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Susceptibility of *Anopheles gambiae sensu lato* (Diptera: Culicidae) to Permethrin, Deltamethrin and Bendiocarb in Ibadan City, Southwest Nigeria

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Abstract: This study was carried out to assess the resistance/susceptibility level of *An. gambiae s.l.*, a major malaria vector in Ibadan metropolis, south west Nigeria, to two classes of insecticides approved by World Health Organization (WHO) for vector control. *Anopheles* larvae were collected from two communities (Ojoo and Bodija) within the metropolis and reared to adults. Two to three-day old, non-engorged female mosquitoes were exposed to discriminating dosages of 0.05% deltamethrin, 0.75% permethrin and 0.1% bendiocarb using WHO protocols and susceptibility test kits. Knockdown effect was recorded every 10 min and mortality scored 24 h after exposure. Species identification was by morphological characteristics only. The study revealed that the major malaria vector in Nigeria, *An. gambiae s.l.*, were susceptible (mortality rate of 98.8%) to deltamethrin in Ojoo community, while Bodija recorded marginal susceptibility (87.5%). Both sites showed reduced susceptibility to permethrin and resistance to bendiocarb with 24 h post exposure mortalities ranging from 83.5 to 87.7% and 70.0 and 52.5%, respectively. The median Knockdown Time (KDT₅₀) from 22-44.2 min for the pyrethroids (permethrin and deltamethrin), while that of carbamates (bendiocarb) range from 35.2 to 49.5 min. Resistance recorded in the field populations of *An. gambiae s.l.* in Ibadan to bendiocarb was not observed in same population exposed to the pyrethroids (deltamethrin and permethrin) but with differential susceptibility. Therefore Bendiocarb may not be suitable as alternatives to mitigate pyrethroid resistance. A rationalised use of these insecticides coupled with regular monitoring of resistance status is essential to improve and evaluate the efficacy of the current vector control tools (ITN and IRS).

Keywords: *An. gambiae s.l.*, insecticides, malaria vectors, Nigeria, resistance, susceptibility

INTRODUCTION

In the absence of a vaccine, malaria control programmes rely on the combination of effective vector control and efficacious treatment of clinical cases (WHO, 2008). Indoor Residual Spray (IRS) of insecticides and Long Lasting Insecticide treated Nets (LLINs) are the two most important malaria vector control tools in the tropical world (Yakob *et al.*, 2011).

Both methods have shown to be very effective not only in reduction of adult anopheline vector population densities (Service and Davidson, 1964; Magesa *et al.*, 1990) but also preventing malaria parasite transmission (Kamau *et al.*, 2007). High coverage with either of these interventions can result in a dramatic reduction in malaria associated morbidity and mortality (Lengeler, 2004; Pluess *et al.*, 2010). Progress in malaria control in African countries in the past decade can be attributed largely to the massive scale up of insecticide-based interventions targeting malaria carrying mosquitoes. Through the support of developing partners such as

Global fund World Bank, UNICEF, USAID and Canadian Red Cross, Nigeria is currently scaling-up its malaria control programme through free mass distribution of Long-Lasting Impregnated Nets (LLINs) and Indoor Residual Spraying (IRS) (USAID/PMI/CDC, 2011). In 2008, the overall ITNs ownership coverage was 8% and reached 42% in 2010 (USAID/PMI/CDC, 2011). Similarly, the proportion of the vulnerable groups (pregnant women and children) sleeping under ITNs has increased from 6% in 2008 to 29% in 2010 and IRS has been piloted in selected areas in the country (NPC *et al.*, 2012). Both methods depend heavily on the use of safe and effective insecticides. 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 public health insect pests. In addition, lack of available alternative insecticides for vector control has

also been an issue (Coleman *et al.*, 2006). For example, the current control tools are highly dependent on a single class of insecticides, the pyrethroids. Out of the four classes of insecticides (organochlorine, pyrethroids and carbamates and organophosphates) approved for malaria vector control (Devine and Ogusuku, 2009) pyrethroids are the most compounds for IRS and the only insecticide class recommended for use in ITNs (WHO, 2010a). This is for safety, efficacy, acceptability and cost reasons (WHO, 2010b). Unfortunately, resistance to pyrethroids in *An. gambiae s.l* and *An. funestus* has emerged and it is spreading rapidly (Etang *et al.*, 2003; Corbel *et al.*, 2004). This has constituted an impediment to vector control initiatives in African countries (Hemingway *et al.*, 2004; WHO, 2005). In Nigeria, the development of resistance to DDT and other classes of insecticides including organochlorine, organophosphate, carbamates and recently pyrethroid 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 (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). Also in North-central Nigeria, Permethrin and DDT resistance in *An. gambiae s.l* has been reported (Ndams *et al.*, 2006; Olayemi *et al.*, 2011).

