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Susceptibility Status of *Anopheles* Mosquitoes Exposed to Three Test Papers in Adamawa, Northeast Nigeria

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ABSTRACT

This study was carried out to assess the resistance/susceptibility status of *Anopheles* mosquitoes, a major malaria vector in tropical and subtropical regions of the world, on three classes of insecticides approved by World Health Organization (WHO) for vector control. *Anopheles* larvae were collected from three communities in Adamawa State and reared to adults. Two to three-day old, non-engorged (fed) female *Anopheles* mosquitoes were exposed to discriminating dosages of 0.075% permethrin, 4% DDT and 0.1% bendiocarb using WHO protocols and susceptibility test kit. Knockdown effect was recorded after 60 min (1hr) of insecticide exposure and mortality scored 24h after the exposure. Using WHO (2013) criteria for evaluating resistance /susceptibility of mosquitoes, it was discovered that *Anopheles* mosquitoes from Yola South were susceptible to three test papers, while in Mubi, they were all resistance. *Anopheles* mosquitoes from Numan also showed resistance to DDT and permethrin while, susceptible to bendiocarb. The continual confirmation of resistance in *Anopheles* mosquitoes to the available insecticides especially carbamates will further aggravate problems to the National Malaria Control Program who are tipping carbamates as the possible alternative for pyrethroids in the control of malaria vectors.

KEY WORDS: *Anopheles*, malaria, resistance, susceptibility, test papers.

Introduction

The World Health Organization (WHO) estimates that there were 655,000 malaria deaths in 2010, with 86% occurring in children under 5 years (WHO, 2010). Malaria deaths are declining with the massive scaling up of control measures, of which insecticide-treated bed net (ITNs) are major components. ITNs (Insecticide Treated Nets) reduce death in children (Lengeler, 2004) and provide personal protection to the users and at scale they provide community-wide protection by reducing the number of infective mosquitoes in the vicinity where ITNs are used (Jones *et al*, 2012). Between 2008 and 2010, 254 million ITNs were supplied to countries in sub-Saharan Africa, and the proportion of African Households in possession of a net rose from 3% in 2000 to 50% by 2010 (WHO, 2010). Nets, when in good condition and used correctly, are effective, simple to use, easy to deliver to rural communities and cost effective when used in highly endemic malarious area. On account of their low mammalian toxicity, speeds of action, and high insecticidal activity, pyrethroid are the only insecticide class currently recommended by WHO for use in ITNs (Hougard *et al.*, 2003). ITNs are effective with the African vectors *A. gambiae* s.s. and *A. funestus* in part because these species are endophagic (feed indoors) and endophilic (rest indoors after feeding). Aside from their insecticidal activity, pyrethroids also exert an excite-repellency effect, which leads to fewer mosquitoes entering a home where ITNs are used (Okumo *et al.,* 2013). Because of excite-repellency property of ITNs, these nets retain their personal protection properties for users even after the nets become holed (Darriet *et al.*, 1984).

The emergence and spread of insecticides (pyrethriods, organochlorines, organophosphate, and carbamates) are threating the effectiveness of ITNs and indoor residual house spraying. Currently, 27 countries in sub-Saharan Africa have reported pyrethroid resistance in *Anopheles* vectors (Ranson *et al.*, 2011). Because of their pyrethroid dependency, ITNs are especially vulnerable to insecticide resistance, unlike indoor residual house spraying where there are readily available alternative insecticides. To prevent amplifying pyrethroid resistance, the WHO recommends that pyrethroid insecticides should not be used for indoor residual house spraying in areas with high long-lasting insecticide treated bed net (LLIN) coverage (WHO, 2010). In a recent study the extensive deployment and the use of LLINs was blamed in part of selecting resistance in *Anopheles* vectors in Senegal, where malaria morbidity also increased (Trape *et al*, 2011). The trend of resistance has led the WHO and member of the roll back malaria partnership to produce the "Global Plan for Insecticide Resistance Management in Malaria Vectors" which stresses the urgency with which these problems need to be addressed (WHO, 2010). In Adamawa State currently, there is lack of information on the current susceptibility/resistance status of malaria vector (*Anopheles gambiea* complex) to common classes of insecticides. Hence, compared with the increasing health concern over malaria, which continue to cause mortality and morbidity among children in the sub-sahara Africa, especially in Nigeria, there is need to know the susceptibility/ resistance status of these vector for proper control measures.

MATERIALS AND METHODS

Description of the study area

The study was conducted in three Local Government Areas of Adamawa State Viz; Mubi North, Numan and Yola South Local Government of Adamawa State (Figure 1). Adamawa lies between 8 ^{0}00N and 11^{0}00N and longitude 11^{0}50E and 13^{0}50 E (Adebayo and Tukur, 1999). It is characterized with a tropical regime, with a period of high water from May to September during the rainy season, and a period of low water – or even complete dryness – from October to April (Adebayo and Tukur, 1999).

