ORIGINAL RESEARCH

HIGH LEVEL OF DDT RESISTANCE IN THE MALARIA MOSQUITO: *ANOPHELES GAMBIAE S.L.* FROM RURAL, SEMI URBAN AND URBAN COMMUNITIES IN NIGERIA

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ABSTRACT

Objective: To investigate the susceptibility status of the major malaria mosquito in selected rural, semi-urban and urban areas of Nigeria to DDT and Permethrin based insecticides used in vector control. Methods: Two to three day old unfed adult female *Anopheles* mosquitoes raised from larvae collected across three ecological zones in Nigeria were exposed to 4 % DDT impregnated papers. To find out the possibility of DDT – pyrethroid cross-resistance, susceptibility tests were carried out using 0.75% Permethrin. Mosquitoes tested were identified to species level using PCR assays. Knockdown resistance assays were also carried out on all specimens. Results: At all three locations, the principal species was *A. gambiae s.s* constituting more than 80% of mosquitoes analysed. The 24 hour post-exposure mortality for Permethrin ranged from 51.7 to 100% with resistance observed in five out of the 19 communities tested. DDT resistance was observed at all of the 12 communities tested with mortality ranging from 9.8% to 80%. Incidence of Permethrin resistance was associated with urban communities compared to DDT resistance which was widespread in all the communities. Resistance was recorded in the *A. gambiae s.s,* however DDT resistance was observed in *A. arabiensis* from five study communities. Conclusions: Indications of differential susceptibility to Permethrin and high frequency of DDT resistance in *A. gambiae* was observed which highlights the need for baseline information prior to the use of DDT for indoor residual spray in Nigeria. It also highlights the need for an effective insecticide resistance management programme.

Key Words: Rural; Urban; Semi-urban; Susceptibility test; Permethrin; DDT; *A. gambiae;* Nigeria.

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INTRODUCTION

Indoor residual spray (IRS) with Dichlorodiphenyltrichloroethane (DDT) was a major strategy of the global malaria elimination programme of the late 1960's (Musawenkosi et al., 2004). However, among other things, the development of DDT resistance led to the decline of the Global Malaria Eradication Campaign in sub-Saharan Africa (Litsios, 1996); except for Southern Africa where it was scaled up to include a larger part of the country (Musawenkosi et al., 2004). The wide use of DDT in agriculture and the following growing concern about environmental issues and safety led to a rapid decline in its use in the 1970s, thus affecting its availability for public health purposes (Sadasivaiah et al., 2007). DDT was finally enlisted as one of the persistent organic pollutants targeted for phase out and elimination (Taverne, 1999). Recently, there is a reawakened interest in the adoption of DDT because of the WHO recommendation which suggests its use in IRS for malaria control (WHO, 2006; Coleman et al., 2008). However widespread reports of pyrethroid resistance in *A. gambiae* in West and East Africa (Vulule et al., 1999; Chandre et al., 1999) and its cross-resistance with DDT are major challenges to its adoption for vector control purposes. Report on widespread DDT resistance is not entirely new and its role in undermining malaria control efforts was already reported by WHO in 1975 (Hemingway and Ranson, 2000).

The need of providing current baseline information to guide operational malaria vector control programs in Nigeria was a justification for this study. Of the four classes of insecticides approved for indoor residual spray, DDT remains the cheapest having a longer residual efficacy (6 to 12months) but requiring at least two spray cycles per year (WHO, 2000). The use of DDT remains a possible choice for IRS in terms of cost-effectiveness when compared to other alternatives (Walker, 2000). Permethrin belong to the pyrethroid class which is commonly used in the treatment of bed-nets and also for IRS. In Nigeria, there has been a recent campaign for the re–introduction of DDT for IRS. This study was carried out at potential IRS sites in Nigeria to investigate the susceptibility status of the major malaria mosquitoes to DDT and Permethrin based insecticides.

MATERIALS AND METHODS

Study communities

Anopheles larvae were collected from 19 communities in three states in Nigeria: Oyo (N 07.3500, E 4.4833), Lagos (N 6.4500, E 3.8333) and Niger (N 9.4200, E 4.4999). Two of these states, Oyo and Lagos, are within the mangrove and transitional forest ecological zones of the south western area while Niger state lies in the savannah forest of the north central area. The study sites included larval collection points in nine urban, four semi–urban and six rural communities (Figure 1). The description of the communities in terms of the rural–urban classification is given in Table 1.

