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Full Length Research

Susceptibility and resistance status of malaria vectors in five selected communities of Emohua LGA, Rivers State

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ABSTRACT: Malaria is one of the public health problems in many parts of Nigeria. To effectively implement malaria control program, baseline studies of mosquito species abundance and their susceptibility status to insecticides is required. The aim of the study was to determine the susceptibility and resistance status of indoor malaria vectors in five communities of Emohua Local Government Area of Rivers State. Mosquito larvae were collected from different breeding sites and reared in the insectary and identified morphologically. The knockdown effect was recorded every 10 minutes and mortality scored 24 hours after exposure. Data collected were analysed using statistical software (SPSS) version 20.0. Results of the insecticide susceptibility assay showed that there was comparable progressive knockdown as exposure time increased. Mosquito vectors were found to show resistance to Permethrin (0.75%) with 62% mortality, Deltamethrin (0.05%) showed 47% mortality and Lambdacyhalothrin (0.05%) showed 47% mortality while possible resistance in local mosquitoes against Alphacypermethrin (0.75%) with 97% mortality was suspected in the study areas. This study contributed to the understanding of the susceptibility and resistance of local malaria mosquitoes to insecticides. It is therefore suggested that there is a need for collaboration with the farmers before decision on the choice of insecticides to be used in their communities to avoid overuse of a particular insecticide and the repetition of the same in malaria control.

Keywords: Emohua, insecticides, resistance, rivers state, susceptibility.

INTRODUCTION

Malaria is the most important parasitic disease of public health importance caused by five species of *Plasmodium* parasites namely: – *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. According to WHO (2013a), malaria is presently considered endemic in a total of 104 countries and territories. Globally, an estimated 3.4 billion people are at risk of malaria. Most cases (80%) and deaths (90%) occurred in Africa, and most deaths (77%) were in children under 5 years of age. Malaria remains a major public health problem in many countries of the world despite the progress in reducing cases and deaths. The global burden of mortality is dominated by countries in sub-Saharan Africa, with the Democratic Republic of Congo and Nigeria together accounting for more than 35% of the global total of estimated malaria deaths.

In Nigeria, malaria remains a public health problem and is a risk for 97% of the population. It is known to contribute to over 300,000 deaths per annum and 11% of maternal mortality (Onwuemele, 2014). Nigeria has the largest burden of malaria in Africa, yet not much is known about the vectors, how the species interact, overlap or differ across the country (Okorie *et al.,* 2011).

Malaria is transmitted through the bite of an infected female mosquito of the genus *Anopheles*. There are about 400 different species of *Anopheles* mosquitoes, but only 30 are vectors of major importance for malaria. Each species of *Anopheles* mosquito has its own preferred aquatic habitat; for example, some prefer small, shallow collections of freshwater, such as puddles and hoof prints, which are abundant during the rainy season in tropical countries (Okwa *et al*., 2007). It is an established fact that mosquitoes are the most important insects affecting human health (Woodbridge and Edward, 2006) chiefly in the spread of malaria the most common lethal disease second only to HIV/AIDS (Scott et al., 2005). Malaria transmission is variable from one area to another and this has an impact on its epidemiology and control (CDC, 2004). In another study in Badagry by Okwa *et al*. (2007), a coastal area of southern Nigeria, several species of *Anopheles* occur in sympatry.

This study was carried out to investigate the susceptibility and resistance status of principal malaria vectors in five communities in Emohua LGA of Rivers State. Although the findings of Gillies and De Mellion (1968) and Sinka *et al. (*2010) suggested that members of *An. gambiae* and *An. funestus* complexes being Africa's most important malaria vectors because of their widespread distribution, can be found in sympatry and present across all geographical zones in the country, only *An. gambiae* was found in this present study. The finding that the *An. gambiae* complex is the most abundant is consistent with the findings of Sinka *et al*. (2012). He concluded that *An. Gambiae. s.s.* is omnipresent in Nigeria because of its indiscriminate breeding habits. Morphological identification revealed that *An. gambiaes. l*. is predominant in this study area compared to other *Anopheles* groups.

