Monitoring Insecticide Resistance among Malaria Vectors in Coastal Kenya

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DECLARATION

I, James Edward Msami, hereby declare that this thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I dedicate this to my dearest wife Rehema J. Msami, my son Jeifa and my daughters Doroth & Jessie J. Msami for their prayers and support for the whole period of my studies. God bless you excessively and abundantly in life.

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ACRONYMS AND ABBREVIATIONS

DDT	Dichlorodiphenlytrichloroethane
GST	Glutathione S-transfereses
HCH	Hexachlorocychlohexane
IRS	Indoor Residual house Spraying
ITN	Insecticide Treated Nets
IPT	Intermittent Presumptive Therapy
KDR	Knockdown Resistance
LLINs	Long Lasting Insecticidal Nets
KDT	Knockdown time
KEMRI	Kenya Medical Research Institute
PCR	Polymerase Chain Reaction
PT	Permethrin tolerance
RBM	Roll Back Malaria
WHO	World Health Organization
RR	Resistance ratio

ABSTRACT

Long Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS) are effective measures of malaria vector control. Pyrethroid insecticides are recommended for use in LLINs and IRS due to their low mammalian toxicity and fast action. Currently pyrethroid resistance has been reported in western and eastern Africa, therefore monitoring of resistance is important in all malaria endemic countries. The overall goal of this study was to monitor resistance levels in malaria vectors along the Kenyan coast. Susceptibility of malaria vectors to pyrethroids and use of LLINs was determined in Kilifi, Malindi and Taveta districts of Coastal Kenya. Three sentinel sites from each district were selected and mosquitoes were sampled from each sentinel site in the three districts. The collected *Anopheles* mosquitoes were reared to adults in the insectary. Two to five days old *An. gambiae* mosquitoes were assessed for resistance levels to Deltamethrin (0.05%), Lambdacyhalothrin (0.05%), Dichlorodiphenlytrichloroethane (DDT 4%), Bendiocarb (0.1%) and Fenitrothion (0.1%). Knockdown time (KDT) was recorded up to 60 minutes and maintained for 24hrs post-exposure on 10 % sucrose solution, after which mortality was recorded. Furthermore, in each sentinel site, a questionnaire on use of LLINs and other anti-mosquito tools was evaluated. The susceptibility test showed that mosquito mortality after 24 hrs for deltamethrin was 97%, 93.5%, and 100% in Malindi, Kilifi and Taveta, respectively, while for Lambdacyhalothrin mosquito mortality was recorded at 97% (Malindi), 95.67% (Kilifi), and 97.5% (Taveta). In addition, the study found that use of LLINs was below 80%. This study revealed development of resistance to deltamethrin and Lambdacyhalothrin in *An. gambiae s.l.* in Kilifi, Malindi and Taveta. It is therefore strongly recommended that the impact of this development on malaria control efforts be closely monitored before this problem becomes widespread in the East African Region.

1.0 CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Malaria is one of the most important vector borne diseases, estimated to cause between 300-500 million clinical episodes and 1.4-2.6 million deaths each year, of which tropical Africa contributes 80-90% (WHO 1995 2009). Currently, there is a trend of malaria clinical cases reduction across Africa. The most important tools for malaria control in recent times have been the introduction of insecticide impregnated nets (ITNs), long lasting insecticide treated nets (LLIN) and indoor residual spraying (IRS). In a series of trials supported by WHO in Africa, child mortality from all causes has been reduced between 17 to 63% as a result of the introduction of permethrin impregnated nets and LLIN (Alonso 1991; D'Allessandro *et al.,* 1995; Nevill *et al.* 1996; Binka *et al.,* 1996).

A major strategy and component of the WHO in preventing transmission of malaria parasite is by expanding the extensive rapid roll out of long lasting insecticide treated bed nets and indoor residual spraying in highly endemic areas. (Hinzoumbe *et al.*, 2008; Ranson *et al.*, 2009). This has shown a positive impact in reduction of morbidity and mortality (Stump *et al.*, 2004, WHO 2004a; Lengeler *et al.*, 2007). Therefore, WHO recommended ITNs/LLINs as the key strategy for malaria control in most vulnerable group i.e. children under five and pregnant women in their first trimester. Other strategies include proper management of malaria cases, intermittent preventive treatment (IPTp) to pregnant and early warning and containment of malaria epidemic (WHO 2006 b).

Insecticide resistance has a long history with its first demonstration recorded in the San Jose scale in 1908 where apples were treated with lime-sulphur in orchards. By 1970 most of the synthetic classes of insecticides we use today in vector control had experienced resistance

problems. There were already 91 cases of resistance to DDT just 22 years after its introduction, 135 resistance cases to cyclodiene 18 years since its first use, and 54 species had showed resistance to organophosphates (OP) only 15 years after its first use in the field, there were 3 cases of carbamate resistance and 3 cases of pyrethrin resistance. Great impacts of resistance were witnessed during the malaria eradication campaigns. As early as 1951 there was already a pronounced failure of DDT and cyclodiene against *An. sacharovi* in southern Greece nearly 15 years after beginning of these pesticides for house spraying operations. In 1956-1958 dieldrin experienced a great failure to control *An. gambiae* in a campaign to eradicate malaria through IRS in northern Nigeria, inland Liberia and several other parts of West Africa. The consequences of the failure due to resistance have been very serious in control of *An. stephensi* in Iraq, Iran and parts of India. Since intensive and continual use of insecticide for malaria control may result in development of insecticide resistance in exposed mosquito populations which cause threat to vector control (Betson *et al.,* 2009, Matowo *et al.*, 2010). Therefore, resistance to pyrethroid and other insecticides in mosquitoes is significant threat to the control of malaria in Africa.

