International Journal of Mosquito Research

ISSN: 2348-5906
CODEN: IJMRK2
IJMR 2018; 5(1): 106-111

Received: 09-11-2017
Accepted: 10-12-2017

Isaac Olayinka Oyewole
a) Osun State College of Education, Ila Orangun, Osun State, Nigeria
b) Babcock University, Ilisan Remo, Ogun State, Nigeria

Mustapha
Babcock University, Ilisan Remo, Ogun State, Nigeria

Abdur-Rahman Kolawole
Osun State College of Education, Ila Orangun, Osun State, Nigeria

Oluwakemi Christianah Adedeji
Osun State College of Education, Ila Orangun, Osun State, Nigeria

Dapo Adeogun
Lead City University, Ibadan, Nigeria

Sam Awolola
Nigerian Institute of Medical Research, Lagos, Nigeria

Correspondence
Isaac Olayinka Oyewole
a) Osun State College of Education, Ila Orangun, Osun State, Nigeria
b) Babcock University, Ilisan Remo, Ogun State, Nigeria

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

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

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

Keywords: anophelinae, *Anopheles gambiae* s.s., susceptibility, insecticides

1. Introduction
The level of morbidity and mortality, especially in children and pregnant women due to malaria attack in sub-Saharan Africa is still a great cause for concern. Despite advances in control, treatment and preventive measures adopted in the past and recent times, malaria still threatens lives of millions of people in African countries. Success of the control measures adopted in the past decades, using indoor residual spray, insecticide treated nets and treatment with Artemisinin-based Combination Therapies (ACT's) have been limited by both the mosquito and parasite resistance respectively. In tropical countries, prevailing environmental conditions such as high humidity and warmth which support mosquito growth have contributed to malaria transmission in this part of the world. Other contributing factors include socio-cultural and economic attributes such as education, income, housing patterns, social groups, water storage also play major role in malaria transmission[1].Generally, malaria is known to be more prevalent in rural and peri-urban settlements in sub-Saharan African due to availability of favourable conditions for the breeding of anopheline vector. Dwellers in these areas are often bedevilled with poverty, poor housing, poor water supply, poor environmental conditions which are often laden with swamps, gutters and thick vegetations to enhance the breeding of mosquito. The population in this region are usually more vulnerable to mosquito bites, hence high malaria transmission intensity.
In Nigeria, malaria is endemic throughout the year in the entire country including the urban areas where more than 90% of the approximately 132 million people are at the risk of the infection. According to the Federal Ministry of Health [2], half of the population in Nigeria suffers one or more malaria attacks annually. In the recent times, efforts directed towards the control of malaria include the interruption in the disease transmission by reducing man-

mosquito contact. In Nigeria, the two major approaches adopted are the use of long lasting insecticide treated nets (LLINs) and indoor residual spray (IRS). In 2014, Elimination Programme (NMEP) in Nigeria scaled up indoor residual spraying (IRS) in some regions to supplement LLINs. However, the efficacy of those malaria control strategies has been limited with the development of resistance by *Anopheles* mosquitoes to all classes of WHO-recommended adult insecticides, particularly pyrethroids [2, 3]. Four classes of insecticides are currently recommended for malaria control includes Pyrethroids, Organochlorine, Organophosphate and Carbamate, out of which only pyrethroids is currently approved for LLINs due to its safety, residuality and cost effectiveness. However, Pyrethroid resistance to *Anopheles* species has been an issue since it was first reported in Nigeria in 2002 [4] and this has been on a growing trend ever since [6-14]. Resistance in malaria vectors to Pyrethroids has also been reported in 23 out of 49 African countries [15-17, 5, 18-31]. The efficacy of interventional insecticides is germane to the successful implementation of both IRS and LLINs which invariably is a factor of availability of insecticide(s) susceptible *Anopheles* mosquitoes in the local environment. Therefore, regular monitoring of susceptibility status of local *Anopheles* vectors to insecticides is essential to determining the effectiveness of malaria control programmes which rely solely on LLINs and IRS interventions.
The present study tested the susceptibility status of *Anopheles gambiae* to Lambdacyhalothrin, Permethrin, Deltamethrin, DDT, Fenitrothion and Bendiocarb.

