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Insecticide Susceptibility Status of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Awka South Local Government Area, Anambra State, Nigeria

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ABSTRACT

Insecticide resistance in *Aedes spp* is a major concern to yellow fever vector control programmes, in particular in Awka, Nigeria owing to the dense population of monkeys and the tradition that barns the killing of monkeys in this area. The study was aimed to determine the insecticide susceptibility status of *Aedes aegypti* and *Aedes albopictus* in Awka South, Anambra state, Nigeria. Locally modified American Centre for Disease Control (CDC) ovitraps were used for the collection of *Ae. aegypti* and *Ae. albopictus* eggs from Ifite, Awka. The eggs were reared to adult stage at the insectary unit of National Arbovirus and Vectors Research Centre, Enugu and the first generation progeny (F1) exposed to WHO insecticide impregnated papers. The treatments used for the two species consist of carbamates (0.1% propoxur), organophosphates (0.25% pirimiphos-methyl), pyrethroids (0.05% deltamethrin) and organochlorines (4% DDT). The result showed *Ae. aegypti* to be susceptible to primiphos-methyl (98.75%) and deltamethrin (100%), tolerant to propoxur (97.3%) and resistant to DDT (10.55%) while *Ae. albopictus* were susceptible to propoxur (100%), resistant to DDT (62.5%) and showed possibility of resistance to primiphos-methyl (97.5%) and deltamethrin (93.6%). Knock down times (KDT50 and KDT95) are as follows; For *Ae. Aegypti-* propoxur (4.26 and 39.79mins), pirimiphos-methyl (4.18 and 41.35mins), deltamethrin (3.77 and 17.27mins), DDT (53.33 and 248.53mins) and for *Ae. albopictus-* propoxur (30.19 and 54.76mins), pirimiphos-methyl (1.12 and 195.02mins), deltamethrin (5.88 and 46.39mins), DDT (45.94 and 176.16mins). This study clearly demonstrates that both *Ae. aegypti* and *Ae. albopictus* are resistant to DDT. Also, frequent use of these insecticides for vector control interventions (long lasting insecticide nets and indoor residual spraying) in Nigeria may result to resistance in deltamethrin and pirimiphos-methyl. There is therefore urgent need to implement proper insecticide resistance management strategies in line with international best practices in this area.

Keywords: *Aedes aegypti*; *Aedes albopictus*; Insecticide susceptibility; Resistance; Nigeria

INTRODUCTION

Insecticide resistance in the major tropical arbovirus vector, *Aedes aegypti* and *Aedes albopictus* (*Diptera: Culicidae*) is common in West Africa. This presents a major challenge in the control of yellow fever and other arboviral diseases in this region [1]. *Ae. aegypti* is a tropical mosquito that is believed to have originated from Central Africa where it is found in greatest abundance [2]. From Africa, it was widely distributed by slave trade to other parts of the world through water barrels in ships [3]. *Ae. albopictus* was first discovered by Skuse in 1894 from specimens collected in the city of Calcutta on the Indian subcontinent [4]. This species has since expanded its distribution to other continents except Antarctica [4]. It is an invasive vector [5] and its expansion into other sub-continents likely occurred as a consequence of trading used tyres between various countries [6,3]. The breeding of *Ae. albopictus* was first noticed in Nigeria

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in 1991 after the outbreak of sylvatic (jungle) fever in many rural communities in Delta state in which the species were incriminated in the viral transmission of Sylvatic fever [7].

Ae. aegypti and *Ae. albopictus* are container breeding mosquitoes [7] and sympatric existence of both species has been reported in densely urbanized but shaded habitats [8]. Rochlin [3] was of the opinion that occupancy of artificial containers, human facilitated transport, desiccation-resistant eggs and associations with human habitats were among the predisposing factors that made *Ae. aegypti* and *Ae. albopictus* cosmopolitan. These two mosquitoes are disease vectors for many important human viral diseases. *Ae. aegypti* for instance, is a known vector of several viruses including yellow fever virus, dengue virus, chikungunya virus, rift valley fever and Zika virus [9].

