INSECTICIDE SUSCEPTIBILITY STATUS OF *AEDES AEGYPTI* IN UMUDIKE, IKWUANO LGA ABIA STATE, NIGERIA

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ABSTRACT

This study was conducted in order to evaluate the insecticide susceptibility status of Aedes aegypti in Umudike, Abia State, Nigeria. Larval and pupal stages of the mosquitoes were collected from different points within Umudike, and reared to adulthood in the laboratory. The adults that emerged were tested on 4 % DDT (organochlorines), 0.1 % bendiocarb (carbamates), 0.25 % primiphos-methyl (organophosphates) and 0.05 % deltamethrin (pyrethroids) procured from National Arbovirus and Vector Research Institute, Enugu. Twenty sugar fed female Aedes aegypti mosquitoes aged 3 – 5 days were used for the bioassay which was replicated four times with two control. Knockdown was recorded at five minutes, and then 10 minutes interval for 1 hour and then maintained for 24 hours post-exposure on 7 % sugar solution, after which a final mortality was recorded. The Knockdown times (KDT_{50} and KDT_{90}) were determined by Probit analysis. Aedes aegypti was susceptible to all the insecticides but DDT, with 24-hour post exposure percentage mortalities of 62.85, 100, 97.50 and 93.75 in DDT, bendiocarb, primiphos-methyl and deltamethrin, respectively. It is necessary that the mechanism behind this resistance displayed by Ae. aegypti mosquitoes in Umudike to DDT be investigated. Routine surveillance of insecticide susceptibility/resistance in wild mosquito population is also advocated in line with integrated vector control strategy in Umudike.

Keywords: *Aedes aegypti*, Insecticide, DDT, Bendiocarb, Primiphos-methyl, Deltamethrin, Knockdown times, Susceptibility, Umudike

INTRODUCTION

Mosquitoes are found all over the world, except in the regions near the two poles and altitudes beyond 2000 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 (Annadurai, *et al*., 2015), and of recent Zika virus. Several mosquito species belonging to the genera *Anopheles*, *Culex* and *Aedes* are vectors of these diseases (Mittal, 2003).

Aedes aegypti is the vector for yellow fever, dengue fever and many other mosquito-borne viruses. The eggs are usually black, more or less ovoid in shape and are always laid singly. Careful examination shows that the eggshell has a distinctive mosaic pattern. Eggs are laid on damp substrates just beyond the water line, such as on damp mud and leaf litter pools, on the damp walls of clay pots, rock-pools and tree-holes (WHO, 1982). Larvae of *Aedes* species usually have a short barrel-shaped siphon, and there is only one pair of sub ventral tufts which never arise from less than one quarter of the distance from the base of the siphon. Many, but not all, *Aedes* adults have conspicuous patterns on the thorax formed by black, white or silvery scales, in some species yellow scales are present. The legs often have black and white rings. *Aedes aegypti*, often called the yellow fever mosquito, is readily recognized by the lyre-shaped silver markings on the lateral edges of the scutum. Scales on the wing veins of *Aedes* mosquitoes are narrow and are usually more or less all black, except may be at the base of the wing. According to Braks *et al.* (2003), they are found chiefly in clear and turbid waters. *Ae. aegypti* primarily develops in containers and its larval density tends to be high (hundreds of larvae in a container). *Aedes aegypti* is especially abundant in urbanized and densely populated neighbourhood (Braks *et al.,* 2003). It is found breeding in natural receptacles such as holes but always near human habitation (Rathor, 1996). They are usually found in water containers in houses, discarded tire and coastal sailing boats, but occasionally occur in rocks and tree holes (Abdalmagid and Alhusien, 2008).

Vector control is one of the major elements of the World Health Organisation (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.

Jirakanjanakit *et al.* (2007) reported the resistance of *Aedes aegypti* to some pyrethroid insecticides in Thailand. In Nigeria, the development of resistance to DDT and other classes of insecticides including organochlorine, organophosphate, carbamates and pyrethroid has been reported in many mosquitoes 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).

The successful implementation of IRS program partly depends on availability of information on insecticides susceptibility of mosquitoes in the local environment. It is therefore imperative to periodically conduct bioassay tests to assess the susceptibility status of local mosquito species to IRS interventional insecticides. The susceptibility of mosquitoes against 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 southwest Nigeria (Kristan *et al*., 2003; Awolola *et al*., 2005; 2007; Djouaka *et al*., 2008; Oduola *et al*., 2010; Okorie *et al*., 2011). In the Northern part of Nigeria, 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-east of Nigeria. Apart from the little work in Enugu, and Ebonyi states, no documented evidence on the susceptibility status of *Ae. aegypti* 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 of *Ae. aegypti in* Umudike, Abia State, Nigeria. It is hoped also that 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-eastern Nigeria. Ikwuano, is located in the tropical rain forest zone of Nigeria (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 is host to National Root Crops Research Institute, and Michael Okpara University of Agriculture both of which utilize agricultural pesticides.

