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Dynamics of knockdown pyrethroid insecticide resistance alleles in a field population of *Anopheles gambiae s.s.* in southwestern Nigeria

T.S. Awolola[a], A.O. Oduola[a], I.O. Oyewole[b], J.B. Obansa[a], C.N. Amajoh[c], L.L. Koekemoer[d,e] & M. Coetzee[d,e]

[a]*Public Health Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria;* [b]*Department of Pure and Applied Sciences, Babcock University, Ilishan, Ogun State, Nigeria;* [c]*National Malaria and Vector Control, Federal Ministry of Health, Abuja, Nigeria;* [d]*Vector Control Reference Unit, National Institute for Communicable Diseases, Sandringham, South Africa;* [e]*Division of Virology and Communicable Disease Surveillance, School of Pathology of the National Health Laboratory Service and the University of the Witwatersrand, Johannesburg, South Africa*

Abstract

Background & objectives: Pyrethroid insecticide resistance in the malaria vector *Anopheles gambiae* Giles is mainly associated with reduced target site sensitivity arising from a single point mutation in the sodium channel gene, often referred to as knockdown resistance (kdr). This resistance mechanism is widespread in West Africa and was reported for the first time in Nigeria in 2002. Here we present changes in the susceptibility/resistance status of the molecular 'M' and 'S' forms of *An. gambiae* and the frequency of the kdr alleles from 2002–05.

Methods: Adult anophelines were sampled quarterly inside human dwellings from January 2002 to December 2005 and adults reared from wild larvae were identified using morphological keys. Samples belonging to the *An. gambiae* complex were subjected to PCR assays for species identification and detection of molecular 'M' and 'S' forms. Insecticide susceptibility tests were carried out using standard WHO procedures and test kits only on 2–3 days old adult *An. gambiae s.s.* reared from larval collections. The kdr genotypes were determined in both live and dead specimens of *An. gambiae s.s.* using alleles-specific polymerase chain reaction diagnostic tests.

Results: The overall collection showed that the molecular 'S' form was predominant (>60%) but the proportions of both forms in the mosquito populations from 2002–05 were not statistically different. Both forms also occurred throughout the period without apparent relationship to wet or dry season. Insecticide susceptibility tests did not show any significant increase in the resistance status recorded for either Permethrin or DDT from 2002–05, rather, an improvement in the susceptibility status of the mosquitoes to these insecticides was observed from 2004–05 relative to the tests performed in 2002–03.

Conclusion: The proportion of the molecular 'M' and 'S' form of *An. gambiae* and the kdr frequencies have not increased significantly from 2002 when it was first reported in Nigeria. However, the findings on susceptible mosquitoes exhibiting the kdr gene need further investigation. Further monitoring of this may provide additional information on the ongoing debate on the possibility of restriction in gene flow and reproductive barriers in these sympatric taxa.

Key words *Anopheles gambiae* – DDT – insecticide resistance – molecular forms – permethrin

Introduction

Anopheles gambiae Giles (Diptera: Culicidae) is one of the most important malaria vectors in Africa[1–2]. In West Africa, it exists as two distinct molecular forms, referred to as 'M' and 'S' based on the variation observed in molecular markers[3,4]. Both forms are anthropophagic and effective vectors of human malaria parasites. In sub-Saharan Africa, insecticide-treated nets (ITNs) and indoor residual insecticide spraying (IRS) are the cornerstones of malaria vector control[5]. Currently, pyrethroids are the only class of insecticides approved for treating bednets or curtains because of their high effectiveness and strong excito-repellent effect on mosquitoes, yet low mammalian toxicity[6]. However, pyrethroid resistance in *An. gambiae s.s.* has been described in West, East and Central Africa[7–12]. This resistance is mainly associated with reduced target site sensitivity arising from a single point mutation in the sodium channel gene, often referred to as knockdown resistance (*kdr*) characterised by a leucine-phenylalanine mutation in West Africa[13] and leucine-serine mutation in East Africa[11]. Both West and East African *kdr* mutations have recently been reported in a population of *An. gambiae s.s.* from Central Africa[14]. The *kdr* mutation has also been used as a marker for monitoring introgression between the 'M' and 'S' molecular forms of *An. gambiae*[15].