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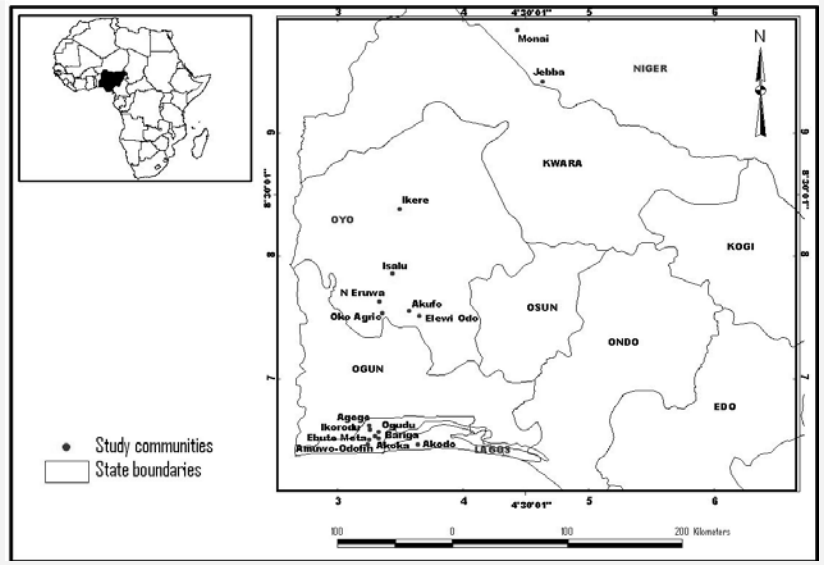
Figure 1: Map showing study communities in three states in Nigeria, West Africa.



Larval collection, rearing and insecticide susceptibility bioassays

Anopheles larvae were collected from both temporary and permanent breeding sites found within the study locations during the rainy season, May to August, between 2007 and 2009. The breeding sites included abandoned roadsides, vehicle tracks, puddles and ground pools in public water places. All larval samples collected were transported to the insectary and reared to adulthood. Insecticide susceptibility tests were carried out following WHO standard procedures (WHO, 1998).

Two to three day old 20 to 25 unfed female mosquitoes were exposed to two types of WHO bioassay test papers each impregnated with DDT (4%) and Permethrin (0.75%). The knockdown rates were recorded at 10 minute intervals for a period of one hour and the final mortality was assessed 24 hours post exposure. To ensure that the test papers remained effective, they were pretested against the Kisumu susceptible strain (from Kenya) known to be 100% susceptible to DDT and Permethrin. All susceptibility tests were carried out at 26 to 29^{o}C and 74% to 82% relative humidity. Permethrin tests were carried out on *A. gambiae* populations from 19 communities as compared to 12 communities where the DDT based insecticide was tested.

Species identification and detection of the pyrethroid knockdown resistance (*kdr*) gene

All field mosquito samples were identified using morphological keys (Gillies and de Meillon, 1968; Gillies and Coetzee, 1987). Mosquito samples belonging to the *Anopheles gambiae* group were identified to species level and molecular forms using PCR assays (Fanello *et al*., 2002). Molecular characterizations of the *kdr* mutations in mosquito samples were determined following the procedure of Martinez –Torres and co-workers (1998).

Statistical Analysis

The WHO criteria for evaluating the insecticide resistance/susceptibility status of mosquitoes was used (WHO, 1998). The criteria indicate resistance when 24 hours post exposure mortality rate is lower than 80%. Mortality rates greater than 98% indicate susceptibility while rates between 80% and 97% suggest the possibility of resistance which needs to be re-confirmed independently. Species composition of *Anopheles* mosquitoes were tested for significant differences using Chi-square tests. Significant differences in species composition of unexposed samples and populations surviving insecticide exposure were tested using the Wilcoxon Signed Rank Test. Statistical analysis was conducted using SPSS version 15.0 (SPSS Inc, Chicago, Illinois). P-values less than 0.05 indicated significance.

Ethical approval

This study required no ethical approval. However informed consents of household and community heads were obtained.

RESULTS

Species composition of *Anopheles gambiae* group from the study locations

A total of 1937 *A. gambiae* were analysed by the Polymerase Chain Reaction. Out of these, 1917 (98.9%) were identified up to species level. *A gambiae s.s* and *A. arabiensis* were found to co-exist in 70% (n = 13) of the study communities (Table 1). *A. gambiae s.s* was predominant (91.7%) and presen int all the communities. In contrast, *A. arabiensis* was absent in 30% (n = 6) of the study communities. There was significant difference in species composition at the study communities (χ^2 = 99.45; df = 18; P = 0.00).

