

Anopheles gambiae s.l. insecticide susceptibility status in Umudike, Ikwuano LGA, Abia State, Nigeria

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Abstract

This study was conducted in order to determine the insecticide susceptibility status of *Anopheles gambiae* in Umudike, Abia State, Nigeria. Larval and pupal stages of *An. gambiae* mosquitoes were collected from different points within Umudike, and reared to adult stage in the laboratory. The adults that emerged were tested on four types of WHO bioassay test papers impregnated with recommended diagnostic concentrations of 4% DDT (Organochlorines); 0.1% bendiocarb (Carbamates); 0.25% Primiphos-methyl (Organophosphates); and 0.05% Deltamethrin (Pyrethroids) procured from National Abovirus and Vector Research Institute Enugu. Twenty 10% sugar solution fed female *Anopheles gambiae* mosquitoes aged 3-5days were used for the bioassay which was replicated four times with two control of same population without insecticide treatment. Knockdown was recorded at five minutes post-exposure, and then at 10 minutes interval for 1 hour and then maintained for 24 hours post-exposure on 10% sugar solution, after which a final mortality was recorded. The Knockdown Times (KDT_{50} and KDT_{90}) were determined by Probit analysis. *An. gambiae* was resistant to DDT, primiphos-methyl and Deltamethrin with 24 hours post exposure percentage mortalities of 4.65, 11.85, and 24.83 respectively. In contrary the mosquitoes were susceptible to bendiocarb which killed 98.88% of the mosquitoes 24 hours after exposure. Bendiocarb is hence recommended as the most effective insecticide for the control of *Anopheles gambiae* in Umudike, while there were need for the investigation of the mechanism behind the resistance displayed by this mosquito. Furthermore, routine surveillance of insecticide susceptibility/resistance in mosquito population is advocated in line with integrated vector control strategy.

Keywords: Insecticide; susceptibility; *Anopheles gambiae*; Umudike; Abia State; Nigeria,

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Introduction

Mosquitoes are present all over the world, except the regions near the two poles and Altitudes beyond 2,000 meters. Out of about 3,500 mosquito species, 100 species are capable of transmitting diseases to humans including malaria, dengue fever, chikungunya fever, yellow fever, filariasis, Japanese encephalitis, rift valley fever, and other viral encephalitis (Kalaivani *et al* 2015), and Zika virus. Several mosquito species belonging to the genera *Anopheles*, *Culex* and *Aedes* are vectors of these diseases (ICMR, 2003).

Among the mosquito-borne diseases, majority of the morbidity and mortality are attributed to malaria transmitted by *Anopheles gambiae* s.l. This mosquito has slightly spotted legs, palps with long white apex and narrow sub-apical white band. Currently, about 100 countries are reported to have active transmission of malaria. In 2012, there were an estimated 207 million malaria cases, with more than one case per 1,000 population in high-risk areas and 627,000 deaths due to malaria (WHO, 2012). Out of all the mosquito-borne diseases, malaria remains a major

public health burden in Nigeria, which carries a quarter of all cases of malaria in the 45 malaria-endemic countries in Africa and in which over 90 million people are at risk of malaria every year (NMCP, 2012).

Vector control is one of the major elements of the World Health Organization (WHO) global mosquito-borne diseases control strategy, which primarily focuses on indoor residual spraying and the use of Insecticide Treated Nets (ITNs). However these control measures have drawbacks including insecticide resistance and difficulties in achieving high coverage (Killeen *et al* 2002). In many parts of the world, mosquitoes have developed resistance to almost all insecticides. In addition, rapid urbanization, unplanned cities, industrialization are posing threat to further increase in mosquito's population.

Indoor Residual Spray (IRS) of insecticides and Long Lasting Insecticide Nets (LLINs) are the two most important malaria vector control tools in the tropical world (Yakob *et al*, 2011). The Federal Government Policy on Malaria Control in Nigeria focuses on LLINs, IRS, Intermittent Preventive Treatment (IPT) and environmental



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management (NMCP, 2014). However, the emerging and rapid spread of insecticide resistance in major malaria mosquito vectors across the African countries threatens the efficacy and sustainability of these control tools. The extensive use and abuse of common insecticides for agriculture has contributed immensely to the development of resistance in malaria vector.

