MALARIA CONTROL AND INSECTICIDE RESISTANCE: A REVIEW

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ABSTRACT

Introduction: Malaria has been referred to as a global health concern and it's the main mosquito-borne disease that results to global human mortality. The primary vector control interventions that include the use of insecticides are responsible for the decrease in malaria burden worldwide. Malaria control programmes were reported to have challenges due to insecticide resistance. Numerous studies have shown that the strength and distribution of this resistance has increased in recent years.

Methods: Improved knowledge of current resistance levels and underlying mechanisms are important in designing proper management strategies and to avoid future selection for resistance. Vector control is regarded as one of the major methods that can be used to decrease the transmission of malaria at local level, it can bring down malaria transmission from extremely high levels to about zero level.

Results: Malaria is a disease that can be prevented and treated with proper implementation of recent recommended interventions. Currently, there are four classes of chemical insecticides used for vector control programmes. Insecticide is known as a substance that is toxic which eliminates or kills insects or vectors/pests that transmit diseases. Resistance in mosquito can be in form of target-site insensitivity that occur as result of mutations in the target proteins, metabolic resistance which is due to an increased breakdown of the insecticide by enhanced detoxification activities, or a decrease penetration or sequestration of the chemical insecticide.

Conclusion: Malaria can be controlled by effective vector control measures that can be achieved by proper use of insecticides as means of eliminating the disease vector. Knowledge and early identification of insecticide resistance can provide assurance for the eradication and elimination of malaria through vector control strategy.

Key Words: Malaria, Insecticide resistance, Insecticides, Vector control, Mosquito.

INTRODUCTION

Malaria is the main mosquito- borne disease well known among other major causes of human deaths globally[1]. Malaria is a global public health concern, approximately 3.2 billion people are at risk of malaria infection worldwide. World Health Organization (WHO) database recorded about 92% of the global mortalities in Africa, most were recorded in children under the age of five [2,3]. About 228 million malaria cases were recorded globally in 2018. Most of these cases (93%) were in the African region, 3.4% from the South East Asia Region and 2.1% from Eastern Mediterranean Region. Nineteen countries in sub- Saharan Africa and India take about 85% of the malaria burden worldwide. Six countries are responsible for more than half of all the global malaria cases, these countries include Nigeria, The Democratic Republic of Congo and Uganda with 25%, 12% and 5% of cases respectively[4]. Malaria contributes to poverty and annually cost the economies of the African continent an estimated range of 0.5% to 9%

of GDP[5], it also results in loss of productivity. Malaria, together with tuberculosis and HIV, are important causes of morbidity and mortality, especially in children[6]. It also has a strong negative impact on developmental stages in children leaving many that survive the disease disabled for life[7].

In Nigeria, about 97% of its population are at risk of malaria[8], about 51 million cases and 207,000 deaths are recorded annually. Nigeria had the highest malaria cases than any other country across the globe with an estimated 25% of the total malaria burden within Africa[9]and up to 27% of the total African malaria burden in 2016[10]. Malaria cases in pregnancy is an important public health issue in Nigeria due to their implication in maternal mortality. The major complications of malaria in pregnant women include high placental plasmodia burden, foetal complications, low birth weight in new born babies, and sometime new born death[11,12.]

Malaria Burden

Malaria burden worldwide is reduced mostly due to the improved core vector- control interventions, that include: indoor residual spray (IRS) with insecticides and long-lasting insecticidal nets (LLINs)[13, 14]. However, insecticide resistance and climate change threaten the achievement made by IRS in malaria control. Insecticide resistance has been observed in all the four classes of insecticides approved by the WHO for vector control intervention namely Organophosphates, organochlorines, pyrethroids and carbamates[15] Change in environmental temperature among other causes of resistance is implicated, worsening the resistance condition through alteration in the genetic structure, protein profiles and enzymes of mosquitoes[3]. Malaria control programmes are having a challenge of rising insecticide resistance in the main anopheline vectors in Africa and beyond. This affects the main malaria vector control interventions[7].

Since the first established relationship between malaria vectors and transmission of pathogens to humans and other vertebrates in the late nineteenth century[16], a key control strategy against major mosquito-borne diseases such as malaria, dengue, yellow fever, Zika virus infection and chikungunya fever, is targeting mosquito vectors to interrupt the transmission of diseases[17]. Preventing infection, prompt diagnosis and effective treatment are other measures used to control malaria and its complications[18].