Early detection of insecticide resistance can enable a proper selection of insecticides to be used in the area for the scaling up of long-lasting insecticide-treated nets and indoor residual spraying as malaria prevention tools (Hargreaves *et al.,* 2000 , WHO 2006 a, 2006 b, Henry *et al.,* 2005). In sub-Saharan Africa, the major malaria vectors (*An. gambiae s.s.* and *An. arabiensis)* have developed resistance to DDT, dieldrin and Hexachlorocyclohexane (HCH) in several regions (Yewhalaw *et al.,* 2010). In some areas, resistance to multiple insecticides has been reported. This grab considerable attention in public health workers as ITNs, IRS and LLINs are currently the most effective control measure against malaria vectors. There is already bad news concerning spread of resistance and there has been increasing reports from different parts of Africa which

suggest IRS and ITNs are losing their effectiveness due to increased resistance (Chandre *et al.,* 1999, N'Guessan *et al.,* 2007). Sustainability of ITNs and IRS depends much on the continued susceptibility of mosquitoes to insecticides. In the past few years, reports on the efficacy to ITNs in western Kenya showed high levels of susceptibility of *Anopheline* species to the 4 classes of insecticides recommended for vector control. However, current resistance tests using the WHO bio-assays in areas with high coverage of ITNs have detected a gradual decrease in susceptibility levels giving alert on the efficacy of ITNs and IRS with pyrethroids (Kamau *et al.,* 2007). The resistance reported from East Africa is associated with elevated levels of oxidases in the vector (Stump *et al.,* 2004*).*

Development of resistance may necessitate switching to an alternative class of insecticide to enable recommencement of control (Hargreaves *et al.,* 2000). So early detection of resistance facilitates more rational selection of insecticides or may enable timely introduction of resistance management strategies (Hemingway *et al.,* 2004). To achieve the main Kenya National Malaria Control Program objective to have a "malaria free Kenya" by 2017 in line with the Roll Back Malaria (RBM's) recommendations, the Division of Malaria Control advocates the use of long lasting treated nets in malaria endemic areas and indoor spraying in epidemic prone areas. The insecticides of choice in both strategies were synthetic pyrethroids and on the other hand it has been noted that, the high resistance occurs in areas of intensive mosquito control as compared to non intervention areas (Brogdon and Mc Allister, 1998). This habitually raises the fear of development of insecticide resistance in the target vectors in the areas. However, the presence of resistance in East Africa is still intermittent emergence resulting in fear of spread to other places. This calls for effective early detection monitoring of insecticide resistance including detection of resistance problem as early as possible and rapid assimilation of information of rational pesticide

choice. Furthermore, at the coastal region where there has been long time use of ITN and LLINs, the status of resistance is unknown. Thus, the aim of this study was to establish the status of insecticide resistance data associated with LLINs/ IRS coverage along Coastal Kenya that will help in monitoring resistance and control of malaria vector.

1.2 LITERATURE REVIEW

1.2.1 Malaria infection and vector biology

Malaria is a disease caused by a protozoan parasite of the genus *Plasmodium*, which is transmitted by mosquito vectors of the Genus *Anopheles* mosquitoes (WHO, 2000). *Plasmodium falciparum* is the greatest species that causes the greatest illness and death in the Africa (WHO 2004a). Epidemiology of malaria depends on many factors including climate, topography, hydrology and housing (Environmental factors), land use and occupation, daily activities and human habits, migration (human movement), and infection rate (malaria prevalence and entomological factors) (Laumann 2010). In coastal Kenya (Kilifi district), the hospital admissions for malaria decreased from 18·43 per 1000 children in 2003 to 3·42 in 2007 (O'meara *et al.*, 2008).

Anopheles gambiae complex and *Anopheles funestus* complex are the most important vectors of malaria in sub-Saharan Africa. Member of the *Anopheles gambiae* complex includes *Anopheles gambiae sensu strict*, *An.gambiae arabiensis*, *An.gambiae quadrannulatus, An.gambiae merus, An. gambiae melas*, *An.gambiae bwambae, An.gambiae coluzzii* and *An.gambiae amharicus.* (Coetzee *et al.,* 2013). Member of the *An. gambiae* complex cannot be distinguished morphologically. However *An. gambiae ss* prefers wet or humid environments where as *An. arabiensis* prefer dry savannah and is in the most cases associated with water development

project e.g. rice irrigation schemes. (Gillies and Coetzee 1987; Coetzee *et al.,* 2000; Service 2004). *Anopheles merus* is associated with brackish water (salty water) along the coastal area of East Africa. While *An. melas* breeds under similar conditions in West Africa, *Anopheles quadriannulatus* is found in isolated areas along the coast of Zanzibar (Service 2004). Members of the *An. gambiae* complex prefer to breed in open water (unshaded), which are well exposed to sun light e.g. rice paddies, small pools and puddles, animal hoofs print etc (Minakawa *et al.,* 199; Service 2004)**.** *Anopheles funestus* also a species of the complex is wide spread in sub Saharan Africa. It is the most important vector of malaria after *An. gambiae ss* and *An arabiensis.* It prefers breeding in shaded habitat more or less permanent water, especially with vegetation such as swamps, marshes edges of streams, ditches etc. (Minakawa *et al.,* 1999, Coetzee *et al.,* 2000, Service 2004).

