International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2015; 2(3): 56-63

Received: 01-06-2015
Accepted: 08-08-2015

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Insecticide resistance to *Anopheles* spp. mosquitoes (Diptera: Culicidae) in Nigeria: A review

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Abstract
Monitoring of insecticide resistance is indispensable to acquire comparable baseline data on insecticide resistance. Hence, there is need to understand its past and present status. An investigation was carried out on the insecticide resistance to *Anopheles* spp. mosquitoes in Nigeria which is on the increase and spreads across Africa. Various methods of detecting insecticide resistance were reported. The studies showed existence of DDT resistance and reduced susceptibility to permethrin in *Anopheles gambiae* sensu stricto (s.s.) a major vector of malaria in Nigeria. Resistance to permethrin was recorded in all the geographical zones but highest levels were found in the forest savannah, Mosaic and Guinea savanna. Similar results were obtained in Ghana where resistance in all classes of insecticides was recorded except organophosphates. In Cameroun, DDT and permethrin also showed high levels of resistance. A number of causes of resistance were highlighted and factors influencing their development were also enumerated. Available methods of detecting insecticide resistance were discussed. Various measures for the management of resistance in different continents of the world were also highlighted. Non insecticidal methods of mosquito control which include the use of repellant plants, environmental management, biological control and mechanical controls were enumerated. Recommendations were also made to the Nigerian Government to incorporate some of the successful techniques applied in other parts of the world. The study has implications for the control of mosquito populations and the spread of human, livestock and poultry diseases.
Keywords: *Anopheles gambiae*, Cameroun, Ghana, insecticides, Nigeria, resistance

Introduction

Insecticide resistance is defined as "an inherited characterization that allows the development of ability in some individuals of a given organism to tolerate doses of a toxicant which would prove lethal to a majority of individuals in a normal population of the same species" [1, 3]. Insecticides play a central role in the control of mosquito vectors and will continue to do so for the foreseeable feature. However, the ubiquitous use of a limited number of insecticides for both agricultural pests and vector of human and livestock diseases has led to resistance making insecticides used ineffective and limiting the available option for disease control [4]. Vector borne diseases are among the major causes of illness and death, particularly in tropical and subtropical countries; vector control through the use of insecticide plays a key role in the prevention and control of infectious diseases [4]. In Nigeria, mosquitoes are regarded as public health enemies because of their biting annoyance and noise nuisance, sleeplessness, allergic reaction and disease transmission due to their bites [5, 6]. They transmit human diseases such as malaria, yellow fever, dengue, hemorrhagic fever, filariasis and encephalitis [7, 9]. Mosquitoes also transmit animal diseases like the fowl pox of poultry, myxomatosis of rabbits, rift valley fever of sheep, encephalitis of horses and birds, dirofilariasis of dogs [10, 12]. Mosquitoes also transmit the relatively new but deadly threat of west nile virus and while the disease in humans is been deadly but rare, it has quickly become established as a real threat to horses with 40% of horses that contact the disease dying of illness [13, 14]. All these diseases cause great suffering to man and livestock. They do not only cause high morbidity and mortality in human and animal population, but also lead to huge economic losses [10, 15]. One of the approaches for the control of these mosquito borne diseases is the interruption of diseases, transmission by killing or preventing mosquitoes from biting man and animals [16, 18]. Mosquito control remains an important component of human and animal diseases [19, 20]. However, this has been limited by the development and spread of resistance and limited knowledge of mosquito biology [21, 22].