In recent years, Indoor Residual Spraying (IRS) has been adopted to protect entire households and other community members who do not have access to or who choose not to use ITNs. Out of the four classes of insecticides that WHO has approved for IRS malaria vector control (organochlorine, pyrethroids, carbamates and organophosphates), only pyrethroids are recommended for both the treatment of bed nets and IRS. Deltamethrin has been deployed by the President's Malaria Initiative (PMI)-funded Africa Indoor Residual Spray (AIRS) project in Nasarawa Eggon and Doma in Nasarawa State, while other pyrethroids and an organophosphate (Actellic) has been deployed in Lagos and 6 other states supported by the World Bank. This includes; Epe in Lagos State, Kirikashama in Jigawa State, Shendam/Barkin Ladi in Plateau State, Enugu in Enugu State, Ikwere in Rivers State and Nasarawa Eggon/Doma in Nasarawa State (AIRS project, Nigeria 2014).

MATERIALS AND METHODS

Study area

This study was conducted in Emohua Local Government Area in Rivers State, South-South Nigeria (Figure 1). It is one of the 23 local government areas of the state. The major ethnic groups include the Kalabaris, Ikwerres, Ogonis, Ekpeyes, Ogbas, Engenes, Ibanis and Okrikas. Emohua has an estimated population of 201,901 at the 2006 census (NPC, 2006). The geographical coordinates of Emohua LGA are 4°52'44''N latitude and 6°51'40''E longitude. It rains always with annual rainfall averaging 1500 mm, and relative humidity over 80%. The climate is characterized by two distinct seasons, the wet and dry seasons, the former taking place from April to October and the dry season between November and March. Although Rivers State experiences rainfall no matter how little every month of the year, this does not fall on every part of the state at the same time. The expanse of freshwater swamps, dense rain forest and intricate network of creeks and coastal ridges promote the breeding of malaria vectors (Tobin-west *et al*., 2011). Over 70% of the inhabitants reside in rural areas and are engaged in subsistence fishing and farming, with the involvement of some in petty trading.

Selection of communities and households

Communities and households sampled were selected based on areas where IRS (Indoor Residual Spraying) intervention program had not taken place.

Collection of mosquito larvae

Collection, processing, rearing and identification of mosquito larvae were as described by Adeleke *et al.* (2010). Larvae were collected from puddles, shallow wells, gutters and farms. The larvae were collected using the dipping technique in which the dipper was lowered gently about 10 times at an angle of 45° just below the surface so that water flows in together with any larvae that were present. All larvae collected were transported in a container to the insectary at the Rivers State University of Science and Technology (RUST) and reared for a period of one week in mosquito cages during which they were fed with a mixture of yeast and biscuits (10% yeast and 90% low-fat biscuits). Water traps were placed in the cage near the larvae bowl to prevent ants from destroying the mosquito that emerge from the larvae. Emerged adult mosquitoes used for the susceptibility test were left in the cage and fed with a 10% glucose solution. The adult female mosquitoes were morphologically identified using identification keys; palps are as long as proboscis, the wings are spotted with dark and pale areas and legs with dark and pale scales as described by Gillies and Coetzee (1987).

