

Research Report

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Insecticide Resistance in *Anopheles gambiae* s.l Mosquitoes in Awka, Anambra State, Southeast Nigeria

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Abstract Long-lasting insecticide nets (LLINs) and indoor residual spraying (IRS) are the main methods used for malaria vector control. However, the success of these methods has been hampered by the development and spread of insecticide resistance in major malaria vectors. The emergence of insecticide resistance in *Anopheles* mosquitoes in Nigeria has enormous implications for vector control interventions in the country. This study aimed to investigate the insecticide susceptibility levels of wild *Anopheles gambiae* mosquitoes from Southeast, Nigeria to the four main classes of insecticides used for vector control. Larval mosquitoes were collected from different breeding sites and reared in the insectary. Mosquitoes were identified morphologically and two to five day old adult female mosquitoes were used to conduct WHO susceptibility assays against pyrethroid (0.75% permethrin and 0.05% deltamethrin), organochlorine (4% DDT), organophosphate (0.25% pirimiphos-methyl) and carbamate (0.1% propoxur and 0.1% bendiocarb) insecticides. All mosquitoes collected were identified as members of the *Anopheles gambiae* s.l. The mosquitoes were completely susceptible to bendiocarb (100% mortality). Resistance to permethrin, pirimiphos-methyl and DDT was recorded with percentage mortalities of 26.5%, 17.5% and 1.3% respectively. The KDT₅₀ recorded were 36.7 minutes (0.1% Bendiocarb), 39.8 minutes (0.1% propoxur), 50.9 minutes (0.05% deltamethrin), 91.4 minutes (0.75% permethrin), 116.3 minutes (0.25% Pirimiphos-Methyl) and 119.1 minutes (4% DDT). The results show that there is very high frequency of insecticide resistance in the study area and calls for a need for insecticide resistance management strategies to be implemented in the area.

Keywords *Anopheles gambiae*; Nigeria; Insecticide resistance; Insecticide susceptibility; Mosquitoes

Background

Malaria is a major cause of morbidity and mortality in Africa and Nigeria together with Congo account for over 40% of malaria cases in Africa (WHO, 2015). Various methods used to control malaria including; the use of prophylaxis, repellents have not resulted in the desired outcome (Coetzee *et al.*, 2006; Sharp *et al.*, 2007). Generally, malaria vector control is achieved largely through the use of Long-lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS) (Yakob *et al.*, 2011). The key to the success of these measures is knowledge of the local vector populations, the species identity, role in transmission and the susceptibility of the local mosquitoes to insecticides used to control them. Baseline surveys to collect this information need to be carried out prior to implementation of malaria vector control interventions and during on-going susceptibility surveys in order to guard against the development of increasing insecticide resistance. This is becoming more important as insecticide resistance increases and spreads across Africa.

Although four classes of insecticides (organochlorines, pyrethroids, carbamates and organophosphate) are recommended by WHO (WHO, 2011) for use against adult mosquitoes in public health programmes, in practice, modern-day malaria vector control has become more dependent on just one class of insecticide-the pyrethroids. Currently, pyrethroids are used on all approved LLINs and are the basis of the vast majority of IRS programmes worldwide (WHO, 2011). This near ubiquitous use of just one class of insecticide has given rise to fresh concerns about the problem of resistance to insecticides in malaria vectors. The pyrethroids offer several advantages over other insecticides in terms of cost, safety (low mammalian toxicity) and duration of residual action. These

insecticides are now widely used, both in agriculture and as household pesticides; their use as larvicides is limited because of their toxicity to non-target aquatic organisms. Wide-scale use of insecticide-based malaria control strategies over the past decade has been associated with the development of resistance in several important vector species, including *Anopheles gambiae* and *An. funestus* complexes (WHO, 2013). Resistance to at least one class of insecticide has been identified in many countries with ongoing malaria transmission, with resistance to the pyrethroids being the most common (WHO, 2015).

The mechanisms responsible for widespread levels of resistance are of two main types: those mediated by changes at the target site of the insecticide (e.g. *kdr* mutations) and those caused by an increase in the rate of insecticide metabolism (Hemingway and Ranson, 2000; Liu, 2015). In the past decade, increased deployments of indoor residual spraying (IRS) and insecticide treated nets have made tremendous contributions towards decreasing the number of malaria cases. Unfortunately, these gains are threatened by the rapid development and spread of insecticide resistance among major malaria vectors in Africa.