Presently, there are 12 insecticides recommended by the WHO insecticide evaluation scheme for indoor residual spraying (IRS) against mosquitoes, out of this only dichloro-diphenyltrichloroethane (DDT) which has the longest residual effect (> 6 months) is not yet used in Nigeria, because of environmental concerns [23]. The re-introduction of DDT into the mosquito control is expected to produce mosaic defense against the development of resistance [24]. *Anopheles* vector control relies heavily on a single class of insecticides, the pyrethroids. These insecticides are the only class approved for use on insecticide treated nettings [25, 26] and are being increasingly deployed in Indoor residual spray (IRS) programmes in Africa and Long lasting insecticide treated nets (LLINs) [26, 27]. A rise of pyrethroids resistance by mosquitoes has become the latest threat to combating malaria in Nigeria, where roughly up to 300,000 people die each year from the killer disease [28]. The problem of insecticide resistance is very real and growing in Nigeria [29], there are signs that it might worsen due to the effects of climate change and there is concern that the mosquitoes are becoming resistant to the entire classes of insecticide in use [30, 31, 7], reported the studies on the distribution of *Anopheles* in Nigeria between 1900 to 2010 as follows; *An. gambiae s.1* (181),65.2%, *An. gambiae* s.s (156),6.5%, *An. Arabiensis* (122), 5.0%, *An. funestus* complex (95) 17.3%, *An. funestus* s.s (21), 2.5% while other species (57) constitute 4.5%. In a related investigation carried out in the Southern part of Nigeria *An. gambiae s.1* constitutes 77.7% of the total number of mosquitoes caught followed by *An. funestus* 22.3% which confirms it as the most common mosquito in the country [32]. Knowledge on insecticide resistance in target species is a basic requirement to guide insecticide use in malaria control programs. It is the purpose of this research therefore to determine the causes, detection, factors responsible, current status, mode of action, mechanisms of resistance and enumerate alternative methods for the control of mosquitoes, which will assist in future decisions on insecticide usage made by authorities responsible for malaria vector control.

Classes of insecticides

Based on their chemical nature and origins, insecticides have been classified into the following groups namely; organochlorines, organophosphates, carbamate and pyrethroids, [33, 35] (Figure 1).

Figure 1: History of WHO approved insecticides for adult malaria mosquito control. Adapted from [36].

Year	Class of insecticide	Name
1940-45	Organochlorines	DDT Lindane
1951-55	Organophospahtes	Malathion Fenitrothion Chlorpyrifos-methyl Pirimiphos-methyl
1961-65	Carbamates	Propoxur Bendiocarb
1971-75	Pyrethroids	Permethrin Cypermethrin
1976-80		Alpha-Cypermethrin Cyfluthrin
1981-85		Lambda-cyhalothrin Deltamethrin Bifenthrin
1986-90		Etofenprox

Causes of insecticide resistance

The two major causes of insecticide resistance are alterations in the target sites and increase in the rate of insecticide metabolism [37, 39]. However, the major emphasis on research focuses in molecular mechanisms and rational resistance management with a view of controlling the spread and development of resistance in mosquito population [40, 41].

Brief history of insecticide resistance in Nigeria

Dieldrin was found to be resistant to *An. gambiae* in the Western Sokoto region of Northern Nigeria[42]. Another similar strain resistant to dieldrin and BHC was reported from Kano, some 300 miles eastward [43] but none were found in Lagos, Ibadan (Oyo state) or Ilaro town (Ogun state) in the Southern forest belt of Nigeria [42, 44]. In Kano, the use of insecticides for public health purposes had taken place on an extremely limited scale only being confined to protection of the international airport with DDT and BHC, but in the immediate post war year’s quantities of BHC amounting to several tons were used to protect the “pyramids” of ground nuts awaiting transport [45]. In Ibadan, relatively limited use has been made of DDT, almost entirely as larvicide. The tolerance appears to be non-specific, as some relative tolerance to DDT appeared to be present [45]. The dieldrin- resistance of *An. gambiae* in Northern Nigeria extends to aldrin, chlordane and gamma-BHC, but not to DDT [46]. In crosses with a normal strain, the F1 hybrid is intermediate in resistance, when this is back crossed with either parental strain, two phenotypes appear in 50:50 ratio. These results indicate dieldrin–resistance to be due to a genetic factor showing no dominance, the homozygotes can survive 4% dieldrin and the heterozygotes 0.4% dieldrin according to Busvin and Nash test [47].

Pyrethroid resistance to *Anopheles* species has also been reported in 23 out of 49 African countries. It was first reported in Cote d’ ivore in 1993 and is now widespread throughout the western [26, 48, 49] and central regions of Africa [26, 50, 51]. The resistance is mainly associated with reduced target sensory arising from a single point mutation in the sodium channel gene, often referred to as knock down resistance (kdr). This mechanism is as well widespread in West Africa and was first reported in Nigeria in 2002 [52].

Current status of insecticide resistance in Nigeria

[53] studied the ecological zones in Nigeria (Figure 2) on the insecticide resistance and the results showed > 78% susceptibility to the diagnostic concentrations of insecticides

(DDT, Permethrin and Deltamethrin) 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.