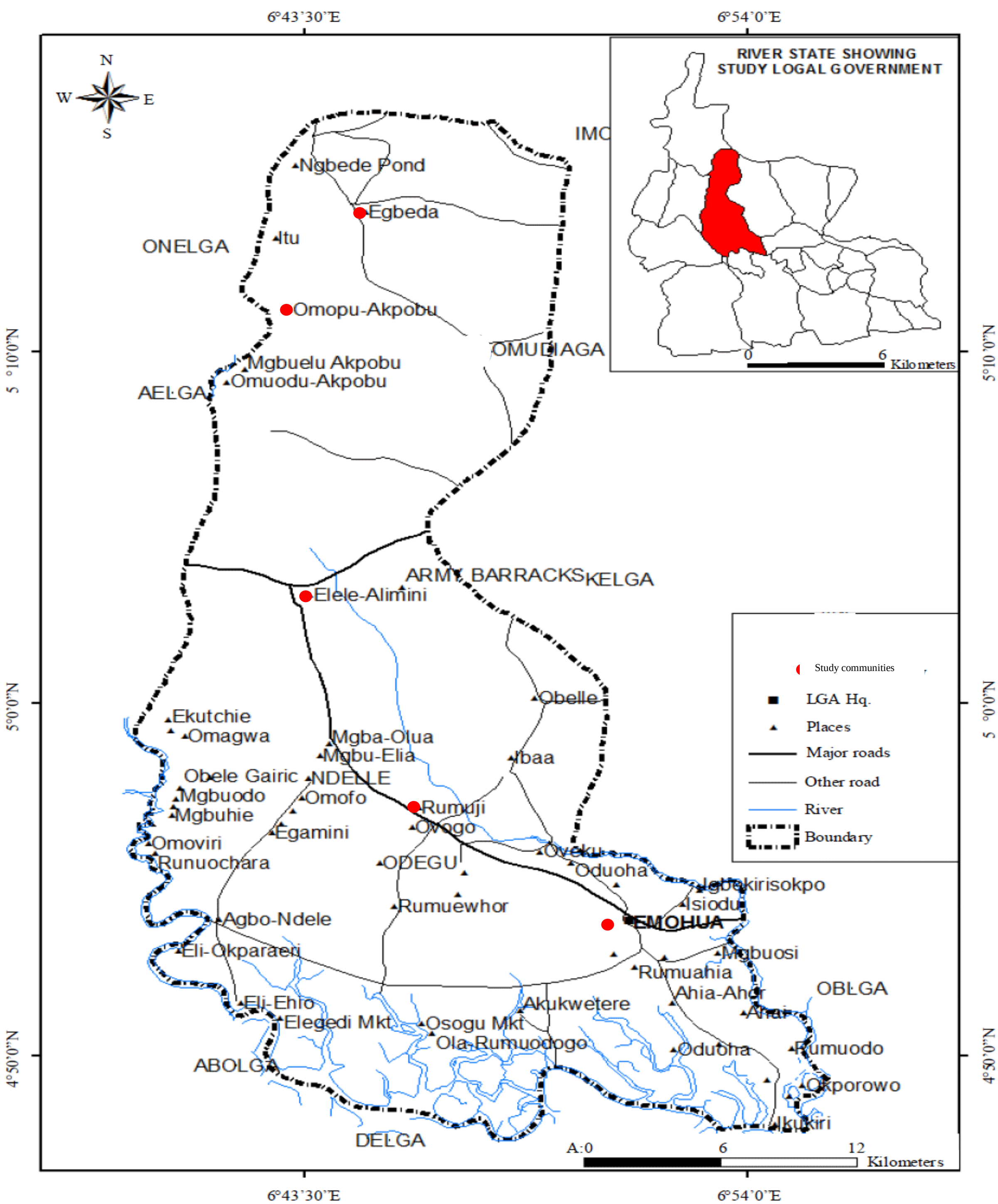

Figure 1. Map of Emohua (study area) showing study sites (Source: *Edu et al*., 2015).

Determination of the effect of exposure time on knockdown rates and susceptibility of malaria vector to insecticides

Insecticide susceptibility bioassay tests were carried out using insecticide susceptibility test kits according to World Health Organisation standard procedures (WHO, 2013a). Four replicates of 25 adult female mosquitoes per test tube were used in the assay. Mosquitoes were exposed to papers impregnated with the WHO-recommended concentrations (v⁄w) of 0.05% Deltamethrin, 0.05% Lambdacyhalothrin, 0.75% Permethrin and 0.75%

Alphacypermethrin. The controls were exposed to clean paper impregnated with silicone oil (pyrethroids control). During the exposure period, knock-down (KD) rates were recorded after 10, 20, 30, 40, 50 and 60 minutes. Mosquitoes were then transferred into another set of holding tubes with untreated papers to check mortality after 24 hours and fed on glucose solution. Mortalities were recorded 24 hours post-exposure and the number of dead mosquitoes was counted and recorded. The susceptibility status of the population was graded according to WHO recommended protocol (WHO, 2013a). All susceptibility tests were carried out at different temperatures and relative humidity.