The natural transmission cycle of yellow fever involves tree-hole breeding mosquitoes and a wide array of monkeys, apes and marmosets [10]. Primate species in Africa rarely develop fatal disease following yellow fever virus infection [11] but several species of New World monkeys in the Americas are susceptible to severe, fatal yellow fever disease. According to the Pan American Health Organization [12], in the Americas, deaths of susceptible non-human primate act as a guard that may indicate presence of yellow fever virus in a specific geographic location or environment and surveillance for such events is an important tool to prevent human disease. The goal of surveillance for deaths among non-human primates is to provide an early warning of risk of yellow fever virus transmission to humans, for rapid implementation of vaccination and prevention strategies [13]. However, there have been few reports in Africa on how epizootic surveillance has been used to inform yellow fever vaccination in human populations.

Vector control is a major component of the World Health Organization (WHO) global malaria control strategy and when implemented properly, it can be an effective strategy for preventing mosquito-borne diseases [3]. Chemical control remains the most widely used approach for vector control [14,15]. However, many populations of mosquito vectors have developed resistance to synthetic organic insecticides used mostly during the last half of the 20th century [16]. The development and spread of insecticide resistance represents a serious threat as it can lead to a reduction in the efficacy of larvicide or adulticide-based control programs as demonstrated in the control of the main dengue vector, *Ae. aegypti* [3]. Agricultural and household use of insecticides in Nigeria has been implicated as the reason for the development of resistance in mosquitoes [17].

The mechanisms responsible for resistance to insecticides used in mosquito control has also been identified and are of two main types namely; those mediated by changes at the target site of the insecticide (e.g. kdr mutations) and those caused by increase in the rate of insecticide metabolism resulting in increased metabolic detoxification [9,18]. Resistance to multiple insecticides (pyrethroids and organophosphates) has been reported in *Ae. aegypti* in South-East Asia [19]. However, the insecticide susceptibility status of *Ae. albopictus* and *Ae. aegypti* is poorly documented in Nigeria [20] especially *Ae. albopictus* owing to its recent introduction in Africa. There is also dearth of information on the insecticide susceptibility status of yellow fever virus in Awka South area of Anambra State, Nigeria, despite the dense population of monkeys in this area. Yellow fever disease is known to occur in certain cercopithecid monkeys inhabiting the forests in Africa [21] and these monkeys play significant role in the epidemiology of yellow fever in Africa.

Resistance monitoring therefore should be an integral part of vector and public health control programs in Nigeria where currently almost all the four classes of insecticides are used in vector control interventions and for agricultural purposes [1]. Considering the latter, knowledge of vector susceptibility to insecticides, changing trends in resistance and other operational implications are basic requirements that will guide insecticide use for vector-borne disease control programs [9]. Yet this baseline information is lacking in the study area, hence the present study.

MATERIALS AND METHODS

Study Area

Awka is the capital of Anambra state, Nigeria with an estimated population of 301,657 as at 2006 Nigerian census (National population commission, 2006). It is located approximately between latitude 60°, 14" and, 60°, 18" North and between 70°, 5"and 70°, 9" East longitude. Awka is in the tropical rainforest zone of Nigeria and experiences two distinct seasons: wet and dry seasons. There are seven months of heavy tropical rain which occurs between April and October and are followed by five months of dryness from November to March.

It has a relative humidity of 70% reaching 80% during rainy season and an annual rainfall of about 2000 mm. The temperature in Awka ranges from 27°-30°C between June and December but rises to 32°-34°C between January and April [22]. Awka has a high number of monkeys compared to the whole of Anambra state, due to their long age tradition that barns its occupant from killing the monkeys around this area.

Collection of *Ae. aegypti* and *Ae. Albopictus*

Eggs of *Ae. aegypti* and *Ae. albopictus* were collected using locally modified American Centre for Disease Control (CDC) ovipostion traps. The traps consist of coloured plastic cups about a litre in volume. Each cup was lined with a strip of white calico cloth measuring 9 cm in width and 30 cm in length and half-filled with water. The traps were then placed strategically in the study area especially in shaded areas such as underneath low growing shrubs and under plantain trees.