Mosquito Larval Collection and Rearing: Immature stages of *Aedes* mosquitoes (eggs, 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 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, while the eggs were collected with ovitraps. The bowls were scrutinized for presence of unwanted organisms or predators and a pipette was used to remove any that was found. The *Aedes* mosquito larvae collected were transported in well labelled plastic bottles to the insectaria in the Entomology unit of the National Arbovirus and Vector Research Institute, Enugu, where they were maintained and reared at 26 ± 3 °C and 74 ± 4 % relative humidity to adult stage, and ready to be used for bioassays following the World Health Organisation (WHO) standard. Larvae were fed on ground biscuits and adults were provided with 10 % sugar solution. The resulting adults were identified according to the morphological keys of 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 Arbovirus and Vector Research Institute, Enugu, were used for the bioassay. Tests were carried out using 3 – 5 day old, sugar-fed female *Aedes aegypti* mosquitoes. A maximum of 100 female mosquitoes in four replicates were tested for each insecticide. Accordingly, 4% DDT, 0.05% deltamethrin, 0.1% bendiocarb and 0.25% primiphos-methyl 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 *Ae. aegypti* mosquitoes into the four holding tubes with an aspirator and allowed to stand for one hour. Thereafter, 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 one hour. Records of mortalities were taken at intervals of 0, 15, 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 7% 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, for preservation. All susceptibility tests were carried out at 26 ± 3 °C temperature and 74 ± 4 % 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 (Abbott, 1987). The resistance status of the *Aedes aegypti* mosquito samples was 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 possibility of 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 AND DISCUSSION

The present study presents for the first time, baseline data on the susceptibility status of *Ae. aegypti* to World Health Organization Pesticide Evaluation Scheme (WHOPES) (WHO, 2006) approved indoor residual spray (IRS) insecticides in Umudike, Abia State, Southeastern Nigeria to guide procurement of IRS in the State.

Out of all the insecticides that *Ae. aegypti* was exposed to, the highest mortality was recorded in bendiocarb (100%), followed by primiphosmethyl (97.50%), Deltamethrin (93.75%) and then DDT (62.85%). There was no significant difference in the mortalities observed in bendiocarb, primiphosmethyl and Deltamethrin ($p>0.05$), But there was significant difference between their mortalities and that observed in DDT ($p<0.05$). During the knockdown assessment for *Ae. aegypti,* the highest KDT_{50} was recorded in DDT and bendiocarb, at 50 minutes for both insecticides (Table 1). There was no significant difference ($p>0.05$) between the KDT_{50} of DDT and bendiocarb. But there was significant difference ($p<0.05$) between their KDT_{50} and that of primiphosmethyl and deltamethrin which both had a value of 30 minutes (Table 1). There was also no significant difference ($p>0.05$) between the KDT_{50} values of primiphosmethyl and deltamethrin. From the Table it can be seen that a KDT_{90} value was recorded at 60 minutes for *Ae. aegypti* across all the insecticides, hence there was no significant difference ($p>0.05$) between the KDT_{90} values. The results of the knockdown assessment showed that the tested insecticidal papers induced knockdown of the adult *Ae. aegypti*, suggesting that knockdown mechanisms could be operating in the local mosquito populations of Umudike (Table 2). This confirmed earlier studies which indicated the knockdown 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 indicated the presence of knock down resistance (KDR) (Table 2) mechanism operating in populations of *Ae. aegypti* mosquitoes of Umudike (Kristan *et al.,* 2003; Awolola *et al.*, 2007; Ibrahim *et al.,* 2014; Umar *et al.*, 2014). This could have been responsible for the level of resistance displayed by this mosquito to the various insecticides evaluated.

