

INSECTICIDE RESISTANCE STATUS OF *ANOPHELES GAMBIAE* BREEDING IN STAGNANT WATER BODIES IN LAGOS, NIGERIA

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Abstract

Anopheles gambiae, the major Afro-tropical malaria vector is resistant to pyrethroid insecticide and this is threatening the impact of malaria control efforts using insecticides.

In this study, the major malaria vector species from stagnant water bodies and their susceptibility status to pyrethroid insecticide was determined in some areas of Lagos state, Nigeria. *Anopheles* larvae were sampled from three areas of Lagos, namely Akoka, Idiaraba and Okobaba and raised to adulthood in the insectary. Two to three days old adult female mosquitoes were exposed to 0.75% permethrin and 0.05% lambda-cyhalothrin using the World Health Organization (WHO) insecticide susceptibility test kits. All samples exposed were identified morphologically and by Polymerase chain reaction (PCR) using the *Anopheles gambiae* species specific PCR assay. All mosquito samples tested belong to the *A. gambiae* complex. The PCR assay showed a mixture of *A. gambiae* and *A. arabiensis*: (32%: 68%) at Akoka, (51%: 49%) at Idiaraba, and 100% *A. arabiensis* at Okobaba. The 24 hr post exposure mortality rates in permethrin and lambda-cyhalothrin were 60% and 100% at Akoka, 49% and 60% at Idiaraba, 69% and 71% at Okobaba respectively. The Knock down time, KDT_{50} and KDT_{95} (Time taken for 50% and 95% of the test population to be knocked down) for the entire sample tested was between 23-31 minutes and >1hr respectively. This result showed that the *A. gambiae* s.l used were more resistant to permethrin than to lambda-cyhalothrin. Although *Anopheles* resistance to permethrin has been reported in other sites in Lagos, this is the first report on resistance of *Anopheles gambiae* s.s to lambda-cyhalothrin in Nigeria. This study also provides early evidence that *A. gambiae* is adapting to stagnant water. The level of insecticide resistance is a concern and could be of interest in the epidemiology of urban malaria and hopefully a target for larviciding.

Key words: *Anopheles gambiae*, stagnant water, insecticide resistance, Lagos, Nigeria.

INTRODUCTION

Malaria is a disease of public health importance affecting an annual estimate of 216 million people globally (WHO, 2011). 174 million (81%) cases were reported in Africa region and global annual mortality rate stands at 655 000 with Africa having the highest rate of 596 000 (91%). This figures shows a reduction in malaria transmission. There have been reports of reduction in malaria cases of more than 50% between 2000 and 2010 in 43 of the 99 countries with ongoing transmission, while downward trends of 25%–50% were seen in 8 other countries (WHO, 2011). However approximately 86% of malaria deaths globally were of children under 5 years of age. Malaria is responsible for much of the disease burden in Nigeria with more than 85% of the approximately 140 million at risk. It accounts for 30% of deaths in children under the age of 5 and 11% maternal mortality (FMOH, 2005).

In the absence of a much needed malaria vaccine, vector control remains a major component of the global strategy for reducing malaria related deaths. Vector control interventions such as the use of insecticide treated net (ITNs) and indoor residual spray (IRS) are the major strategies of the Roll back malaria (RMB) program to fight malaria (Scott *et al.*, 2005). Most vector control interventions rely on the use of pyrethroid insecticides and insecticide resistance remains a major challenge in Africa as it is reported to threaten the effectiveness of malaria vector control programs (N'Guessan, *et al.*, 2007). Furthermore *A. gambiae* is adapting to polluted water bodies in urban settings and this could account for the rise in incidence of urban malaria as literature has shown that samples collected from water polluted with petroleum

products show a high resistance to insecticide (Rousseau *et al.*, 2007).

The ability of the mosquito vector to adapt to polluted waters around human habitat is dangerous as this could serve as potential breeding grounds for the vector which can increase malaria transmission. Urban malaria cases are becoming common in Africa as more people move into cities and industrialization proceeds. Contrary to previous reports that city centers are less likely to harbor malaria mosquitoes as a result of decreased open space and improved socioeconomic conditions (Keating *et al.*, 2004), poor housing, lack of sanitation inadequate drainage, stagnant water in areas around human habitation and pollution of surface water have increased mosquito breeding and human vector contact creating a unique challenge for malaria vector control (Keiser *et al.*, 2004). This study reports the breeding and resistant status of malaria vector collected from stagnant water bodies in Lagos, Nigeria.