Pyrethroid *kdr* has been found in *An. gambiae s.s.* in Nigeria[16–18], and, therefore we initiated studies to determine the spread and dynamics of the *kdr* mutation. Here, we report changes in the proportion of the molecular 'M' and 'S' forms, their susceptibility status to insecticides and the frequency of the *kdr* alleles in field populations of *An. gambiae* from 2002–05.

Material & Methods

Study site: The study was carried out at "Alakia" in the suburbs of Ibadan (03°54'E, 07°26'N) situated in the forest area of southwestern Nigeria. It is an agrarian community where there is considerable agricultural use of insecticides against maize and cocoa pests. The study site is among the few places in Ibadan where there is usage of insecticide treated nets (ITNs), introduced by the National Malaria and Vector Control Programme in 2001, with a low coverage of <5%. The climate in the area is tropical with a well marked dry season from November to April. The rainy season extends from May to October with a short break in August.

Mosquito collections: Adult anophelines were sampled quarterly from January 2002 to December 2005 inside the same human dwellings reported earlier[16], using mouth aspirator, flash light and paper cups[19] between 0600 and 0900 hrs. The characteristics of the houses were the same throughout the study period as none of the occupants had bednets. During the same period, anopheline larvae were collected from natural breeding sites, reared to adulthood in an insectary.

Species identification: All anophelines collected were identified using morphological keys[1,20]. All belong to the *An. gambiae* complex and were subjected to the *An. gambiae* species specific PCR assays for species identification[21]. Aliquots of DNA extracted from PCR positive specimens of *An. gambiae s.s.* were subjected to PCR assays for identification of the molecular 'M' and 'S' forms[3].

Insecticide susceptibility tests: Insecticide susceptibility tests were carried out using standard WHO procedures, diagnostic test kits and impregnated papers[22] only on 2–3 days old adult *An. gambiae s.s.* reared from larval collections. The test papers included 1% permethrin and 4% DDT to assess whether there was cross-resistance between DDT and permethrin. To check that the test papers remained insecticidal, all were tested prior to the exposure of the mosquitoes, against a reference laboratory colony of *An. gambiae s.s.* named 'AGIB' that had been set up in 2001 from mosquitoes collected at Ibadan, southwestern Nige-

ria. The colony is maintained in our laboratory insectaries at the Nigerian Institute of Medical Research, Lagos, and is 100% susceptible to the diagnostic concentration of the insecticides used. After one hour exposure, the mosquitoes were maintained on 10% sucrose solution and the final mortality was recorded after 24 h. Samples were preserved individually on desiccated silica gel for further analysis.

Detection of the kdr mutation: The *kdr* genotypes were determined in all live and dead specimens of *An. gambiae s.s.* from the bioassay test. Similarly, the *kdr* alleles were investigated in the adult mosquitoes collected indoor using an allele-specific polymerase chain reaction diagnostic test designed for the West African leucine-phenylalanine mutation[13]. Owing to recent reports of the presence of both the East and West African *kdr* mutations in *An. gambiae* populations in a neighbouring country[14], we conducted an investigation of the East African *kdr* mutation on 1400 mosquito samples comprising of both 'M' and 'S' forms of specimens preserved on desiccated silica and from bioassay tests conducted in between 2004 and 2005.

Statistical analysis: Mosquitoes collected in the wet (May–October) and dry season (November–April) for each year were analysed separately. The mean values for the two periods were compared using the Student's *t*-test. Differences in the numbers of mosquitoes collected and the proportion of the molecular M and S forms over the period were analysed using the Chi-square test. One way analysis of variance was performed to compare the seasonal frequencies of the *kdr* alleles and Fisher's exact test for the frequency of the *kdr* gene in the mosquito population.