In light of ongoing scaling-up of control tools (LLINs and IRS) with the goal to reduce by 50% the malaria related morbidity and mortality in Nigeria by 2013 (USAID/PMI/CDC, 2011). It is therefore crucial that information on the susceptibility of major malaria vectors to common insecticide used in public health and the underlying mechanism are investigated nationwide. This will properly informed control programs of the most suitable insecticide to use and facilitate the design of appropriate resistance management strategies (Djouaka *et al.*, 2011). Furthermore, there is a need to look into alternatives to pyrethroid insecticides to manage resistance. Reports from the neighboring country, Republic of Benin has identified carbamates bendiocarb as a potential alternative to combat pyrethroid-DDT resistance in *An. gambiae* populations (Corbel *et al.*, 2007; Akogbeto *et al.*, 2010), mainly because of its separate mode of action (Oduola *et al.*, 2012).

However, there is dearth of information on the insecticide resistance status of the field strain of *An. gambiae* in Nigeria to carbamate insecticide (Olayemi *et al.*, 2011; Oduola *et al.*, 2012).

In this study, we investigated susceptibility status of *An. gambiae s.l* population in Ibadan city Southwest Nigeria to pyrethroids (permethrin and deltamethrin) and alternative insecticide, carbamate (bendiocarb).

MATERIALS AND METHODS

Study area: This study was conducted in Ibadan. Ibadan (07°22'N, 03°58'E) is the capital city of Oyo state in south western Nigeria. It belongs to transitional forest-savannah climatic domain with average temperature ranges from 25 to 38°C and mean annual rainfall is 131.42 cm. The city has an estimated population of 3.57 million people on an estimated land area of 3080 km² (Fig. 1).

Mosquito larval collection and rearing: Immature stages of anopheles mosquitoes were collected from two sites, Bodija and Ojoo, within Ibadan metropolis during the rainy season July to September 2011. At each locality chosen, Anopheline larvae were collected from various natural breeding sites including ground pools, gutters, tire tracks and puddles. 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 instars larvae in order to have sufficient adult emergence of the same physiological age. The bowls were scrutinized for presence of unwanted organisms or predators; if any were found, a pipette was used to remove them. The Global Positioning System (GPS) was used to establish the coordinates of the study sites. The mosquito larvae collected were transported in well labeled plastic bottles to the insectary in the Entomology unit of the Department of zoology, University of Ibadan, where they were maintained at 26±3°C and 74±4% relative humidity. The resulting adults were identified as *An. gambiae s.l* according to the morphological keys (Gillies and Coetzee, 1987). Female adult mosquitoes belonging to the *Anopheles gambiae* complex were blood-fed and placed in breeding tubes, three days post-feeding to lay eggs. Emerging adult females (F1 generation) were fed on sugar 10% solution and used in the bioassays.

Insecticide susceptibility tests: Insecticide susceptibility tests were carried out using the WHO standard procedures and test kits for adult mosquitoes (WHO, 1998). Three types of WHO bioassay test papers impregnated with recommended diagnostic concentrations of 0.05% deltamethrin, 0.75% permethrin and 0.1% bendiocarb were used. The Kisumu reference strain susceptible to all insecticide classes was used to establish the potency of the treated papers before the exposures were carried out using wild samples. Tests were carried out using 2-3 day old, sugar-fed female mosquitoes. For each insecticide, four replicates of 20 female mosquitoes were exposed by tarsal contact to insecticide-impregnated test papers in the test tubes for 1 h. Controls included batches of mosquitoes from each site exposed to untreated papers. The knockdown effect of each insecticide was recorded