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

2.2 Sample collection, identification and susceptibility test
Anopheline larvae collected from the natural breeding habitat in the selected areas using the standard (350ml dipper) dipping method were reared to adulthood. Bioassays were conducted on non-blood fed, 2 to 3 days old female mosquitoes using the standard WHO procedures and susceptibility test kits [32]. Samples were identified morphologically [33, 34] and later subjected to susceptibility test. Bioassays were conducted by exposing the mosquitoes to six test papers impregnated with Lambdacyalothrin (0.05%), Permethrin (0.75%), Deltamethrin (0.05%), Bendiocarb (0.1%), Fenitrothion (1.0%) and DDT (4%) and untreated control using the WHO standard procedures and test kits [32].
For each insecticide paper and the control, a three replicates of 20 adult mosquitoes were exposed to toxicant tubes containing insecticide impregnated papers for 60 min and cumulative knocked-down was recorded at intervals of 10 min. Mosquitoes were then transferred into clean WHO observation tubes, fed with 10% glucose solution and final mortality recorded after 24h pre-exposure.
The resistance status was defined according to WHO guidelines [35] which states as follows: 98-100% mortality indicates susceptibility, 90-98% indicates possibility of resistance for confirmation, and <90% indicates resistance. The dead and survived mosquitoes were later separated and kept individually in Eppendorf tubes containing desiccated silica gel for further tests.

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

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

Table 1: Knock-down rate for different insecticides during one hour to exposure in the study area

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

						319.68)	
Carbamate	Bendiocarb	0.1	100	24.91 (22.45-26.09)	1.13 (0.8)	31.69 (29.82-38.98)	0.38 (0.7)
Organochlorin	DDT	4.0	86	189.56 (94.97-272.66)	7.78 (0.005)	234.06 (104.7-356.8)	9.45 (0.005)
Organophophate	Fenitrothion	1.0	105	89.76 (48.09-108.3)	4.11 (0.08)	123.39(24.60-288.92)	6.83 (0.02)