Collection of Larval Mosquitoes

Immature larval stages of *Anopheles* mosquitoes were collected from three Local Government Areas of the Adamawa State, during the rainy season between August and September, 2014. At each locality, anopheline larvae were collected from various natural breeding sites including ground pools, gutters, tires track and puddles. Water was scooped using a plastic scoop and poured into small transparent plastic bowls. *Anopheles* larvae were separated from culicines larvae by their resting positions in their natural breeding sites during collection. The *Anopheles* larvae collected were transported in well labeled plastic bottles to the Laboratory of the Biological Sciences Department, Adamawa State University Mubi, and were reared to adult stage in an insectary, which were locally constructed using plastic buckets. 10% sugar solution soaked in cotton wool in a small plastic container was kept as food in the insectary. The set up was maintained under Laboratory conditions.

Insecticide Susceptibility Tests

Insecticide susceptibility test was carried out using the WHO standard procedures and test kits for adult mosquitoes (WHO, 1998). Three test papers impregnated with recommended WHO diagnostic concentrations of 4% DDT, 0.75% permethrin and 0.1% bendiocarb were used. The test was carried out using 2-3day old female *A. gambiae* mosquitoes maintained on 10% sugar solution. For each insecticide, four replicates of 20 female mosquitoes were exposed to the test papers, using WHO susceptibility/resistance test kit for 60 minutes. The knockdown effect of each insecticide was recorded every 60 minutes' exposure period. After the exposure, mosquitoes were then transferred to a recovery tube and provided with 10% sugar solution. Mortality was also noted after 24h post-exposure.

Data Interpretation and Analysis

The percentage knockdown of *Anopheles* mosquitoes exposed to the three test papers and the percentage mortality after 24hrs post-exposure were analyzed using descriptive statistics with bar charts. The susceptibility/resistance status of *Anopheles* mosquitoes was determined using the WHO criteria, 2013 (WHO, 2013). By the criteria, mortality ranges between 98-100% indicate susceptibility; while mortality less than 98% is an indication of the existence of resistance.

Results and Discussion

Knockdown effect of the three insecticides on Anopheles mosquitoes after 60 minutes of exposure

Figure 1 shows the percentage of knockdown after 60 minutes of insecticide exposure. The highest knockdown was observed in the populations of *Anopheles* mosquitoes exposed to 0.75% permethrin in Numan. Similar knockdown effects were seen in the populations of *Anopheles* from Yola exposed to 4% DDT and 0.75% permethrin as well as the populations from Mubi, exposed to 0.1% bendiocarb. Generally, there was higher knockdown effects of the three insecticides tested in the populations of *Anopheles* mosquitoes from Numan, followed by the populations from Yola, and the least was in the populations from Mubi. Generally, 0.75% permethrin (pyrethroid) recorded the highest knockdown and the least was in 4% DDT (organochlorine). The order of the efficacy of the three insecticides tested is: 0.75% permethrin (pyrethroid)> 0.1% bendiocarb (carbamate)> 4% DDT (organochlorine).

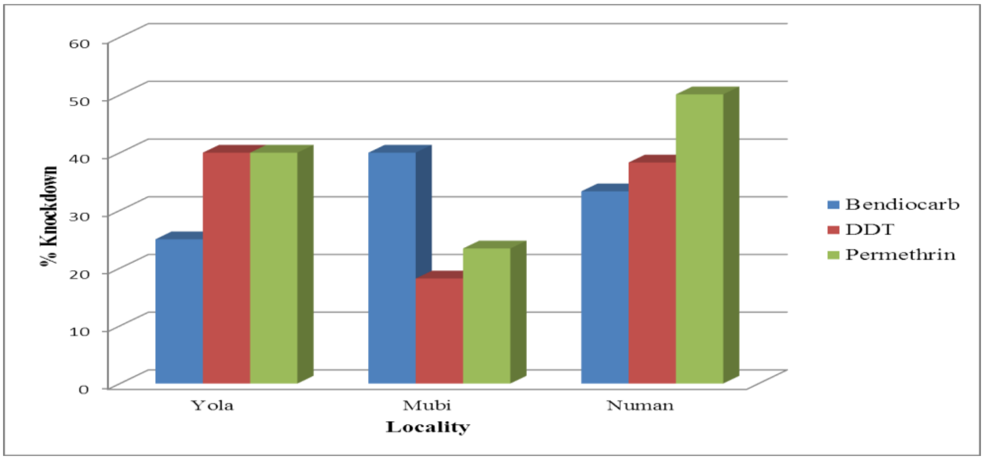

Figure 1: Percentage knockdown of *Anopheles* mosquitoes after 60 minutes of insecticides exposure.