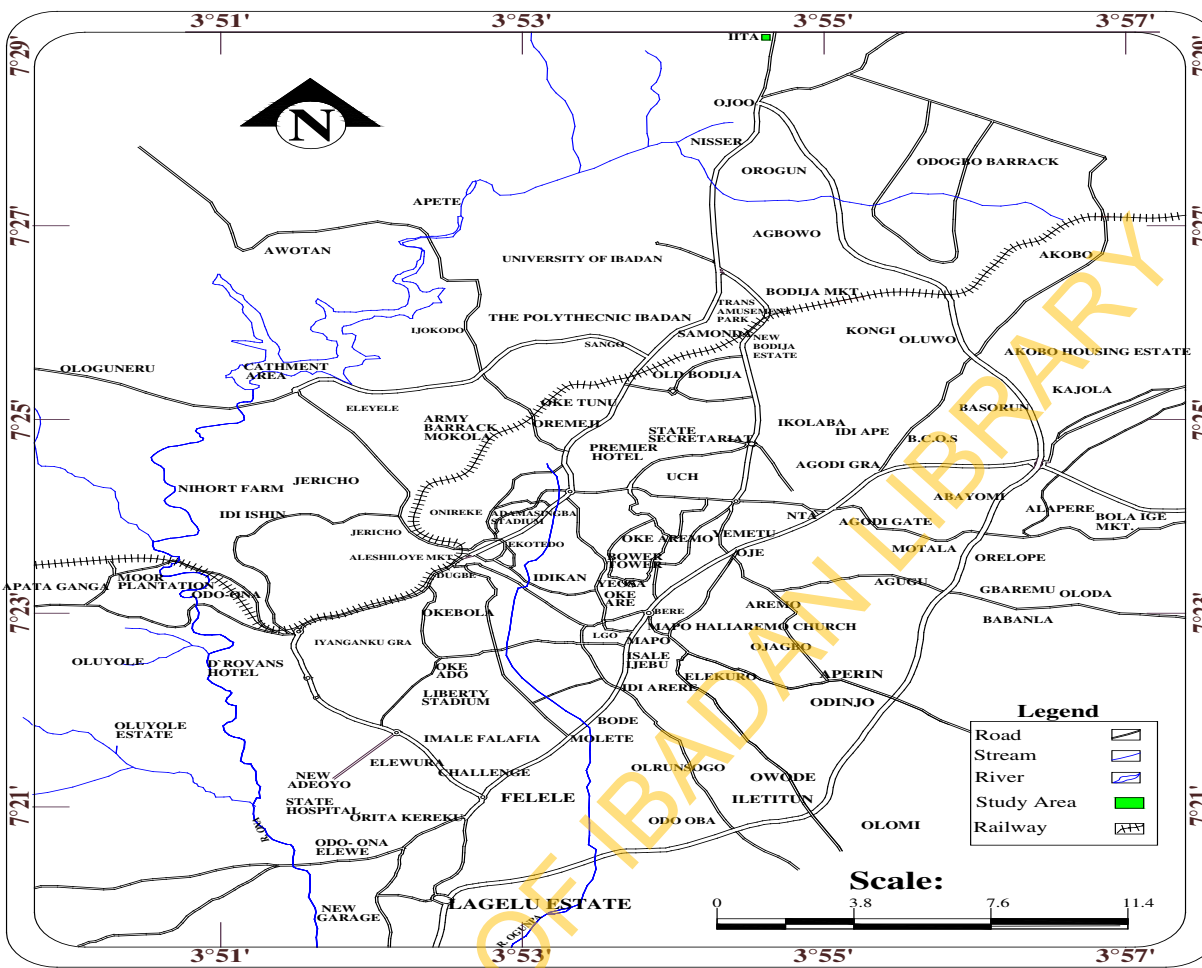


Fig. 1: Map of Ibadan metropolis showing the larval collection sites

every 10 min over the 1 h exposure period. After the exposure, mosquitoes were then transferred to a recovery tube and provided with 10% honey solution. Final mortalities were recorded 24 h post-exposure and the susceptibility status of the population was graded according to WHO recommended protocol (WHO, 1986). Dead and survived mosquitoes from this bioassay were separately kept in clearly labeled 1.5 mL tubes containing silica gel, for species identification. All susceptibility tests were carried out at $25 \pm 3^\circ\text{C}$ temperature and $75 \pm 6\%$ relative humidity.

