Susceptibility pattern of Anopheles mosquito to different classes of insecticides in selected communities in Ila-Orangun, Southwest Nigeria

Isaac Olayinka Oyewole, Mustapha, Abdur-Rahman Kolawole, Oluwakemi Christianah Adedeji, Dapo Adeogun and Sam Awolola

Abstract
Malaria remains a public health issue and it is endemic throughout Nigeria, where it accounts for about one million episodes annually. In recent times, distribution of long lasting insecticide nets (LLINs) was scaled up in Nigeria to curb the menace of the disease. Successful implementation of this strategy depends on the susceptibility of the local anopheline mosquitoes to the insecticides used in treating the LLINs. In the present study, we investigated the susceptibility status and knock-down data of local Anopheles mosquito species using World Health Organization Pesticide Scheme (WHOPES) recommended insecticides. Anopheles species larvae were collected in naturally infested water bodies using the standard (350ml dipper) dipping method from four communities in Ila-Orangun. The unfed 2-3 days old adult females were subjected to susceptibility test following WHO recommended protocol against six insecticides (0.05% Lambdacyhalothrin, 0.75% Permethrin, 0.05% Deltamethrin, 4% Dichloro-Diphenyl-Trichloroethane (DDT), 1% Fenitrothion and 0.1% Bendiocarb) using diagnostic kits. Anopheles gambiae were found to be resistant to Lambdacyhalothrin, Permethrin and Deltamethrin, and DDT but susceptible to Fenitrothion and Bendiocarb. The susceptibility pattern observed could be attributed to the types of pesticides/insecticides used for agricultural activities and public health programmes in the study area. The implication of this study to the success of vector control programmes is discussed.

Keywords: anophelinea, Anopheles gambiae s.s., susceptibility, insecticides

1. Introduction
The level of morbidity and mortality, especially in children and pregnant women due to malaria attack in sub-Saharan Africa is still a great cause for concern. Despite advances in control, treatment and preventive measures adopted in the past and recent times, malaria still threatens lives of millions of people in African countries. Success of the control measures adopted in the past decades, using indoor residual spray, insecticide treated nets and treatment with Artemisinin-based Combination Therapies (ACT’s) have been limited by both the mosquito and parasite resistance respectively. In tropical countries, prevailing environmental conditions such as high humidity and warmth which support mosquito growth have contributed to malaria transmission in this part of the world. Other contributing factors include socio-cultural and economic attributes such as education, income, housing patterns, social groups, water storage also play major role in malaria transmission[1]. Generally, malaria is known to be more prevalent in rural and peri-urban settlements in sub-Saharan African due to availability of favourable conditions for the breeding of anopheline vector. Dwellers in these areas are often bedevilled with poverty, poor housing, poor water supply, poor environmental conditions which are often laden with swamps, gutters and thick vegetations to enhance the breeding of mosquito. The population in this region are usually more vulnerable to mosquito bites, hence high malaria transmission intensity.

In Nigeria, malaria is endemic throughout the year in the entire country including the urban areas where more than 90% of the approximately 132 million people are at the risk of the infection. According to the Federal Ministry of Health[2], half of the population in Nigeria suffers one or more malaria attacks annually. In the recent times, efforts directed towards the control of malaria include the interruption in the disease transmission by reducing man-
mosquito contact. In Nigeria, the two major approaches adopted are the use of long lasting insecticide treated nets (LLINs) and indoor residual spray (IRS). In 2014, Elimination Programme (NMEP) in Nigeria scaled up indoor residual spraying (IRS) in some regions to supplement LLINs. However, the efficacy of those malaria control strategies has been limited with the development of resistance by *Anopheles* mosquitoes to all classes of WHO-recommended adult insecticides, particularly pyrethroids [2, 3]. Four classes of insecticides are currently recommended for malaria control includes Pyrethroids, Organochlorine, Organophosphate and Carbamate, out of which only pyrethroids is currently approved for LLINs due to its safety, residuality and cost effectiveness. However, Pyrethroid resistance to *Anopheles* species has been an issue since it was first reported in Nigeria in 2002 [4] and this has been on a growing trend ever since [6-14]. Resistance in malaria vectors to Pyrethroids has also been reported in 23 out of 49 African countries [15-31]. The efficacy of interventional insecticides is germane to the successful implementation of both IRS and LLINs which invariably is a factor of availability of insecticide(s) susceptible *Anopheles* mosquitoes in the local environment. Therefore, regular monitoring of susceptibility status of local *Anopheles* vectors to insecticides is essential to determining the effectiveness of malaria control programmes which rely solely on LLINs and IRS interventions.