A period of 48 hours elapsed before the ovitraps were retrieved. A total of twenty oviposition traps were set and retrieved twice a week. Subsequently, the calico strips were examined under compound microscope and the strips containing the eggs of *Ae. aegypti* and *Ae. albopictus* were separated from the strips without eggs.

Rearing of *Ae. aegypti* and *Ae. albopictus*

This was carried out in the insectary section of National Arbovirus and Vectors Research Centre. The strips with the eggs

were soaked in a bowl containing one litre of water and 0.2 g of yeast which serves as stimulant. The emerged larvae were fed with oats and the pupae that emerged from the larvae were picked using pipette and were transferred into plastic cups. The larvae were reared at a temperature and humidity of 24.6-36.3oC and 70-91% respectively. They were subsequently transferred in labeled cages for adult emergence.

The emerged adults were separated into males and females using the antenna as the taxonomic key. The males and females were identified as either *Ae. aegypti* and *Ae. albopictus* species using standard morphological keys. Males and females of each species were placed in separate cages and females were fed with blood. Oviposition traps were placed in each of the cages and eggs collected from each species were reared to adult. Eventually, 3-5 day old F1 generation adult mosquitoes were used for the susceptibility test.

Insecticide Susceptibility Test

Laboratory reared active female *Ae. aegypti* and *Ae. albopictus* mosquitoes held for one hour in resting tubes were later supplied with 10% glucose solution before testing. The tests were performed using WHO diagnostic bioassay kits and protocol for measuring mosquito susceptibility. Non-blood fed, 3-5 day old females were exposed to filter papers impregnated with one insecticide from each class of insecticide. Insecticides used were: 0.1% Propoxur (carbamate), 0.25% Pirimiphos-methyl (Organophosphates), 0.05% Deltamethrin (Pyrethroids) and 4% DDT (Organochlorines). Cohorts of 20-25 mosquitoes per replicate were exposed for one hour to papers impregnated with discriminating dose of each insecticide and exposure tubes held in a vertical position.

Each experiment had 3 replicates and two controls. Control mosquitoes were exposed to non-treated papers. The number of mosquitoes knocked down after 5, 10, 15, 20, 30, 40, 50 and 60 minutes were recorded. After the exposure, all knock-down and surviving mosquitoes were transferred to the holding tubes of the WHO test kits with untreated papers and allowed a 24-hour recovery period. The mosquitoes were maintained with a 10% glucose solution and kept in insulated coolers during the recovery period. Mosquitoes were recorded as either dead (susceptible) or alive (resistant) and stored individually over silica gel.

Data Analysis

Knock-down times (KDT50 and KDT95) were analyzed using Probit analysis [23] in software package for the Social Sciences (SPSS 20.0). The mortality of test samples was calculated by summing the number of dead mosquitoes after 24 hours across all four exposure replicates and expressing this as a percentage of the total number of exposed mosquitoes.

$$\text{Observed mortality} = \frac{\text{Total number of dead mosquitoes}}{\text{Total sample size}} \times 100$$

Susceptibility was determined using the WHO criteria for susceptibility test in which 98-100% mortality indicates susceptibility; mortality of <98% suggests existence of resistance and points to a need for further investigation. The presence of resistant genes in the vector population must be confirmed if the observed mortality rate is between 90-97% but confirmation is not necessary if mortality is <90% [24]. Experiments that had more than 5% death in control were corrected using the Abbott's formula [24].

RESULTS

Mortality of *Aedes aegypti* to Determine Susceptibility

Mortality of *Aedes aegypti* : A total of 332 female adult mosquitoes were reared from eggs collected and morphologically identified as *Ae. aegypti*. The result showed that propoxur, pirimiphos-methyl, deltamethrin and DDT recorded mortalities of 97.50%, 98.75%, 100% and 12.50% respectively (Table 1).