Insecticides are significant in malaria control through vector control strategies. As the efforts to control the disease intensifies, so the selection pressure on mosquitoes to develop resistance to the available insecticides. The strength and distribution of this resistance has increased in present years and presently threatens the success of malaria control programmes[19]. Improved knowledge of current resistance levels and underlying mechanisms is important in designing proper management strategies and to curtail future selection for resistance[20].

MALARIA CONTROL

Malaria is a disease that can be prevented and treated with proper implementation of the present recommended interventions. WHO recommendations include vector control measures, diagnostic testing, preventive therapies, intensive malaria surveillance, artemisinin-based therapies. These are part of measures required to control and eradicate malaria[21]. The choice of malaria control measure in a particular region is being affected by numerous factors that include seasonality, vector specie, vector behaviour, health facilities, disease pattern, endemicity among others[3].

Vector control is a primary method that can be used to decrease the transmission of malaria at local level, it can reduce malaria transmission from extremely high levels to about a zero level[3]. Malaria prevention by vector control leads to decrease human-vector contact and also reduce the average lifespan of the mosquito population at local level. Residual insecticides are applied to the inner surfaces of living areas with the aim of targeting Anopheles mosquitoes that rest indoors[21]. ITMs (Insecticide Treated Materials), when used at large scale are expected to have major effect on

vector populations and this help the whole community including those areas without nets. Populations not making use of ITMs, but within areas with increased ITMs coverage, have been shown to be at lower risk of infection due to decrease in overall malaria transmission in such areas[22,23]. Many studies have reported that malaria-related morbidity and mortality were reduced by the use of ITMs[24,25]. Antimalarial drugs can be used in malaria prevention especially for individuals travelling to malaria endemic regions. Also, intermittent preventive treatment with sulfadoxine-pyrimethamine is recommended by WHO for pregnant women and infants living in high malaria transmission regions[3].

Insecticides use in vector control

Use of insecticides for vector control is a significant component of numerous vector-borne disease control measures[9]. Currently, there are four classes of chemical insecticides used in vector control programmes consisting of the organochlorines (DDT exclusively), the pyrethroids, the carbamates and the organophosphates. However, only pyrethroids are recommended insecticide by WHO for treating bed nets due to their relatively low toxicity to humans and fast knock-down effect[26].

Dichlorodiphenyltrichloroethane (DDT)'s insecticidal efficacy was identified around 1940 and was used worldwide for eradication of malaria between the years 1950 and 1978 to eliminate malaria in 37 of the 143 malaria endemic countries[9].Other insecticide groups that include benzyl phenyl urea and biological control agents like *Bacillus thuringiensis* are used less often against mosquitoes. Pyrethroids have better advantage of multiple modes of action on the vector. They affect sodium channels by opening the channels resulting in continuous nerve excitation, paralysis and death of the vector. They also cause irritant effect, rapid knockdown, that lead to hyperactivity, shorter landing times, undirected flight, and feeding inhibition, all of which reduce the biting ability of the vector[27].

Organochlorines (DDT) are used for Indoor Residual Spray (IRS) in vector control. The persistent use of DDT for vector control measures is approved based on condition under the Stockholm Convention on Persistent Organic Pollutants in line with WHO guidelines and recommendations[18]. Pyrethroids and DDT have identical modes of action, and hence cross resistance to these two classes of insecticides mayoccur[28]. Carbamates and organophosphates are also used for IRS, they are very effectivebut with relatively short residual activity when compared to DDT and pyrethroids. Encapsulation (CS) technology is now applied to increase the residual performance of some carbamate and organophosphates insecticides[29].

Carbamates and organophosphates inhibit cholinesterase, this prevents the breakdown of neurotransmitter acetylcholine, and this leads to neuromuscular overstimulation and death of the vector[27].

INSECTICIDE RESISTANCE

Insecticide is regarded as a substance that is toxic, it eliminates or kills insects or vectors/pests that transmit diseases. Insecticides are regarded as silver bullets or strong agents used in both agricultural practices and public health sectors in the developing countries[30].