Statistical analysis

Statistical software SPSS version 20.0 was used and data recorded was subjected to *chi*-square analysis. The susceptibility of female *Anopheles* mosquitoes was assessed using the current WHO (2013b) criteria. The susceptibility of female *Anopheles* mosquito samples collected and tested was determined according to WHO current criteria (2013b). The mortality rate of less than 80% indicates resistance while those greater than 98% indicate complete susceptibility. Mortality rates between 80 to 98% suggest a possibility of resistance that requires further clarification.

RESULTS

Exposure time and knockdown time

The knockdown times recorded for the different insecticides are presented in Table 1. The results showed that Lambdacyhalothrin and Deltamethrin with 47% knockdown each in 24 hours had the lowest percentage knockdown, followed by Permethrin and Alphacympermethrin with 62% and 97% knockdown after 24 hours respectively.

The knockdown effect of the four insecticides determined over a one-hour period indicated that, in all cases, mosquitoes were resistant (Figure 2) to all the insecticides, all mosquitoes showed various percentage (%) knock-down within the one hour exposure period, The results show that Lambdacyhalothrin and Deltamethrin with 47% mortality each in 24 hours had the lowest percentage mortality, followed by the Permethrin and Alphacympermethrin had 62 and 97% mortality after 24 hours respectively; which indicated resistance to the insecticides according to WHO protocol.

Susceptibility status of mosquito vectors

Results of the insecticide susceptibility assay showed that there was comparable progressive knockdown as exposure time increased. Local mosquitoes were found to show resistance to Permethrin (0.75%), the result showed 18% knockdown after 1 hour and 62% mortality after 24 hours, Deltamethrin (0.05%) showed 85% knockdown after 1 hour and 47% mortality after 24 hours and Lambdacyhalothrin (0.05%) showed 40% knockdown after 1 hour and 47% mortality after 24 hours while possible resistance of local mosquitoes against Alphacypermethrin (0.75%) was suspected in the study area as it showed 100% knockdown after 1 hour and 97% mortality after 24 hours (Table 1).

The WHO interpretation for susceptibility is as follows (At least 80 mosquitoes tested per insecticides):

- Susceptible: Mortality 98-100%
- Resistance suspected (to be confirmed): Mortality 90-97%
- Resistance: Mortality <90% (WHO 2013).

DISCUSSION

Their status as the insecticides of choice for malaria vector control relies on their proven efficacy at low dosage, relative safety for humans and non-target organisms, excito-repellent properties, rapid rate of knockdown and residual killing effects. The knockdown effect of the four insecticides determined over a period of one hour indicated that, in all cases, mosquitoes were resistant (Figure 2). To all the insecticides, all mosquitoes showed various percentage knock-downs within the one hour exposure period. The results showed that Lambdacyhalothrin and Deltamethrin with 47% knockdown each in 24 hours had the lowest percentage knockdown, followed by the Permethrin and Alphacympermethrin with 61 and 97% knockdown after 24 hours respectively (Figure 2); which indicated resistance to the insecticides according to WHO protocol.

The evidence of growing resistance to pyrethroids in *An. gambiae s.l*. populations, which already are resistant to DDT, indicate the need for strategies to combat multiple resistance. Thus, there is a need to consider alternatives for resistance management in Nigeria in line with the recommendations of Zaim *et al.* (2002). Findings from this study clearly indicate the need for a more concerted effort in insecticide resistance management in Nigeria. All the four pyrethroids used lamdacyhalothrin, deltamethrin, alphacyhalothrin and permethrin showed 47, 47, 97 (resistance suspected) and 62% mortality, respectively. This implies that pyrethriods, which are the major insecticide used in malaria vector control in Long Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS) in this part of the world are on the verge of losing their efficacy in these various interventions for malaria control. The main factor driving resistance has been the heavy reliance by vector control programmes on a single class of insecticide, the pyrethroids (GPIRM, 2012).