The present study tested the susceptibility status of *Anopheles gambiae* to Lambdacyhalothrin, Permethrin, Deltamethrin, DDT, Fenitrothion and Bendiocarb.

2. Materials and Methods

2.1 Study area

This study was conducted from May to October 2017 and all samples were collected from four selected sites within Ila-Orangun in Osun Central Senatorial District, South-Western Nigeria. The climate of the area is characteristic of the forest zone with two district seasons, that is, the rainy season from April to October and dry season from November to March. Housing structures consist of both traditional houses (40-70%: mud wall with thatched roof) and modern homes (20-30%: brick houses with corrugated iron roof). The inhabitants are mainly of the Yoruba ethnic group with similar culture and traditions. The population is dominated by farmers who engaged in planting of cash crops such as cocoa, kolanut, cashew and other food crops such as yam, maize, cocoyam and vegetables. They often used synthetic pyrethroids to treat and prevent their crops from pest infestation. Malaria is endemic in these areas where transmission associated with *An. gambiae s.s* occurs year round.

2.2 Sample collection, identification and susceptibility test

Anopheline larvae collected from the natural breeding habitat in the selected areas using the standard (350ml dipper) method were reared to adulthood. Bioassays were conducted on non-blood fed, 2 to 3 days old female mosquitoes using the standard WHO procedures and susceptibility test kits [32]. Samples were identified morphologically [33, 34] and later subjected to susceptibility test. Bioassays were conducted by exposing the mosquitoes to six test papers impregnated with Lambdacyhalothrin (0.05%), Permethrin (0.75%), Deltamethrin (0.05%), Bendiocarb (0.1%), Fenitrothion (1.0%) and DDT (4%) and untreated control using the WHO standard procedures and test kits [32]. For each insecticide paper and the control, a three replicates of 20 adult mosquitoes were exposed to toxicant tubes containing insecticide impregnated papers for 60 min and cumulative knocked-down was recorded at intervals of 10 min. Mosquitoes were then transferred into clean WHO observation tubes, fed with 10% glucose solution and final mortality recorded after 24h pre-exposure.

The resistance status was defined according to WHO guidelines [35] which states as follows: 98-100% mortality indicates susceptibility, 90-98% indicates possibility of resistance for confirmation, and <90% indicates resistance. The dead and survived mosquitoes were later separated and kept individually in Eppendorf tubes containing desiccated silica gel for further tests.

2.3 Statistical analyses

Determination of the knock-down time (KDT$_{50}$ and KDT$_{90}$) and 95% confidence interval (CI) were conducted with probit analysis using the STATA statistical package (STATA Corp LP, USA, Version 9). Comparisons of proportions between categorical variables and determination of fitment of probit were performed using a Chi-square test (P-value 0.05). The mortality rate was calculated as the percentage of individuals that died within 24h of post-exposure. Susceptibility pattern was determined using WHO [35] criteria: an overall mortality ranging from 98-100% indicates susceptibility, 90-98% of resistance, less than 90% indicates resistance.

3. Results

3.1 Knock-down bioassays

The results of knock-down effect of six insecticides as determined against *An. gambiae s.s* in the study sites over an exposure time period of one hour is shown in Table 1. The results indicate that the lowest KDT$_{50}$ and KDT$_{90}$ values of 24.91min and 31.69min was observed in Bendiocarb while highest KDT$_{50}$ and KDT$_{90}$ of 189.56 min and 234.06min was in DDT. In the pyrethroid group, the highest KDT$_{50}$ and KDT$_{90}$ ranging from 58.58min to 102.01min was obtained in Lambdacyhalothrin.