MATERIALS AND METHOD

Study Area – The study was carried out in Lagos (*Lat*-6.4500 and *Long*- 3.3833) South-West of Nigeria. The estimated population of Lagos is about 15.5 Million (NMO, 2007). Lagos with an estimated population increase of about 275,000 persons per annum is one of the fastest-growing cities in the world. There are two rainy seasons with shorter rainy season from February to April and the longer rains from May to October. Monthly rainfall averages over 300 mm (11.81 inches) for the heavy raining season and between 75 mm and 35 mm (1.5 inches) for the shorter raining period. The average temperature in January is 27°C (79°F) and for July it is 25°C (77°F) (Rexparry, 2007).

The study was carried out in three localities namely Akoka, Idiaraba and Okobaba in Lagos between the months of April to October 2006. These sites were selected because they were typical urban settings.

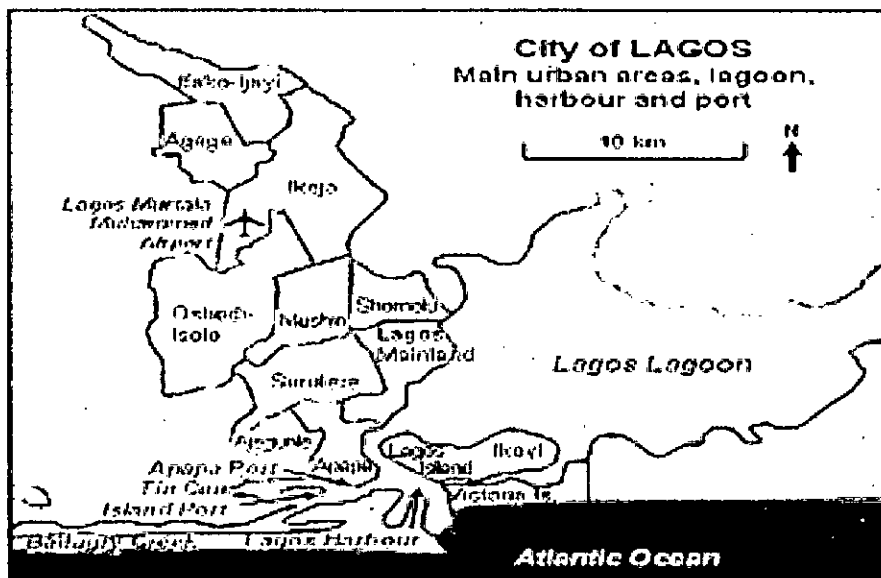
Akoka: Akoka (N06°31.029 E003°23.139) is a typical urban setting and home to the University of Lagos. This area is also close to the Lagos lagoon. Akoka is in Bariga, one of the communities in Somolu Local Government Area, other communities include Anthony Oke Side interchange, Somolu, Bashua, Abule Okuta, Seriki village, Apelehin, Ilaje, Gbagada Phase I and Igbobi. This local government has only one major industrial estate known as the Gbagada Industrial Estate.

Idi-Araba: Idi-Araba (N06° 31.077 E003°21.270) is home to Lagos University Teaching Hospital, (LUTH). Idi-Araba is in Mushin Local Government. Mushin is a suburb of Lagos. It is largely a congested residential area with inadequate sanitation and low-quality housing. Mushin is entirely an urban local government. There are three

towns and nine villages in the local government. Among them are 21 autonomous communities such as Shogunle, Mafoluku, Ejigbo, Isolo, Ilasamaja, Ewutuntun, Itire, Idi-Araba, Idi Oro, Eire, Ojuwoye, Babalosa, Atewolara, Palmgroove, Ilupeju, Olowu, and Maforita Ajao.

Okobaba: This site (N06° 29.412 E003°23.418) is in Ebute Metta Local Government area. A part of Lagos that is mainly residential and overcrowded. Ebute Metta is also a main access route that links mainland Lagos to the 3 main islands of Victoria Island, Ikoyi and Lagos Island. Ebute Metta is also known for production and sales of local food and cloths. Many of the houses in this area are over 60 years old and were built during the colonial eras (Rexparry, 2007).

Fig1: Map of Lagos showing study sites (marked with white arrow) (<http://www.answers.com/topic/lagos>)



Sample Collection and Processing

Sampling

Larval samples were collected from three different locations in Lagos. All larval samples collected were transported to the insectary attached to the Molecular Entomology and vector control unit of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos.