In Nigeria, the development of resistance to DDT and other classes of insecticides including malathion, propoxur and recently permethrin has been reported in *Anopheles gambiae* s.s., *An. arabiensis* and *An. funestus* from different zones (Awolola *et al*, 2005, 2007). In south-west Nigeria, the first case of pyrethroid resistance in *Anopheles gambiae*, the major malaria vector, in Nigeria was documented by (Awolola *et al*, 2002) and since then the phenomenon has been well established in this region (Awolola *et al.*, 2003; Kristan *et al*, 2003; Awolola *et al*, 2005, 2007; Oduola *et al*, 2010, 2012). In North-central Nigeria, permethrin and DDT resistance in *An. gambiae* s.l. has been reported (Ndams *et al*, 2006; Olayemi *et al*, 2011).

Successful mosquito control programmes depend on a good knowledge of the species, its abundance and distribution, levels and dynamics of insecticide resistance in the local mosquito population, followed by continuous resistance monitoring in order to detect resistance to insecticides.

Constant monitoring of the susceptibility status of mosquito vectors is essential to predict the development of resistance (Ranson *et al*, 2009). The Federal Ministry of Health has scaled up vector control interventions for malaria control in Nigeria and knowledge of the susceptibility status of mosquitoes to the principal insecticides in vector populations will provide useful information for mosquito-borne disease control and for future research work on the major disease vectors in these areas.

The successful implementation of IRS programme partly depends on availability of information on insecticides susceptibility of mosquitoes in the local environment. Therefore, it is imperative to periodically conduct bioassays tests to assess the susceptibility status of local mosquito species to IRS intervention programme.

The susceptibility of Anopheline mosquitoes to insecticides has to a large extent been evaluated in the south-western part of Nigeria (Olayemi *et al*, 2011; Oduola *et al*, 2012). Also resistance to the four classes of insecticides has been found previously in *An. gambiae* s.l. in south-west Nigeria (Kristan *et al*, 2003; Awolola *et al*, 2005, 2007; Djouaka *et al*, 2008; Oduola *et al*, 2010; Okorie *et al*, 2011). In the north Ndams *et al*, 2006 and Umar *et al*, 2014 have evaluated the susceptibility of various mosquito species to different insecticides, but there is dearth of information in the south-eastern Nigeria. Apart from the little work in Enugu, and Ebonyi States, no documented evidence on the susceptibility status of mosquitoes to guide the procurement of IRS insecticides in the south-eastern part of Nigeria is available. Hence this

study has been conducted to provide baseline data on the insecticide susceptibility status *Anopheles gambiae* sl in Umudike, Abia State, Nigeria. Findings from this study will promote and improve effective vector control decision making.

Materials and methods

Study area

The study was carried out in Umudike, Ikwuano LGA of Abia state, south-east Nigeria. Ikwuano, is located in the tropical rain forest zone of Nigeria on Latitude 05°26'-5°29'N and Longitude 07°34'-7°36'E. It has a mean annual rainfall of 2238 mm, minimum and maximum temperatures of 23 and 32°C, respectively, with a relative humidity range of 63-80% (NRCRI, 2003).

Umudike is situated in Abia Central Senatorial District and host to National Root Crops Research Institute, and Michael Okpara University of Agriculture both of which utilize agricultural pesticides.