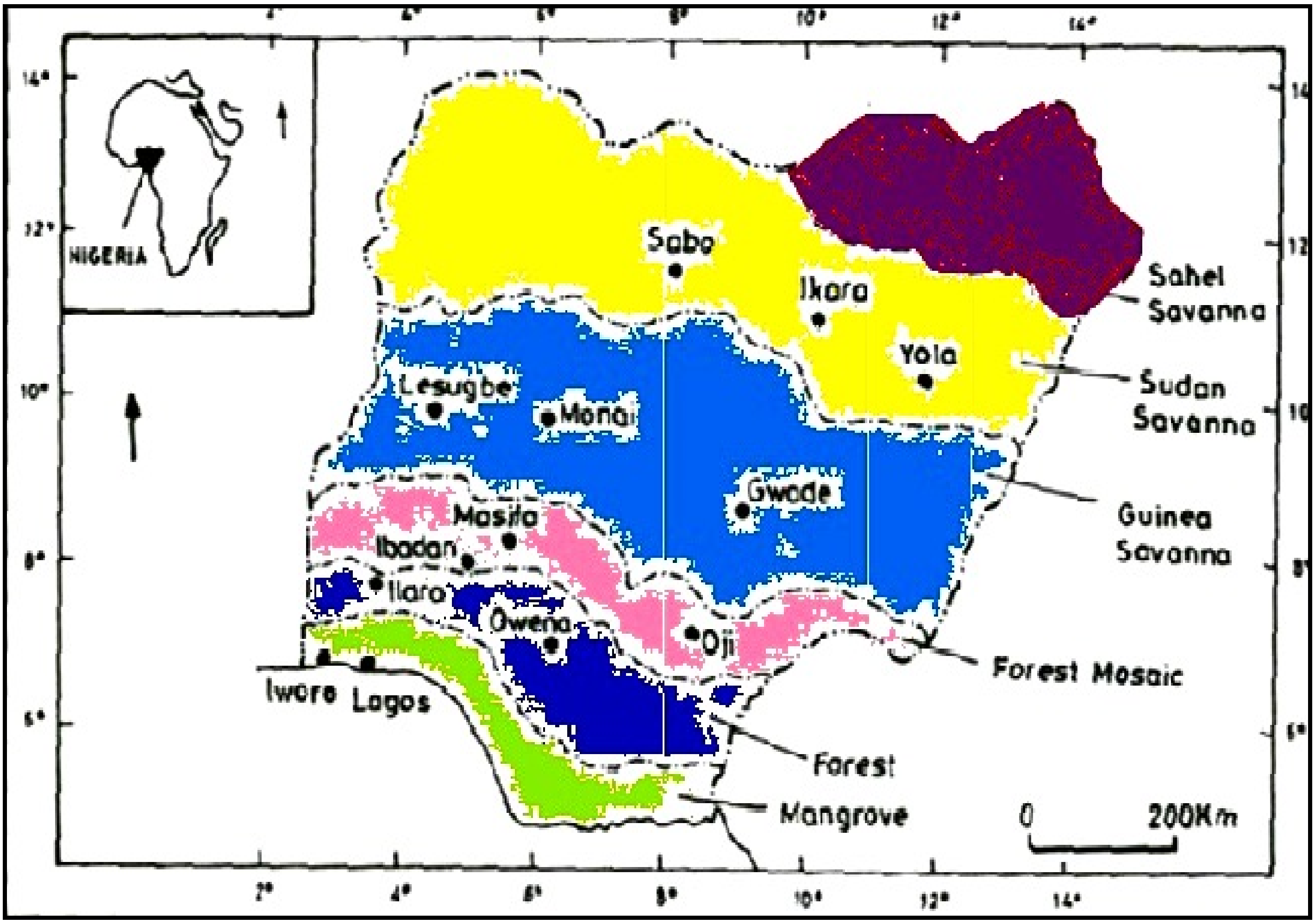


Figure 2: A map of Nigeria showing the six ecological Zones. Dark orchid represents Sahel savanna, Yellow represents Sudan savanna, Royal blue represents Guinea savanna, Pink represents Forest mosaic, Blue represents Forest whilst Green represents Mangrove. Adopted and modified from [53].