3.2 Percentage knock-down

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

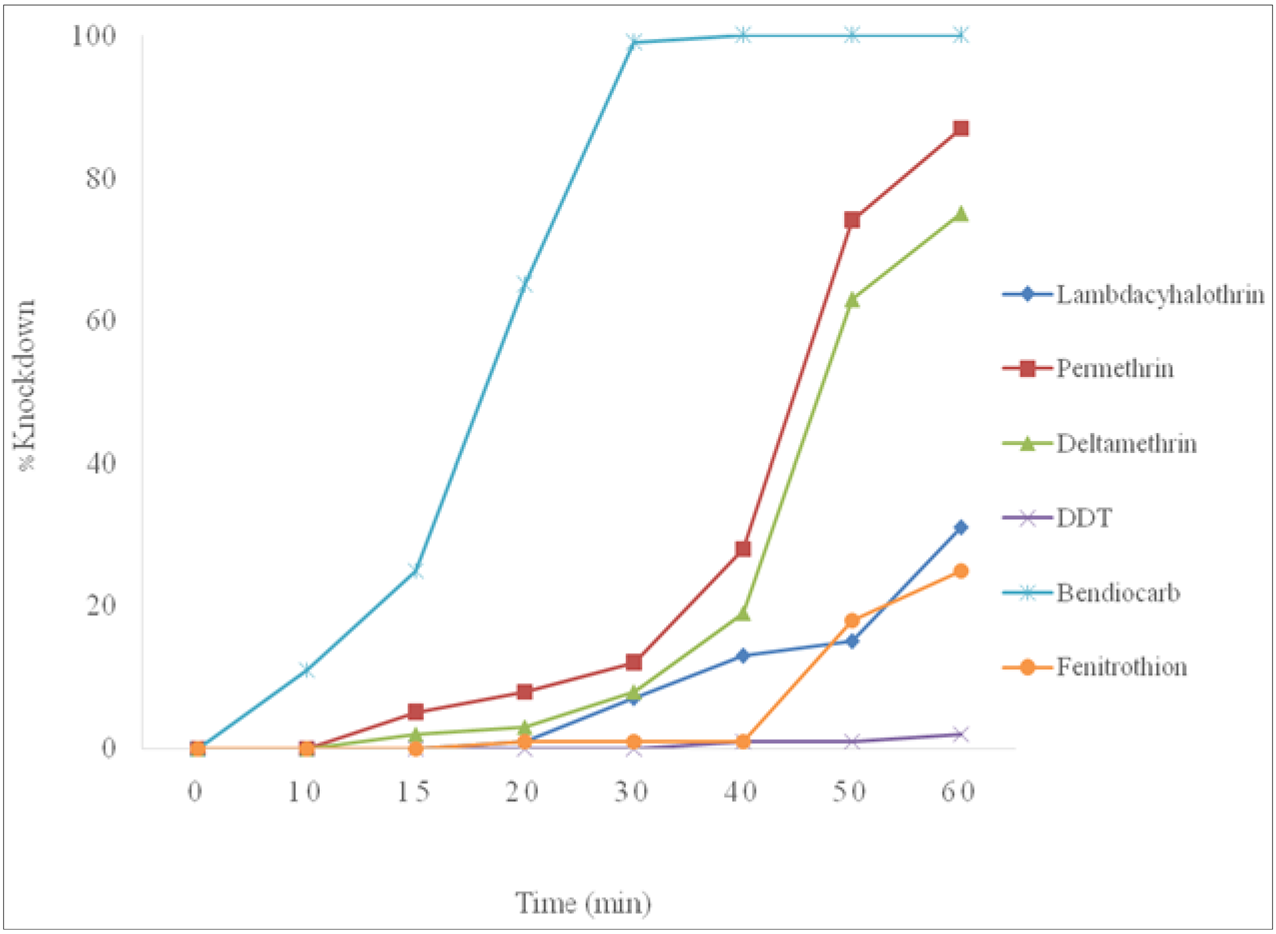

Fig 1: Knock-down rate for different insecticides during 1 hour of exposure in the study area

3.3 Susceptibility pattern

The susceptibility pattern of the *Anopheles* mosquitoes against the six insecticides tested was assessed based on the mortality rate during the 24h post-exposure. Following the WHO protocols (WHO, 2013b) *Anopheles* mosquitoes were highly susceptible to Bendiocarb, observed mortality rate was 99% (CI: 97.6-99.9) and Fenitrothion with mortality rate of 98% (CI: 90.9-97.7). Reduced susceptibility to Lambdacyhalothrin, Permethrin and Deltamethrin was observed with the corresponding mortality rate of 47% (CI: 38.4-52.3), 55% (CI: 47.9-63.4) and 53% (CI: 52.9-64.5) respectively. The mortality of *Anopheles* mosquitoes due to DDT in all the study sites was below 10% (CI: 8.7-12.6) indicating a high level of resistance to DDT (Table 2).

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

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

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

4. Discussion
4.1 Aim and procedure of bioassays
Assessment of the successful implementation of LLINs and IRS for vector control requires periodic monitoring of resistance in the insecticides used for their treatment [36]. In the present study, WHO insecticide bioassays were carried out to investigate the susceptibility pattern of *Anopheles* mosquitoes to Lambdacyhalothrin, Permethrin, Deltamethrin, Bendiocarb, DDT and Fenitrothion in the selected area. To achieve the aim of this study two to three days old female anopheline mosquitoes were used for the bioassay according to WHO [35] guidelines.

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

