain before being applied to field populations. For *An. gambiae s.l.*, the diagnostic dose of 21.5 µg per bottle for permethrin was used for a diagnostic exposure time of 30 minutes. The solution was prepared and the bottles coated according to the CDC protocol (Brogdon and Chan, 2010).

Then, these coated Wheaton bottles was carried out from Centre de Recherche Entomologique de Cotonou (CREC) laboratory to mono department. Fifteen to 20 unfed female *An. gambiae s.l.* mosquitoes were introduced into four 250 ml Wheaton bottles coated with permethrin and one control bottle coated with acetone only.

The number of dead or alive mosquitoes was monitored at different time intervals (15, 30, 35, 40, 45, 60, 75, 90, 105, 120 minutes). This allowed us to determine the total percent mortality (Y axis) against time (X axis) for all replicates using a linear scale.

Statistical analysis

The resistance status of the first mosquito sample was determined according to the latest WHO criteria (WHO, 2013) 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

The resistance status of the second mosquito sample was determined according to the CDC criteria (Brogdon and McAllister, 1998; Brogdon and Chan, 2010). The susceptibility threshold at the diagnostic time of 30 minutes for pyrethroids is:

Mortality rate = 100%: the population is fully susceptible

Mortality rate < 100%: 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 either the test-tubes or test-bottles because the mortality rates in all controls was always less than 5% (Abbott, 1987). Analysis using Fisher's exact test and test of proportion was performed on the data sets gathered from the localities surveyed to compare the tested mosquito samples and assess the insecticide resistance status of each tested *An. gambiae* population using WHO method for the first sample and CDC method for the second sample.

Results and Discussion

The analysis of table 1 showed that all female mosquitoes of *Anopheles gambiae* Kisumu which were exposed to WHO papers impregnated with permethrin 0.75% were knocked-down after 30 minutes whereas a non-neglected proportion of *An. gambiae s.l.* Grand Popo, Comè and Lokossa populations; 62.9%, 54.5% and 85.7% respectively continue again to fly in the WHO cylinder plastic tubes which contained these impregnated papers. The analysis of table 2 showed that after 24 hours mortality recording Kisumu strain (control) confirmed its susceptibility status as a reference strain whereas *An. gambiae s.l.* Grand Popo, Comè and Lokossa populations were resistant to permethrin 0.75%. According to Zaim *et al.*, (2000), pyrethroids have unique modes of action such as fast knockdown and excito-repellent effects.

In the same way, the analysis of table 1 shows that all female mosquitoes of *Anopheles gambiae* Kisumu which were exposed to CDC

bottles treated with permethrin 21.5µg/bottle were died after 30 minutes, which represents susceptibility threshold time or diagnostic time clearly defined by CDC protocol. That showed Kisumu strain (control) confirmed its susceptibility status as a reference strain. A non-neglected proportion of *An. gambiae s.l.* Grand Popo, Comè and Lokossa populations; 13,3%, 15% and 11,1% respectively after 30 minutes exposure to CDC bottles treated with permethrin, continue again to fly in these bottles. That showed these populations were resistant to this product.

Although two different mosquito samples were used with two different protocols for the determination of insecticide susceptibility in malaria vectors, the susceptibility status to permethrin recorded with WHO and CDC

methods were the same (Table 2). The mortality rates recorded with CDC method were slightly higher than those obtained with WHO method (Table 2). This slight increase should be explained by the age of the second sample used. Otherwise, the age of mosquitoes which entered the rooms by the doors or others windows where traps were not put, was not known. Then, these mosquitoes entered the window traps by going out through windows early in the morning.

They were likely old mosquitoes. In fact, a previous study by Aizoun *et al.*, (2014k) showed that the mortality rate obtained when female *An. gambiae s.l.* Sekandji populations were unfed and aged 20 days old was higher than the one obtained when these populations were unfed and aged 2-5 days old.

Table.1 Knock-down at 30 minutes with WHO method and mortality at the susceptibility threshold with CDC method

Populations	Permethrin			
	WHO		CDC	
	Number tested	% Kd at 30 min	Number tested	% Mortality at 30 min
Kisumu (Control)	100	100	100	100
Grand Popo	100	37.1	19	86.7
Comè	100	45.5	22	85
Lokossa	100	14.3	14	88.9

Table.2 Susceptibility status to permethrin in *Anopheles gambiae s.l.* populations

Populations	Permethrin					
	WHO		CDC		WHO	CDC
	Number tested	% Mortality	Number tested	% Mortality	Resistance status	Resistance status
Kisumu (Control)	100	100	100	100	S	S
Grand Popo	100	73.7	19	86.7	R	R
Comè	100	72.7	22	85	R	R
Lokossa	100	78.6	14	88.9	R	R

A previous study which used the same mosquito sample or female *An. gambiae s.l.* mosquitoes collected by using the dipping method on several breeding sites, with both WHO and CDC methods also showed the same susceptibility status to insecticides (Aizoun *et al.*, 2013a). The age of tested female *An. gambiae s.l.* mosquitoes collected in the window traps put on windows of four rooms in each district surveyed was not known whereas the age of tested female *An. gambiae s.l.* mosquitoes collected by 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) was known. But, that had no impact on susceptibility results recorded with both methods in the current study. However, Aizoun *et al.*, (2014k), showed that many factors influence vector susceptibility to insecticide. Among these factors, there are mosquito sex, physiological status and mosquito age. That is for this reason, it is useful to respect the WHO criteria in the assessment of insecticide susceptibility tests in malaria vectors. Otherwise, susceptibility testing is conducted using unfed female mosquitoes aged 3-5 days old. Tests should also be carried out at (25+/-2) °C and (80+/-10) % relative humidity ((WHO, 2013). In the current study, the temperature recorded during the assessment of insecticide susceptibility tests on the field or in Mono department ranged from 25 to 30°C whereas the relative humidity ranged from 70 to 80%. Sometimes, when the insectary is very far from the field or localities surveyed, it is useful that the collected larvae and pupae are reared up to adult emergence on the field but that is not easy because of field conditions. This way to process may be better than using samples from window traps. That also depend on the number of consecutive days the surveys take on the field. For that, Aizoun *et al.*, (2014l), had studied the efficacy of a

WHO impregnated paper with bendiocarb in field conditions.

The current study shows that mosquito samples from dipping method on several breeding sites or larvae and pupae collections and those from the window traps put on windows of rooms in districts surveyed gave similar results with regard to susceptibility status to permethrin recorded with both WHO and CDC methods. However, the use of the mosquito samples from larvae and pupae collections may be better than using samples from window traps in the assessment of insecticide susceptibility tests in malaria vectors in field conditions.

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