<table>
<thead>
<tr>
<th>Insecticide group</th>
<th>Insecticide paper</th>
<th>% Conc</th>
<th>Number exposed</th>
<th>KD$_{50}$ (95% CI) (min)</th>
<th>x$^2$ (p)</th>
<th>KD$_{90}$ (95% CI)(min)</th>
<th>x$^2$ (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrethroids</td>
<td>Lambdacyhalothrin</td>
<td>0.05</td>
<td>100</td>
<td>58.58 (43.59-164.92)</td>
<td>1.67 (0.7)</td>
<td>102.01 (65.46-156.10)</td>
<td>0.44 (0.6)</td>
</tr>
<tr>
<td></td>
<td>Permethrin</td>
<td>0.75</td>
<td>97</td>
<td>41.84 (29.97-47.67)</td>
<td>5.17 (0.02)</td>
<td>71.19 (13.47-184.49)</td>
<td>0.14 (0.9)</td>
</tr>
<tr>
<td></td>
<td>Deltamethrin</td>
<td>0.05</td>
<td>95</td>
<td>53.79 (49.16-95.32)</td>
<td>0.31 (0.9)</td>
<td>59.44 (41.70-107.14)</td>
<td>0.18 (0.8)</td>
</tr>
</tbody>
</table>

Table 1: Knock-down rate for different insecticides during one hour to exposure in the study area
3.2 Percentage knock-down
The percentage knock-down achieved from all the study sites is represented in Fig. 1. Among the six insecticides tested, the least knock-down percent range (1-2%) was recorded for DDT while the highest knock-down percent range (11-100%) was recorded for Bendiocarb. In the pyrethroids group, the least knock-down percent range (1-31%) was observed for Lambdacyhalothrin within one hour of exposure period.

![Fig 1: Knock-down rate for different insecticides during 1 hour of exposure in the study area](image)

### Table 2: Susceptibility pattern of *Anopheles* mosquitoes against different insecticides in the study area

<table>
<thead>
<tr>
<th>Insecticide group</th>
<th>Insecticide paper</th>
<th>% Conc</th>
<th>% Mortality</th>
<th>Mortality rate (%) SD</th>
<th>Mortality rate (95% CI)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrethroids</td>
<td>Lambdacyhalothrin</td>
<td>0.05</td>
<td>47(100)</td>
<td>47±2.63</td>
<td>47 (38.4-52.3)</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>Permethrin</td>
<td>0.75</td>
<td>55(97)</td>
<td>56.70±2.08</td>
<td>56.70 (47.9-63.4)</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>Deltamethrin</td>
<td>0.05</td>
<td>53(95)</td>
<td>55.78±1.89</td>
<td>55.78 (52.9-64.5)</td>
<td>R</td>
</tr>
<tr>
<td>Carbamate</td>
<td>Bendiocarb</td>
<td>0.1</td>
<td>99(100)</td>
<td>99±0.5</td>
<td>99 (97.6-99.9)</td>
<td>S</td>
</tr>
<tr>
<td>Organochlorin</td>
<td>DDT</td>
<td>4.0</td>
<td>9(86)</td>
<td>10.47±2.06</td>
<td>10.47 (8.7-12.6)</td>
<td>R</td>
</tr>
<tr>
<td>Organophosphate</td>
<td>Fenitrothion</td>
<td>1.0</td>
<td>98(105)</td>
<td>93.33±0.58</td>
<td>93.33 (90.9-97.7)</td>
<td>S</td>
</tr>
</tbody>
</table>

Number of tested mosquitoes in parentheses; % Mortality: Mortality rate 24 h post exposure; R: suggests resistance; S: indicates susceptibility.
4. Discussion

4.1 Aim and procedure of bioassays
Assessment of the successful implementation of LLINs and IRS for vector control requires periodic monitoring of resistance in the insecticides used for their treatment [16]. In the present study, WHO insecticide bioassays were carried out to investigate the susceptibility pattern of *Anopheles* mosquitoes to Lambdacyhalothrin, Permethrin, Deltamethrin, Bendiocarb, DDT and Fenitrothion in the selected area. To achieve the aim of this study two to three days old female anopheline mosquitoes were used for the bioassay according to WHO [15] guidelines.