Several countries in Africa including Nigeria have evaluated the efficacy of insecticides used in IRS and bed-nets distributed through mass campaigns. Anambra state has witnessed two LLINs mass distribution campaigns (2009 and 2014) and indoor residual spraying. Amansea is a beneficiary of the two rounds of IRS deployed in Awka North and South LGAs in 2013 and 2014 respectively. The community had also received batches of LLINs in the past decade. Until date, there is paucity of information on the susceptibility status of *Anopheles gambiae* s.l. mosquitoes to insecticides used in these interventions. Against this background, the present investigation is aimed at evaluating the efficacy of the four classes of insecticides recommended by WHO against *Anopheles* mosquitoes in Amansea, Awka Anambra State.

1 Materials and Methods

1.1 Study area

This study was conducted in Amansea community; a town in Awka North LGA, Anambra State. It lies between latitude 6°15'13.17"N and longitude 6°55'02.93"E. It has a population of about 2,965 people. Amansea is largely an agrarian community. Its outskirts are still hugely forested, creating conducive environment for the malaria vectors.

1.2 Mosquito sampling

Potential breeding sites of *Anopheles* mosquitoes were surveyed between June 2015 to August 2015 using the standard dipping method. Larvae were placed in loosely capped plastic containers. They were then taken to the insectary of National Arbovirus and Vectors Research Centre (NAVRC), Enugu, and reared to adults. The larvae were reared at room temperature and fed with a mixture of biscuits and yeast. All adults were fed on 10% sugar solution. For the bioassay, 2-5 days old non-blood fed female adult mosquitoes were used.

1.3 Insecticide susceptibility assays

Insecticide susceptibility assays were carried out on *Anopheles gambiae* s.l using the WHO standard method (WHO 2013) against the four classes of WHO approved for public health. The insecticides used were 4% DDT (organochlorine), 0.1% Bendiocarb and 0.1% Propoxur (carbamate), 0.75% Permethrin and 0.05% Deltamethrin (pyrethroids) and 0.25% Pirimiphos-methyl (organophosphate). Between 80 and 100 mosquitoes were tested for each insecticide depending on the availability of mosquitoes. This was done in four replicates of 20-25 mosquitoes each. For each test, a control of 20-25 mosquitoes was exposed to untreated papers. The knockdown effect of each insecticide was recorded every 10 min over a 1h exposure period. Final mortality was recorded 24h post exposure and each mosquito was scored as either dead (susceptible) or alive (resistant).

1.4 Statistical analysis

The knockdown times for 50% and 95% of the tested mosquitoes (KDT_{50} and KDT_{95}) were analyzed using probit analysis (Finney, 1971). The resistant status of the mosquito samples was determined according to the WHO protocol (WHO, 2013). According to the protocol, 98-100% mortality means the population is susceptible.

Mortality between 90-97% means resistance is suspected and should be confirmed. Mortality less than 90% confirms the presence of resistance genes and additional bioassays may not be necessary, however mechanisms and distribution of resistance must be investigated. None of the insecticides recorded more than 5% control mortality hence; Abbott's formular was not applied in any of the tests.

2 Results

2.1 Mortality

A total of 660 female adult mosquitoes were reared from larvae collected from Amansea, Awka North LGA, Anambra State and morphologically identified as *An. gambiae* s.l. The mortality rates and resistance status of the field populations of *An. gambiae* s.l against the insecticides are shown in Table 1.

The mosquito populations were resistant to 4% DDT (1.3% mortality), 0.25% Pirimiphos-methyl (15.6% mortality), 0.75% permethrin (26.3% mortality), 0.05% deltamethrin (38.8% mortality) and 0.1% propoxur (87.5%) 24 h post exposure mortalities respectively. However, they were fully susceptible to 0.1% bendiocarb.

2.2 Knockdown time effect

The knockdown times recorded for the different insecticides are presented in Table 2. The results show that the carbamates had the lowest KDT₅₀ and KDT₉₅ with values of 36.7 and 52.3 min recorded for bendiocarb while 39.8 and 62.3 min recorded for propoxur respectively. The highest KDT₅₀ was recorded for DDT (119.1 min) while the highest KDT₉₅ values were recorded for Pirimiphos-methyl.