Results

Species identification: Table 1 shows the proportion of the molecular 'M' and 'S' forms out of 2,850 wild adult female *An. gambiae s.s.* collected indoor from 2002–05. The analysis showed that the molecular 'S' form was predominant. The proportion of both forms from 2002 to 2005 was similar ($\chi^2 = 0.3$, df = 3, p = 0.96). The number of *An. gambiae s.s.* collected in the wet season in each year was significantly higher ($p < 0.001$), both 'M' and 'S' forms, however, occurred throughout the period without apparent relationship to wet or dry season (Table 1). The proportion of 'M' and 'S' forms in the wild adult and adult reared from larval collections were also similar. No hybrids of 'M' and 'S' forms were found during the study period.

Insecticide resistance status: The susceptibility of adult mosquitoes (reared from larval collection) to permethrin and DDT from 2002–05 is presented in

Table 1. Proportion of the molecular 'M' and 'S' forms in the wild adult *An. gambiae* during the wet and dry season in the study area

Period	N	Molecular forms	Proportion (%) of the molecular 'M' and 'S' forms/year				
			2002	2003	2004	2005	% Total*
Wet season	681	M	210 (26)	143 (23)	165 (23.4)	163 (22.8)	23.9
(May–Oct)	1251	S	354 (43.9)	280 (45)	316 (44.8)	301 (42.1)	43.9
Dry season	324	M	91 (11.3)	77 (12.4)	75 (10.6)	81 (11.3)	11.4
(Nov–Apr)	594	S	152 (18.4)	122 (19.6)	150 (21.3)	170 (23.8)	20.8
Total	2850		807	622	706	715	100

*Overall, 67.8% (1932/2850) were collected in the wet and 32.2% (918/2850) in the dry season; 64.7% (1845/2850) were of the molecular 'S' form and 35.3% (1005/2850) were of the 'M' form. Figures in parenthese are percentages.

Table 2. The resistance status of the mosquitoes was based on the decrease in the mortality rates according to WHO criteria[22]. The overall results showed no significant increase in the level of permethrin and DDT resistance from 2002–05, although the 'M' form showed a trend of increase in DDT resistance (Table 2). The 24 h post-exposure mortality rate using permethrin also showed a slight decrease in the number of susceptible mosquitoes from 78.6% in 2002 to 72.1% in 2003, yet it remained stable thereafter. The corresponding data for DDT was 75.0% in 2002, 71.4% in 2003 and >78% for both 2004 and 2005. There were no significant differences in the proportions of 'M' or 'S' form that were resistant to permethrin or DDT over the period of the study. The highest level of mortality (and, therefore the lowest resistance) was recorded among the mosquitoes in 2005. In all, an improvement in the susceptibility status of the mosquitoes to permethrin and DDT was observed during 2004–05 relative to the tests performed during 2002–03 (Table 2).

Frequency of the kdr mutation: The results of the *kdr* PCR assay detected only the leucine-phenylalanine *kdr* mutation in the wild adult and in those adults reared from larval collections. The leucine-serine *kdr* mutation was negative in all (1400) samples tested. The *kdr* PCR assay showed three genotypes, identi-

Table 2. 24 h post-exposure mortality rate and frequency of the *kdr* alleles in the molecular 'M' and 'S' forms in 2–3 days old adult *Anopheles gambiae* reared from wild larvae after one hour exposure to 1% permethrin and 4% DDT in WHO test kits

Variables	2002		2003		2004		2005	
	Permethrin	DDT	Permethrin	DDT	Permethrin	DDT	Permethrin	DDT
No. exposed	98	112	398	378	210	250	225	180
No. (%) of knock-down after 1hour	76 (77.6)	90 (80.4)	297 (74.6)	295 (78.0)	158 (75.2)	196 (78.4)	178 (79.1)	148 (82.2)
No. (%) mortality 24 h post-exposure	77 (78.6)	84 (75.0)	287 (72.1)	270 (71.4)	162 (77.2)	206 (82.4)	176 (78.2)	146 (81.1)
No. (%) survivors 24 h post-exposure	21 (21.4)	22 (19.6)	111 (27.9)	108 (28.6)	48 (22.8)	44 (17.6)	49 (21.8)	34 (18.9)
% M and S forms in survivor population								
'M' form	38.1	31.8	40.5	41.7	36.5	40.7	36.7	44.1
'S' form	61.9	68.2	59.5	58.3	63.5	59.3	63.3	55.9
Kdr frequency (%) in survival population								
'M' form	0	0	0	7.4	0	0	0	0
'S' form	19.1	36.4	34.2	38.8	26.9	25.9	28.6	29.4
% M and S forms in susceptible specimens								
'M' form	32.5	33.3	39.4	40.7	42.0	44.7	39.8	41.1
'S' form	67.5	66.7	60.6	59.3	58.0	55.3	60.2	58.9
Kdr frequency (%) in susceptible specimens								
'M' form	0	0	0	0	0	0	0	0
'S' form	0	0	0	0	8.6	10.6	12.5	8.2