Insecticides are strongly efficient when used appropriately[31], however, numerous factors affect its efficacy including operational capability and inadequate resources[32], insecticide resistance[33], and the use of poor-quality or adulterated insecticides[27]. These factors need to be properly taken care of by Governments, and all the stake holders concern. Insecticide resistance can be genetic, or natural ability of an organism to withstand exposure to a chemical substance that would ordinarily kill a member of that specie[34].

Resistance of mosquitoes to the four classes of insecticide primarily used in Insecticide Treated Nets or Indoor Residual Spray pose threats to efforts in malaria prevention and control. Insecticide resistance to at least one insecticide in a study site was recorded in 61 countries, while in 50

countries the resistance was to two or more insecticide classes in a site.

Insecticide resistance to the four classes of insecticides was observed in malaria vectors from all WHO regions with the exception of Europe, but the level of monitoring differed from one region to another. Most of the data (about 70%) were recorded in countries of the WHO African Region[10].

Different types of resistance include cross resistance, multiple resistance and metabolic resistance. Cross resistance is when a pest population have resistance to more than one member of a chemical family. Multiple resistance include multiple, independent resistance mechanisms, and can result into resistance to pesticides/chemicals from different chemical families. Metabolic resistance involves detoxification enzymes that can function in the biotransformation of xenobiotics to less or non-toxic substances[34].

Major methods in preventing malaria by vector control are still based on chemicals, these methods include larviciding, personal protection, environmental management, biological control among others. Indoor Residual Spray (IRS) is an important measure to control malaria vector in regions with significant number of housing facilities having enough sprayable areas and most of the vectors rest indoors (endophilic). Insecticide Treated Nets (ITNs) can be used in places where coverage rates are much and a high proportion of vector human-biting occurs at late night when people are asleep. These methods require susceptibility of vectors to the chemical insecticides. The use of ITNs and IRS has shown promising effect in reduction of mortality and severe disease as a result of malaria in endemic regions[24,35].

Insecticide resistance evolution in insect population can arise due to a rise in the frequency of one or more resistance genes in the insect population as a result of exposure to insecticides. Genetic drift and natural selection act on genetic differentiation in the population that is created by genetic recombination, mutation and gene flow[36].

Insecticide Resistance Management

Insecticide resistance management is an effort to reduce or eliminate the development of resistance[34]. Resistance management can easily be basedprimarily on insecticides, this can take various forms such as combinations, rotation, mosaic, or mixtures. Reducing the selection pressure posed by a certain insecticide or a particular mode of action is of major significance in order to attain success in resistance management[37]. Insecticide resistance of mosquitoes is a growing threat in the African region with few insecticides recommended for public health purposes, and drawbacks in the development of new chemical molecules in years to come. Therefore, application of resistance management strategies is important to maintain the efficacy of control programmes[38].

Insecticide Treated Nets with mixture of pyrethroid and carbamate were endorsed as tools for insecticide resistance management[39]. It has been reported that synergists have the ability to delay control failure, as a result of insecticide resistance in agricultural practices. Synergists are substances that improve the toxicity of some insecticides, although having limited toxicity when used alone. These substances include S,S,S-tributylphosphoro-trithioate (DEF), piperonylbutoxide (PBO), and N-Octylbicycloheptenedicarboximide (MGK-264), etc. Synergists improve the efficacy of various classes of insecticide, including the pyrethroids, carbamates and organophosphates, they achieve their actions by inhibiting the insecticide metabolizing enzymes like Cytochrome P450s, Glutathione S Transferases and esterases in the insects' system. Now, there are Insecticide Treated Materials (ITMs) like Permanet® 3.0 that contain a mixture of a pyrethroid (insecticide) and PBO (synergist) to defeat pyrethroid resistance in mosquitoes. This technique has been reported to be more effective against mosquitoes with multiple resistance mechanisms when compared to other ITMs treated with only pyrethroid insecticide[40].

Insecticide Resistance Mechanisms

To develop resistance management strategies, knowledge of factors that influence insecticide resistance in addition to characterizing the insecticide resistance mechanisms are of primary importance. Factors that are likely to cause insecticide resistance in mosquitoes include: agriculture, industrialisation and urbanization. These factors are related to some environmental parameters; use of insecticides/pesticides in agriculture, natural or anthropogenic xenobiotic, and interactions of vectors with other organisms[38].