1.2.2 Mosquito life cycle

Normally the female mosquitoes mate once in their life time and require blood meal for egg development which takes 2 to 3 day after blood meal before can they lay batch of eggs. As in other insects *Anopheles* mosquitoes have a four stage life cycle namely egg, larvae, pupae and adults, and the time taken for larval development depends on the temperature and the nutritional factors in their environments, higher temperatures shorten development time (Service, 2004; WHO 2004a). About 100-150 eggs are laid on the water surface during oviposition. The oviposition site vary from small hoofs print and rain pool to streams, swamps, canals, rivers, ponds, lakes and rice field. The average life span of female *Anopheline* in the tropical climate is about three to four weeks (21- 30 days). Female mosquitoes lay between one and three batches of eggs during their life time, though some may lay as many as seven batches. Eggs hatch into larvae after one or two days and generally these larvae float parallel on the water surface, since

they need to breathe, they feed by taking up nutrients from the water. There are four larval stages or instars; first, second, third and fourth instars before they can turn to pupae which take eight to ten days to emerge into adult at normal tropical water temperature (25-33˚C). At low temperature (6-8˚C) larval development ceases. The pupa is shaped like a coma and it is at this stage where the transformation takes place from living in water to the flying adult mosquitoes. The newly emerged adults rest temporarily on the water surface until they are able to fly. The flight range of mosquito is usually up to three kilometers from their breeding places. (Gillies and Coetzee 1987; Service 2004).

1.2.3 Mosquito feeding habits

Knowledge of the mosquito feeding habits is very important because it is through the feeding process, that malaria parasites are transmitted as a result of man- vector contact. Only female mosquitoes take blood meal for their eggs development which occurs once every 2 to 3 days in tropical temperature area and takes longer interval in temperate countries (WHO 2002a, Service 2004). The majority of *Anopheline* mosquitoes bite at night, after the blood meal they usually rest on the wall, under furniture or on hanging clothes for indoor resting mosquitoes while outdoor resting mosquitoes usually rest on plants, holes, in tree leaves, in ground or in other cool dark place for a short period (Chandler *et al.,* 1975; Boreham *et al.,* 1979; Charlwood *et al.,* 2000; Mathenge *et al.,* 2001; Service 2004). Some of the *Anopheles species* prefer to feed outside (exophagic) while others feed inside dwellings (endophagic). When they are blood fed, some prefer to rest indoor (endophilic) while others prefer to stay outside (exophilic). In this respect ITN/LLINs, indoor residual spraying (IRS) and improved houses can reduce mosquito biting nuisance and infection from endophilic mosquitoes, while source reduction remains best intervention for exophagic and exophilic mosquitoes. However, for the mosquito to rest inside

the house it depends on factors such as condition of the building, its surroundings, number of occupants and conditions favorable for mosquito survival (Service, 2004).

1.2.4 Malaria control and insecticide resistance

According to WHO strategies for controlling malaria via Roll Back Malaria initiative, identified main interventions of reducing morbidity and mortality, particularly among children, these include detection of malaria cases, early and prompt treatment, promotion of insecticide treated bed nets especially at risk groups (children and pregnant women), preventing malaria in pregnancy using intermittent presumptive therapy (IPTp) and making sure that during malaria epidemics all cases are detected early as an emergency. The use of insecticides such as insecticide treated bed nets and indoor residual spraying can be highly efficacious when used properly (WHO, 1993). But this control strategy of malaria will be affected when the level of malaria vector resistance is high. In this case the frequency of surveillance and monitoring of the resistance should be conducted periodically to identify factors that lead to less susceptibility of mosquitoes in the respective area, and to give advice and implement efficient and sustainable vector control strategies (Brogdon and Mc Allister ,1998; WHO 2006b; Hinzoumbe et al., 2008), This is important since mosquitoes resistance to pyrethroid and DDT have been reported in various countries in Africa since 1950s and Kenya (Vulule *et al.,* 1994; 1999). It has been noted that both agricultural setting and public health use of insecticides may contribute to the development of resistance in mosquito population. For example, in Kenya reduced susceptibility to permethrin was due to distribution and use of insecticide treated nets (Vulule *et al.,* 1994) whereas, agricultural use of pyrethroid has contributed to selection for resistance in Benin and Burkina Faso (Diabate *et al.,* 2002b) . The resistance caused by the level of control of high coverage of ITNs is not clear though the resistance in pyrethroid was reported in Uganda

whereby the L1014S *kdr* allele frequency varied from 3% to 48% in *An gambiae s.s* (Chandre *et al.,* 2000, Verhahgen *et al.,* 2010) . In Western Kenya the knockdown resistance has been reported where reduced susceptibility to pyrethroid and *kdr* gene was identified respectively. The target site resistance observed by Vulule *et al.,* 1999, was increased permethrin tolerance (PT) due to elevated level of oxidases and esterases among *Anopheles gambiae* following the introduction of permethrin impregnated bed nets in some village in Kisumu western Kenya. However in Central Kenya has shown no evidence in insecticide resistance for *An. arabiensis* (Vulule *et al.,* 1994; Kamau *et al.,* 2007).