fied by the characteristic 293bp band common to both susceptible and resistant specimens, a 137bp band associated with susceptible (SS) specimens and a 195bp *kdr* band (R). The presence of all three bands in a single specimen indicates heterozygosity (RS). Analysis of the wild adult population showed that the *kdr* alleles (RR + RS) were found only in the molecular 'S' form with an overall frequency of 20.4, 30.9, 28.6 and 27.6% for 2002, 2003, 2004 and 2005, respectively (Fig. 1). Although there was more than a 10% increase in the kdr frequency from 2002 to 2003, the overall increase from 2003 to 2005 was not statistically significant (p = 0.08 by Fisher's exact test) and was similar to the trend observed in the *kdr* frequencies in the survival 'S' population exposed to insecticide tests. The highest *kdr* frequency in the wild adult (Fig. 1) and adult reared from larval populations (Table 2) was obtained in 2003. A general decline in the homozygous (RR) frequency was observed for 2003–05, no such trend was evident for 2002 (Fig. 1). The overall *kdr* frequency in the dry season was significantly lower than that recorded during the rest of the years in the wild adult (Fig. 2) (F = 2.85, p = 0.02) and those reared from larval populations (F = 2.04, p = 0.03), but in each case, the proportion of the homozygous (RR) and heterozygous (RS) *kdr* alleles were similar. Only eight (7.4%) mosquitoes of the 'M' form reared from larval collections and resistant to DDT in 2003 and none for permethrin were positive for the *kdr* mutation (Table 2). All were heterozygous for the kdr allele and were recorded in the dry season. The corresponding *kdr* frequency in the 'S' form for the same period was >30%. Further analysis of the bioassay test showed that 9.8% (36/368) and 10.6% (34/322) susceptible specimens (all belonging to the 'S' form) in between 2004 and 2005 respectively exhibited the heterozygous *kdr* genotype.

Discussion

The distribution of the molecular 'M' and 'S' forms of *An. gambiae* and the *kdr* mutation associated with pyrethroid resistance in this species is still being determined for much of West and East Africa. The present study showed that the molecular 'M' and 'S' forms occurred in sympatry in southwestern Nigeria

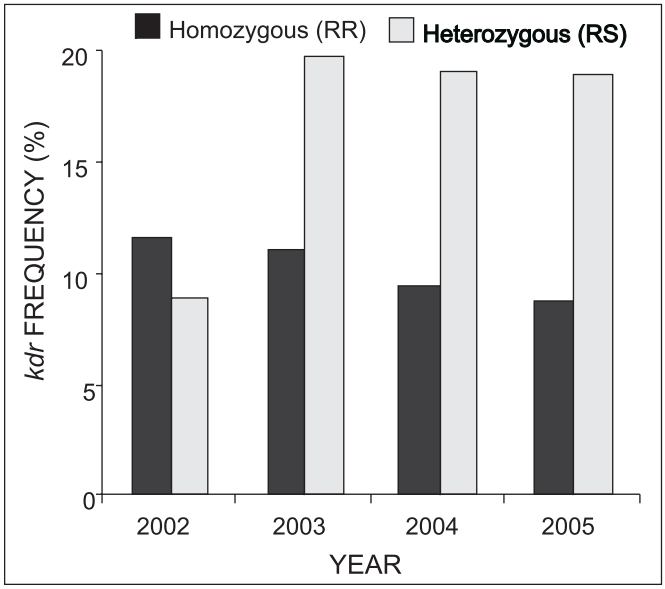

Fig. 1: Frequencies of the homozygous (RR) and heterozygous (RS) *kdr* alleles in the wild adult population of the molecular 'S' form of *An. gambiae*