Table 1 Percentage mortality of *Anopheles gambiae* s.l mosquitoes from Amansea, Awka North L.G.A. of Anambra State, Nigeria exposed to four classes of insecticides

Insecticides (conc)	Number exposed	Number dead	24 h Mortality (%)	Susceptibility status
DDT (4%)	160	2	1.3 ± 0.6	Resistant
Pirimiphos-methyl (0.25%)	160	25	15.6 ± 5.6	Resistant
Permethrin (0.75%)	80	21	26.3 ± 2.1	Resistant
Deltamethrin (0.05%)	80	31	38.8 ± 0.7	Resistant
Propoxur (0.1%)	80	70	87.5 ± 0.9	Resistant
Bendiocarb (0.1%)	100	100	100 ± 0.0	Susceptible

Table 2 Knockdown times of *Anopheles gambiae* s.l mosquitoes from Amansea, Awka North L.G.A. of Anambra State, Nigeria exposed to four classes of insecticides

Insecticides (conc)	Number exposed	KDT ₅₀ (min)	95% CI	KDT ₉₅ (min)	95% CI
DDT (4%)	160	119.1	89.7-292.8	170.1	119.7-472.0
Pirimiphos-methyl (0.25%)	160	116.3	94.6-166.5	185.3	114.0-282.0
Permethrin (0.75%)	100	91.4	75.3-127.0	171.1	133.2-257.1
Deltamethrin (0.05%)	80	50.9	47.8-54.6	85.6	78.3-95.7
Propoxur (0.1%)	80	39.8	36.1-44.0	62.3	56.1-72.3
Bendiocarb (0.1%)	80	36.7	30.5-44.1	52.3	44.7-72.0

3 Discussion

This study has provided baseline information on the susceptibility status of *Anopheles gambiae* s.l. mosquitoes to DDT (4%), Pirimiphos-methyl (0.25%), Permethrin (0.75%), Deltamethrin (0.05%), Propoxur (0.1%) and bendiocarb (0.1%) in Amansea community. The mosquito populations tested were resistant to DDT, pirimiphos-methyl, permethrin, deltamethrin and propoxur. However, they were susceptible to bendiocarb.

High level of resistance was found in both DDT and pyrethroids in this study. Cross-resistance between DDT and the pyrethroids have been reported in *An. gambiae* (Corbel and N'Guessan 2013; Protopopoff *et al.*, 2013, Luisa *et al.*, 2013). Awolola *et al.* (2014) confirmed resistance of *Anopheles* mosquitoes to DDT and the pyrethroids (deltamethrin, permethrin and lambda-cyhalothrin) in Southwest, Nigeria. This alludes to an earlier notion of the presence of multiple pyrethroid resistance mechanisms in the malaria vector *An. gambiae* s.s in Nigeria. Oduola *et al.* (2010) also reported high level of DDT resistance in malaria vectors collected from rural, semi-urban and urban areas in the Southwestern part of the country. Okorie *et al.* (2015) showed that *Anopheles* mosquitoes from Ibadan, Southwest, Nigeria were resistant to DDT and pyrethroids, but completely susceptible to the organophosphates (malathion and fenitrothion) and carbamate (bendiocarb).

The resistance level of the mosquito population tested here was higher for permethrin compared to deltamethrin. The *An. gambiae* s.l mosquitoes studied here may be less susceptible to permethrin than to deltamethrin because permethrin has been widely used for both agricultural and public health purposes than deltamethrin (Adasi and Hemingway, 2008). Also, deltamethrin is a type II pyrethroid which contains an alpha-cyano group in their chemical structure and results in better kill against insects (WHOPES, 2005; Davies *et al.*, 2007). The use of LLINs are known to provide important health benefits such as increase in protective efficacy, reduction in malaria incidence and child mortality in malaria endemic countries in sub-Saharan Africa (Matowo *et al.*, 2015). The use of LLINs has also led to reduced low birth weight and miscarriages/stillbirths as well as reduced placental parasitaemia amongst pregnant women (Gamble *et al.*, 2007). Thus, LLINs have been widely promoted for use as a vector control tool (WHOPES 2005; Kesteman *et al.*, 2014; WHO, 2015). Since pyrethroids are the only class of insecticide currently being used for LLINs, resistance to this class of insecticides by malaria vectors could hamper against their usability.