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Table 1: Species composition of the *Anopheles gambiae* group from 19 study communities in Nigeria.

Study communities	Community types	Coordinates	Numbers assayed (N)	Spieces composition (%)	
				A. gambiae s.s	*A. arabiensis*
OYO STATE					
Elewi Odo	urban	N 07º 25.058 ' E 003º. 56 528'	77	62 (80.5)	15 (19.5)
N Eruwa	semi urban	N 07º 32. 918' E003º. 26 639'	199	174 (87.4)	25(12.6)
Isalu	urban	N 07º 58. 811' E003º. 35 655'	112	95(84.8)	17(15.2)
Akufo	rural	N 07º 29 059' E003º. 49 050'	155	147(94.8)	8(5.2)
Ikere	rural	N 08º 10. 257' E003º. 41 633'	264	235(89.0)	29(12.3)
Oko Agric	rural	N 07º 31. 703' E003º. 27 411'	178	161(90.4)	17(10.6)
Kisumu	Control		100	100	
LAGOS					
Akodo	semi urban	N 06º 24.999 ' E 003º.55 999'	70	70(100)	0
Amuwo –Odofin	semi urban	N 06º 28.000 ' E 003º. 18 000'	78	74(94.9)	4(5.1)
Ebute Meta	urban	N 06º 28.999 ' E 003º. 18 528'	91	91(100)	0
Ikorodu	urban		48		
Akoka	urban	N 06º 30.969 ' E 003º. 24 000'	89	89(100)	0
Ilaje	urban	N/A	82	74(90.2)	3(3.6)
Agege	urban	N 06º 37.999 ' E 003º. 18 999'	88	85(96.6)	0
Bariga	urban	N 06º 31.999 ' E 003º. 22 999'	94	87(92.6)	4(4.3)
Ogudu	urban	N 06º 34.000 ' E 003º. 24 000'	75	73(97.3)	0
NIGER STATE					
Monai	rural	N 09º 52. 665' E 004º. 32 331'	56	51(91.1)	3(5.4)
Tamanai	rural	N/A	33	29(87.9)	2(6.1)
Jebba	semi urban	N 09º 07.999 ' E 004º. 49 999'	60	48(80)	12
Awuru	rural	N/A	88	88(100)	0

N/A not available

The 24 hour post-exposure mortality of *A. gambiae* exposed to Permethrin ranged between 51.7% and 100%. According to the WHO criteria for evaluating resistance, the possibility of Permethrin resistance was found in *Anopheles gambiae s.l.* from nine sites: Monai and Awuru in rural; Elewi-Odo, Isalu, Akoka, Agege and Ogudu in urban; and Akodo and Jebba in semi-urban areas. Resistance to Permethrin was found in five communities: Ebute-metta, Ikorodu, Ilaje, Bariga in urban and Amuwo–Odofin in semi-urban areas. *Anopheles* mosquitoes from Akufo, Ikere, Oko Agric in rural and Tamanai in semi-urban areas were fully susceptible to Permethrin (Figure 2).

In this study, the 24 hour post exposure mortality indicating Permethrin resistance ranged between 51.7% and 73.1% for urban communities. This was observed to be lower when compared to the least mortality value in rural communities which was 87.5%.

Mosquitoes exposed to DDT test papers showed a high frequency of DDT resistant *A. gambiae* populations at all 12 communities where the test was carried out (Figure 3). The level of DDT resistant individuals recorded was high in Lagos, including Ikorodu, Amuwo - Odofin and Bariga where 24 hour post-exposure mortality was less than 10%. Out of a total of 1189 *A. gambiae s.l* exposed to Permethrin, 168 (14.13%) survived, of which, 162 (96.4%) were successfully identified as *A. gambiae s.s.* None of the populations surviving Permethrin exposure tested positive for *A. arabiensis*. Similarly, of a total of 899 adult *Anopheles gambiae s.l* exposed to DDT based insecticide 403 (44.8%) survived to 24 hours post-exposure.

The PCR assay on survivors revealed that the composition of *A. gambiae s.s and A. arabiensis* were 383 (97%) and 12(3%) respectively. In contrast to Permethrin exposure, *A. arabiensis* was identified in the *Anopheles* populations that survived DDT exposure in five communities: Elewi Odo, Isalu, Amuwo Odofin, Bariga and Monai (Figure 4). The proportion of *A. arabiensis* in the surviving *A. gambiae s.l* exposed to DDT ranged between 2.6% and 11.1% (Figure 3). The proportion of *A. gambiae s.s* in the DDT resistant population was significantly higher (P = 0.016) compared to the unexposed population in the communities where both species occurred together.

