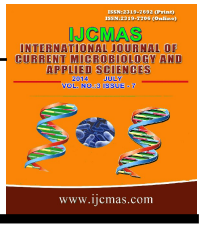
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 9 (2014) pp. 224-229**
http://www.ijcmas.com



Original Research Article

Propoxur resistance in *Anopheles gambiae s.l.* populations from N'dali district in northern Benin, West Africa

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A B S T R A C T

Because of the Indoor Residual Spraying (IRS) with carbamate in northern part of the country since 2011, it is important to investigate susceptibility to propoxur, a carbamate compound in *Anopheles gambiae s.l.* populations from surrounding departments of Atacora department in northern Benin. Larvae and pupae of *Anopheles gambiae s.l.* mosquitoes were collected from the breeding sites in Borgou department. WHO susceptibility tests were conducted on unfed female mosquitoes aged 2–5 days old. WHO bioassays were performed with impregnated papers with propoxur 0.1%. PCR techniques were used to detect species and *Ace-1* mutations. *Anopheles gambiae* N'dali populations were resistant to propoxur. The mortality rate observed was 89.53%. PCR revealed that all specimens tested were *Anopheles gambiae s.s.* The presence of *Ace-1R* at very low frequency (0.01) was observed in *Anopheles gambiae* N'dali populations. This study shows that propoxur resistance detected in *An. gambiae* populations from N'dali was first in this district. Therefore, these populations of *An. gambiae* need to be monitored for insecticide resistance in this area.

Keywords

Anopheles gambiae, Ace-1, Resistance, propoxur, Benin

Introduction

National programmes reported that 135 million people representing 4% of the global population at risk were protected by IRS in 2012. The proportion of the population protected by IRS increased substantially in the African Region during 2006–2008, and the increased coverage was maintained during 2009–2011, at 10%–12% of the population at risk. In 2012, a total of 58 million people, or 8% of the population at risk, were protected (WHO, 2013a). IRS involves the application of residual insecticides to the inner surfaces of dwellings targeting *Anopheles* mosquitoes that rest on walls after having taken a blood meal. IRS programmes can rapidly reduce local malaria incidence and mortality, provided that most houses and animal shelters in targeted communities are sprayed. WHO recommends the spraying of at least 80% (and ideally100%) of houses, structures and units in the targeted area in any round of spraying (WHO, 2013b).

Achieving universal coverage with effective vector control interventions requires timely and sustained programme-delivery operations. In turn, this requires specialized personal at national, provincial, district and community levels. These teams are best achieved through free mass distribution campaigns every 3 years or less. However, to ensure that coverage is maintained, it is essential to complement these campaigns with continuous distribution programmes (e.g. through antenatal and routine immunization services) before, during and after mass campaigns (WHO, 2013a).

Organophosphates and carbamates (OP and CX) insecticides are competitive inhibitors that irreversibly inhibit the AChE enzyme, blocking nervous transmission and leading to the death of the insect. So, acetylcholinesterase is a key enzyme in the nervous system, terminating nerve impulses by catalysing the hydrolysis of the neurotransmitter acetylcholine. It (AChE) is the major target for organophosphate (OP) and carbamate insecticides, which inhibit enzyme activity by covalently phosphorylating or carbamylating the serine residue within the active site gorge (Corbett JR, 1974).

The Benin National Malaria Control Programme has implemented indoor residual spraying (IRS) campaign under the financial support of the PMI (President's Malaria Initiative) using bendiocarb in the north of the country since 2011. The same product was previously used to control *Anopheles gambiae s.l.* populations from Ouemé department in southern Benin (2008–2010).

The present study proposes was to assess the resistance status of malaria vectors from N'dali to propoxur. Moreover, this study evaluated the presence of the *ace-1R* mutation within and among these *An. gambiae s.l.* populations in the north Benin.

Materials and Methods

Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Borgou. Borgou department is located in the north of Benin and the study was carried out more precisely in N'dali district, a rice growing area. 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. The northern zone (N'dali) is characterized by a Sudanian climate with only one rainy season per year (May to October) and one dry season (November–April). The temperature ranged from 22 to 33°C with the annual mean rainfall of 1,300 mm.

Mosquito collection

An. gambiae s.l. mosquitoes were collected from March to July 2012 during the rainy season in N'dali district selected in the northern part of the country. *Anopheles* pre-imaginal stages (L1 to L4 instars) were collected via ladles within rice farms from N'dali using the dipping method on several breeding sites. Once, larvae and pupae collected, they were then kept in labeled bottles related to the N'dali district 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. The 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. 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 CREC laboratory at 25+/-2°C and 70 to 80% relative humidity.

Testing insecticide susceptibility

Females *An. gambiae* aged 2 to 5 days old were exposed to WHO diagnostic dosage of propoxur 0.1% according to the WHO protocol (WHO, 1998). 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 60 minutes and mortalities at 24 hours were recorded following the WHO protocol (WHO, 1998). Dead and surviving mosquitoes were separately stored in individual tubes with silicagel and preserved at -20°C in the laboratory, for further molecular characterization. We used propoxur, an insecticide of same class as bendiocarb to check if there was already resistance to this product in the district surveyed.