These values were significantly different (P< 0.001) from the control mortalities that recorded mortalities of 3.75 and 1.25 respectively. The mosquito populations were susceptible to propoxur (97.50% mortality), Pirimiphos-methyl (98.75% mortality) and deltamethrin (100% mortality) respectively. However, they were resistant to DDT (12.5% mortality) (Table 1).

Table 1: Percentage mortality of *Aedes aegypti* mosquitoes.

Insecticides (conc.)	Number exposed	Number dead	Mean Mortality (± s.e)	24 h % mortality	Susceptibility status
Propoxur -0.1%	80	78	19.50 ± 0.29	97.5	Susceptible
Pirimiphos-methyl -0.25%	80	79	19.75 ± 0.25	98.75	Susceptible
Deltamethrin (0.05)	80	80	20.00 ± 0.00	100	Susceptible
DDT -4%	80	10	2.50 ± 1.50	12.5	Resistant
Means of three replicates (± s.e); P value < 0.001;LSD = 1.988					

Effect of exposure time on the knockdown rate of *Aedes aegypti* mosquito : The result showed that the knockdown rate increased with increase in exposure time (Table 2). Deltamethrin had the highest percentage knockdown rate of 100% after 60 minutes, followed by Pirimiphos-methyl (98.75%), and Propoxur (97.50%) while DDT had the least knock rate of 60% (Table 2).

Deltamethrin was the most effective of the four insecticides with KDT50 and KDT95 of 3.77 and 17.27 minutes (Table 3). DDT was the least effective as it took the highest timing (53.33 and 248.53 minutes) to knockdown 50% and 95% of the test population (Table 3). The knockdown values were significantly

different (P< 0.001) from the control that recorded no knockdown after 60 minutes (Table 2).

Table 2: Mean and Percentage effect of exposure time on the knockdown rate of *Aedes aegypti* mosquito.

Exposure Time (Minutes)								p value	LSD
	10	15	20	30	40	50	60		
Propoxur	13.50 ± 0.87	17.00 ± 0.91	17.75 ± 1.38	18.25 ± 1.60	18.75 ± 1.60	19.00 ± 1.47	19.50 ± 1.56	<0.001	3.53
% KDT	67.5	85	88.75	92.35	93.75	95	97.5		
Pirimiphos-methyl	15.00 ± 1.58	16.75 ± 1.11	17.00 ± 0.91	17.75 ± 1.11	18.25 ± 1.18	19.25 ± 0.48	19.75 ± 0.25	<0.001	2.73
% KDT	75	83.75	85	88.75	91.25	96.25	98.75		
Deltamethrin	16.00±0.82	19.25±0.25	19.50±0.29	19.75±0.25	19.75±0.25	19.75±0.25	20.00±0.00	<0.001	1.19
% KDT	80	96.25	97.5	98.75	98.75	98.75	100		
DDT	1.00 ± 0.58	1.00 ± 0.58	2.50 ±1.26	5.50 ±1.89	6.50 ± 2.22	9.00 ± 3.00	12.00 ± 4.00	<0.001	5.78
% KDT	5	5	23.75	27.5	32.5	45	60		
Control	0	0	0	0	0	0	0		

Means of three replicates (±s.e)

Table 3: Knockdown times of *Aedes aegypti* mosquitoes.

Insecticide	KDT50 (mins)	KDT95 (mins)
Propoxur	4.26	39.79
Pirimiphosmethyl	4.18	41.35
Deltamethrin	3.77	17.27
DDT	53.33	248.53

Mortality of *Aedes albopictus* to determine susceptibility

Mortality of *Aedes albopictus* : Of the 324 adult female mosquitoes collected and exposed to diagnostic doses, a total of 286 mosquitoes died after 24hr exposure period. The mortality rate of *Ae. albopictus* exposed to the four insecticides are as follows; propoxur (100%), pirimiphos-methyl (97.50%), deltamethrin (93.75%) and DDT (61.25%) (Table 4).