Mortality due to permethrin was < 95% in all the zones, At the forest savanna mosaic and Guinea savanna zones, mosquitoes exposed to permethrin recorded the lowest mortality (<74%). The corresponding knockdown value of the deltamethrin and DDT was > 80% in all zones. Higher percentage (> 90%) of *An. gambiae* exposed to all insecticides were knocked down at the Forest mangrove and Sudan savanna were susceptibility was high, but <85% of those exposed to permethrin in forest savanna mosaic and Guinea savanna were knocked down during the 1 hour exposure. Resistance to permethrin was recorded in all the zones but highest level of resistance found in forest savanna mosaic and Guinea savanna. Meanwhile, high level of DDT resistance was also observed in Guinea savanna. Applying the [54] criteria for determining resistance or susceptibility, 98-100% mortality indicates susceptibility; < 80% mortality suggests resistance while 80-97% mortality requires confirmation of resistance. Overall, mosquitoes from the forest savanna mosaic showed high resistance. The kdr polymerase chain reaction (PCR) assay showed that kdr allele was present in most of survivor mosquitoes previously exposed to the three test insecticides. At Guinea savanna for example, 87% of the 25 are resistant to deltamethrin, 90.06% 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% (25), 87.5% (22) and 76.4% (20) at forest savanna mosaic, 80% (of 25), 82.7% (of 22) and 72.7 % (of 20) a% at Sudan savanna and 55.2% (of 25), 61.4% (of 22) and 58.6% (of 20) at mangrove (Tables 1, 2 and 3).

Table 1: 24 h post exposure mortality rate of 2 to 3 day old *An. 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		115 (92)	111 (89.2)
Sudan – savanna	100	97 (97)	100 (100)
	610	578	557

Table 2: 24 -h post exposure mortality rate of 2 to 3 day old *An. 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)
	610	537	506

Table 3: 24-h post exposure mortality rate of 2 to 3 day old *An. 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)
	610	553	506

Summarily, *An. 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 origin of insecticide resistance in the study areas could either be due to agricultural or public health use of insecticide. Survey of literature revealed decrease levels of susceptibility of *An. gambiae* to permethrin [24, 55, 56]. The result therefore has implications for the current long lasting insecticidal nets (LLINs) and IRS for *Anopheles* spp. mosquitoes control program in Nigeria. It has also been demonstrated elsewhere that carbamate insecticide bendiocarb and oranophosphates are useful alternative chemical classes to DDT and pyrethroids for vector control programmes [53, 57]. This reduces the spread of cross resistance between DDT and pyrethroids which has been established in *An. gambiae* [53, 58]. This phenomenon is closely associated with kdr allele in *An. gambiae* [59]. Here there was correlation between the frequency of the kdr allele and frequency of resistance among the survivor and exposed samples. Similar report has been documented from the neighboring West African countries [51]. This may indicate that kdr is associated with DDT resistance and strongly contribute to resistance to certain pyrethroids such as permethrin. However, there is possibility of additional resistance mechanism in both cases [52, 56, 60].

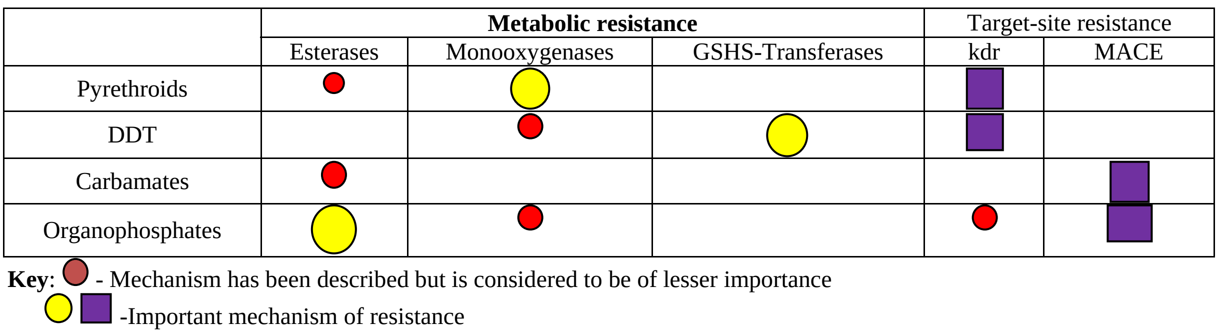
In Ghana, [61] reported a wide spread of insecticide resistance. The highest resistance an hour exposure to paper treated with organochlorines (40% DDT and 4.0% Dieldrin with mortality at 5.4% and 0 % (Tarkwa), Akyem (5.7%) and 0% and Ahufo 3.1% 9.2% and Obuasi DDT 31.7% respectively. Permethrin also showed resistance in Obuasi at 0.75% permethrin, 38.5% mortality were recorded 24 hours post exposure in all cases.