PCR detection of species and *Ace-1* mutations

Specimens of *An. gambiae* from the WHO bioassay tests were subjected to the *An. gambiae* species specific PCR assays for species identification (Scott *et al.*, 1993). The PCR-Restricted Fragment Length Polymorphism (PCR-RFLP) diagnostic test was used to detect the presence of G119S mutation (*ace.1R* gene) as described by Weill *et al.* (2003). Mosquito genomic DNA was amplified using the primers Ex3AGdir 5′GATCGTGGACACCG TGTTCG3′ and Ex3AGrev 5′AGGAT GGCCCGCT GGAA CAG3′according to Weill *et al.* (2003). One microlitre of total DNA extracted from a single mosquito was used as a template in a 25 µl PCR reaction containing Taq DNA polymerase buffer, 0.2 mM dNTP and 10 pmol of each primer. The PCR conditions were 94°C for 5 min and then 35 cycles of (94°C for 30 s, 54°C for 30 s and 72°C for 30 s) with a final 5 min extension at 72°C. Fifteen microlitres of PCR product were digested with 5U of AluI restriction enzyme (Promega) in a final volume of 25 µl. The PCR fragments were fractionated on a 2% agarose gel stained with ethidium bromide and visualized under UV light.

Statistical analysis

The resistance status of mosquito samples was determined according to the latest WHO criteria (WHO, 2013c) 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 all control tubes was less than 5% (Abbott, 1987).

To compare the status of insecticide resistance, Fisher's exact test was carried out to determine if there was any significant difference between mortality rates of

populations of *An. gambiae s.s.* of districts using Statistica 6.0.

Allelic frequency of G119S mutation was analysed using the version 1.2 of Genepop (Raymond and Rousset, 1995). The software R-2.15.2 (R Development Core Team, 2011) was used for the statistical analysis.

Ethical approval

This study was approved by the Ministry of Health and the Center for Entomological Research of Cotonou, Benin.

Result and Discussion

Susceptibility of *An. gambiae s.l.* populations to propoxur

Kisumu strain (control) confirmed its susceptibility status with 100% mortality as a reference strain. All female mosquitoes of *Anopheles gambiae* Kisumu, which were exposed to WHO papers impregnated with propoxur 0.1% showed a total mortality with no survivors after 24 hours mortality recording. This result confirmed that they were susceptible to this product. Regarding *An. gambiae* N'dali populations, they were resistant to propoxur 0.1% with the mortality rate of 89.53% (Table 1).

Species of *Anopheles gambiae* and *Ace-1* genotype

PCR revealed 100% of mosquitoes tested were *Anopheles gambiae s.s.* The frequency of *Ace-1R* in *Anopheles gambiae* N'dali was 1% (Table 2).

Acetylcholinesterase is a key enzyme in the nervous system, terminating nerve impulses by catalysing the hydrolysis of the neurotransmitter acetylcholine. It (AChE) is the major target for organophosphate (OP) and carbamate insecticides, which inhibit enzyme activity by covalently phosphorylating or carbamylating the serine residue within the active site, gorge (Corbett, 1974).

An. gambiae N'dali populations were resistant to propoxur in northern part of the country. According to N'Guessan *et al.* (2003), *An. gambiae* breeds in rice fields (at early stage of the cultivation) and habitually rests in houses of urban and peri-urban areas, and it may be exposed to considerable selection pressure by agricultural insecticides and/or domestic aerosols and mosquito coils. Carbamate resistance was also recently reported in *An. gambiae* populations from Atacora department in north-western Benin (Aïkpon *et al.*, 2013; Aïzoun *et al.*, 2013a) with the implication of biochemical resistance mechanisms mainly the esterases activity in bendiocarb resistance (Aïzoun *et al.*, 2013b).

In addition, Djogbenou *et al.* (2008, unpublished data) has shown that *Anopheles gambiae* Kandi populations from Alibori department in northern Benin were also resistant to carbosulfan, a carbamate, with a mortality rate of 73%. There are no previous published studies about the resistance status of *An. gambiae* populations from N'dali district to carbamates until the current study was carried out. Therefore, these populations of *An. gambiae* need to be monitored for insecticide resistance in this area.

Propoxur resistance in *An. gambiae* N'dali populations was corroborated with *Ace-1* mutation frequency as it was 1%. This result confirmed that there was really resistance of these populations to this carbamate compound.

Table.1 Percentage of dead *Anopheles* mosquitoes observed after 1hour exposure to WHO papers impregnated with propoxur in N'dali district

Population	Insecticide	Number tested	% Mortality	Resistance status
Kisumu (Control)	Propoxur	101	100	S
N'dali	Propoxur	96	89.53	R

Table.2 *Ace-1* mutation frequency in *An. gambiae* populations issue from WHO bioassays tests

			Ace-1 mutation			
Locality	Number tested	Species Ag	RR	RS	SS	F(*Ace-1*)
N'dali	49	49	0	1	48	0.01

Ag: *An. gambiae s.s.*

In the current study, PCR revealed that 100% of mosquitoes from N'dali tested were *Anopheles gambiae s.s.* No *An. arabiensis* mosquitoes were found. Conversely, Djogbenou *et al.* (2008, unpublished data) has shown that *Anopheles arabiensis* populations were also present in N'dali in the proportion of 1.2% within *An. gambiae* complex. This result showed that *An. arabiensis* populations from N'dali district tend to decline after four years. That could likely be due to the susceptibility of these populations to carbamates four years ago.

This study shows that propoxur resistance detected in *An. gambiae* populations from N'dali was first in this district. Therefore, these populations of *An. gambiae* need to be monitored for insecticide resistance in this area.

Acknowledgements

We are grateful to the President's Malaria Initiative of the U.S. Government through USAID which supported in part this study. We are also grateful to the Ministère de l'Enseignement Supérieur et de la Recherche Scientifique (MESRS) of Benin which supported the second part of this study and the doctoral training of Nazaire. The authors would like to thank Frederic OKE-AGBO for statistical analysis and Damien TODJINOU for providing technical assistance.

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