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Full Length Research Paper

Epidemiology of malaria and insecticide resistance burden in Nigeria

I. O. Oyewole[1]*, A. A. Ogunnowo[1], C. A. Ibidapo[2], H. I. Okoh[3], T. S. Awolola[3] and M. A. Adedayo[2]

[1]Babcock University, Ilisan Remo, Ogun State, Nigeria.
[2]Lagos State University, Lagos, Nigeria.
[3]The Nigerian Institute of Medical Research, Yaba, Lagos, Lagos State, Nigeria.

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Anopheles **mosquito larvae were collected from the natural breeding sites in five of the six ecological zones in Nigeria between 2002 and 2004. The larvae were reared to adulthood in a standard insectary. Susceptibility tests were conducted on non - blood fed, 2 to 3-day- old emerged adult female mosquitoes using standard WHO procedures, diagnostic kits and test papers (WHO, 1998). PCR assays were used for the identification of the species and for characterization of the kdr allele. The mosquito samples from all the zones were susceptible to the diagnostic doses of insecticides tested, although, a significant level of resistance was recorded particularly in forest- savanna mosaic and Guinea savanna. However, there was no significant difference in knock down effects of insecticides in all the zones ($F_{4, 15}$=6.49, P=0.0001) . There was a level of correlation between the frequency of the kdr allele and frequency of resistance among the survivor and exposed samples (F = 22.05; P = 0.0037). This may indicate that kdr is associated with resistance in *Anopheles* mosquito to the tested insecticides. This study forms a baseline data for insecticide resistance status of the local anopheline mosquitoes which can be used to formulate control programmes in Nigeria.**

Key words: *Anopheles*, malaria, pyrethroid resistance, Nigeria.

INTRODUCTION

Malaria still kills more people than HIV/AIDS or any other killer disease and it is endemic throughout Nigeria accounting for 25% of infant mortality and 30% of childhood mortality (FMoH, 2005) . About 50% of the population has at least one episode of malaria each year. The economic impact of malaria has been estimated to cost Africa US$12 billion every year (WHO 1998). The economic impact includes costs of health care, working days lost due to sickness, day lost in education, decreased productivity due to brain damage from cerebral malaria, loss of investment, tourism and diversion of household resources (Greenwood et al., 2005). In Nigeria, the economic impact of malaria can be attributed to low gross national income per capital (GNI) of US$260 (FMoH, 2005).

Opting for vector control seems more feasible in preventing the disease in that it protects individual from mosquito bite. In 1998 the World Health Organization (WHO), in collaboration with United Nations Children's Fund (UNICEF), United Nations Development Program (UNDP), and the World Bank, launched the Roll Back Malaria (RBM) Global Partnership to coordinate efforts in fighting malaria. Insecticide treated nets (ITNs) was introduced as one of the strategies in malaria control. However, control efforts through the use of insecticides and treated bed nets is becoming problematic, as the mosquitoes that transmit the parasite are becoming resistance to the pyrethroid insecticides, the only class of bed net insecticide approved for malaria control, in West and East Africa (Elissa et al., 1993; Awolola et al., 2003). The emergence of pyrethroid and DDT resistance in *Anopheles gambiae* s.s, the major Afro tropical malaria vector *would* have considerable implications for the success of vector intervention and the monitoring of ongoing

*Corresponding author. E-mail: oyewoleio@gmail.com.

control programs. Hence, there is a strong need for the development of appropriate tools to monitor resistance in field populations of anopheline mosquitoes. In the present study, we evaluate the pyrethroids and DDT resistance status of the local anopheline species in Nigeria as a baseline to formulate control programs.

MATERIALS AND METHODS

Study area

Nigeria constitutes of six ecological zones which range from forest, forest–savanna mosaic (transitional zone) and narrow belt of mangrove in the south to a Guinea–savanna, Sudan and arid (Sahel) savanna in the north (Figure 1). The southern part experienced a long period of rain from April to October and a short dry season from November to March. The Northern region is much drier with a short period of rain while dry season often extends between 6 and 8 months.