Data interpretation and analysis: The resistant status of mosquito samples was determined according to WHO criteria (WHO, 1986a). Mortality rates of less than 80% indicate resistance while those greater than 98% indicate full susceptibility. Mortality rates between 80-98% suggest the possibility of resistance that needs to be clarified. Mortality rates were corrected using Abbott formula when control mortality was above 5% (Abott, 1987). The Student t test in SPSS version 15.0 was used to compare the KDT_{50} and KDT_{95} between the insecticides.

RESULTS

Mortality: Overall, 1,335 adult mosquitoes were reared from larvae collected from two localities (Ojoo and Bodija) within Ibadan city. Of these, 600 female adult were morphologically identified as *An. gambiae s.l* and exposed to diagnostic doses of three insecticides; permethrin (0.75%), deltamethrin (0.05%) and bendiocarb (0.1%). The mortality and knock down rates and resistance status of field populations of *An. gambiae s.l* against the insecticides were shown in Table 1. The mosquito populations from the both Ojoo and Bodija were resistance to 0.1% bendiocarb with 24 h post exposure mortalities of 70.0 and 52.5% respectively. However, they showed reduced susceptibility to 0.75% permethrin with 24 h post exposure mortalities ranging from 83.5 to 87.7% indicating suspected resistance that needs investigation. Susceptibility to 0.05% deltamethrin was found among mosquito populations in Ojoo community with mortality rate of 98.5%. Mosquito species from Bodija were less susceptible with mortality rate of 87.5%. Difference in mosquito mortality rates between the two

Table 1: Knockdown times and percentage mortality (with 95% confidence intervals) of *Anopheles gambiae s.l* mosquitoes exposed to dosages of insecticides for 60 min

Locality	Insecticides	N	KDT ₅₀ (min)	95% CI	KDT ₉₅ (min)	95% CI	Knockdown after 1 h exposure (%)	Mortality (%) after 24 h	Susceptibility status
Ojoo	Permethrin (0.75%)	80	34.00	(16.4-21.6)	64.60	(61.3-64.6)	85.02	87.65	Suspected
	Deltamethrin (0.05%)	80	44.20	(41.1-47.7)	117.30	(92.6-157.4)	98.75	98.53	Full susceptible
	Bendiocarb (0.1%)	80	49.50	(44.7-51.8)	85.41	(64.5-122.7)	71.25	70.03	Resistance
Bodija	Permethrin (0.75%)	80	22.50	(17.1-31.9)	42.81	(38.9-46.7)	56.25	83.52	Suspected
	Deltamethrin (0.05%)	80	27.70	(25.2-30.6)	61.91	(56.9-74.1)	92.50	87.52	Suspected
	Bendiocarb (0.1%)	80	35.20	(26.5-42.3)	70.32	(67.4-82.9)	70.00	52.51	Resistance

CI: Confidence intervals

Table 2: Progressive mean number of knockdown and mean total mortality rates of anophelines population from Ojoo and Bodija communities in Ibadan metropolis, during exposure to insecticides

Sites	Insecticides (conc)	Mean number of Knockdown (KD) mosquitoes							Mean mortality @ 24 h
		0 min	10 min	20 min	30 min	40 min	50 min	60 min	
Ojoo	Control (0.0%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.00±7.94
	Bendiocarb (0.1%)	2.25±0.96	4.25±2.50	5.50±1.92	8.75±3.40	10.25±3.59	11.25±2.87	14.25±0.96	14.00±1.83
	Deltamethrin (0.05%)	9.00±3.16	13.50±1.73	15.25±0.96	18.25±2.22	19.50±0.58	19.50±0.58	19.75±0.50	19.75±0.50
	Permethrin (0.75%)	4.00±2.80	5.75±1.71	7.25±2.63	13.25±2.87	15.25±2.22	17.00±2.16	19.75±0.50	18.00±1.41
Bodija	Control (0.0%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.33±3.51
	Bendiocarb (0.1%)	2.25±1.50	2.75±1.26	3.75±1.50	5.75±0.96	8.75±3.78	9.50±2.38	14.00±1.83	10.50±2.65
	Deltamethrin (0.05%)	5.50±1.73	5.75±1.50	10.50±1.29	12.00±1.82	14.75±1.26	18.00±1.41	18.50±0.58	17.50±1.29
	Permethrin (0.75%)	2.50±1.29	1.75±0.96	1.75±1.50	6.00±0.82	7.00±0.82	11.00±1.83	11.25±1.71	17.00±1.83

CI: Confidence intervals

communities for each insecticide treatments was not significant ($p > 0.05$), using t-test.