In this study, resistance to Permethrin occurred in five communities, out of which four (80%) were urban and one (20%) was semi-urban. The number of communities where the possibility of Permethrin resistance existed were in four (44%) urban, three (33.3%) rural, and two (22.2%) semi-urban communities. In contrast, four (80%) out of the five communities which where fully susceptibility to Permethrin were rural while the remaining community was semi-urban. Result of this study showed that the incidence of Permethrin resistance was higher in urban communities compared to DDT resistance which was widespread in all the community types.

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Figure 2: Post-exposure mortality of *A.gambiae* populations from study communities exposed to DDT and Permethrin based insecticides. DDT tests were carried out in only 12 of the 19 communities (r=rural; u=urban; su=semi-urban).

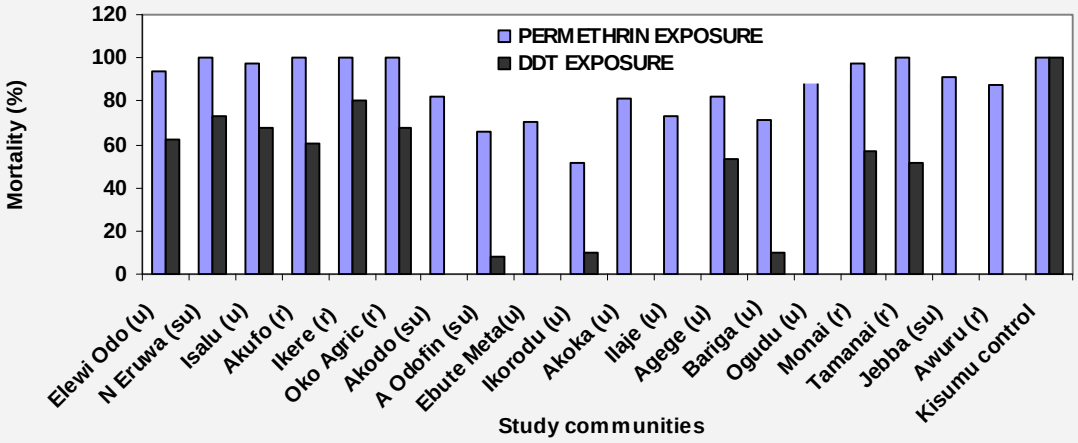
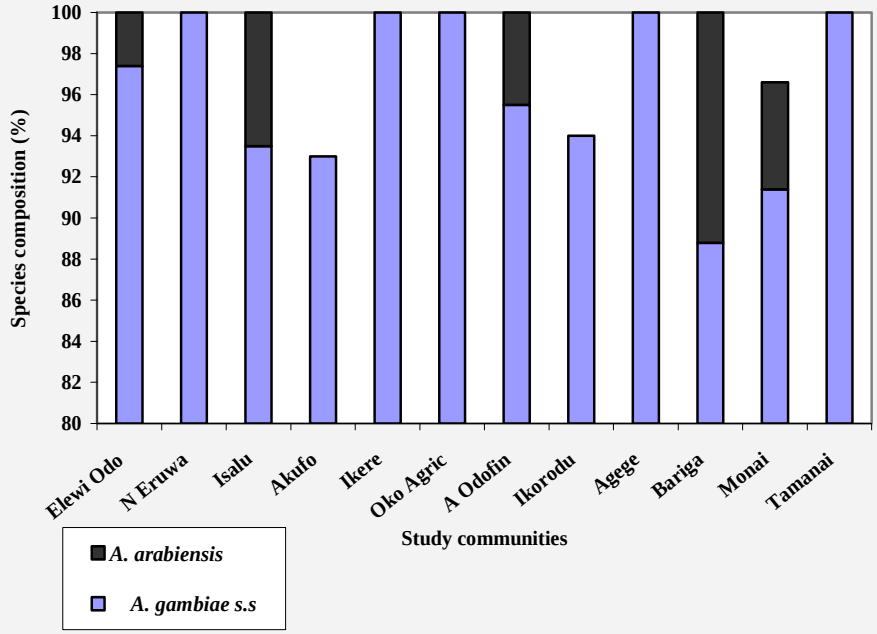


Figure 3: Species composition of *A. gambiae s.l* survivors of DDT exposure.