Epidemiological data

Existing data from 1990 to 2002 was compiled to assess the epidemiologic implication of malaria on morbidity and mortality on Nigeria population.

Mosquito sampling

Some localities were randomly selected from five of the six major ecological zones (except the Sahel-savanna) in Nigeria. Larvae were collected from different breeding habitat ranging from shallow well, gutters, tires, to standing water between 2002 and 2004 with the aim to evaluate the pyrethroids and DDT resistance status of the local anopheline species. These were fed with mashed biscuit of low fat (12.30g) plus yeast capsules (7.59g) and maintained under a standard insectary conditions. The emerged adults were maintained on 10% sucrose solution for 24 h prior to further tests.

Insecticide Bioassays

Bioassays were carried out on non-blood fed 2 to 5 day old female mosquitoes emerged from the larvae collected from the field. Standard procedures were followed for susceptibility tests using WHO test kits and insecticide- impregnated papers (WHO, 1998). Three test papers impregnated with deltamethrin, permethrin, and DDT at 0.025, 0.25 and 4% concentrations respectively were used to assess the presence of cross resistance between DDT and the pyrethriods used. The mosquitoes from each location were divided into four to six groups of 20 to 25 samples per zone; these were then exposed for 60 min to each of the three insecticides or to untreated paper (control) . The number of mosquitoes knocked down was recorded every ten minutes of exposure after which the specimen were maintained for the next 24 h on 10% sucrose solution and the number of mortality was recorded. Morphological identification of the survivor and dead mosquitoes was carried out with the aid of identification keys (Gillies and Coetzee, 1987). These were preserved individually on silica gel in Eppendorf tube for molecular identification.

Molecular assays for species identification and characterization of kdr mutation

PCR assays were used for the identification of the species and for characterization of the kdr allele.

Species identification

Samples from survivor and dead mosquitoes used for insecticide exposure were identified to species level using species- specific PCR assay following procedure described by Scott et al. (1993) with little modifications by Van Rensburg et al. (1996).

Characterization of kdr mutation

PCR-based assay was carried out to check for knock- down resistance (kdr) gene in the survived mosquitoes. Extraction of DNA follows the procedure of Collins et al., (1987). Characterization of kdr mutation frequency was determined by assaying the specimens that were detected susceptible and resistant to DDT and pyrethroids using the two-step PCR and primer sequences methods described by Martinez-Torres et al. (1998) and Ranson et al. (2000).

STATISTICAL ANALYSES

Coefficients of correlation were used as test statistics to find level of relationship between the frequencies of the kdr allele and resistance among the survivor and exposed samples. Analysis of variance (ANOVA) was used to establish differentiation (variation) between zones. Significance in comparisons was assumed if $P < 0.05$ is obtained in the appropriate test.

RESULTS

Epidemiological data

The summary of the data for the assessment of the epidemiologic implication of malaria on morbidity and mortality on Nigeria population between 1990 and 2002 is presented in Table 1.

Collection and molecular identification of anopheline species

A total of 3254 larvae collected from the sampling sites were reared to adult stage and these were identified as members of *A. gambiae* complex with the aids of morphological identification key (Gillies and Coetzee, 1987). A representative sample of these was subjected to species-specific PCR, indicating that 74.6% belong to *A. gambiae* while the remaining 26.4% were *A. arabiensis* (Figure 2).