At each study site, mosquito larvae were collected from pools of small standing water bodies. The larvae were scooped with the aid of a 35cm long spoon and emptied into small 6 by 6cm in length and 7cm deep plastic containers. It was turned into appropriate containers and taken to the insectary where they were emptied into a 26 by 26cm in length and 12.5cm deep plastic bowls covered with nylon mesh. They were fed with larvae food (yeast and cabin biscuit blended together at ratio 1:2) and reared to adult. The adults were collected with the aid of mouth aspirator and transferred to adult mosquito cages where they were maintained on 10% sucrose solution in the insectary before being assayed.

Insecticide Susceptibility Test

This was carried out on all the samples collected using the WHO susceptibility test kit and insecticide impregnated papers (WHO, 1998). The test papers: 0.75% permethrin and 0.05% lambda-cyhalothrin. Four replicates were exposed to each test paper and the knockdown rate (KDR) taken. Observation of the number of knocked-down mosquitoes was made during an hour-long exposure period. A mosquito is considered knocked down if it is unable to stand or fly in a coordinated way; it will usually fall to the bottom of the exposure tube. Observations were made at regular intervals, usually after 10, 15, 20,

30, 40, 50 and 60 minutes in the exposure period; with the last observation just before transfer to the observation tube. The mosquitoes were placed on 10% sucrose and observed mortality rates were taken 24 hours post exposure. Control samples were exposed to plain papers. Both dead and living mosquitoes were identified to species level using species-specific PCR. In order to check that the test papers had been correctly impregnated and that they remained insecticidal, all were tested, both prior to and after the exposure of the wild mosquitoes, against *A. gambiae* s.s from a laboratory colony (AGIB). The colony was established in 2003 from field samples collected in Ibadan. The colony is 100% susceptible to insecticides.

***Anopheles gambiae* identification**

Morphological identification- All the specimens collected were identified morphologically using the morphological keys of Gillies and Coetzee (Gillies and Coetzee, 1987).

PCR identification of the samples - Specimens belonging to the *Anopheles gambiae* complex were further analyzed using a multiplex polymerase reaction (PCR) assay (Scott, 1993).

WATER ANALYSIS

To study the magnitude of water pollution at the breeding sites, seven abiotic factors including pH, dissolved oxygen, conductivity, total hardness, oil, nitrate turbidity were analyzed from replicate water samples collected over three months. Water pollution associated with heavy metals (Zinc, Copper, Iron, Lead, Mercury, Nickel and Manganese) were measured using the atomic Absorption Spectrophotometry. Controls included water samples from natural breeding sites of *A. gambiae* in a rural area of Lagos.

Statistical Analysis

Mean mortality was determined across 4 replicate of mosquitoes tested for each insecticide and the WHO criteria used to evaluate the resistance/ susceptibility status of the mosquitoes tested. By this criteria, resistance is indicated when mortality rate is less than 90% after 24h post exposure to insecticide. Mortality rates greater than 98% are indicative of susceptibility while mortality rate between 80%-90% suggests the possibility of resistance that need to be confirmed with additional bioassay test. From the observed KD counts, knock-down rates for 50%, as well as 95%, of mosquitoes KD50 and KD95 was calculated graphically using log-probit paper (WHO, 2012).

RESULTS

Morphological identification showed all 597 samples to belong to the *A. gambiae*

complex. The species specific PCR assay (Fig. 2) revealed a mixture of *A. gambiae* and *A. arabiensis*: 129(68%): 60(32%) at Akoka, 99(51%): 96(49%) at Idi-Araba, and pure collection of *A. arabiensis* (213) in Okobaba (Table 1). Mortality rate of the samples exposed to permethrin and lambdacyhalothrin using the WHO insecticide susceptibility test were 60%, 100% for Akoka, 49%, 60% for Idi-Araba and 69%, 71% for Okobaba (Table 1). Apart from the samples from Akoka which were susceptible to lambdacyhalothrin every other sample showed resistance to both permethrin and lambdacyhalothrin. The Knock down time KDT50 and KDT95 (Time taken for 50% and 95% of the test population to be knocked down) for the entire sample tested was between 23-31 minutes and >1hr respectively (Table 1).