4.2 Insecticide knockdown assay
The results of the insecticide knockdown assay showed that the test paper induced knockdown of the anopheline mosquitoes which indicates the presence of kdr mutation as previously reported [7-8, 10-13, 37]. The KDT$_{50}$ and KDT$_{90}$ values for DDT were very high, ranging between 189.56 (CI: 94.97-272.66) and 234.06 (CI: 104.70-356.80) respectively. Previous studies have also reported high KDT values from the regions with high level of DDT resistance [38-40].

4.3 Susceptibility pattern
Records from the susceptibility test of the local anopheline species to insecticides during the 24h post-exposure indicate that the organisms were susceptible to Fenitrothion and Bendiocarb. This is in contrast to the earlier studies in Nigeria where resistance of *Anopheles* species to Bendiocarb were reported [12, 13, 41] and elsewhere in Benin Republic, resistance to Fenitrothion and Bendiocarb was also documented [29]. However, in another studies conducted in Ibadan (Oyo State), Nigeria, a neighbouring state to Osun State, Nigeria, where this present study was conducted, susceptibility of anopheline species to Fenitrothion and Bendiocarb was also reported [14]. These results may indicate a stable susceptibility to these insecticides in this part of south western Nigeria, hence, a possibility of replacing Permethrin which are becoming resistance with Bendiocarb particularly for indoor residual spray (IRS). Elsewhere in Africa (Tanzania) susceptibility of *An. gambiae* s.l to Bendiocarb has also been documented [31] while at the borderline of Iran, Armenia, Azerbaijan and Turkey susceptibility of *An. sacharovi* to Propoxur and Bendiocarb was also reported [42]. This may indicate that susceptibility of anopheline species to Fenitrothion and Bendiocarb varies from one region to another. This implies that there is the need for periodic monitoring of the susceptibility pattern of these insecticides in order to guarantee the effectiveness and success of the control programmes.

In the present study, the local anopheline species showed resistance to all the pyrethroids and DDT tested. The previous studies in Nigeria and elsewhere in Africa have also shown evidences of resistance of anopheline mosquitoes to Permethrin [8, 30, 36, 43-45], Deltamethrin [13, 30, 31, 38, 41, 43, 46, 47] Lambdacyhalothrin [13, 30, 43, 48]. The susceptibility pattern of the local mosquito species as observed in this study could be attributed to the agricultural activities in the area where pyrethroids are used extensively for pest control. Previous studies have also associated resistance to pyrethroid with the intensity of agricultural practices in those areas where resistance level increases with the increase in agricultural spread [49-52]. In the recent years, pyrethroid based long lasting insecticidal net (LLIN) has also been distributed on a large scale by the government agencies in Nigeria. All these activities might have increased the selection in malaria vector to this class of insecticides. Resistance of *Anopheles* mosquitoes to pyrethroids and DDT is a serious cause for concern as this may have serious implications on the success of malaria control programmes in the country.

5. Conclusion
This study showed that *Anopheles gambiae* was resistance to Lambdacyhalothrin, Permethrin and Deltamethrin, and DDT but susceptible to Fenitrothion and Bendiocarb. The susceptibility pattern observed could be attributed to the types of pesticides/insecticides used for agricultural activities and public health programmes in the study area. There is the need for periodic monitoring of susceptibility pattern of insecticides used in malaria vector control strategies in order to delay the expansion of insecticide resistance and to guarantee success of the intervention programmes.

6. Acknowledgements
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7. Conflict of interests
The authors declare that they have no conflict of interests.

8. Ethics approval and consent to participate
This is not applicable. But we obtain verbal consent from the community leaders and household heads after the purpose of the study was clearly explained to them.

9. References