Characterization of kdr mutation and susceptibility to insecticides

Figure 3 shows the characterization of the kdr allele using

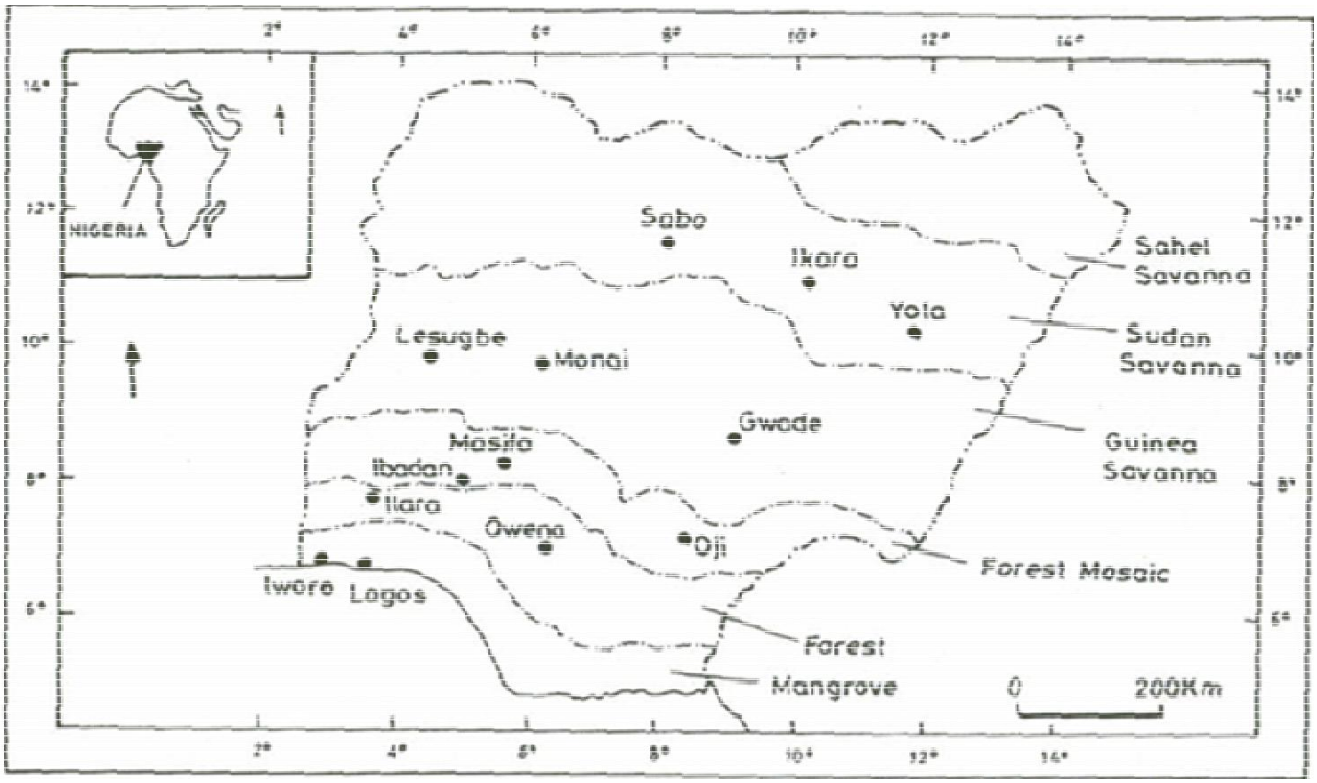

Figure 1. Ecological zones in Nigeria showing the sampling sites.

PCR assays. The mosquito samples (*A. gambiae s.s*) from all the zones showed >78% susceptibility to the diagnostic concentrations of insecticides tested, however, not less than 27% of the exposed specimen showed resistance. More than 97% mortality was recorded for samples from mangrove and Sudan-savanna when exposed to deltamethrin and DDT respectively. Mortality due to permethrin was < 95% in these zones. At the forest-savanna mosaic and Guinea-savanna zones, mosquitoes exposed to permethrin recorded the lowest mortality (<74%) . The corresponding knock down value of the deltamethrin and DDT was > 80% in all the zones. At the forest, mangrove and Sudan – savanna where susceptibility was high, >90% of *An. gambiae* exposed to all the insecticides were knocked down but <85% of those exposed to permethrin in forest- savanna mosaic and Guinea savanna were knocked down during the 1 h exposures. However, there was no significant difference in knock down effects of insecticide in all the zones ($F_{4, 15}$=6.49, P=0.0001) . The knock down effect of deltamethrin was more pronounced in the entire zones than recorded for other two insecticides (Tables 2 to 4). Resistance to permethrin was recorded in all the zones but highest levels of resistance were found in forest-savanna mosaic and Guinea savanna (Table 3). Meanwhile, high level of DDT resistance was also observed in Guinea savanna (Table 4).