These mortality values were significantly different (P< 0.001) from the control mortalities that recorded mortalities of 5.00% and 3.75% for control 1 and control 2 respectively (Table 4). The mosquito populations were susceptible to Pirimiphos-methyl (97.50% mortality), Deltamethrin (93.75%) and propoxur (100% mortality) 24hr post exposure. However, they were resistant to DDT (61.25% mortality) (Table 4).

Table 4: Percentage mortality of *Aedes albopictus* mosquitoes.

Insecticides (conc)	Number exposed	Number dead	Mean Mortality (±s.e)	% mortality	Susceptibility status
Propoxur -0.1	80	80	20.00 ± 0.00	100	Susceptible
Pirimiphos-methyl (0.25%)	80	79	19.50 ± 0.50	97.5	Susceptible
Deltamethrin (0.05)	80	75	18.75 ± 0.95	93.75	Resistant
DDT -4%	80	49	12.25 ± 1.03	61.25	Resistant

Means of three replicates (± s.e), P value < 0.001, LSD = 2.019

Effect of exposure time on the knockdown rate of *Aedes albopictus* : The result showed that the knockdown rate increased with increase in exposure time (Table 5). Propoxur had the highest knockdown rate of 100% after 60 minutes, followed by Deltamethrin (95.00%), and Pirimiphos-methyl (88.75%) while DDT had the least knock rate of 75% (Table 5).

Propoxur showed to be the most effective of the four classes because it took the least number of time of exposure (30.19 and 54.76 minutes) to knock down 50% and 95% of *Ae. albopictus* mosquitoes tested respectively (Table 6). Conversely, DDT was

the least effective because it had the highest exposure time (45.94 and 176.16 minutes) (Table 6).

Table 5: Mean and percentage effect of exposure time on the knockdown rate of *Aedes albopictus* exposed to four classes of insecticides.

Exposure Time (Minutes)								p value	LSD
	10	15	20	30	40	50	60		
Propoxur	0.00 ± 0.58	0.00 ± 0.00	0.25 ± 0.25	0.50 ± 0.50	3.25 ± 1.25	11.50 ± 0.87	20.00 ± 0.00	<0.001	1.588
% KDT	0	0	1.25	2.5	16.25	57.5	100		
Pirimiphos-methyl	14.25 ± 1.03	16.50 ± 0.65	16.50 ± 0.65	17.00 ± 0.91	17.25 ± 1.03	17.50 ± 1.19	17.75 ± 1.11	<0.001	2.543
% KDT	71.25	82.5	82.5	85	86.25	87.5	88.75		
Deltamethrin	8.50 ± 1.19	17.75 ± 0.75	18.00 ± 0.58	18.25 ± 0.48	18.50 ± 0.50	18.75 ± 0.25	19.00 ± 0.00	<0.001	1.668
% KDT	42.5	88.75	90	91.25	92.5	93.75	95		
DDT	1.00 ± 0.58	1.75 ± 0.75	2.25 ± 0.85	5.25 ± 0.25	7.25 ± 0.63	10.00 ± 0.91	15.00 ± 1.58	<0.001	2.319
% KDT	5	8.75	13.75	26.25	36.25	50	75		
Control	0	0	0	0	0	0	0		

Means of three replicates (±s.e)

Table 6: Knockdown times of Aedes albopictus mosquitoes.

Insecticide	KDT50 (mins)	KDT95 (mins)
Propoxur	30.19	54.76
Pirimiphosmethyl	1.12	195.02
Deltamethrin	5.88	46.39
DDT	45.94	176.16

DISCUSSION

Based on the WHO criteria for characterizing insecticide resistance/susceptibility, results from the study site showed that *Ae. aegypti* and *Ae. albopictus* developed resistance to DDT. However, while *Ae. albopictus* was resistant to deltamethrin, *Ae. aegypti* was susceptible to deltamethrin. It is interesting to note that the two Aedes species *Ae. albopictus* and *Ae. aegypti* were susceptible to both propoxur (carbamate) and pirimiphos-methyl (organophosphate) insecticides. The possible reason(s) for the two different species of Aedes to be susceptibility to two different insecticides may be due to their similar mechanism of action since both carbamate and organophosphate insecticides are synaptic poisons that inhibit the acetylcholinesterase enzyme in insects [25,26]. It is also known that the habitats of these two Aedes species do overlap in many areas and they may be exposed to similar selection pressure.