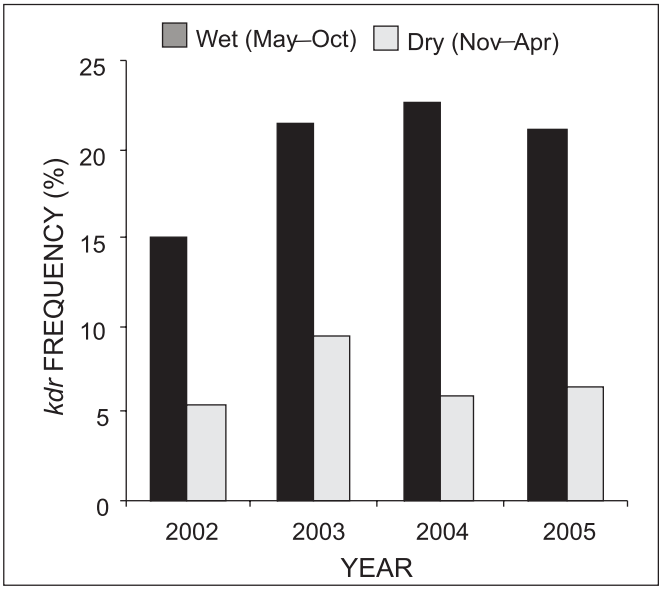

Fig. 2: Kdr frequencies in the wild adult population of the molecular 'S' from of *An. gambiae* in the wet and dry seasons

and were most abundant in the wet season, although they both occurred throughout the year. The overall collection showed that the 'S' form was predominant and corroborates findings from elsewhere in Nigeria[17,23]. The absence of hybrid 'M' and 'S' forms over the four-years period contrasts with reports from Burkina Faso[15], but strongly suggests restriction in gene flow between the two forms[24], indicating positive assortative mating and supporting their status as good biological species.

Anopheles gambiae s.s. is highly endophilic, making it a target for malaria vector control by indoor residual insecticide spraying or the use of insecticide treated bednets. The results showed that both molecular forms of *An. gambiae* are resistant to permethrin and DDT, but that the *kdr* gene was found mainly in the 'S' form. The allelic frequency of the *kdr* gene in the 'S' form was proportionate to the resistant level in the mosquito population. However, only eight DDT resistant specimens of the 'M' form in 2003 and none in permethrin resistant populations were positive for the *kdr* alleles strongly suggesting additional mechanisms conferring resistance. The presence of the *kdr* mutation in the 'M' form in other parts of West Africa, have been attributed to recent introgression of the *kdr* mutation from the 'S' to the 'M' form[25–27]. However, our data do not support this hypothesis since the *kdr* allele was absent in the wild adult population and more so that it was not found again in the 'M' form two years after it was recorded in adult reared from larval collections. The absence of the *kdr* allele in the 'M' form under field conditions also provides additional support for the hypothesis of restricted gene flow between the two forms and, therefore, positive assortative mating. However, the results on susceptible mosquitoes having the *kdr* genotype need further investigation because of the over emphasis on *kdr* as the major resistance mechanisms in pyrethroid resistant *Anopheles gambiae s.s*. in West Africa.

The East and West Africa *kdr* mutations have evolved independently in *An. gambiae s.s.* The leucine-phenylalanine *kdr* mutation, first reported in Cote D'Ivoire was attributed to the extensive use of agricultural insecticides[7]. Although the origin of pyrethroid resistance in the Nigerian mosquito population used in the present study could not be ascertained due to agricultural and public health use of insecticides in the study site, the long history of agricultural use of insecticides in the area suggests that the *kdr* mutation must has been in existence in the mosquito population prior to the introduction of ITNs in the area. Even so, the considerable stability of the *kdr* frequency and the reduction in the number of individual mosquitoes carrying the homozygous *kdr* allele in wild adult collections from 2002–05 demonstrates that at least in the short-term, resistance levels will drop when insecticide pressure is lessened in the area. The absence of the East African *kdr* mutation in our study is similar to reports from neighbouring Benin and Cote D'Ivoire and to date; this mutation has not been recorded in West Africa[28].