The WHO (1998) criteria for determining resistance or susceptibility were applied: 98 to 100% mortality indicates susceptibility, <80% mortality suggests resistance while 80 to 97% mortality requires confirmation of resistance. Overall, mosquitoes from mangrove were most susceptible while those from forest-savanna mosaic showed highest level of resistance (Tables 2 to 4) . The kdr PCR assay showed that kdr allele was present in most of the survivor mosquitoes previously exposed to the three test insecticides. At Guinea-savanna for example, 87% of the 25 resistant to deltamethrin, 90.6% of the 22 resistant to permethrin and 80% of the 20 resistant to DDT carried the kdr allele. The indicative values in other zones are as follows: 84% (of 25), 87.5% (of 22) and 76.4% (of 20) at forest savanna mosaic, 80% (of 25), 82.7% (of 22) and 72.7% (of 20) at forest, 63.3% (of 25), 67.4% (of 22) and 65.2% (of 20) at Sudan-savanna and 55.2% (of 25), 61.4% (of 22) and 58.6% (of 20) at mangrove. Overall, *A. gambiae s.s* resistance to the three insecticides was more pronounced in forest-mosaic and Guinea savanna. In these zones lowest level of mortality was recorded among the mosquitoes exposed to all the insecticides with high level of resistance to permethrin.

The results of coefficients of correlation between the frequency of the kdr allele and frequency of resistance among the survivor and exposed samples showed a level of significance (F = 22.05; P = 0.0037).

Table 1. A summary of epidemiologic implication of malaria episode in Nigeria between 1990 and 2002.

Population data in thousands[a]	1990	1997	1998	1999	2000	2001	2002
Total population	85953	104793	107664	110614	113862	117141	120515
Annual population growth rate (per 100)	2.88	2.74	2.74	2.74	2.88	2.88	2.88
Population < 5 years	15815	18758	19272	19800	19698	20265	20849
Population < 5 years	70138	86035	88392	90814	94164	96876	99666
Women population (15-49 years)	37905	46633	47911	49223	51579	53065	54593
Number of clinical malaria cases reported[b]							
Total	1047292	616466	19034	21181	31685	-	-
Under five years	-	-	4948	7797	13225	-	-
Five and above	-	-	13275	12675	17504	-	-
Pregnant women	-	-	811	709	956	-	-
Number of admitted malaria cases reported[b]							
Total	-	-	4319	5828	2205	-	-
Under five years	-	-	220	2038	1177	-	-
Five and above	-	-	4033	3659	949	-	-
Pregnant women	-	-	66	131	79	-	-
						-	-
Number of malaria deaths reported							
Total	-	-	52	40	58	-	-
Under five years	-	-	18	14	28	-	-
Five and above	-	-	33	26	28	-	-
Pregnant women	-	-	1	0	2	-	-

Sources: [a]United Nation Population Division - 2000 World Population Prospects: Population Database: The 2002 Revision, http://esa.un.org/unpp., [b]Federal Ministry of Health.