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Out of total of 160 *A. gambiae* s.s. that survived Permethrin exposure and were analyzed for the molecular M and S forms, 154 samples were successfully analysed. The two molecular forms were found to occur in the Permethrin and DDT resistant populations. However, the molecular M form was found to be predominant 153 (99.4%) in the Permethrin resistant samples irrespective of the originating community (Table 2). Out of the 379 samples identified as survivors from DDT exposure, 346 (91.3%) belonged to the M form compared to the 33 (8.7%) in the S form. The molecular M form was found to be significantly more frequent in the DDT resistant samples from urban ($P<0.001$) and from rural ($P=0.001$) communities. All samples tested negative on the *kdr* gene assay.

Table 2: Composition of the molecular 'M' and 'S' forms of *A. gambiae s. s.* surviving Permethrin and DDT based insecticide exposure.

	Permethrin (0.75%)			DDT (4%)			
Study sites	Survivals	M (%)	S (%)	Survivals	*A. arabiensis*	M (%)	S (%)
Elewi Odo	7	6 (83.3)	1(16.7)	39	1 (2.6)	33 (84.6)	5 (12.8)
N Eruwa	-	0	0	21	0	15 (71.4)	6 (28.6)
Isalu	2	2 (100)	0	31	2 (6.5)	22 (70.9)	7 (22.6)
Akufo	-	-	-	29	0	27 (100)	0
Ikere	-	-	-	20	0	15 (75.0)	5 (25.0)
Oko Agric	-	-	-	27	0	19 (70.4)	8 (29.6)
Akodo	8	7 (87.5)	0	-	-	-	-
A. Odofin	21	21 (100)	0	44	2 (4.5)	42 (95.5)	0
Ebute Metta	12	12 (100)	0	-	-	-	-
Ikorodu	27	27 (100)	0	64	0	60 (93.8)	0
Akoka	10	10 (100)	0	-	-	-	-
Ilaje	32	29 (100)	0	-	-	-	-
Agege	14	14 (100)	0	17	0	15 (88.3)	2 (11.7)
Bariga	20	20 (100)	0	36	4 (11.1)	32 (88.9)	0
Ogudu	2	2 (100)	0	-	-	-	-
Monai	1	1 (100)	0	58	3 (5.2)	53 (91.4)	0
Tamanai	0	0	0	17	0	13 (76.5)	0
Jebba	2	0	0	-	-	-	-
Awuru	2	2 (100)	0	-	-	-	-
Total	160	153 (95.6)	1 (0.63)	403	12 (3.0)	346 (85.9)	33 (8.2)

Overall compositions in the molecular 'M' and 'S' forms did not sum up to 100% because of amplification/digestion failure in some samples.

DISCUSSION

There is a renewed commitment for the global partnership for malaria elimination (Snow et al., 2002). Indoor residual spraying has been indicated to play a major role in this effort and the choice of insecticide should be evidence based. The lower mortality values obtained from Permethrin exposure in this study (53.1% to 73.1%) contrasts with higher mortality values (72.1% to 79.6%) from previous studies from Nigeria (Awolola et al., 2002). This result might indicate a reduction in the susceptibility status of the *A. gambiae* population to Permethrin based insecticides. Similarly, the present study found lower mortality figures (less than 10%) after DDT exposure compared to values of 72% to 100% which were previously reported from Nigeria (Awolola et al., 2002, Kristan et al., 2003). Our results suggest a trend towards DDT resistance. The scale of DDT resistance in Nigeria is largely unknown, however its prevalence is obvious considering the high number of study sites from where DDT resistance is presently being reported. These results emphasize the need for constant monitoring of the susceptibility status of vector populations in view of vector control programmes which are employing the use of these insecticides for the treatment of bed-nets or for the purpose of indoor residual spraying.