1.2.5 The role of insecticide treated nets, long lasting nets and Indoor residual spraying;

Insecticide treated nets (ITNs) impregnated with pyrethroid insecticide have become of the most talented interventions to prevent malaria in highly endemic areas. (Eisele *et al.,* 2006). However the Roll Back Malaria Partnership has recently set the target of protecting 80% of children and pregnant women at risk for malaria with ITNs by the year 2015 (Eisele *et al.,* 2009). The impact of reducing morbidity and mortality due to malaria will only be seen if there is a proper and steady use of ITNs in the area (WHO 2004a). It is estimated in malaria endemic settings with a high coverage of ITNs, lives of between 6 and 35 under five children could be saved each year per 1000 population (Schellenberg *et al.,* 2001). Apart from reducing exposure to children and pregnant women, the LLINs/ITNs kill other insects and pests like fleas, mites and bed-bugs. It also provides some kind of privacy and allows the user to sleep happily (WHO, 1996). Since mosquitoes are night feeders, proper use of nets may provide physical barriers to humans against mosquito bites, malaria and other mosquito-borne disease transmission. ITNs reduce human host seeking mosquito population by repelling and killing mosquitoes (RBM 2001-2010; Takken 2002; Gimnig *et al.,* 2003). Various studies in The Gambia (Lindsay *et al.,* 1989, Betson *et al.,*

2009) have demonstrated effectiveness of ITNs in reducing human vector contact. A similar study (Mathenge *et al.,* 2001) in Kenya indicated that *An gambiae ss* and *An arabiensis* avoided entering bedroom with ITNs in comparison to house with untreated nets.

Indoor Residual Spraying (IRS) is the application of long acting insecticide on the walls, ceilings and roofs of a house-hold structure and domestic animal shelters in order to kill the adult female mosquito malaria vectors that land and rest on these surfaces (Brogdon and Mc Allister, 1998). These chemicals have persistent effect for a certain period of time (3- 9 months) after spraying. The method relies on the fact that most malaria infected mosquitoes enter houses during the night to feed on the occupants and rest on the walls or roofs prior to and after feeding. The treated walls and roof with effective residual insecticide, the mosquito will pick up a lethal dose (WHO 2002b). DDT (Dichloro- diphenyltrichloroethane) is among insecticides used in IRS application, it is an organochlorine compound which is highly effective and persistent organic compound. It can stay in the sprayed surface for long period of time after its initial application, above 12 years (WHO 2006). Other insecticides used in IRS are synthetic pyrethroids, Organophosphate (Malathion and Fenithrothion) and Carbamates (Propoxur, Bendiocarb) (WHO, 2002b).

Out of these four chemical groups, currently the recommended insecticides for IRS are twelve, one Organochlorine, 6 pyrethroids, 3 Organophosphate and 2 Carbamets. The selection of these compounds is based on its susceptibility to the malaria vectors, behavior and safety for human and environment as well as cost effectiveness (WHO, 2006a). The contribution of IRS to malaria control has highly shown in 1950s and 1960s where malaria was almost eradicated from many parts of the world (WHO 1998a; 2006b). The malaria incidence was reduced by 90% or more in

major area of tropical Asia and Southern America by IRS and other measures of malaria control during the eradication programme (WHO, 2006 b).

In Africa between 1950s and 1970s, the pilot study for malaria eradication was conducted at Benin, Bukina Faso, Burundi, Cameroon, Kenya, Liberia, Madagascar, Nigeria, Rwanda Senegal, Uganda and Republic of Tanzania and it was revealed the possibilities of controlling malaria vectors with IRS (WHO, 2006b). However, large scale application of insecticide is not sustainable because of the high cost (insecticide purchasing and operational costs), vector resistance to insecticide and environmental concerns (Brogdon and Mc Allister, 1998; WHO 2000). Despite many advantages of IRS the development of resistance to insecticide constitutes the major threat to the chemical malaria vectors control.

1.2.6 Insecticide resistance

Insecticide resistance refers to the ability of insect population to tolerate doses of insecticide that would be lethal to majority of individuals in a normal population of that species, therefore resistance should be suspected in an insect population when the new normal dose rate of insecticide is not able to control the pest (WHO, 2002a). This has happened in malaria vectors because of using the same insecticide for crop protection, which may contaminate the breeding habitat when sprayed. This direct exposure has resulted in development of vector resistance worldwide (WHO, 2007).