The present study also revealed mortality rates of 10.55% and 62.65% for *Ae. aegypti* and *Ae. albopictus* respectively indicating a very low percentage mortality as against WHO (2013). Similar resistance to DDT was observed in both farm and non-farm sites in Lagos, Nigeria [27] as well as in Cameroun [28]. Also, a study carried out in Benin showed mortality rates ranging from 4-12% for DDT in four study sites [22].

The resistance to DDT may not be unconnected with the historical use of this insecticide in vector control activities in Nigeria. The extensive use and abuse of DDT for agricultural purposes could have contributed immensely to the development of resistance in insect pests [22,8]. IRS using DDT for malaria control has been reported to favour the selection of DDT resistance in Anopheles as well as Aedes vectors [28]. The contamination of larval breeding places by insecticides used in agriculture has also been shown to select for DDT resistance in malaria vectors [28]. This insecticide has been used in the past for agricultural purposes and IRS programmes carried out in those areas which could have resulted in selection of DDT resistance in Anopheles as well as Aedes mosquitoes. In Ifite Awka, Awka South where mosquitoes for the present study were collected, Indoor Residual Spraying (IRS) operation has been concluded by the Ministry of Health, Anambra State in 2013 and 2014. Also, Ifite people are predominantly farmers; therefore, it may be possible that mosquito populations in this community have acquired resistance as a result of routine application of insecticides.

Resistance to deltamethrin was also suspected for *Ae. albopictus* from Yaoundé in Cameroun (83%) [29]. This is consistent with

the findings of this study that shows resistance of *Ae. aegypti* and Ae. albopictus to DDT and the possibility of resistance to deltamethrin by *Ae. albopictus*.

Hadura et al. [8] carried out a study on pyrethroid and organophosphate susceptibility status of *Ae. aegypti* and *Ae. albopictus* in Penang, Malaysia. The study evaluated the susceptibility to lamba-cyhalothrin and pirimiphos-methyl of both species in a traditional community at Bagan Dalam, Penang, Malaysia. Results showed that both species were highly resistant to lambda-cyhalothrin but were susceptible to pirimiphos-methyl showing 100% mortality recorded 24 hours after treatment. The present study when compared to the study in Malaysia shows that *Ae. aegypti* was susceptible to pirimiphos-methyl but *Ae. albopictus* shows the possibility of resistance with a mortality of 97.5% 24 hours after exposure. Also, the result showed that increase in exposure time led to increase in percentage mortality and knockdown of both mosquitoes. This is consistent with the work done by Parakrama et al.[1], where it was shown that increase in time led to the deactivation of the enzyme, epsilon class GST (GST) which confers resistance to Anopheles gambiae mosquito. The selection pressure exerted by insecticides for more than five decades on the populations of *Ae. aegypti* and *Ae. albopictus* in Nigeria, has generated widespread resistance to DDT and variability in susceptibility to organophosphates, carbamates and pyrethroids.

CONCLUSION

This study has clearly shown that *Ae. aegypti* and *Ae. albopictus* populations in Ifite Awka, Awka South were both resistant to DDT. However, deltamethrin and propoxur were very effective against *Ae. aegypti* and suggest existence of resistance in *Ae. albopictus*. This has implications for malaria and dengue vector control programmes in the area and is really a threat for the efficacy of LLINs, ITNs and other forms of vector control such as Indoor Residual Spraying which may be carried out in the study area. There is therefore need to diligently implement proper insecticide resistance management strategies in line with international best practices.

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