Knock down: The median Knockdown Time (KDT₅₀) obtained from time-mortality regression using probit analysis range from 22.5 to 44.2 min, while the KDT₉₅ ranged from 42.8 to 117.3 min for the pyrethroids (permethrin and deltamethrin). The knock-down effect of deltamethrin was more pronounced than that of permethrin (Table 2) for the carbamates bendiocarb, the KDT₅₀ and KDT₉₅ ranges from 35.2 to 49.5 min and 70.3 to 85.4 min, respectively. The longest median Knockdown Time (KDT₅₀) of 49.5 min was observed in Ojoo mosquito population exposed to 0.1% bendiocarb, while the shortest knockdown of 22 min was recorded in Bodija mosquito population exposed to 0.75% deltamethrin.

None of the three insecticides recorded a complete knock down within 60 min exposure. Considerably it took a long time for mosquitoes to be knockdown by bendiocarb compared to other two insecticides.

Table 2 shows the mean total number of adult female *An. gambiae s.l* knocked down at 10 min interval during an hour exposure to three insecticides and the mean total mortality rates recorded after 24 h recovery exposure. In both sites, all the three insecticides showed gradual knockdown abilities at various times during the 1 h exposure. Only deltamethrin was able to knockdown over 60% of the mosquito population within 30 min of exposure, while appreciable knockdown owing to permethrin and bendiocarb occurred at the latter times (40-60 min). While the significant (>50%) knockdowns due to deltamethrin exposure were restricted to last 30 min, that of bendiocarb were limited to last 20 min in both Ojoo and Bodija communities. All the insecticides tested show prolong knockdown activities but the knockdowns owing to deltamethrin was time after time

significantly higher ($p < 0.05$) than other two insecticides throughout the exposure time. At Ojoo and Bodija survivors were found, >90% of *An. gambiae s.l* exposed to deltamethrin but <75% of those exposed to bendiocarb were knock-down during the 1 h exposure. The knock-down owing to 1 h exposure to permethrin was 85 and 56.25% in Ojoo and Bodija respectively. The corresponding mortalities at each of these sites, recorded 24 h after exposure, were also higher for deltamethrin than for other two insecticides (Table 1).

DISCUSSION

The resistance/susceptibility status of *Anopheles gambiae sensu lato* mosquitoes to 0.75% permethrin, 0.05% deltamethrin and 0.1% bendiocarb was investigated in Ibadan city, southwest Nigeria. Based on the conventional criteria for characterizing insecticide resistance/susceptibility, where susceptibility is defined by mortality rates greater than 98% 24 h post exposure, there was evidence of resistance among the mosquitoes tested from the two study sites (Ojoo and Bodija) in the city to bendiocarb and marginal resistance to permethrin. While the Ojoo mosquito populations were susceptible to deltamethrin, the Bodija collections recorded partial susceptibility indicating suspected resistance that needs investigation. The level of permethrin resistance (12.5%) in *An. gambiae* populations at the present study sites was much lower than the previous resistance levels of 72.2-79.6% previously reported by Awolola *et al.* (2003) in Alakia, a locality which is less than 10 km from the study sites. This signifies the focal nature of insecticide resistance which is not unusual at relatively short distances (Ranson *et al.*, 2009). These different levels of insecticide susceptibility may reflect differential insecticide selection pressure exerted on field mosquito populations (Kerah-Hinzoumbé *et al.*, 2008).