The mosquitoes in this study were completely susceptible to bendiocarb, a carbamate. Complete susceptibility to bendiocarb has been recorded in Nigeria (Okorie *et al.*, 2015) and other African countries (Aikpon, 2013). Carbamate resistance has spread in malaria vectors especially in West Africa and has been reported in Cote d'Ivoire, Burkina Faso and Benin (Manguin *et al.*, 2013). In Nigeria, propoxur resistance was found in Lagos with mortality rates ranging between 25-77% (Oduola *et al.*, 2012). Increased level of carbamate resistance in African mosquito populations is a concern for malaria control because these chemicals are increasingly used alone or in combination with pyrethroids for IRS. Studies have shown that the use of organophosphates for IRS in combination with pyrethroid treated can increase protection against malaria (Rowland *et al.*, 1997; Corbel *et al.*, 2013). The mosquito populations in this study were resistant to pirimiphos-methyl an organophosphate which has been reported to be effective in IRS field trials although with a short lived activity against anophelines and culicines (Rowland *et al.*, 2013). Knockdown time in the course of susceptibility test showed that bendiocarb, propoxur and deltamethrin took 36.7, 39.8 and 50.9 minutes, respectively, to knock down 50% of the exposed mosquitoes. Only 1.3% of the mosquitoes were killed by DDT in the 60 minutes exposure period.

The mosquito population studied was resistant to multiple classes of insecticides used for vector control. Resistance of *An. gambiae* s.l to multiple classes of insecticides has been reported elsewhere in Nigeria (Oduola *et al.*, 2010; 2012; Riveron *et al.*, 2015). The detection of multiple resistances in this mosquito population has serious consequences for the Nigeria malaria control programme as it has implications on the effectiveness of the LLINs and IRS currently used in the country.

Insects become resistant either through metabolic detoxification of the insecticide or through a decrease in the insecticide binding affinity to their target site (Hemingway and Ranson 2000; Cisse *et al.*, 2015). However, this study did not include study of resistance mechanisms and therefore could not associate the susceptibility tests with the *kdr* mutation or the detoxifying enzymes (cytochrome P450s, carboxylesterases and glutathione S-transferases). Further characterization on the resistance mechanisms employed by the vectors is necessary in future studies to aid the implementation of adequate vector control strategies.

Insecticide resistance in malaria vectors is increasing worldwide due to the increasing selection pressure on mosquito populations caused by the presence of urban, domestic and/or agricultural pollutants in the environment. This may be the case in Amansea, as most of the inhabitants revealed that they made use of insecticides such as aerosol and mosquito coil for personal protection against mosquitoes. The use of these products may have placed selection pressure on the local mosquito population. It has been suggested that large scale distribution of LLINs may have an effect on insecticide resistant selection pressure (Aizoun *et al.*, 2014). This may be a possible cause in this study as this community has received two rounds of IRS done in the entire LGA in 2013 and 2014 respectively.

4 Conclusions

This study has provided baseline information on the susceptibility status of *Anopheles gambiae* s.l. mosquitoes to DDT (4%), Pirimiphos-methyl (0.25%), Permethrin (0.75%), Deltamethrin (0.05%), Propoxur (0.1%) and bendiocarb (0.1%) in Amansea community. This base-line data on insecticide resistance for the community would enable follow-up of trends in susceptibility status of the local mosquito population and would serve as basis for insecticide resistance management. High level of resistance was found in both DDT and pyrethroids in this study. The mosquitoes in this study were completely susceptible to bendiocarb (a carbamate), however, they were resistant to pirimiphos-methyl (an organophosphate). The pyrethroid and DDT resistance reported in this study could have implications in this region for the current reliance on LLINs and IRS for vector control, particularly for the recent campaign of LLINs distribution in Nigeria. Although this data serves to highlight the resistant status of the local mosquito population, further studies on the underlying mechanisms of insecticide resistance is required for the development of insecticide resistance management strategies.

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