Mortality of Anopheles mosquitoes exposed to three WHO test papers after 24h of exposure

After the exposure, the exposed mosquitoes transferred to the holding tube were noted for mortality after 24h. The mortality observed over the 24h post-exposure period shows that it was higher in the populations from Yola as shown in Figure 2. Although 0.1% bendiocarb recorded higher mortality rate in *Anopheles* population from Numan, the least mortality was also recorded in the same populations exposed to 4% DDT. At the end of the holding period after the insecticide exposure to the three test papers, there was higher mortality observed after 24h in populations from Yola exposed to 0.75% permethrin and 0.1% bendiocarb, while the least was in the populations from Numan exposed to 4% DDT. This shows the efficacy of the pyrethroids on *Anopheles* mosquitoes.

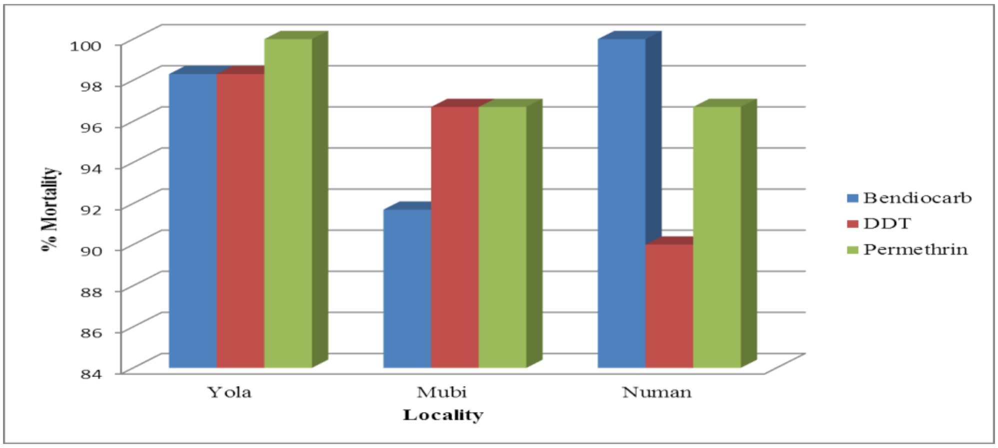

Figure 2: Percentage mortality of *Anopheles* mosquitoes after 24h of insecticide exposure

Resistance/susceptibility status of Anopheles mosquitoes exposed to three WHO test papers

Table 1 shows the resistance/susceptibility status of *Anopheles* mosquitoes exposed to three insecticides in the three study localities in Adamawa State. This was determined using the criteria of WHO (2013) for evaluating *Anopheles* mosquitoes resistance. By the criteria, mortality in the range 98-100% indicates susceptibility, and a mortality of less than 98% is a suggestive of the existence of resistance as shown in Table 1. The populations of *Anopheles* mosquitoes from Yola were all susceptible to the three insecticides tested, while the populations from Mubi were all resistance to the three test papers. Mosquito populations from Numan were only susceptible to 0.1% bendiocarb. The resistance observed in this study could probably be due to the extensive use of carbamates, DDT and pyrethroids for crop protection (Chandre *et al*., 1999). The high level of DDT resistance in this study agrees with Oduola *et al.* (2010), who reported a high level of DDT resistance in *A. gambiae* from rural, semi urban, and urban communities in Nigeria. The study also agrees with Oduola *et al.* (2012) and Kolade *et al* (2013), who also reported a carbamate resistance in the population of *A. gambiae* in the Southwest of Nigeria. In search for a suitable replacement of pyrethroids for Indoor Residual Spray (IRS) which is part of the insecticide management strategy because of the high resistance being reported in *A. gambiae* to pyrethroid and DDT, bendiocarb based IRS are being introduced for malaria vector control in West Africa (Djouaka *et al.*, 2011). But unfortunately, carbamate may no longer be an alternative candidate for malaria vector control, especially in Nigeria, where carbamate resistance has subsequently been reported.

TABLE 1: Resistance/Susceptibility status of *Anopheles gambiae* mosquitoes exposed to three insecticides

Insecticide	Locality	Number exposed	% Mortality	Resistance status
Bendiocarb	Yola	60	98.3	Susceptible
DDT	Yola	60	98.3	Susceptible
Permethrin	Yola	60	100	Susceptible
Bendiocarb	Mubi	60	91.7	Resistant
DDT	Mubi	60	96.7	Resistant
Permethrin	Mubi	60	96.7	Resistant
Bendiocarb	Numan	60	100	Susceptible
DDT	Numan	60	90	Resistant
Permethrin	Numan	60	96.7	Resistant

Conclusion

Pyrethroids, carbamates and organochlorines resistance may seriously jeopardize the efficacy of IRS and LLINs on which most African countries including Nigeria rely on, to reduce malaria transmission. This study reveals the susceptibility/ resistance status of *Anopheles* populations from Adamawa State to

0.1% bendiocarb, 0.75% permethrin and 4% DDT. Therefore, further studies will help for decision making in the National Malaria Control Program particularly in the choice of insecticide to use during campaigns of Indoor Residual Spraying in Adamawa State, Nigeria.

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