Insecticides impair the role of molecular targets that are important for survival. Mutation of any kind that changes the physiology or the behaviour of the insect in a way by which the target becomes less functional will be able to induce resistance. As such, insecticide resistance may be caused by one or more than one of these mechanisms: increased detoxification or excretion, target insensitivity, decreased penetration, or behavioural avoidance of the insecticide by the insect species[41]. Insecticide resistance by insects can be as a result of numerous physiological changes that include mutations of the targeted proteins termed as target-site insensitivity[42], or an increased breakdown of the insecticide due to enhanced detoxification activities termed as metabolic resistance, or a decreased penetration or sequestration[43]. Resistance to pyrethroids has been reported to occur due to target-site mutations termed as the 'knock down resistance' (kdr) and metabolic resistance mechanisms, however, other mechanisms like cuticle alteration have also been reported[44]. Knock down resistance (kdr) mutations and increased levels of detoxification enzymes also result in resistance to DDT while resistance to carbamate can be due to ace1 mutation and detoxification[45].

Metabolic Resistance

Metabolic resistance is a more diverse phenomenon that involves active regulation of detoxification system of the mosquito as such counteracting the effect of chemical insecticide. Metabolic resistance include elevated levels or enhanced activities of insecticide-detoxifying enzymes. This results in development of resistance in insects due to sufficient amount of insecticide substances being degraded before getting to their target in the nervous system of the insect[46].

Detoxification enzymes initially linked to insecticide resistance involve three main gene families: the cytochrome P450 (monooxygenases), glutathione S transferases and the carboxyl/choline esterases, but other enzyme families such as UDP glucosyl-transferase(UGTs) may also be included[47].

Insecticide degradation can be as a result of the increased production or structural modification of one or more of these enzymes either from common or distinct family. These enzymes can work sequentially or simultaneously to bring resistance. Many studies are aimed at the increased production of detoxification enzymes while few are on the selection of particular detoxification enzyme alleles which results in significant insecticide degradation in mosquito vectors[48]. High throughput sequencing approaches like RNAseq can be used to fill this knowledge gap, RNAseqprovides information on gene expression and nucleotide variations over the whole transcriptome from an experiment[49,50].

These enzymes metabolise insecticides to less toxic or non-toxic chemicals or, sequester them. Enhanced detoxification is a known resistance mechanism that stops inhibition of the targets[51].

Cytochrome P450 monooxygenases

Cytochrome P450 monooxygenases are Phase I detoxification enzymes. They catalyse many reactions, but are mostly identified for their role as monooxygenases by adding polar or reactive groups into endogenous compounds or xenobiotics[52]. CYP enzymes' catalytic cycle require electron supply to the heme iron in presence of oxygen. Most of CYP enzymes only accepts electrons fromNAD(P)H. But for some other CYPs another pathway has been identified with peroxides as donors of activated oxygen[53]. This pathway is called “peroxide shunt” and provide significant features for not only the preparative but also

analytical applications[54]. The problem of this pathway results from the faster inactivation of the enzyme by the peroxide. To avoid the inactivation of the protein, continuous photochemical or electrochemical generation of peroxide has been applied for preparative substrate conversion[55].

The Cytochrome P450 gene family was identified to have large expansion of more than 100 CYPs identified in *An. gambiae* according genomes of mosquito[56], more than 200 genes in *Culex quinquefasciatus*[57]and 160 in *Ae. Aegypti*[58]. Increased levels of P450 activity have mostly been identified in mosquitoes with pyrethroid-resistant[43]. These same enzymes can also be induced by most xenobiotics[52]. After the development of the 'Anopheles Detox Chip' microarray[59], most over-transcribed mosquito CYPs were observed in either laboratory colonies or resistant field mosquito populations by microarray analysis[60, 61, 62]. Numerous CYP genes were also associated to pyrethroid resistance by QTL approaches and positional cloning[63, 64]. *An. gambiae* CYP6P3 and CYP6M2 among others were identified as pyrethroid metabolizers[65, 66], *Ae. aegypti* CYP9J32, J24, J26 and J28 and *Anopheles minimus* CYP6P7 and CYP6AA3[67,68].