Conclusion

The proportion of the molecular 'M' and 'S' forms of *An. gambiae* and the *kdr* frequencies have not increased significantly from 2002 when it was first reported in Nigeria. This provides additional support for the hypothesis of restricted gene flow between the two forms. Although, the operational impact of pyrethroid *kdr* resistance on the efficacy of insecticide-treated nets on malaria vector control is not fully clear, the persistence of *kdr* alleles in the mosquito populations should be of concern to control programmes because of the potential for failure of pyrethroid-based vector control interventions. Resistance management strategies will need to be considered in order to contain the spread of the resistant populations.

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References

1. Gillies MT, Coetzee M. *A supplement of the Anophelinae of Africa South of the Sahara (Afrotropical Region)*. Johannesburg: The South African Institute of Medical Research 1987.

2. Coetzee M, Craig M, Le Sueur D. Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitol Today* 2000; *16:* 74–7.

3. Favia G, Dimopoulos G, Della Torre A, Toure YT, Coluzzi M, Louis C. Polymorphisms detected by random PCR distinguishes between different chromosomal forms of *Anopheles gambiae*. *Proc Nat Acad Sci* 1994; *91:* 10315–9.

4. Della Torre A, Fanello C, Akogbeto M, Dossou-yovo J, Favia G, Petrarca V, Coluzzi M. Molecular evidences of incipient speciation within *Anopheles gambiae s.s*. in West Africa. *Insect Mol Biol* 2001; *10:* 9–18.

5. Curtis CF, Myamba J, Wilkes TJ. Comparison of different insecticides and fabrics for antimosquito bednets and curtains. *Med Vet Entomol* 1996; *10:* 1–11.

6. Mittal PK, Adak T, Sharma VP. Acute toxicity of certain organochlorine, organophosphorus, synthetic pyrethroid and microbial insecticides to the mosquito fish *Gambusia affinis* (Baird and Girard). *Indian J Malariol* 1991; *28:* 167–70.

7. Elissa N, Mouchet J, Riviere F, Meunier JY, Yao K. Resistance of *Anopheles gambiae s.s*. to pyrethroids in Cote D' Ivoire. *Ann Soc Belg Med Trop* 1993; *73:* 291–4.

8. Vulule JM, Beach RF, Atieli FK, Mcallister JC, Brogdon WC, Roberts JM, Mwangi RW, Hawley WA. Elevated oxidase and esterase levels associated with permethrin tolerance in *Anopheles gambiae* from Kenyan villages using permethrin impregnated nets. *Med Vet Entomol* 1999; *13:* 239–44.

9. Akogbeto M, Yakoubou S. Resistance of malaria vectors to pyrethroids used for impregnated bednets in Benin, West Africa. *Bull Soc Pathol Exot* 1999; *92:* 123–30.

10. Chandre F, Darriet F, Manguin S, Brengues C, Carnevale P, Guillet P. Pyrethroid cross-resistance spectrum among populations of *Anopheles gambiae s.s*. from Cote D'Ivoire. *J Am Mosq Control Assoc* 1999; *15:* 53–9.

11. Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH. 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* 2000; *9:* 491–7.

12. Etang J, Manga L, Chandre F, Guillet P, Fondjo E, Mimpfoundi R, Toto JC, Fontenille D. Insecticide susceptibility status of *Anopheles gambiae s.l.* (Diptera : Culicidae) in the Republic of Cameroon. *J Med Entomol* 2003; *40:* 491–7.

13. Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Berge JB, Devonshire AL, Guillet P, Pasteur N, Pauron D. Molecular characterisation of pyrethroid knockdown resistance (*kdr*) in the major malaria vector *Anopheles gambiae s.s*. *Insect Mol Biol* 1998; *7:* 179–84.