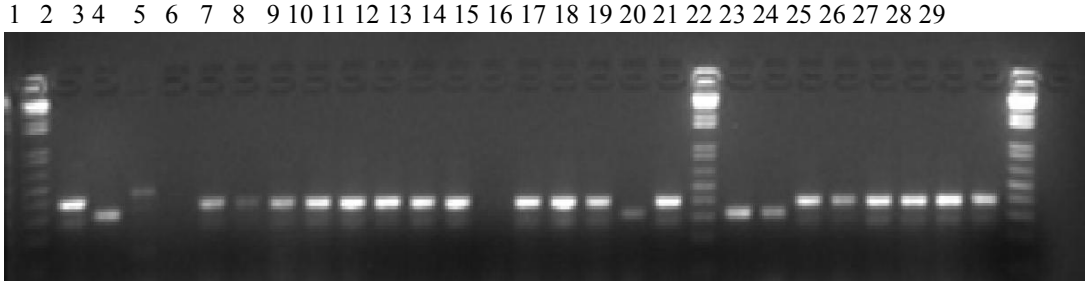

Figure 2. Species – specific PCR assay showing the amplified fragments for the identification of the members of *A. gambiae* complex from all the study sites. Lane 1, 20 and 29: 1kb molecular makers, Lanes 2 to 4: positive controls (*A. gambiae* s.s: 390bp, *A. arabiensis*: 315bp, *A. merus/melas*: 364bp respectively), Lane 5: negative control, Lanes 6-13, 15-17, 19 & 23-28: (*A. gambiae*), Lanes 18, 21and 22 (*A. arabiensis*). Samples were run on a 2.5% Tris-acetate-EDTA agarose gel.

to malaria attack and constitutes the highest number of hospitalized patients. This is an irony since the general impression is that the risk of malaria attacks in residents of malaria endemic areas falls as they become older (Koch et al., 1900; Baird et al., 1991), indicating that protection is a function of age. Nevertheless, previous report showed that clinical attacks of malaria also occur in adults living in areas of high endemicity (Miller, 1958) while a recent record indicated the role of malaria as a cause of death in adults from West Africa (Adjuik et al., 2006). In the present study, evidence of resistance to all the insecticides tested was more pronounced in two of the five zones studied. This could be due to extensive use of agricultural insecticides particularly in the forest-mosaic and Guinea savanna zones. The presence of leucine-phenylalanine kdr mutation was first reported in Cote D'Ivoire and this was attributed to the extensive use of agricultural insecticides (Elissa et al., 1993). However,

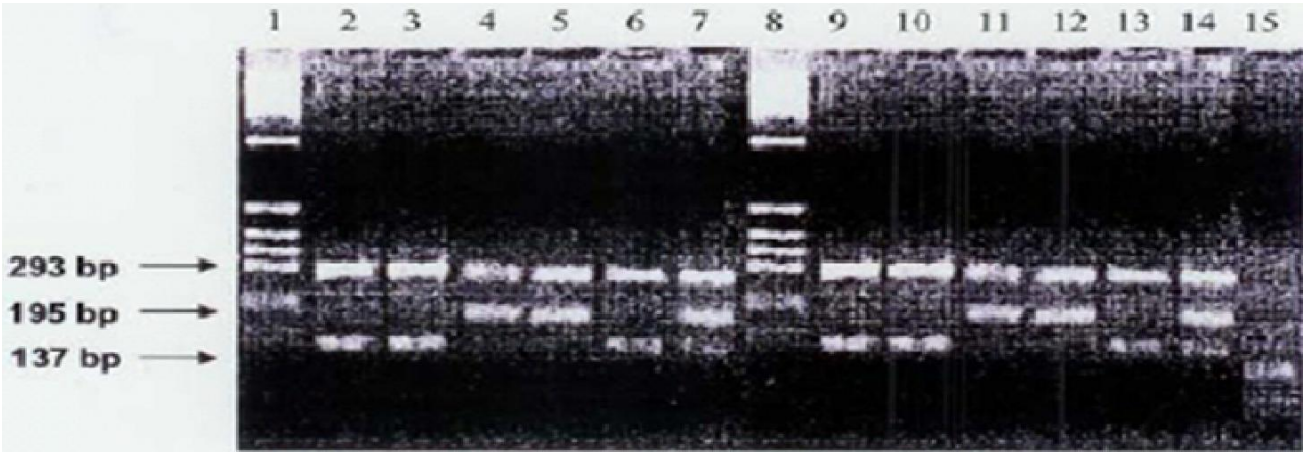

Figure 3. Detection of the kdr gene in a wild population of *A. gambiae* "S" form. Lanes 1 and 8: molecular weight maker: Lanes 2, 3, 6, 9, 10 and 13: susceptible, Lanes 4, 5, 11 and 12: homozygous resistant, Lanes 7 and 14: heterozygous resistant, Lanes 15: negative control.