Many studies done in West Africa reported on the two major forms of biochemical resistance (Brogdon and Mc Allister, 1998); these are target site resistance which occurs when the insecticide no longer binds to its target (Corbel *et al*., 2007) and detoxification enzymes-based (Metabolic) resistance, which occurs when enhanced levels or modified activities of estarases, oxidases or glutathione S-transferases (GST) prevent the insecticide from reaching its site of

action (Hemingway and Hilary, 2000). Any kind of mutation in the target site of a gene caused by a given insecticide usually induces cross-resistance to all insecticides acting on the same site (Brogdon and Mc Allister, 1998). Knockdown resistance mutation(*kdr)* in sodium channel induce a change of one of the amino acids on the target site for DDT and all pyrethroids, including the related pseudo-pyrethroids such as etofenpron, where by mutation induced by a change in acetlycholinesterase will induce cross resistance to all organophosphates and carbamates insecticides. When such resistance mechanisms are involved there is no need to test a wide range of insecticide to know more about the resistance spectrum. In regular monitoring of insecticide resistance, it can be easy to recognize if there is resistance such as *kdr* or not. It is thus recommended to test DDT when the pyrethroid is being tested (Brogdon and Mc Allister, 1998; WHO 1998b; Hemingway and Hilary, 2000), so that if there is resistance to pyrethroids and DDT then *kdr* is likely to be involved. Another good indicator for *kdr* is evaluation of the knockdown rate, expressed as the time taken for 50% or 90% of individual mosquito to be knocked down. This is because of application of a discriminating concentration which separates the susceptible from resistant malaria vectors allowing accurate detection of resistance when the gene is dominant whereas, when resistance is recessive or present in small amount, the discriminating dose test based on mortality may lose its precision (WHO, 1998a; Matowo *et al.,* 2010). However, the simple and practical tool that can be used in daily monitoring resistance to determine the other resistance mechanism is Polymerase Chain Reaction (Brogdon and Mc Allister 1998).

1.2.7 Groups of insecticides

There are four classes of chemical insecticides available for malaria control. These include organochlorines, organophosphates, carbamates, and pyrethroids**.** The first group consists of

organochlorines (OC) such as DDT and its metabolites, BHC, Dieldrine, and Endosulphan (Thiodan). These have high chlorine content, soluble in organic solvents including fats, less soluble in water and long persistence of its residue on sprayed surfaces. It causes adverse effect to human health and environment and have been carried through environmental media across borders to regions where they have never been used or produced (WHO, 2000). Organophosphates (OP) e.g. fenitrothion, tetrechlorvinphos, fenthion lack sufficient toxicity and persistence and have never been used in large scale. Carbamates which are acid esters, somehow like OP insecticides are biodegradable and not persistent in the environment. The mode of action is similar to OP, which may affect acetylcholinesterase (AChE) receptors. Carbaryl and propoxur (Baygon) and Bendiocarb are an example of this group (Mittal *et al.,* 2004). Pyrethroid insecticide (PY) is a new generation of highly potent synthetic insecticide derived from a group of insecticide esters, the pyrethrins, extracted from the flower heads of certain Chrysanthemum species (*Crysanthemum cinerariaefolium*) which are neurotoxins and target insects' central nervous system (Orose *et al.,* 2005). The synthetic pyrethroids originally have been made to mimic insecticidal compounds in pyrethrum to the reason that the natural pyrethroids are not stable to use as a residual insecticide (WHO, 1996). It has so many advantages compared to other groups of chemical compounds, that have excite repellent properties are effective and act very fast even in small quantities. Furthermore the compound is friendly to the environment (WHO, 1996).

1.2.8 Mode of action of insecticide

It is better to understand the mode of action of the insecticide and the targeted pest system so that we are able to elucidate the mechanism of resistance and to control it. These insecticides generally target the nervous system, growth and development, energy production or water

balance. The most important target of some insecticides is the neurotransmitters which carry the incoming signal. In humans and insects acetylcholine (A*ch*) and gamma- butyric acid (GABA) are important neurotransmitters (Brown, 2006). When insects have been poisoned by cholinesterase inhibitor, the cholinesterase is not accessible to assist in breaking down the *Ach.* As a result, the neurotransmitter can continue to cause the neuron to fire or send its electrical charges, that cause over stimulation of the nervous system and the insect dies (Brown, 2006). Pyrethrins are natural compounds derived from the plant family Chrysanthemum while pyrethroids are synthetic version of pyrethrin, specifically designed to be more stable in the environment so to provide longer lasting control. Both act on tiny channels through which sodium is pumped to cause excitation of neurons. They cause the sodium channel to stop as a result nerve impulse transmission continues leading to tremors and eventually death (Brown, 2006). Another mechanism is the Acetylcholine mimics whereby the insecticide mimics the action of the neurotransmitter Acetylcholine (Ach) e.g. Imidacloprid and nicotinoid; Chloride channel modulators which bind to the GABA- gated chloride channel and blocks reaction in some nerves, preventing excessive stimulation of the central nervous systems (CNS) e.g. Avemectin and Fipronil (Brown, 2006).

1.2.9 Types of resistance metabolism

There exists two major forms, that is, target site resistance which occurs when the insecticide no longer binds to its target, and detoxification enzyme-based resistance which appear when enhanced level or modified activities of estarases, oxidases or glutathione S-transferases (GST) hinder the insecticide from reaching its site of action (Brogdon and Mc Allister, 1998).