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23



Fig. 2: PCR Identification of members of the *Anopheles gambiae* complex

Lane 1 and 23: 1Kb DNA standard ladder

Lane 2: *Anopheles gambiae* s.s positive control

Lane 3: *Anopheles arabiensis* positive control

Lane 4: *Anopheles melas* positive control

Lane 5: negative control

Lanes 6,7,8,9,11,13,14,15,16,21,22 : *Anopheles gambiae* s.s

Lanes 10, 12, 17- 20 : *Anopheles arabiensis*

Table 1: Knockdown and 24 hrs post exposure mortality of *A. gambiae s.l* population

Study site	Insecticide	No of mosquito	No and % knockdown	24hrs mortality (%)	KdT 50(min)	KdT95(min)
Akoka	Permethrin	93	69 (74)	61	27	>1hr
	Lambdacyhalothrin	96	90 (94)	100	31	>1hr
Idi-Araba	Permethrin	105	69 (66)	61	27	>1hr
	Lambdacyhalothrin	90	69 (77)	60	30	>1hr
Okobaba	Permethrin	108	84 (78)	69	23	>1hr
	Lambdacyhalothrin	105	90 (86)	71	31	>1hr

DISCUSSION AND CONCLUSION

All the mosquitoes exposed to permethrin and lambdacyhalothrin insecticide from the three sites were morphologically identified as *A. gambiae sensu lato*. The results of the PCR assay carried out to identify the mosquitoes to species level show a mixture of *A. gambiae s.s* and *A. arabiensis*. Samples from Akoka and Idiaraba were a mixture of both species while the samples from Okobaba were purely *A. arabiensis*. Although, *A. arabiensis* sometimes occur in sympatry with *A. gambiae* they are mainly found in arid regions. These findings agree with observations from other studies on vector identification in urban communities in forest zones where *A. arabiensis* occurred more than those in rural communities (Coluzzi, 1979).

The susceptibility test conducted showed that samples from Akoka were susceptible to lambdacyhalothrin with 100% mortality after 24hrs exposure. Samples from the same site exposed to permethrin were found to be resistant with a mortality rate of 61%. Resistance to the two insecticides used was recorded for samples from Idiaraba and Okobaba with mortality rates ranging between 60% -61% for permethrin and 69% - 71% for lambdacyhalothrin. This result shows that the *A. gambiae s.l* used were more resistant to permethrin than to

lambdacyhalothrin. Although *Anopheles* resistance to permethrin has been reported in other sites in Lagos, this is the first report on resistance of *Anopheles gambiae s.s* to lambdacyhalothrin in Nigeria. Pyrethroids and DDT are fast-acting insecticides that have a knock-down effect. When knock-down resistance (*kdr*) is involved, the rate of knock down (KD) has been shown to be a sensitive indicator for early detection of resistance. The knockdown rates at 10min interval were not significantly different between the samples from the three sites. The KDT₅₀ for samples exposed to permethrin was 23-27mins while that of lambdacyhalothrin was 30-31mins. Knockdown with permethrin was more rapid compared to lambdacyhalothrin. The 24hr % mortality was also higher with permethrin. In all, the KDT₉₅ was >1hr at all the sites which is an indication of resistance.

This report is consistent with various report of insecticide resistance in urban areas in West Africa. Awolola *et al.*, (2005, 2007) have reported pyrethroid and DDT resistance in Nigeria. Resistance to pyrethroid and organophosphate has also been reported in Abidjan (Cote d'Ivoire) (Girod *et al.*, 2006). Corbel *et al.*, (2007) reported a high resistance of *A. gambiae* to pyrethroid, carbamate, organophosphate and organochlorine in Benin. A study carried

out in 2007 in Nigeria by Rousseau *et al.*, (2007) also reported high level of pyrethroid resistance in *Anopheles* population breeding in water polluted with petroleum products. A very recent report has also confirmed the adaptation of different *Anopheline* species to polluted water bodies in Sri Lanka (Gunathilaka *et al.*, 2013).

As Africa prepares to scale up larviciding activities, it is imperative that water bodies other than clear standing waters (which used to be the breeding sites associated with *Anopheline* species) be considered for treatments as well. With the current reliance on insecticide treated nets/long lasting nets, the resistance level exhibited by *Anopheles gambiae* at the study sites should be of concern. To ensure that insecticide-based vector control interventions are not impaired, appropriate measures should be put in place to check the spread of insecticide resistance. Our ongoing studies could provide additional information on the spread of resistance in Lagos state

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