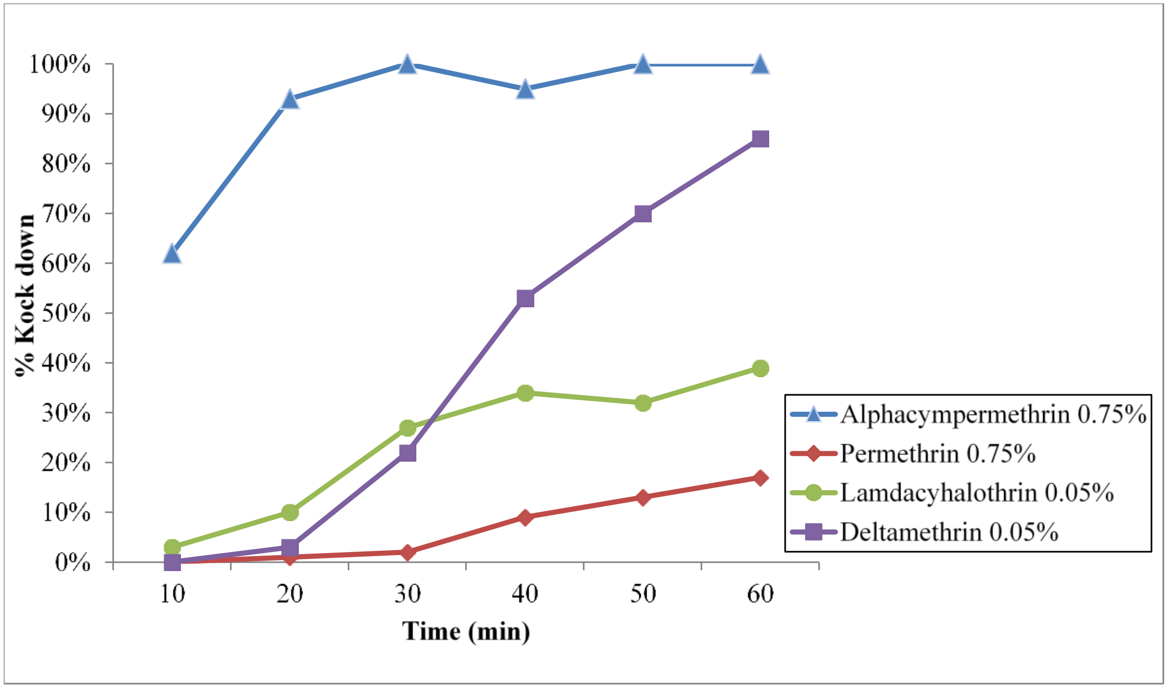

Figure 2. Knockdown Rate of unfed female *Anopheles gambiae s.l.* mosquitoes.

Table 1. Susceptibility test of malaria vector to WHOPES insecticides.

Insecticide (diagnostic concentration)	No. *Anopheles* tested (4 replicates)	No. (%) Knock down after 1 hour	No. (%) 24 hours post exposure mortality	No. *Anopheles* in control	Mortality in control No.	Remarks
Permethrin (0.75%)	100	18(18)	62(62)	25	1	Resistance
Deltamethrin (0.05%)	100	85(85)	47(47)	25	0	Resistance
Lambdacy-halothrin (0.05%)	100	40(40)	47(47)	25	1	Resistance
Alphacy-permethrin (0.75%)	100	100(100)	97(97)	25	1	Resistance suspected

NB. Mortality in the “Control” tube for each of the test paper was between 0 and 1%.

Temu *et al.* (2012) reported that insecticide bioassay results from Bomi County Liberia indicate that the local *An*. *gambiae* has a high prevalence of resistance to the pyrethroid insecticide deltamethrin. This resistance is operationally significant, as IRS with deltamethrin does not consistently achieve the minimum 80% mortality of mosquitoes needed to meet current WHO standards.