Table 2. 24 h post exposure mortality rate of 2 to 3 day old *A. gambiae* and frequency of knock-down after 60 min exposure to deltamethrin [0.025 %(v/w)].

Ecological zone	Number exposed (N)	Number and (%) knock- down	Number and (%) mortality
Forest	130	125 (96.2)	118 (90.8)
Forest-savanna mosaic	130	118 (90.7)	103 (78.8)
Mangrove	125	123 (98.4)	125 (100)
Guinea – savanna	125	115 (92)	111 (89.2)
Sudan – savanna	100	97 (97)	100 (100)
Total	**610**	**578**	**557**

Table 3. 24 -h post exposure mortality rate of 2 to 3 day old *A. gambiae* and frequency of knock-down after 60 min exposure to permethrin [0.25 %(v/w)].

Ecological zone	Number exposed (N)	Number and (%) knock- down	Number and (%) mortality
Forest	130	118 (90.7)	112 (86.2)
Forest-savanna mosaic	130	110 (84.6)	90 (69.2)
Mangrove	125	122 (97.6)	118 (94.4)
Guinea – savanna	125	97 (77.6)	92 (73.6)
Sudan – savanna	100	90 (90)	94 (94)
Total	610	537	506

Table 4. 24-h post exposure mortality rate of 2 to 3 day old *A. gambiae* and frequency of knock- down 60 min after exposure to DDT [4 %(v/w)].

Ecological zone	Number exposed (N)	Number and (%) knock-down	Number and (%) mortality
Forest	130	122 (93.8)	116 (89.2)
Forest-savanna mosaic	130	115 (88.5)	110 (84.6)
Mangrove	125	122 (97.6)	123 (98.4)
Guinea – savanna	125	100 (80)	97 (77.6)
Sudan – savanna	100	94 (94)	97 (97)
Total	610	553	543

the origin of insecticide resistance in the study areas could not be ascertained per se, this could either be due to agricultural or public health use of insecticides.

Nevertheless, the long history of agricultural use of insecticides in the study areas could be more appropriate in explaining the existence of kdr mutation in the study sites ever before the introduction of ITNs. The highest frequency of resistance in this study was found with permethrin. Previous reports have also documented decrease levels of susceptibility of *A. gambiae* to permethrin (Awolola et al., 2007; Abdalla et al., 2007; Ramphul et al., 2009).

This result has implications for the current reliance on ITNs and IRS for vector control in Nigeria. Although the operational impact of pyrethroid resistance on the efficacy of ITNs is not clear but reduction in the efficacy of ITNs and IRS in pyrethroid resistance areas has recently been reported in the Benin Republic with high kdr frequency (Corbel et al., 2007; N'Guessan et al., 2007). There is therefore the need to closely monitor insecticide resistance in malaria control programmes which rely solely on ITNs and IRS inter-ventions across Africa (Awolola et al., 2008). It has been demonstrated elsewhere that carbamate insecticide bendiocarb and organophosphates are useful alternative chemical classes to DDT and pyrethriods for vector control programmes (Abdalla et al. 2007; Ramphul et al., 2009). This will also minimize the spread of cross-resistance between DDT and the pyrethriods which has been established in *A. gambiae* (Chandre et al., 1999; Hemingway and Ranson, 2000). This phenomenon is closely associated with kdr allele in *A. gambiae* (Martinez-Torres et al., 1998; Ranson et al., 2000). Here, there was correlation between the frequency of the kdr allele and frequency of resistance among the survivor and exposed samples and similar report has been documented from the neighbouring West African countries (Martinez-Torres et al., 1998). This may indicate that kdr is associated with DDT resistance and strongly contributes to resistance to certain pyrethroids such as permethrin. However, there is possibility of additional resistance mechanisms in both cases (Brook et al., 1999; Awolola et al., 2003, 2008; Ramphul et al., 2009).