1.2.9.1 Target site resistance

The exoskeleton of insects becomes modified in such a way that the insecticide does not penetrate. Decrease in penetration will permit the detoxifying enzymes to metabolize the chemical compound and as a result become less active. Single amino acid mutation (leu to phe or leu to ser) in the 11S6 membrane spanning region of the sodium channel gene that confers target site DDT and pyrethroid resistance in *Anopheles gambiae* as well as single amino acid changes in the axonal sodium channel insecticide binding site produce a shift in the sodium current activation curve and cause low sensitivity to pyrethroids (Hemingway and Hilary 2000; Ranson 2000; Ranson *et al.,* 2009). The target of organochlorine (DDT) and pyrethroids is the sodium channels of the nerve sheath (Ranson *et al.,* 2009).

1.2.9.2 Metabolic resistance

This involves the metabolic pathways of the insect which becomes modified in ways that detoxify the insecticide or prevent metabolism of the applied insecticide into its toxic form. The change in rate of metabolism is caused by Glutathione S-transferase (GST) (DDT, Pyrethroids, Organophosphate), monooxygenases (Pyrethroids, Carbamates, & DDT), esterase's which include Organophosphate & Carbamates. Sodium channel (*kdr)* includes DDT & Pyrethroids and GABA receptors- Cyclodines & Fipronils (Brogdon and Mc Allister, 1998; Hemingway and Hilary, 2000).

1.3 Technique of resistance mechanism

The ideal task is to make susceptibility data as a baseline data in the area though currently the major effort is on molecular mechanisms of resistance and coherent resistance management so as to detect resistance in the early stages and monitor resistance level (Hemingway and Hilary. 2000). The WHO bioassay method done under laboratory conditions includes susceptibility tests. When it is conducted the dosage needed to kill 50% or 90% of the population can be calculated

as well as the mortality rate changes over the occurrence of time. The method can be used to give a picture of the mechanism conferring resistance in the area.

The biochemical and immunological bioassay method is for detecting resistance based on elevated esterases (Ops and pyrethroids), elevated mixed function oxidases (mfos) (pyrethroids and carbamates), elevated glutathione S-transferases (GSTs) DDT and insensitive acetylcholinesterase (AChE) OP and Carbamate). The ability of carrying out multiple assays on single insect to look for multiple resistances remains the advantages of the methods (Brogdon and Mc Allister, 1998). In molecular assay, DNA and RNA probe are employed to detect resistance genes by Polymerase Chain Reaction (PCR). The easiest resistance mechanism to be detected by this technique is point mutation that cause target site resistance or change in detoxification enzymes specificity. Therefore Polymerase Chain Reaction Restriction Enzymes (PCR- REN) are used to detect target site resistance and the PCR Amplification for specific alleles. In these methods resistance can be detected earlier before it comes out (Brogdon and McAllister, 1998).

1.4 Problem statement

The development of insecticide resistance in malaria vectors remains a serious threat to the implementation of practical and affordable malaria control measures in the Sub-Saharan malaria endemic areas. To date, over fifty *Anopheline species* worldwide have been recorded to be resistant to one or multiple insecticides. In sub-Saharan Africa, the major malaria vectors (*An. gambiae and An. arabiensis)* have developed resistance to DDT, diedrin and HCH in numerous regions. In some areas, resistance to multiple insecticides has also developed (WHO, 1986; Koekemoer *et al.,* 2010). While mosquito vectors are becoming resistant to more insecticides, the options for malaria control become strictly limited, as few new insecticides have been developed in recent years with the most notable are synthetic pyrethroids.

In Kenya, the main malaria control intervention tools are insecticide treated bed nets (ITNs) and indoor residual spraying (IRS) in endemic and epidemic areas respectively. However, the use of insecticides in agricultural activities is low in Coastal Kenya. Since the mass distribution of ITNs to the area was done by the Government in 2006, nevertheless the ITN coverage and the use of indoor residual spraying in the area are not clearly understood. Moreover, the status of insect resistance to pyrethroid insecticide is unknown.

1.5 Justification and significance of the study

The front line malaria control interventions rely heavily on the use of insecticides in the ITNs, currently, long lasting Insecticide nets (LLINs) and indoor residual spray (IRS). Time series monitoring the changes of the susceptibility levels of the local malaria vectors to different insecticides is essential as it allows timely management of resistance and selection of proper insecticides for implementation. Unfortunately this has never been done in this area and therefore highlights the need of this study. The World Health Organization (WHO) guideline indicates that if the population mortality is between 98-100% the mosquito population is susceptible, while between 80-97% the population indicates resistance which needs to be confirmed, but if mortality is less than 80% the population is said to have resistance. This study is anticipated to provide relevant information on the status of insecticide resistance and the use of ITN/IRS in the Coastal area. This information may be useful to the Ministry of Health and public health stakeholders in formulation of sound malaria vector control policies.

1.6 HYPOTHESIS

The long term use of ITNs along the Kenyan coast (Malindi, Kilifi and Taveta) has led to development of significant resistance in *An. gambiae s.l.* population

1.7 OBJECTIVES

1.7.1 Main objective:

To determine insecticide resistance in malaria vectors along Kenyan Coast.