The findings of Aïkpon *et al.* (2013) show that *An. gambiae* s.l populations were susceptible to bendiocarb with mortality rates ranging between 95 and 100%, but it was resistant to deltamethrin with a mortality rate ranging between 27 and 54% and fully resistant to permethrin with a mortality rate which did not exceed 18%. The emergence of resistance to pyrethriod in *An. gambiae* has become a serious concern for the success of malaria control in the last decades (Aïkpon *et al.,* 2013). If nothing is done and insecticide resistance eventually leads to the widespread failure of pyrethroids, the public health consequences would be devastating; much of the progress achieved in reducing the burden of malaria would be lost. It is quite interesting to note that local mosquitoes collected in these communities were found to show high resistance against all these four pyrethriods used. This level of resistance could be due to agricultural insecticides being indiscriminately used by farmers. Further research is needed to confirm this trend.

The incidence of the low toxicity of mosquito populations to pyrethroid in this study is not new, as Umar *et al.* (2008) and Awolola *et al .* (2005, 2007) reported similar cases in *Anopheles* mosquitoes in Lagos, Nigeria. This is due to the fact that pyrethroids have been used for a longer period for agricultural and public health purposes (Hemingway and Jason, 2000).

Conclusion

In conclusion, this study has contributed to the understanding of mosquito vectors and their susceptibility and resistance to insecticides. Evidence of pyrethroid resistance in the *Anopheles* populations is an indication that vector control might be complicated and expensive in future and it calls for the implementation of insecticide resistance management strategies to combat the multiple resistance identified in local mosquitoes.

Pyrethroid usage as an insecticide of choice for malaria vector control purposes relies on their known efficacy at low dosage, relative safety for humans and non-target organisms, excito-repellent properties, rapid rate of knock-down and residual killing effects. Findings from this study clearly indicate the need for a more concerted effort in insecticide resistance management in Rivers State.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

REFERENCES

Adeleke, M. A., Mafiana, C. F., Idowu, A. B., Sam-Wobo, S. O., & Idowu, O. A. (2010). Population dynamics of indoor sampled mosquitoes and their implications in disease transmission in Abeokuta, South-Western Nigeria. *Journal of Vector Borne Disease,* 47, 33-38.

Aïkpon, R., Razaki, O., & Renaud, G. (2013). Entomological baseline data on malaria transmission and susceptibility of *Anopheles gambiae* to insecticides in preparation for Indoor Residual Spraying (IRS) in Atacora, (Benin). *Journal of Parasitology and Vector Biology,* 5(7), 102-111.

Awolola, S. T., Adedapo, O., Adeogun, J. B., Olojede, A. O., Oduola, I. and Amajoh, C. N. (2014). Impact of PermaNet 3.0 on entomological indices in an area of pyrethroid resistant *Anopheles gambiae* in south-western Nigeria. *Parasites & Vectors*, 7, Article number 236

Awolola, T. S., Adeogun, A. O., Olojede, Oduola, A. O., Oyewole, I. O. and Amajoh, C. N. (2014). Impact of permaNet 3.0 on entomological indices in an area of pyrethroid resistant*Anopheles gambiae* in south- western Nigeria. *Parasites and vectors*, *7, Article number* 236.

Awolola, T. S., Oduola, A. O., Oyewole, I. O., Obansa, J. B., Amajoh, O. H., Koekomoer, L. L., & Coetzee, M. (2007). Dynamics of knock down pyrethroid insecticide resistance alleles in a field population of *Anophelesgambiae s.s*. in South western Nigeria. *Journal of Vector Borne Disease,* 44, 181-188.

Awolola, T. S., Oyewale, I. O., Amejoh, C. N., Idowu, E. T., Ajayi, N. B., Oduola, A., Manafa, O. U., Ibrahim, K., Koekomoer, L. L., & Coetzee, M. (2005). Distribution of the molecular forms of *Anopheles gambiae* and pyrethroid knockdown resistance gene in Nigeria. *ActaTropica,* 95, 204-209.