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

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

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

6. Acknowledgements
The authors are grateful to the communities in Ila Orangun where samples were collected for their cooperation and to all our field and technical officers for assistance during the field work. We also thank Tertiary Education Trust Fund (TETFund) for providing TETfund Institution-based Research Intervention to support this research work.

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

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

9. References

1. Anumudu CI, Adepoju A, Adediran M, Adeoye O, Kassim A, Oyewole I, *et al*. Malaria prevalence and treatment seeking behaviour of young Nigerian Adults. Ann. Afr. Med. 2006; 5(2):82-88.
2. Federal Ministry of Health, Federal Republic of Nigeria. National Anti-malarial Treatment Policy 2005. Abuja: Federal Ministry of Health, National Malaria and Vector Control Division, 2005.
3. Curtis CF, Hill N, Kasim SH. Are there effective resistance management strategies for vectors of human disease? Biol. J Linn. Soc. 1993; 48:3-18.
4. Kelly-Hope L, Ranson H, Hemingway J. Lessons from the past: managing insecticide resistance in malaria control and eradication programmes. Lancet Infect. Dis. 20081; 1:45-8.
5. Awolola TS, Brooke BD, Hunt RH, Coetzee M. Resistance of the malaria vector *Anopheles gambiae s.s* to pyrethroid insecticides in south - western Nigeria. Ann. Trop. Med. Parasitol. 2002; 96:849-52.
6. Kristan M, Fleischmann H, della Torrey A, Stich A, Curtis CF. Pyrethroid resistance/susceptibility and differential urban/rural distribution of *Anopheles arabiensis* and *An. gambiae* s.s. malaria vectors in Nigeria and Ghana. Med. Vet. Entomol. 2003; 17:326-