14. Pinto J, Lynd A, Elissa N, Donnelly MJ, Costa C, Gentile G, Caccone A, Do Rosario VE. Co-occurrence of East and West African *kdr* mutations suggests high levels of resistance to pyrethroid insecticides in *Anopheles gambiae* from Libreville, Gabon. *Med Vet Entomol* 2006; *20:* 27–32.

15. Diabate A, Baldet T, Chandre F, Dabire KR, Kengne P, Guiguemde TR, Simard F, Guillet P, Hemingway J, Hougard JM. *Kdr* mutation, a genetic marker to assess events of introgression between the molecular M and S forms of *Anopheles gambiae* (Diptera: Culicidae) in the tropical Savannah area of west Africa. *J Med Entomol* 2003; *40:* 195–8.

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

17. Awolola TS, Oyewole IO, Amajoh CN, Idowu ET, Ajayi MB, Oduola A, Manafa OU, Ibrahim K, Koekemoer LL, Coetzee M. Distribution of the molecular M and S forms of *Anopheles gambiae* and pyrethroid knockdown resistance gene in Nigeria. *Acta Tropica* 2005; *95:* 204–9.

18. Kristian M, Fleischmann H, Della-Torre A, Stich A, Curtis CF. Pyrethroid resistance/susceptibility and differential urban/rural distribution of *Anopheles arabiensis* and

Anopheles gambiae s.s. malaria vectors in Nigeria and Ghana. *Med Vet Entomol* 2003; *17:* 326–32.

19. Service MW. A critical review of procedures for sampling populations of adult mosquitoes. *Bull Entomol Res* 1977; *67:* 343–82.

20. Gillies MT, De Meillon B. *The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region)*. Johannesburg: The South African Institute of Medical Research 1968; p. 343.

21. Scott JA, Brogdon WG, Collins FH. Identification of single specimens of the *Anopheles gambiae* complex by polymerase chain reaction. *Am J Trop Med Hyg* 1993**;** *49:* 520–9.

22. *Test procedures for insecticide resistance monitoring in malaria vectors, bioefficacy and persistence of insecticides on treated surfaces*. WHO/CDS/CPC/MAL/1998.12. Geneva: WHO 1998.

23. Onyabe DY, Vajime CG, Nock IH, Ndamss IS, Akpa AU, Alaibe AA, Conn JE. The distribution of M and S molecular forms of *Anopheles gambiae* in Nigeria. *Trans R Soc Trop Med Hyg* 2003; *97:* 605–8.

24. Fanello C, Petrarca V, Della-Torre A, Santolamazza F, Dolo G, Coulibaly M, Alloueche A, Curtis CF, Touré YT, Coluzzi M. 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* 2003; *12:* 241–5.

25. Weill M, Chandre F, Brengues C, Manguin S, Akogbeto M, Pasteur N, Guillet P, Raymond M. The *kdr* mutation occurs in the Mopti form of *Anopheles gambiae s.s*. through introgression. *Insect Mol Biol* 2000; *9:* 451–5.

26. Gentile G, Slotman M, Ketmaier V, Powell JR, Caccone A. Attempts to molecularly distinguish cryptic taxa in *Anopheles gambiae* s.s. *Insect Mol Biol* 2001; *10:* 25–32.

27. Diabate A, Brengues C, Baldet T, Dabire KR, Hougard JM, Akogbeto M, Kengne P, Simard F, Guillet P, Hemingway J, Chandre F. The spread of the Leu-Phe *kdr* mutation through *Anopheles gambiae* complex in Burkina Faso: genetic introgression and *de novo* phenomena. *Trop Med Int Hlth* 2004; *9:* 1267–73.

28. Chandre F, Darriet F, Duchon S, Finot L, Manguin S, Carnevale P, Guillet P. Modifications of pyrethroid effects associated with *kdr* mutation in *Anopheles gambiae*. *Med Vet Entomol* 2000; *14:* 81–8.

Corresponding author: Dr. T.S. Awolola, Public Health Division, Nigerian Institute of Medical Research, PMB 2013, Yaba, Lagos, Nigeria.
E-mail: awololas@hotmail.com

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