In conclusion, this study showed the existence of DDT resistance and reduced susceptibility to permethrin in *A. gambiae* s.s the major vector of malaria in Nigeria. This may suggest the possibility of a widespread of this phenomenon in the nearest future hence, the need for control programmes to mount management strategies to curb the spread of resistance population.

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REFERENCES

Abdalla H, Matambo TS, Koekemoer LL, Mnzava AP, Hunt RH, Coetzee M (2007). Insecticide susceptibility and vector status of natural populations of *Anopheles arabiensis* from Sudan. Trans. R. Soc. Trop. Med. Hyg., 102 (3): 263-71.

Adjuik M, Smith T, Clark S, Todd J, Garrib A, Kinfu Y, Kahn K, Mola M, Ashraf A, Masanja H, Adazu K, Sacarlal J, Alam N, Marra A, Gbangou A, Mwageni E, Binka F (2006). Cause-specific mortality rates in sub-Saharan Africa and Bangladesh. *Bull World Health Organ.,* 84:181-188.

Awolola TS, Brooke BD, Hunt RH, Coetzee M (2002). Resistance of the malaria vector *Anopheles gambiae s.s*. to pyrethroid insecticides in southwestern Nigeria. Ann Trop Med Parasitol., 96: 849–52.

Awolola TS, Brooke BD, Koekemoer LL, Coetzee M (2003). Absence of the kdr mutation in the molecular 'M' form suggests different pyrethroid resistance mechanisms in the malaria vector mosquito *Anopheles gambiae* s.s. *Trop. Med. Int..Health.,* 8 (5)**:** 420-422.

Awolola TS, Oyewole IO, Amajoh CN, Idowu ET, Ajayi MB, Oduola A, Manafa OU, Ibrahim K, Koekemoer LL, Coetzee M (2005). Distribution of the molecular M and S forms of *Anopheles gambiae* and pyrethroid knockdown resistance gene in Nigeria. *Acta Tropica,* 95: 204–209.

Awolola TS, Oduola AO, Oyewole IO, Obansa JB, Amajoh CN, Koekemoer LL, Coetzee M (2007). Dynamics of knockdown pyrethroid insecticide resistance alleles in a field population of *Anopheles gambiae* in Southwestern Nigeria. J. Vect. Borne. Dis*.,* 44: 181–188.

Awolola TS, Oduola OA, Strode C, Koekemoer LL (2008). Evidence of multiple pyrethroid resistance mechanisms in the malaria vector *Anopheles gambiae* sensu stricto from Nigeria. Trans. R. Soc. Trop. Med. Hyg., 103 (11): 1139-45.

Baird JK, Jones TR, Danudirgo EW, Basri H, Anis BA, Bangs MJ, Wiady I, Masbar S (1991). Age-dependent acquired immunity against *Plasmodium falciparum* among people with 2 years exposure to hyperendemic malaria. Am. J. Trop. Med. Hyg., 45: 65–76.

Brooke BD, Hunt RH, Koekemoer LL, Dossou-Yovo J, Coetzee M (1999). Evaluation of a polymerase chain reaction assay for detection of pyrethroid insecticide resistance in the malaria vector species of the *Anopheles gambiae* complex. J. Am. Mosq. Contro.l Assoc., **15**: 565–568.

Chandre F, Darriet F, Manguin S, Brengues C, Carnevale P, Guillet P (1999). Pyrethroid cross-resistance spectrum among populations of *Anopheles gambiae s.s*. from Cote D'Ivoire. J. Am. Mosq. Control. Assoc., 15: 53–59.

Collins FH, Mendez M A, Razmussen MO, Mehaffey PC , Bensansky N J, Finnerty V (1987). A ribosomal RNA gene probe differentiateds member species of Anopheles gambiae complex, Am. J. Trop. Med. Hyg., 37: 37-41.