332.
7. Awolola TS, Oyewole IO, Amajoh CN, Idowu ET, Ajayi MB, Oduola A *et al*. Distribution of the molecular M and S forms of *Anopheles gambiae* and pyrethroid knockdown resistance gene in Nigeria. Acta Trop. 2005; 95:204-9.
8. Awolola TS, Oduola AO, Oyewole IO, Obansa JB, Amajoh CN, Koekemoer LL, *et al*. Dynamics of knockdown pyrethroid insecticide resistance alleles in a field population of *Anopheles gambiae* in southwestern Nigeria. J Vect. Borne Dis. 2007; 44:181-188.
9. Ndams IS, Laila KM, Tukur Z. Susceptibility of some species of mosquitoes to permethrin pyrethroid in Zaria, Nigeria. Sci world J. 2006; 1(1):15-19.
10. Oduola AO, Obansa JB, Ashiegbu CO, Adeogun A, Otubanjo OA. High level of DDT resistance in the malaria mosquitoe: Anopheles gambiae s.l from rural, semi urban and urban communities in Nigeria. J.Rural Trop Pub Hlth. 2010; 9:114-120.
11. Oyewole IO, Ogunnowo AA, Ibidapo CA. Okoh. Epidemiology of malaria and insecticide resistance burden in Nigeria. J Pub Hlth Epidemol. 2011; 3(1):6-12.
12. Ibrahim Sulaiman S. High frequency of kdr L1014F is associated with pyrethroid resistance in *Anopheles coluzzi* in Sudan savannah of northern Nigeria. BMC Infect Dis. 2014; 14(1):441.
13. Umar A. Kabir BGJ, Amajoh CN, Inyama PU, Ordu DA, Barde AA, *et al*. Susceptibility test of female anopheles mosquitoes to ten insecticides for indoor residual spraying (IRS) baseline data collection in Northeastern Nigeria. J Entomol. Nematol. 2014; 6(xx):98-103.
14. Okorie Patricia N. Insecticide susceptibility of Anopheles coluzzii and *Anopheles gambiae* mosquitoes in Ibadan, Southwest Nigeria. Med. Vet. Entomol. 2015; 29(1):44-50.
15. Elissa N, Mouchet J, Riviere F, Meunier JY, Yao K. Resistance of *Anopheles gambiae s.s*. to pyrethroids in Côte-d'Ivoire. Ann. Soc. Belge Med. Trop. 1993; 73:291-294.
16. Vulule JM, Beach RF, Atieli FK, McAllister JC, Brogdon WG, Roberts JM, *et al*. Elevated oxidase and esterase levels associated with permethrin tolerance in *Anopheles gambiae* from Kenyan villages using permethrin impregnated nets. Med. Vet. Entomol. 1999; 13:239-244.
17. Hargreaves K, Koerkemoer LL, Brooke B, Hunt RH, Mthembu J, Coetzee M. *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. Med. Vet. Entomol. 2000; 14:181-189.
18. Diabate A, Baldet T, Chandre F, Guiguemde RT, Brengues C, Guillet P, *et al*. First report of the kdr mutation in *Anopheles gambiae* M form from Burkina Faso, West Africa. Parassitologia. 2002; 44:157-158.
19. Etang J, Manga L, Chandre F, Guillet P, Fondjo E, Mimpfoundi R, *et al*. Insecticide susceptibility status of *Anopheles gambiae* s.l. (Diptera: Culicidae) in the Republic of Cameroon. J Med. Entomol. 2003; 40:491-497.
20. Fanello C, Petrarca V, Della Torre A, Santolamazza F, Dolo G, Coulibaly M, *et al*. The pyrethroid knock-down resistance gene in the *Anopheles gambiae* complex in Mali and further indication of incipient speciation within *An. gambiae* s.s. Insect Mol. Biol. 2003; 12:241-245.
21. Yawson AE, McCall PJ, Wilson MD, Donnelly MJ. Species abundance and insecticide resistance of Anopheles gambiae in selected areas of Ghana and Burkina Faso. Med. Vet. Entomol. 2004; 18:372-377.
22. Akogbeto M, Djouaka RF, Kinde-Gazard DA. Screening of pesticide residues in soil water samples from agricultural settings. Malar. J. 2006; 5:22-10.
23. Corbel V, N'Guessan R, Brengues C, Chandre F, Djogbenou L, Martin T, *et al*. Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin West Africa. Acta Trop. 2007; 101(3):207-16.
24. Czeher C, Labbo R, Arzika I, Duchemin JB. Evidence of increasing Leu-Phe knockdown resistance mutation in *Anopheles gambiae* from Niger following a nationwide longlasting insecticide-treated nets implementation. Malar. J. 2008; 7:189-10.
25. Santolamazza F. others. Distribution of knock-down resistance mutations in *Anopheles gambiae* molecular forms in west and west central. Africa. Malar. J. 2008; 7(7):74.
26. Yadouleton AW, Asidi A, Djouaka RF, Braïma J, Agossou CD, Akogbeto MC. Development of vegetable farming: a cause of the emergence of insecticide resistance in populations of *Anopheles gambiae* in urban areas of Benin. Malar. J. 2009; 8:103-10.
27. Ahoua-Alou LP, Koffi AA, Adja MA, Tla E, Kouassi PK, Kone M, Chandre F. Distribution of ace-1R and resistance to carbamates and organophosphates in *Anopheles gambiae* s.s. populations from Côte d'Ivoire. Malar. J. 2010; 9:167-10.
28. Ranson R. Pyrethroid Resistance in African Anopheline Mosquitoes: What are the Implications for Malaria Control. Trends in Parasitol. 2011; 27:2.
29. Aikpon R, Agossa F, Osse R, Oussou O, Aizoun N, Oke-Agbo F, *et al*. Bendiocarb resistance in *Anopheles gambiae* s.l. populations from Atacora department in Benin, West Africa: a threat for malaria vector control. Parasit Vect. 2013; 6:192.
30. Matowo J, Kitau1 J, Kaaya1 R, Kavishe1 R, Wright A, Kisinza W, *et al*. Trends in the selection of insecticide resistance in *Anopheles gambiae s.l.* mosquitoes in northwest Tanzania during a community randomized trial of long lasting insecticidal nets and indoor residual spraying. Med. Vet. Entomol. 2015; 29:51-59.
31. Elinas J. Nnko, Charles Kihamia, Filemoni Tenu, Zul Premji, Eliningaya J. Kweka. Insecticide use pattern and phenotypic susceptibility of *Anopheles gambiae* sensu lato to commonly used insecticides in Lower Moshi, northern Tanzania. BMC Research Notes. 2017; 10:443.
32. WHO. Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticide on treated surfaces. WHO/CDS/CPC/MAL/98.12. 1998; WHO press, Geneva, Switzerland.
33. Gillies MT, De Meillon B. The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region). Publ. S. Afr. Inst. Med. Res. 1968; 54.
34. Gillies MT, Coetzee M. A supplement to the Anophelinae of Africa South of the Sahara (Afrotropical region). Publ. S. Afr. Inst. Med. Res. 1987; 55.