In Cameroun, [62] reported insecticide resistance in Douala and Yaoundé where mortality rates for permethrin ranged from 46 to 82% in Douala and from 57 to 90% in Yaoundé with the lowest mortality rates being recorded in cultivated areas in both Douala and Yaoundé. Deltamethrin exposure resulted in 86 to 94% mortality in Douala and 82 to 93% mortality in Yaoundé. The survival rate of mosquitoes exposed to DDT is significantly higher in Douala (81%) compare to Yaoundé (52%) (P<0.0001), whereas survival to permethrin or deltamethrin did not differ significantly between the two sites. Among known and potential factors affecting the evolution of resistance, the operational factors are the only ones open to manipulation by man. Therefore, investigation on the development of resistance should ideally take account of all these factors [1].

Mechanisms of insecticide resistance

Factors that induce resistance are numerous and the mechanisms adopted by organisms depend on the prevailing pressure and on the mode of action of the insecticide in use [1]. Intoxication of mosquitoes by insecticides encompasses different levels of pharmacokinetic interaction: penetration of barrier tissue, distribution, storage, metabolism in internal tissue and molecular interaction with the ultimate target site [1]. A number of resistance mechanisms have been identified (Figure 3).

Figure 3. Major biochemical mechanisms conferring resistance to important classes of insecticides in mosquitoes. Adopted modified from [36].



Insecticide resistance management

Resistance management consists of all measures designed to delay or prevent resistance levels rising to those at which the pesticide must be abandoned, while maintaining effective disease control. Management of resistance can help avoid resistance development in vector populations, slow the rate of resistance development and cause resistant vector to "revert" to a more susceptible level [1]. Tactics for management of resistance in vector populations according to [1, 63] can include the following counter measures: Varying the dose of frequency of pesticide application, using local rather than area wide application i.e. limitation of insecticide use to areas with high levels of disease transmission, using less persistent pesticides and Training only certain life stages of the vectors. For example, use of methods that kill adult females, instead of either sexes or all stages of the life cycle; using pesticide mixture, Using alteration, rotation or sequences, using improved pesticides formulations, using synergists, Exploiting unstable resistance, Avoidance of slow release formulations, Identify new pesticides with alternate site of activity, use of non-chemical control methods alone or an additional measure in seasons or in areas where they are applicable and cost effective.

Non insecticidal methods of mosquito control

The discovery, development and use of synthetic organic chemicals with persistent residual action results in the development of resistance of mosquitoes to synthetic insecticides, undesirable effects on non-target organisms and fostered environmental and human health concern [16, 64]. This has necessitated the need for search and development of environmentally safe, biodegradable, low cost, with minimum care by individual and communities in specific situations [16]. Among these are:

Repellant Plants

Phytochemicals obtained from plants with proven mosquito control potential can be used as alternative to synthetic insecticides or along with other insecticides under the integrated vector control. Plants products can be used either as insecticides or killing larvae or adult mosquitoes as repellant for protection against mosquito bites, depending on the type activity they possess [16].

In Ibadan-Nigeria,[16],used Ten (10) plants traditionally as insecticides namely; *Azadirachta indica, Cymbopogan citrates, Ossimum gratisimum, Ageratum conyzoides, Annona squamosa, Hyptis suavecolens, Tridax procumbens, Citrus sinensis, Lantana camara and Solanum nigrum.* [65] also identified *Ancardium occidentale* (Limaneus), *Myrianthus arboreus* (P. beauv) and *Xylopia aethiopica* (Dunal) were also found to be effective against *An. gambiae*.

Biological control

Biological control involves the use of insects or pathogens to eradicate or manage the aquatic plants. The water lettuce weevil and water hyacinth beetle have been used with limited success. At the present time, there is no effective biological control for cattails. There have been a few successful large-scale biological applications to date. However, more research is needed to adequately address some of the problems found. Biological control has proven to be very cost effective [66]. These methods directed against mosquitoes mostly refer to the use of natural enemies such as predatory fish and invertebrate predators and toxins produced by microbial agents [67, 68].

This largely involves the control of mosquito larvae: biopesticides (e.g. *Bacillus thurigiensis*, *Bacillus sphaericus*) produce proteins that are toxic to mosquitoes. The target site of the toxins is larval midgut cells, which is in the presence of the toxins, undergo tremors, become sluggish and eventually die [1]. Cases of resistance to these alternative insecticides are still limited and the underlying mechanisms are only beginning to be identified [69, 71].