Corbel V, N'Guessan R, Brengues C, Chandre F, Djogbenou L, Martin T et al (2007). Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. Acta Trop., 101: 207-216.

Elissa N, Mouchet J, Riviere F, Meunier JY, Yao K (1993). Resistance of *Anopheles gambiae s.s*. to pyrethroids in Cote D' Ivoire. Ann. Soc. Belg. Med. Trop.,73: 291-294.

Fanello C, Petrarca V, Della-Torre A, Santolamazza F, Dolo G, Coulibaly M, Alloueche A, Curtis CF, Touré YT, Coluzzi M (2003). The pyrethroid knockdown resistance gene in the *Anopheles gambiae* complex in Mali and further indication of incipient speciation within *Anopheles gambiae s.s.* Insect. Mol. Biol., 12: 241–245.

Federal Ministry of Health (FoMH) (2005). Training manual for management of malaria in Nigeria. National malaria and vector control division, Abuja Nigeria. pp. 1-86.

Gillies MT, Coetzee M (1987). A supplement to the Anophelinae of Africa South of the Sahara (Afrotropical region). Publications of the South African Institute for Medical Research p. 55.

Greenwood BM, Bojang K, Whitty CJM, Targett GAT (2005). Malaria. The Lancet 365(9469): 1474-1480.

Hemingway J, Ranson H. (2000). Insecticide resistance in insect vectors of human diseases. Annu. Rev. Entomol., 45: 371-391.

Koch R (1900). Zweiter message thatigkeit über die of malaria expedition. Deutsche Med. Wochenschr 26: 88-90.

Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Berge JB, Devonshire AL, Guillet P, Pasteur N, Pauron D (1998). Molecular characterization of pyrethroid knockdown resistance (*kdr*) in the major malaria vector *Anopheles gambiae s.s*. Insect. Mol. Biol., 7: 179–184.

Miller MJ (1958). Observations on the natural history of malaria in the semi -resistant West African. Trans. R. Soc. Trop. Med. Hyg., 52:152-168.

National Demographic and Health Survey (NDHS) (2003). 2006 Edition. http://www.census.gov.ph/hhld/ndhs_2003.html

N'Guessan R, Corbel V, Akogbeto M, Rowland M. (2007). Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerg. Infect. Dis*., 13: 199-206.

Ramphul U, Boase T, Bass C, Okedi LM, Donnelly MJ, Muller P (2009). Insecticide resistance and its association with target-site mutations in natural population of *Anopheles gambiae* from eastern Uganda. Trans. R. Soc. Trop. Med. Hyg., 103(11):1121-1126.

Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH (2000). Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. Insect. Mol. Biol., 9: 491-497.

Scott JA, Brogon WG, Collins FH (1993). Identification of single specimen of the Anopheles complex by polymerase chain reaction. Am. J. Trop. Med. Hyg., 49: 520-529.

United Nation Population Division - 2000 *World Population Prospects: Population Database*: The 2002 Revision, http://esa.un.org/unpp

Van Rensburg AJ, Hunt RH, Koekemoer LL,Coetzee M, Shiff CJ, Monjas J (1996). The polymerase chain reaction as a tool for identifying members of the *Anopheles gambiae* complex (Diptera: Culicidae) in Northeast Tanzania. J. Am. Mosq. Control. Ass., 12: 271-274.

Vulule JM, Beach RF, Atieli FK, Mcallister JC, Brogdon WC, Roberts JM, Mwangi RW, Hawley WA (1999). Elevated oxidase and esterase levels associated with permethrin tolerance in *Anopheles gambiae* from Kenyan villages using permethrin impregnated nets. Med Vet Entomol., 13: 239-244.

World Health Organization (1998). Fact sheet No. 94 (World Health Organization, Geneva).

World Health Organization (1998). Test procedures for insecticide resistance monitoring in malaria vectors, bioefficacy and persistence of insecticides on treated surfaces. WHO/CDS/CPC/MAL/1998.12. Geneva.