Alakia is a rural community in Ibadan with substantial agricultural use of insecticides (Awolola *et al.*, 2002). Also the high resistance observed may probably be due to the extensive use of DDT and pyrethroids for crop protection (Chandre *et al.*, 1999). The use insecticides for malaria control and of pesticides in agriculture is however by and large low in Ojoo and Bodija. The marginal pyrethroid resistance might have resulted from selection pressure exerted on the *An. gambiae* populations by the extensive use of pyrethroid based aerosol and coils for domestic pest and vector control (Kristan *et al.*, 2003). Furthermore, selection pressure due to increased LLINs coverage or spreading of the resistant mosquito population is other possible causes in urban cities (Kabula *et al.*, 2011). Nevertheless, the relatively low frequencies of resistance found in the present study should not hinder the promotion and the use of LLINs for protection against malaria transmission in this part of southwest Nigeria. The incidence of urban mosquito populations resistant to multiple classes of insecticides used in public health witnessed in this study is not new, as Oduola *et al.* (2010, 2012) reported similar occurrence in *Anopheles gambiae* populations in Lagos city, Nigeria. Similar observations were reported in *An. gambiae* populations in Guinea Conakry (Vezenegho *et al.*, 2009) and Congo (Koekemoer *et al.*, 2011). The detection of multiple resistances in major vectors of malaria in Nigeria may have grave implications for the malaria control programme. It may compromise the efficacy of interventions and potentially lead to the failure of IRS and ITNs based vector control. While this data serves to highlight the threats more information is required on all the underlying mechanisms of insecticide resistance so that a suitable insecticide resistance management plan may be put in place to sustain vector control.

The marginal resistance and resistance recorded in *An. gambiae s.l* populations from the two study sites, to the three insecticides tested is characterized by high KDT₅₀ that ranges from 34.0-49.5 min and 22.5-35.2 min in Ojoo and Bodija communities, respectively. Comparable high KDT₅₀ was recorded in the sites with full susceptibility to deltamethrin. The result from this study is consistent with the higher KDT₅₀ found in urban *An. gambiae* populations exposed to these insecticides in Lagos, southwest Nigeria (Oduola *et al.*, 2012). Higher KDT₅₀ 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). This may be consistent with study carried out in southwest Nigeria by Kristan *et al.* (2003) who reported absence or low frequency of kdr in *An. gambiae s.l*. This calls for further investigation to be carried out to determine the presence of kdr alleles in our mosquito population.

At comparable toxicities, deltamethrin was more efficacious than the permethrin. The higher mortality

owing to exposure to deltamethrin compared to permethrin observed in this study is consistent with those other studies conducted in Nigeria (Awolola *et al.*, 2002, 2003) and Ghana (Adasi and Hemingway, 2008). This is probably due to the fact that permethrin has been used much longer for agricultural and public health purposes than deltamethrin (Adasi and Hemingway, 2008). Also 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 (WHOPES, 2005). In this study, the lowest level of mortality <75% (the highest frequency of resistance) was recorded from *An. gambiae s.l* populations exposed to 0.1% bendiocarb. Resistance of bendiocarb appear to be widespread throughout the study area. This study shows another report of carbamate resistance in *An. gambiae s.l* populations in southwest Nigeria and evidence of its occurrence in populations already harbouring resistance to pyrethroids. This result agrees with previous study which reported high resistance of *An. gambiae* populations to carbamate (Oduola *et al.*, 2012). The bendiocarb resistance recorded in this study raises a special concern for National Malaria Control programs which, because of high resistance to pyrethroids and DDT, are currently introducing bendiocarb based IRS for malaria vector control in West African countries (Djouaka *et al.*, 2011). Therefore in the search of National malaria control programs for a suitable replacement to pyrethroid for IRS as part of an insecticide management strategy, carbamates may not be the right candidate for malaria control in South west Nigeria.

CONCLUSION

Pyrethroid resistance may seriously jeopardize the efficacy of IRS and LLINs on which most African countries including Nigeria, rely to reduce malaria transmission. This study reveals the presence of carbamates bendiocarb resistance and reduced susceptibility/marginal resistance to pyrethroids in *An. gambiae s.l* populations the major malaria vector in Nigeria. The current findings will help for decision-making in the National Malaria control programme particularly in the choice of insecticide to use during campaigns of Indoor residual spraying in south west Nigeria. This suggests multiple suitable alternative insecticides beside carbamates to substitute for the pyrethroid resistance in *An. gambiae* populations in Ibadan metropolis.

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 malaria vectors in Nigeria. Therefore routine surveillance of insecticide susceptibility/resistance in wild anopheline populations across different ecological zones in Nigeria is very critical for effective resistance management strategy.

Author's contributions: KTI conceived and designed the study, supervised field and laboratory procedures, data analysis and interpretation and drafting of manuscript. KOP was involved in the study design, supervised laboratory procedures and reviewed the draft manuscript. ORA, AOA and AKO participated in field and laboratory studies and the analysis of data.

All authors read and approved the final version of the manuscript.

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