1.7.2 Specific Objectives

1. To determine susceptibility status of *Anopheles* mosquitoes to pyrethroid insecticide along Coastal Kenya
2. To determine house-hold coverage of insecticide treated nets (ITN) and indoor residual Spraying (IRS) along the Coastal Kenya

2.0 CHAPETR TWO: MATERIALS AND METHODS

2.1 Study area

The study was conducted along the coastal zone of Kenya where malaria is serious public health concern. The province covers an area of 83,603 km² and a population of 2,487,264 inhabitants (KNBS 2010). The coastal region is largely hot and humid with two rainy seasons, the "long rains" from April to July, and the "short rains" between October and December. The districts of Kilifi, Malindi, and Taveta were selected for the study based on malaria vector species composition, malaria prevalence, epidemiological settings and ecological differences.

2.1.1 Kilifi district

It lies between 3^0 16´south and 4° south and 39°05´east and 40° east. The population of Kilifi was 597,354 people with 90,000 households (census 2009). Kilifi district has 3 seasonal rivers namely Nzovuni, Goshi and Wimbi which create drainage during rainfall, and the permanent Jaribuni river. The annual mean temperature is between 22.5° C and 24.5°C in the months of April, May and June while in the belt of coastal zone, temperatures range between 30°C to 34°C and has the relative humidity of over 60% (Kilifi District Long- Term 2001 – 2015). *Anopheles gambiae s.l.* and *An. funestus* complex are the main malaria vectors (Mbogo *et al.*, 1993, 1995). Three sentinel sites Jaribuni, Shibe and Mavueni villages were selected for entomological sampling. The selection criterion of these sites was presence and abundance of malaria vectors and numerous breeding sites along the existing river streams cutting across the villages. The streams are used in different community activities such as agriculture, fishing and sand harvest. The human activities create many breeding habitats for malaria vectors. The houses are located in groups (homestead) ranging from 5-10 houses per homestead. Most houses are constructed of temporary building materials such as mud, poles, and covered by grass or corrugated iron sheets.

Some small scale agricultural activities such as growing of green vegetables, maize and keeping of domestic animals (goats, poultry, cattle etc) are practiced.

2.1.2 Malindi district

Malindi district covers an area of 7,605 Km^2, with a population of 305,143 (census 2009). Malindi, Marafa and Magarini are the three divisions of the District (CRF 2007- 2008). The main town of Malindi is situated about 120 Km north of Mombasa town. Fishing and agriculture are the main economic activity in the area. The major malaria vectors in this area are *An. gambiae s.l, An. funestus, An. merus* (Macintyre et al. 2002, Mbogo et al. 2003, Keating et al. 2004). Three sentinel sites were selected, Mbogolo, Burangi and Madunguni, because of the presence many breeding sites.

2.1.3 Taveta district

The district is situated to the southwest bordering Tanzania. It is to the leeward side of Mt. Kilimanjaro lying between 2°46′south and 4°10′south and longitude 37°36′east and 30°14′east. The altitude of the area is 481m above the sea level for highlands. This gives two different characteristics: hills experiencing lower average temperature of 18.2°C compared to lower lands with average temperature of 24.6°C. The major rivers are Tsavo, Voi and Lumi which are springs. Jipe and Challa lakes are found in Taveta and are used for small scale irrigation and fishing. Two sentinel sites, Kimundia and Kiwalwa, were selected for the study. Houses in Kiwalwa are close to each other and closely form a village while in Kimundia they are scattered over wide area. Houses are made of stick, mud and grass. The main economic activity is agriculture in crop production, such as banana, maize, beans, sugarcane, arrowroots, tomatoes, etc.