Mosquito larval collection and laboratory rearing

Immature stages of *Anopheles* mosquitoes (larvae and pupae) were collected from various natural breeding sites including ground pools, gutters, tyre tracks and puddles within Umudike from January to July, 2016. Water containing immature stages of mosquitoes was scooped using a plastic scoop and poured into small transparent plastic bowls. A strainer was used to sieve and pool together the third and fourth instar larvae in order to have sufficient adult emergence of the same physiological age. The bowls were scrutinized for presence of unwanted organisms and predators. They were pipette out or scooped with spoon to remove any foreign body that was found. The immature mosquito collected were transported in well labelled plastic bottles to the insectry in the Entomology Unit of the National Abovirus and Vector Research Institute, Enugu State, where they were maintained and reared at 26±3°C and 74±4% relative humidity to adult stage for World Health Organization (WHO) kit bioassays. Larvae were fed on low fat ground biscuits and adults were provided with 10% sugar solution. The resulting adults were identified according to the morphological keys (Gillies and Coetzee, 1987). All bioassays were performed on adult females aged 3–5 days (WHO, 1998).

Insecticide susceptibility test

Insecticide susceptibility tests were carried out using the WHO standard procedures and test kits for adult mosquitoes (WHO, 1998). Four types of WHO bioassay test papers impregnated with recommended diagnostic concentrations of 4% DDT (Organochlorines); 0.05% Deltamethrin (Pyrethroids); 0.1% bendiocarb (Carbamates); and 0.25% Primiphos-methyl (Organophosphates) procured from National Abovirus and Vector Research Institute, Enugu State, were used for the bioassay. Tests were carried out using 3-5 day old, sugar-fed female *Anopheles gambiae* s.l. mosquitoes. A

maximum of 100 female mosquitoes in four replicates were tested on each insecticide. Accordingly, 4% DDT (Organochlorines); 0.05% Deltamethrin (Pyrethroids); 0.1% bendiocarb (Carbamates); and 0.25% Primiphos-methyl (Organophosphates) impregnated paper strips were each introduced into 4 exposure tubes and rolled to line with the wall of the tube and fastened into position by a wire clip for each of the insecticides, while one control was lined with plain sheet of paper. A pre-test was performed by carefully introducing 20 female *A. gambiae* mosquitoes into the four holding tubes with an aspirator and allowed to stand for 1 hour. After which the mosquitoes were transferred into the exposure tubes through a hole on the lid that separates the holding tube and the exposure tube. The exposure tubes were then set upright with the screen-end up and allowed to stand for 1 hour. Records of mortalities were taken at intervals of 0, 5, 10, 20, 30, 40, 50 and 60 minutes. The mosquitoes were then carefully transferred back to the holding (recovery) tubes and kept for 24 hours during which they were fed with 10% sucrose solution. Records of final mortality were taken after 24 hours and the susceptibility status of the population was graded according to WHO recommended protocol (WHO, 2013). Dead and survived mosquitoes from this bioassay were separately kept in clearly labelled 1.5 ml Eppendorf tubes containing silica gel, blocked with paper to prevent direct contact with the silica gel for preservation. All susceptibility tests were carried out at $25\pm3^{\circ}\text{C}$ temperature and $75\pm6\%$ relative humidity.

Data interpretation and analysis

The 24-hours percentage mortality of each insecticide was calculated as the proportion of mosquitoes that died after 24 hours and the total number of mosquitoes exposed using 95% confidence intervals. Mortality rate in the control tubes were not above 5%, and hence were not corrected using Abbott formula (Abott, 1987). The resistance status of the *Anopheles gambiae* *sl* mosquito samples were

determined according to WHO criteria (WHO, 2013). Mortality rates of less than 80% indicated full resistance while those greater than 98% indicated full susceptibility. Mortality rates between 80-98% suggested the suspected resistance that needs to be clarified. The knock down data was subjected to probit analysis using statistical software (Statsdirect, 2013) to compute the KDT_{50} and KDT_{90} (time taken to knock down 50% and 90% of the exposed mosquitoes) and their 95% confidence intervals. Analysis of Variance (ANOVA) was also used to compare the mortalities across the insecticides and Least Significant Difference (LSD) was used to separate the means.