In Sokoto-Nigeria [72], showed that larvicidal efficacy of *Bacillus sphericus* on local species of mosquito had limited activity with risk of early resistance against this biological agent and there was need to search and evaluate others, probably species of bacteria with more effective larvicidal properties. Similar evaluations of *Bacillus thuringiensis* H-14 in the Nigerian soils was also carried out in Nsukka by [73, 74], also considered the potential of using Hypomycetes such as Metarhizium or Beauveria for biocontrol of mosquitoes especially in African mosquito vectors. These vectors can also be cost effectively mass produced locally and many strains are commercially available thereby cutting down cost on the process of registration and the time consumed, which includes risk assessment of ‘new’ control fungal agents.

Environmental management

This refers to activities that reduce larval breeding sites of vector mosquito through temporary changes to the aquatic environment in which the larvae develop [75, 76]. It reduces the availability of the breeding sites and therefore decreases the vector density population [77, 78] reported that construction of dams and bridges, oil drilling and mining activities, urban planning and development, logging activities, road and railway constructions, building of new airports, etc. These projects have been known to inadvertently lead to increase in mosquito breeding sites, thereby increasing human-mosquito contact, and indeed transmission [79, 80]. It is suggested that such projects should include health personnel for proper evaluation of the health risks associated with the projects in their immediate communities. Good drainage systems will reduce mosquito-breeding sites and improve the sanitary condition of urban centers. Proper environmental management will not only lead to sustainable economic resources, but also reduces the creation of new breeding sites for disease vectors.

Mechanical control

This is a method in which equipment or tools are used to physically remove the aquatic vegetation. Examples would include aquatic harvesters, bucket cranes, underwater weed trimmers, and machetes. Mechanical control is limited to areas that are easily accessible to the equipment. It can also be labor intensive and extremely exorbitant [66].

Other global control strategies for mosquitoes

In countries like United States of America (US), Canada, throughout Europe, Brazil and Singapore Larval Source Management (LSM) has been the main focus of malaria control program for decades [81, 83]. This procedure provides the dual benefits of reducing numbers of house-entering mosquitoes, and most significantly, also those that bite outdoors [82]. LSM has recently been tested in a few trials of mosquito control in contemporary Africa. The results from these trials showed that hand-application of larvicides can reduce transmission by 70-90% in settings where mosquito larval habitats are defined but are largely ineffective where habitats are so extensive that not all of them can be covered on foot, such as areas that experience substantial flooding and this may make it difficult to be applied in some parts of Nigeria. More significantly, evidences have shown that LSM can be an effective method of malaria control, especially when combined with long lasting impregnated nets (LLINs) [82, 84]. Studies of three LSM programmes of different sizes and ecological settings in Africa showed the cost per person protected from mosquito bite each year ranges from US$0.94 to US $2.50 [85].

This compares favorably with IRS (range from various African settings US$0.88-4.94 [85] or LLINs (range for LLINs costing US$5 and assumed to last three years US$1.48-2.64 [86] suggesting that LSM presents a viable and cost effective malaria control program that can complement existing malaria control methods in many settings across Africa. And Nigeria with a population of 160 million presents a very good candidate for such laudable program. It is however not a strategy for country-wide application, and should therefore not be the primary tool selected in areas of intensive transmission if the Nigerian government intends to apply this program. Moreover, the LSM program has the potential to be integrated into control programmes after LLINs or IRS have reduced transmission to moderate or low levels of transmission and therefore should be considered in the consolidation phase of control and elimination programmes where it can be targeted in space and time. LSM will further reduce transmission, in a synergistic fashion, and help manage insecticide resistance [82]. Several methods have therefore been used in mosquito control and these depend upon the targeted mosquito species. Integrated vector management techniques such as biological control, pesticide application, public health education and source reduction are usually combined to produce an efficient method of mosquito control [87, 89].

Conclusions and Recommendations

The study showed that there is existence of DDT resistance and reduced permeability to permethrin in *An. gambiae* the major vector of malaria in Nigeria. This may suggest the possibility of widespread of this phenomenon in the nearest feature; hence the need for control programmes as established by a number of research activities in different continents including Africa to mount management strategies to curb the spread of resistant population. The use of multidisciplinary approach (Non insecticidal methods) of mosquito control such as repellant plants most of which are indigenous are potential alternatives to synthetic insecticides. It is therefore imperative to provide a place to the important uses of such plants and extensive investigation into their phytochemical constituents.

Acknowledgements

The authors will like to thank Dr Craig S. Wilding of Liverpool John Moores University, Liverpool, United Kingdom for his helpful comments and suggestions.

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