35. WHO. Test procedures for insecticide resistance monitoring in malaria vectors mosquitoes. Geneva, Switzerland: World Health Organization, WHO press 2013b; 1-39.
36. Awolola TS, Oduola O, Strode C, Koekemoer LL. Evidence of multiple pyrethroid resistance mechanisms in the malaria vector *Anopheles gambiae* sensu stricto from Nigeria. Trans. R. Soc. Trop. Med. Hyg. 2008; 103(11):1139-45.
37. Olayemi IK, Ande AT, Chita S, Ibemesi G, Ayanwale VA, Odeyemi, OM. Insecticidal susceptibility profile of the principal malaria vector *Anophele gambiae* s.l. (Diptera: Culicidae) in North central Nigeria J. Vect. Borne Dis. 2011; 48(2):109-112.
38. Betson M, Jawara M, Awolola TS. Status of insecticide susceptibility in *Anopheles gambiae* s.l. from malaria surveillance sites in The Gambia. Malar. J. 2009; 8:187.
39. Dhiman S, Rabha B, Goswami B, Das NG, Baruah I, Bhola RK, *et al*. Insecticide resistance and human blood meal preference of *Anopheles annularis* in Assam Meghalaya border, Northeast India. J. Vect. Borne Dis. 2014; 51:133-136.
40. Yadav K, Rabha B, Dhiman S, Veer V. Multi-insecticide susceptibility evaluation of dengue vectors *Stegomyia albopicta* and *St. aegypti* in Assam, India. Parasit. Vect. 2015; 8:143
41. Oduola AO, Idowu ET, Oyebola MK, Adeogun AA, Olojede JB, Otubanjo OA, *et al*. Evidence of carbamate resistance in urban populations of *Anopheles gambiae* s.s. mosquitoes resistant to DDT and deltamethrin insecticides in Lagos. Int. Pest Control. 2012; 54:206.
42. Vatandoost H, H Ashraf, SH Lak, RE Mahdi, MR Abai, M Nazari. Factors involved in the re-emergence of malaria in borderline of Iran, Armenia, Azerbaijan and Turkey Southeast Asian J. Trop. Med. Public Hlth. 2003; 34 6-14.
43. Awolola Samson T, Adedapo O Adeogun**,** Judith B Olojede**,** Adedayo O Oduola**,** Isaac O Oyewole**,** Chioma N Amajoh. Impact of PermaNet 3.0 on entomological indices in an area of pyrethroid resistant *Anopheles gambiae* in south-western Nigeria. Parasit Vect. 2014; 7:236.
44. Abdallah H. Insecticide Susceptibility and Vector Status of Natural Populations of *Anopheles Arabiensis* from Sudan. Trans. R. Soc. Trop. Hyg. 2008; 102(3):263-71.
45. Ramphul U. Insecticide Resistance and its Association with Target -site Mutations in Natural Population of *Anopheles gambiae* from Eastern Uganda. Trans. R. Soc. Trop. Hyg. 2009; 103(11):121-12.
46. Kemabota KA, Anikwe JC, Adaezebiora IB. Bioefficacy of Skaeter Abate and Spintor on *Anopheles gambiae and Aedes aegypti* mosquitoes from insecticides resistance areas of Lagos and Oyo states, Nigeria. J. Agric Healthcare. 2013; 3(3).
47. Agossa FR, Gnanguenon V, Anagonou R, Azondekon R, Aizoun N, Sovi A, *et al*. Impact of insecticide resistance on the effectiveness of pyrethroid-based malaria vectors control tools in Benin: Decreased toxicity and repellent effect. PLoS One. 2015; 10(12).
48. Dhiman S, Yadav K, Rabha B, Goswami D, Hazarika S, Tyagi V. Evaluation of Insecticides Susceptibility and Malaria Vector Potential of *Anopheles annularis* s.l. and *Anopheles vagus* in Assam, India. PLoS one. 2016; 11(3).
49. Verhaeghen K, Van Bortel W, Trung H, Sochantha T, Keokenchanh K, Coosemans M. Knockdown resistance in *Anopheles vagus*, *An. sinensis*, *An. paraliae* and *An. peditaeniatus* populations of the Mekong region. Parasit. Vect. 2010; 3:59.
50. Djègbè I, Boussari O, Sidick A, Martin T, Ranson H, Chandre F, *et al*. Dynamics of insecticide resistance in malaria vectors in Benin: first evidence of the presence of L1014S kdr mutation in Anopheles gambiae from West Africa. Malar. J. 2011; 10:261.
51. Dhiman S, Veer V. Culminating anti-malaria efforts at long lasting insecticidal net? J. Inf. Pub. Hlth. 2014; 7:457-464.
52. Nkya TE, Akhouayri I, Poupardin R, Batengana B, Mosha F, Magesa S, *et al*. Insecticide resistance mechanisms associated with different environments in the malaria vector *Anopheles gambiae*: a case study in Tanzania. Malar J. 2014; 13:28.