Results

During the course of this study *Anopheles gambiae* *s.l.* mosquitoes were collected and exposed to four insecticides papers across the four major classes of insecticides, during the investigation. The results of the bioassay are as follows:

Knockdown assessment and percentage mortality of *Anopheles gambiae* *s.l.* mosquitoes exposed to four insecticides

The KDT_{50} and KDT_{90} results of female *Anopheles gambiae* mosquitoes exposed to DDT (organochlorine), bendiocarb (carbamate), primiphosmethyl (organophosphate), and deltamethrin (pyrethroid), as well as the percentage mortality recorded after 24 hours exposure period were presented in Table 1. The KDT_{50} for the four insecticides were all at 50 mins, while the KDT_{90} were all at 60 minutes for the four insecticides respectively. The highest percentage mortality was recorded for bendiocarb (98.88%), 24hours after exposure. This was followed by deltamethrin (24.83%), primiphosmethyl (11.85%), and DDT (4.65%). Comparatively the percentage mortalities of *Anopheles gambiae* across the four insecticides differed significantly ($p<0.05$).

Table 1. Knockdown assessment and percentage mortality 24 hours after exposure of *Anopheles gambiae* *s.l.* mosquitoes exposed to four insecticides.

Class of Insecticide	Type of Insecticide	Mortality after 24 hours (%)	KDT_{50} (Mins)	KDT_{90} (Mins)	Status Analysis
Organochlorine	DDT	4.65 ^c	50 ^a	60 ^a	Resistant
Carbamate	Bendiocarb	98.88 ^a	50 ^a	60 ^a	Susceptible
Organophosphate	Primiphosmethyl	11.85 ^{bc}	50 ^a	60 ^a	Resistant
Pyrethroid	Deltamethrin	24.83 ^b	50 ^a	60 ^a	Resistant

Figures with same letters in columns are not significantly different ($p>0.05$).

Discussion

In the past 40 years mosquitoes have developed resistance to commonly used insecticides such as temephos, malathion, permethrin, propoxur, and fenitrothion in many countries. (Hemingway and Ranson, 2000). The present study presents for the first time baseline data on the susceptibility status of *Anopheles gambiae* *s.l.*

mosquitoes to World Health Organization Pesticide Evaluation Scheme (WHOPES) approved indoor residual spray (IRS) insecticides in Umudike, Abia State, south-eastern Nigeria to guide procurement of IRS in the State. During the knockdown assessment a KDT_{50} -value of 50 mins. and a KDT_{90} value at 60mins were recorded across all the insecticides (Table 1). Hence there was no

significant difference ($p>0.05$) between the KDT_{50} values across the insecticides. There was also no significant difference ($p>0.05$) between the KDT_{90} values across the insecticides. This high KDT_{50} and KDT_{90} (50 mins and 60 mins respectively) values is in agreement with works of Chandre *et al* (1999); Oduola *et al* (2012); and Kolade *et al* (2013).

The results of the knockdown assessment showed that the tested insecticidal papers induced knockdown of the adult *Anopheles gambiae* s.l. mosquitoes, suggesting that knockdown mechanisms could be operating in the local *Anopheles* mosquito populations of Umudike. This confirms earlier studies which indicates the knock down effects of impregnated papers against mosquitoes in Nigeria (Awolola *et al*, 2005; 2007; Oduola *et al*, 2010; Olayemi *et al*, 2011; Ibrahim *et al*, 2014; Umar *et al*, 2014). The knockdown of the mosquitoes exposed to insecticidal papers indicates the presence of knock down resistance (KDR) mechanism (Kristan *et al*, 2003; Awolola *et al*, 2007; Ibrahim *et al*, 2014; Umar *et al*, 2014) operating in populations of *Anopheles* mosquitoes of Umudike. This could have been responsible for the level of resistance displayed by these Anopheline mosquitoes to the various insecticides evaluated.