Center for Disease Control (CDC) (2004). Areas where malaria is no longer endemic. Center for Disease Control.

Edu, E. A. B., Edwin-Wosu, N. L., & Obiechere, N. D. (2015). Monitoring the ecological succession and regeneration status post remediated hydrocarbon impacted site in parts of Ahia oil-field, Omudioga, EMOLGA, Rivers State, Nigeria. *Insight Ecology*, *4*(1), 24-34.

Gillies, M. T., & Coetzee, M. (1987). A supplement to the Anophelinae of Africa South of the Sahara. Publications of the South African Institute for Medical Research, Johannesburg, *55*, 1-143.

Gillies, M. T., & De Meillon, B. (1968). Anophelinae of Africa south of the Sahara. Publication of the South African Institute of Medical Research, 54, Article number 343

Hemingway, J. and Ranson, H. (2000). Insecticide resistance in insect vectors of human disease. *Annual Review of Entomology*, 45, 371-391.

NPC (2006). Landmass compiled from NPC Report, 1991 and field reports.

Okorie, P. N., McKenzie, F. E., Ademowo, O. G., Bockarie, M., & Kelly-Hope, L. (2011). Nigeria Anopheles vector database: An overview of 100 years' research. *Plos one*, *6*(12), e28347.

Okwa, O. O., Carter, V., & Hurd, H. (2007). Abundance, host preferences and infectivity rates of malaria vectors in Badagry local Government Area of Lagos, Nigeria. *Nigerian Journal of Parasitology*, *27*(1), 41-48.

Onwuemele, O.(2014). An assesment of the spatial pattern of malaria infection in Nigeria. *International Journal of Medicine and Medical Sciences*, *6*(2), 80-86.

Scott, J. A., Brogdon, W. G., & Collins, F. H. (1993). Identification of single specimens of the Anopheles gambiae complex by the polymerase chain reaction. *The American journal of tropical medicine and hygiene*, *49*(4), 520-529.

Sinka, M. E., Bangs, M. J., Manguin, S., Coetzee, M., Mbogo, C. M., Hemingway, J., Patil, A. P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Okara, R. M., Van Boeckel, T., Godfray H. C. J., Harbach, R. E., & Hay, S. I. (2010). The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: Occurrence data, distribution maps and bionomic précis. *Parasite Vectors, 3*, Article number 117.

Sinka, M. E., Bangs, M. J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C. M., Hemingway. J., Patil, A. P., Temperley, W. H., Gething, P.W., Kabaria, C. W., Burkot, T. R., Harbach, R. E., & Hay, S. I. (2012). A global map of dominant malaria vectors. *Parasite Vectors,* 5, Article number 69.

Temu, E. A., Maxwell, C., Munyekenye, G., Howard, A. F. V. & Munga, S. (2012). Pyrethroid resistance in *Anopheles gambiae*, in Bomi County, Liberia. *Malaria Vector Control*, 7(9), e44986.

Tobin-west, I., & Seye, C. B. (2011). Community perceptions and practices in the management of malaria in under-five children in Rivers State in Nigeria. *International Journal of Health Research,* 4(3), 111-133.

Umar, A., Kela, S. L., & Ogidi, J. A. (2008). Enhancement of WHO technique for glucose feeding of adult mosquitoes in laboratory under dry and arid environment. *Journal of Entomology*, 5(3), 164-166.

Woodbridge, A. F., & Edward, D. W. (2006). *Medical and veterinary entomology: Mosquito.* Elsevier Science. Pp. 203-256.

World Health Organization (WHO) (2013a). Malaria entomology and vector control Learner's Guide: Procedures for conducting insecticide susceptibility. Geneva: WHO. 192.

World Health Organization (WHO) (2013b). Test procedures for insecticide resistance monitoring in malaria vector Mosquitoes.

Zaim, M., & Guillet, P. (2002). Alternative insecticides: an urgent need. *Trends in Parasitology,* 18, 161-163.