Carboxyl/choline esterases

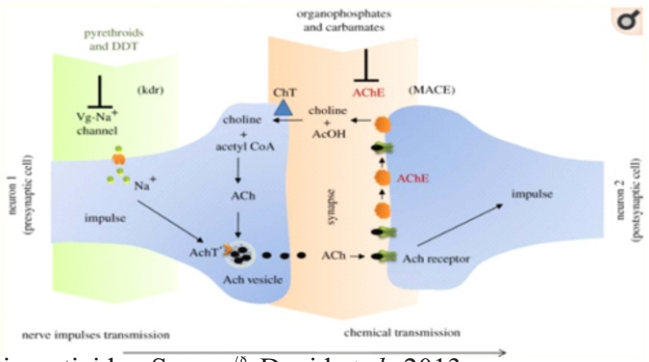
Esterases (E.C. 3.1.1.1) are carboxyl ester hydrolases that catalyse; the hydrolysis of esters having short-chain fatty acids, transesterification reactions and ester synthesis[69]. Carboxyl esterases can be defined as those enzymes catalysing the hydrolysis of acylglycerols with less than 10 carbon atoms (short chains)[70]. Tributyrine is known as the standard substrate for carboxylesterase activity. The enzyme exhibit activity in organic solvents[71]. Carboxyl esterases' active site consist of three residues: an acidic residue (aspartic acid or glutamic acid), a nucleophilic serine residue in a GXSXG motif, and a histidine. These residues work together to catalyse ester hydrolysis. The catalytic triad consists of aspartic acid, serine, and histidine with the serine enveloped in the sequence Gly-X-Ser-X-Gly (where X stands for any amino acid) at the active site[72]. The enzymes have also been reported to show a common α/β hydrolase fold[73] which is common to other hydrolases.

Glutathione S-transferases

The glutathione S-transferases (GSTs) are significant parts of the detoxification pathway in most living organisms. These enzymes work in phase II of the detoxification pathway, they act by conjugating glutathione to products of xenobiotics or metabolism, and these enzymes enhance the excretion of xenobiotics by making them more water soluble. GSTs are currently identified to play major roles in cells' protection against the harmful effects of cell signalingpathways, oxidative stress, intracellular transport, and numerous biosynthetic pathways[74]. About thirty GST genes from various subfamilies have been recognised in mosquitoes[56, 58]. Increased levels of GSTs have been identified mainly in insecticide resistance, they are being over expressed in pyrethroid resistance mosquito populations [58,59,75].

Target-site resistance mechanism

Synthetic insecticides target significant molecules of the nervous system. Organophosphorus (OP) and carbamate (CB) insecticides target synapse acetylcholinesterase (AChE), DDT and pyrethroids (PY) target Na^+ voltage- dependent (NaVdp) channel, while cyclodienes (CYD) targets the GABA receptor of GABA-gated chloride channels (GABAA)[76]. Insecticide resistances as a result of target proteins have been linked to decrease in the targets' binding affinity to insecticide, and not due to increase production of target molecules as was reported for drugs or herbicides[77].



insecticides. Source[78]: David *et al.,* 2013.

Behavioural Resistance

Behavioural resistance is a type of resistance restricted to insects, rodents and mites. This refers to any change in the behaviour of an organism that can help that organism to avoid the harmful effects of chemical pesticides. This mechanism of resistance has been shown for numerous classes of insecticides. The insects might simply avoid feeding after coming across some insecticides, or avoid sites that have been sprayed[79]. This type of resistance does not have same significance as the physiological resistance mechanisms but could be regarded as a factor that contributes to the avoidance of lethal doses of a chemical pesticide[79].

Sequestration

In insects there is significant amplification of metabolic enzymes to about 15% of the total body protein and these enzymes bind to the insecticide, but this binding does not permit the metabolism of the insecticide. The insecticide is sequestered by the enzymes and this renders it harmless to the target organism[79].

CONCLUSIONS

Malaria can be controlled by effective vector control measures that can be achieved by proper use of insecticides as a way of eliminating the disease vector. This should include; education/awareness programmes on the ways to effectively use insecticides in a proper manner so that insecticide resistance can be prevented or reduced. Knowledge and early identification of insecticide resistance can provide assurance for the eradication and elimination of malaria through vector control strategy since the disease is transmitted by the disease vectors. Also, knowing the mechanisms of action of the insecticide detoxifying enzymes will aid in preventing malaria.

New innovations for vector control are recommended in order to maintain the use, and efficacy of chemical insecticides. Eliminating the transmission of malaria through vector control is feasible.

DISCLOSURE OF CONFLICT OF INTEREST

Authors hereby declare that they have no competing interests.

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