Using the WHO (2013) criteria for insecticides susceptibility or resistance assessment of mosquitoes, the 24 hour post exposure results indicates that the *Anopheles gambiae* s.l. mosquitoes were susceptible to bendiocarb with a mortality rate of 98.88% (Table 1). This is in variance with documented works on the resistance of *Anopheles* mosquitoes to bendiocarb (Ndams *et al*, 2006; Ahoua Alou *et al*, 2010; Ibrahim *et al*, 2014; Umar *et al*, 2014). Susceptibility to bendiocarb in Umudike goes on to prove that *Anopheles gambiae* s.l. mosquito in this area have not developed resistance to carbamates.

An. gambiae s.l. was also resistant to DDT and deltamethrin (Table 1). This is in line with other documented evidence on resistance of Anopheline mosquitoes to DDT (Ndams *et al*, 2006; Santolamazza *et al*, 2008; Ranson *et al*, 2009; Yadouleton *et al*, 2010; Oduola *et al*, 2010, 2012; Ranson *et al*, 2011; Umaret *et al*, 2014); and deltamethrin (Awolola *et al*, 2002; Awolola *et al*, 2005; Awolola *et al*, 2007; Djouaka *et al*, 2007, 2008; Oduola *et al*, 2012). This is quite strange because deltamethrin belong to the sub-group of pyrethroids containing an alpha cyano-group in their chemical structure and are extremely potent against insects even at much lower concentration (WHOPES, 2011), although the finding is in agreement with many authors who have posited the growing resistance of many mosquito species to pyrethroid insecticides which are predominantly used in IRS and LLITNS (Sunaiyana *et al*, 2006; Nuananong *et al*, 2007 and McAllister *et al*, 2010). The marginal resistance and resistance (Table 1) recorded in *An. gambiae* populations to three of the insecticides tested are characterized by high KDT_{50} value of 50 mins. The result from this study was consistent with the higher KDT_{30} found in urban *An. gambiae* populations exposed to these insecticides in

Lagos, south-west Nigeria (Oduola *et al*, 2012). Higher KDT_{50} values in the field population have been suggested to give an early indication of the involvement of kdr mechanism of resistance (Chandre *et al*, 1999).

The multiple resistances of *Anopheles gambiae* mosquitoes in Umudike to the tested pyrethroid, organophosphate and organochlorine insecticides may have grave implications for the malaria control programmes. It may compromise the efficacy of interventions and potentially lead to the failure of IRS and ITNs based vector control (Awolola *et al*, 2008; Umar *et al*, 2014). Some of these insecticides would have been used in IRS hence leading to resistance. It is established that prior exposure of mosquitoes to insecticides may induce selection pressure (Kerah-Hinzoumbe *et al*, 2008). Pyrethroids-based aerosols and coils are used for control of mosquitoes and domestic pests and it might contribute to the development of resistance as reported elsewhere (Kristan *et al*, 2003). The farmers in the community also use deltamethrin for agricultural crop protection. Previous researchers have reported that exposure of malarial vectors to crop protection insecticides could result to development of insecticide resistance (Etang *et al*, 2003; Awolola *et al*, 2007; Philbert *et al*, 2014). LLINs have been in use for protection against mosquitoes for a long time in Umudike, and this may induce selections to pyrethroid insecticides.

Previous studies revealed that the use of LLINs could result in development of insecticides resistance in *Anopheles* mosquitoes (Kabula *et al*, 2011; Umar *et al*, 2014). There was no significant difference ($p>0.05$) between the effects of DDT and primiphosmethyl, as well as between primiphosmethyl and deltamethrin on *Anopheles gambiae* s.l. On the other hand, the effect of bendiocarb on *Anopheles gambiae* differed significantly ($p<0.05$) from that of the other three insecticides. The current findings go a long way to show insecticide based malaria control programmes are already under jeopardy in this locality, except there is a quick and timely response. This stems from the fact that the *Anopheles gambiae* s.l. mosquitoes in Umudike are already resistant not just to the pyrethroid class, but have also developed resistance to both the organochlorine and organophosphate classes, with only the carbamate class having potency on them.

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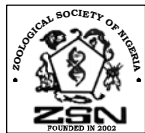
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