Using the WHO (2013) criteria for insecticides susceptibility or resistance assessment of mosquitoes, the 24 hour post exposure results indicated that the *Ae. aegypti* mosquitoes were only susceptible to bendiocarb (100 %). This was contrary to documented evidence on the resistance of mosquitoes to bendiocarb (Canyon and Hii, 1999; Ocampo *et al.*, 2011). This could be attributed to low use of carbamate based insecticides in Umudike, unlike in those other areas where resistance was noted (Canyon and Hii, 1999; Ocampo *et al.*, 2011). On the other hand, the *Ae. aegypti* mosquitoes were fully resistant to DDT (62.85 %), and are suspected to be resistant to primiphosmethyl (97.50 %) and deltamethrin (93.75 %) (Table 1). The DDT resistance in *Ae.* mosquitoes was in tandem with the works of Canyon and Hii (1999), Somboon *et al.* (2003), Ocampo *et al.* (2011) and Ibrahim *et al.* (2013). DDT resistance in Umudike could be attributed to the heavy use of organochloride insecticides in the two agricultural centres located in Umudike, which could have led to the development of resistance by mosquitoes to the organochloride class. Cross-resistance to DDT and pyrethroid has been reported in most species of mosquitoes of public health importance resulting from knockdown resistance (kdr) gene (Hemingway and Ranson, 2000).

Table 1: Knockdown assessment and percentage mortality 24 hours after exposure of *Aedes aegypti* mosquitoes exposed to four insecticides

Insecticides	Mortality after 24 hours (%)	KDT_{50} (Minutes)	KDT_{90} (Minutes)
DDT (Organochlorine)	62.85b	50a	60a
Bendiocarb (Carbamate)	100a	50a	60a
Primiphosmethyl (Organophosphate)	97.50a	30b	60a
Deltamethrin (Pyrethroid)	93.75a	30b	60a

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

Table 2: Susceptibility status* of *Aedes aegypti* exposed to four different insecticides

Insecticides	Class	*Aedes aegypti*
DDT	Organochlorine	Resistant
Bendiocarb	Carbamate	Susceptible
Primiphosmethyl	Organophosphate	Suspected Resistance
Deltamethrin	Pyrethroid	Suspected Resistance

**WHO scoring for resistance (WHO, 2013).*

Resistance to pyrethroids generally confers cross-resistance to other insecticides, and that limits the alternative choices of effective insecticides (Brengues *et al*., 2003). The resistance of *Aedes* mosquitoes to deltamethrin and other pyrethroids was opined by Sathantriphop *et al.* (2006), Jirakanjanakit *et al.* (2007) and McAllister *et al.* (2012), although the work of Ocampo *et al*. (2011) gave a contrary view. The slight resistance of *Ae.* to the organophosphate primiphosmethyl agrees with the report Ibrahim *et al.* (2013). Comparatively, the percentage mortalities of *Ae. aegypti* between bendiocarb, primiphosmethyl and deltamethrin insecticides did not differ significantly (p>0.05), but differed significantly (P<0.05) between the three insecticides and DDT.

The gross resistance of this mosquito to DDT (Table 1) goes a long way to prove the inefficacy of organochlorines in the control of *Ae. aegypti* mosquitoes in Umudike. This is in agreement with other researchers who have lamented on the growing resistance of many mosquito species to DDT (Sathantriphop *et al.,* 2006). This raises a grave concern on the effectiveness of organophosphate insecticides in Umudike.

Furthermore, Table 1 revealed that was suspected resistance to deltamethrin (93.75% mortality). This is quite strange because deltamethrin belong to the subgroup of pyrethroids containing an alpha cyano-group in their chemical structure and are extremely potent against insects even at much lower concentration (WHO, 2006), although the finding is in agreement with many authors who have posited the growing resistance of many other mosquito species to pyrethroid insecticides which are predominantly used in IRS and LLITNS (Sathantriphop *et al.,* 2006; Jirakanjanakit *et al*., 2007; McAllister *et al.,* 2012). The current findings go a long way to show that these programmes are already under jeopardy in this locality, except there is a quick and timely response. This stems from the fact that the *Ae. aegypti* mosquitoes in Umudike are already resistant to the organochlorine class, and are likely to develop resistance to pyrethroid and organophosphate classes, with only the carbamate class having potency on them.

Conclusion: The finding of this research suggests that bendiocarb (carbamate) may be used to substitute the pyrethroid, organophosphate, and organochlorine to prevent resistance in *Ae. aegypti* populations in Umudike. It is recommended that further work be done to find out the mode of resistance existent in this mosquito species of Umudike. In view of the limited numbers of insecticides available for vector control, a rational use of insecticides or mosaic strategy can be adopted to delay development of resistance in Ae. aegypti in Umudike and Nigeria at large. In addition, routine surveillance of insecticide susceptibility/resistance in wild mosquito populations across different ecological zones in Nigeria is very critical for effective resistance management, while integrated vector control strategy is advocated for.

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