In this study, *A. gambiae* populations were found to be susceptible to Permethrin in most of the rural communities compared to the high frequency of resistance observed in urban settings. Selection for pyrethroid resistance in *A. gambiae* has been associated with the use of agricultural insecticides (Akogbeto et al., 2006) but not with DDT because of the restricted use of DDT since it was banned (Sadasivaiah et al., 2007). Permethrin resistance in the urban study communities may not be associated with agricultural insecticide use since most of the urban communities surveyed are located in a highly urbanised centre where agricultural practices are generally uncommon. However, increased use of household insecticide and availability of xenobiotics for larval breeding sites in the urban (Elissa et al., 1993, Djouaka et al., 2007) may be one of the possible factors selecting for pyrethroid resistance in *A. gambiae* in urban areas. Similar reports of high levels of resistance in urban populations of *Culex quinquefasciatus* compared to rural populations have been attributed to ongoing intense control activities (Brogdon and McAllister 1998). Hence, the disparity in the rural to urban susceptibility status *of A. gambiae* maybe dependent on differential insecticide pressure acting in the study communities. A clear pattern of Permethrin resistance in semi-urban communities could not be established because the possibility of resistance was recorded in two out of the four semi-urban communities considered, and *A gambiae* populations showed both resistance and susceptibility to Permethrin in each of the other two communities. There is a need for further investigations in a larger number of communities to substantiate our findings.

The present high frequency of DDT resistance in the *A. gambiae* population suggests the existence of compounds maintaining a strong selection pressure in these populations. There exists documented evidence in Nigeria on the use of DDT in the control of *Simulium* larvae (Elliot and Barnes 1963). It is unlikely that the current selection pressure being observed may have

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originated from the historical use of DDT. This is due to the reported half life of DDT in soils which is between 2 and 15 years (Augustijn-Beckers et al., 1994). Another possible source of DDT use in Nigeria could be in agriculture. However, the ban on DDT use in agriculture and the non-agricultural nature of Lagos' urban communities indicated other sources. Occurrence of resistance of *A. gambiae* to DDT in areas of non-agricultural insecticide use has also been reported from the Côte-d'Ivoire (Tia et al., 2006).

The large differences in DDT resistance in *A. gambiae* observed in Lagos compared to the other locations may have emerged as a result of uncontrolled and illegal usage of DDT or other unspecified and unbranded locally made pesticides from urban centres (Akhiwu and Aligbe, 2000) to control cockroaches and urban mosquitoes. The continued usage of these pesticides in the study areas may have maintained a selection pressure for DDT resistance in the malaria vector population. However, this explanation is pure speculation and requires further investigations.

A. gambiae s.s was the only species predominant in the population that survived exposure to Permethrin. This suggests that *A. arabiensis* which was observed in the unexposed *A. gambiae s.l* population were mostly susceptible to Permethrin. Any Permethrin based control strategy will be adequate to control *A. arabiensis*. The occurrence of DDT resistance in *A. gambiae s.s and A. arabiensis* has not been previously reported in any of these locations in Nigeria. However, similar occurrences of pyrethroids and DDT resistance in *A. gambiae s.l* have been reported in several countries in sub-saharan Africa. This study presents an update on Permethrin and DDT resistance and was able to report DDT resistance in *A. arabiensis* in some of the study communities. Similar results of DDT resistance in *A. arabiensis* population have been reported from South Africa and Sudan (Hargreaves et al., 2000, Balkew et al., 2010). *A. arabiensis* is a major vector in Nigeria and it is the predominant vector species in Sahel Nigeria (Awolola et al., 2003, Samdi et al., 2006). This study has shown that target site mutation (*kdr*) was not responsible for DDT and Permethrin resistance in these populations. This is interesting considering that the *kdr* gene had previously been reported in the S form of *A. gambiae* in Nigeria (Awolola et al., 2005). Findings from this study are consistent with several reports from sub-Saharan Africa (Brooke et al., 1999, Awolola et al., 2009) which have suggested the involvement of metabolic resistance mechanisms. Hence, these findings provide possible evidence of other resistance mechanisms outside the previously reported *kdr*.

Conclusion

Evidenced based selection of insecticides prior to IRS and the need to monitor changes in the response of major vector species to insecticide pressure are key components of a successful IRS programme. The absence of *kdr* point mutations in the resistant populations emphasize the need to carry out further studies to identify the operative resistance mechanisms. The differential susceptibility to Permethrin depending on the community type, the incidence of DDT resistance in *A. arabiensis* and the widespread occurrence of DDT resistance in *A. gambiae s.s* further highlights the need to incorporate insecticide resistance management practices in future control programme. It is important that decision for operational malaria control activities such as IRS in Nigeria will be guided not only by the cost of procurement but more importantly on the level of susceptibility of malaria vectors to these insecticides.

AUTHOR'S CONTRIBUTIONS

AOO conducted the field and laboratory study and drafted the manuscript, JBO, COA and AOA participated in the field and laboratory study and the analysis of the data. OAO and TSA were involved in the study design and they both reviewed the draft manuscript. All authors have read and agreed on the final manuscript.

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