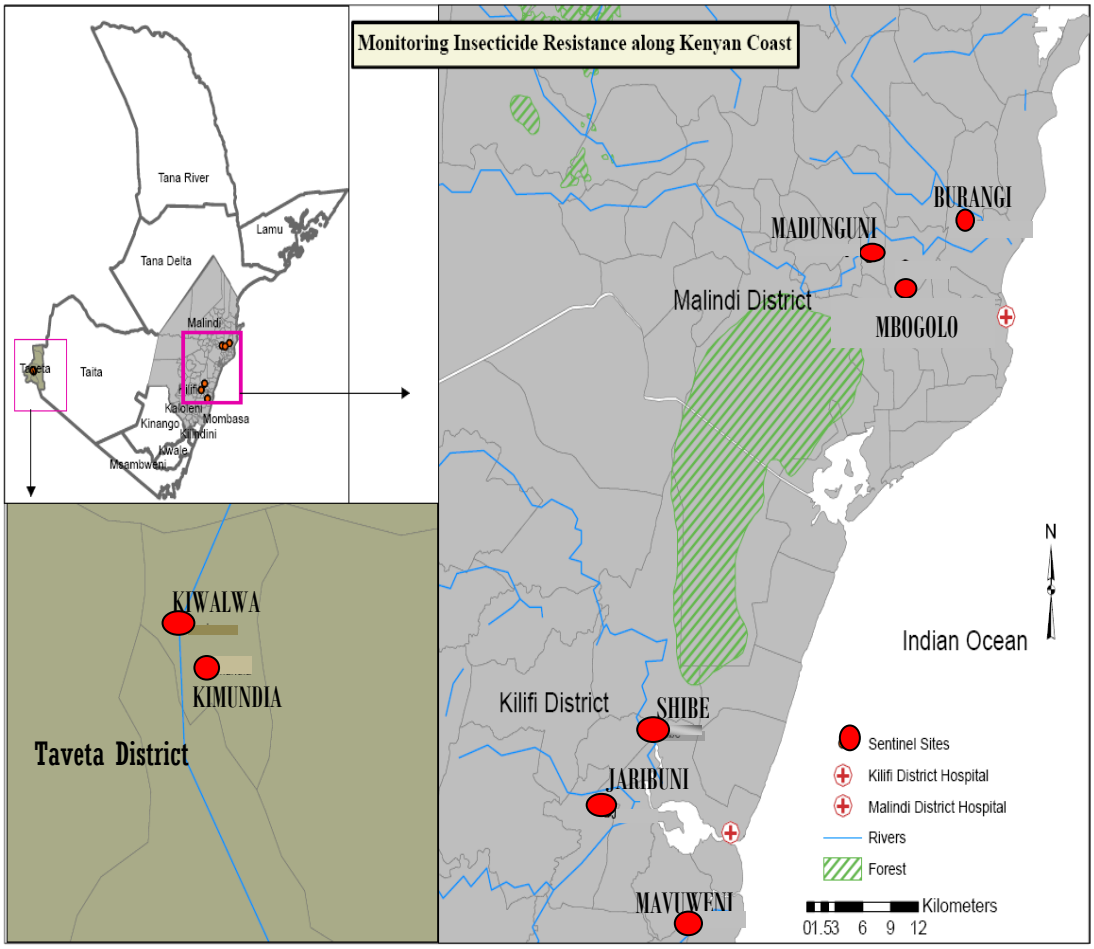

Figure 2.1 Map of the Coastal region of Kenya showing the location of mosquito collection.

(Sentinel sites)

2.2 Study population

2.2.1 Mosquito population

Unfed female mosquitoes aged 2 to 3 days (F_1generation) were used in the test because the physiological status of female mosquitoes such as blood feed, semi gravid or gravid have an effect on susceptibility to insecticide(WHO 1998b)

2.2.2 Households

The use of vector control interventions including ITN and IRS coverage were assessed for each household by use of a questionnaire which was conducted by trained interviewers.

2.3 Sampling method

- The sample size for ITNs coverage was calculated by the formula,

$$n = Z^2 \frac{P(1-P)}{e^2} \quad \text{or} \quad Z^2 \frac{P(100-P)}{e^2}$$

Where

n = sample size

Z = Critical value at 95% (1.96)

P = Proportion of household slept under ITNs (in this case we will take 0.5)

e = Allowable error (0.098)

$$n = (1.96)^2 \times \frac{0.5(1 - 0.5)}{(0.098)^2} = 100 \text{ households}$$

Sample size in one sentinel site = 100 households

Then, systematic random sampling was used to select houses in the sentinel site.

- Following WHO recommendations, the study aimed to use a minimum of 100 female mosquitoes for each insecticide per bioassay.

2.3.1 Adult mosquito sampling

Collections of indoor resting adult mosquitoes were done by aspiration method between 06.00 to 10.00 am, inside houses (Fig.2a). Sampled adult mosquitoes were put into a paper cup covered with netting materials and were provided with 10%glucose soaked in the cotton wool, placed in a cool box and transferred to the laboratory for further processing. In the laboratory, the mosquitoes were identified morphologically into species and sorted out into physiological status. All the blood fed, gravid or half gravid mosquitoes were separated and provided with oviposition media in the insectary. They were kept in the insectary until oviposition was completed (see section 2.5.1below)

2.3.2 Larval sampling

In order to increase the sample size of getting enough F_1 generation to perform the susceptibility tests, larval sampling was done in the nearby breeding sites. Larval collection was done using standard dipping technique (WHO, 1975 , Service, 2004) by scooping in the habitats within the selected villages. The *Anopheles* larvae were collected from a wide range of breeding sites, representative of the diversity of the mosquito population in the study area, such as marshes, ponds, shallow wells, and river banks (Fig. 2). In each location larvae collection was performed in at least 25 breeding sites with an average of 40 larvae of all instars per breeding habitat were collected and reared to adult in the insectary. (Fig.5) *Anopheles* larvae were separated from the *culicines* by the use of a pipette (Fig.4D) and kept in a whirlpak. The whirlpaks containing larvae were kept in a cool box for transportation to the insectary in Kilifi.

2.3.3 Mosquito collection and rearing

The adult mosquitoes from each sentinel site (as stated on 2.3.1 above) were identified into species level and clearly labeled in separate cages, made up of metal frame and netting materials. The cage has cube shape of 30 x 30 x 30 cm with opening of 14 x 14 cm to which a white cloth sleeve of 30 cm long is attached. The eggs were collected on plastic petri dishes of about 6 cm diameter lined with a filter paper on top of wet cotton wool. All the laid eggs from the collected adult were placed in the rearing tray until pupation.

Pupae were collected every morning then transferred into holding cages until they emerged into adults (Fig.5). Upon emergence, mosquitoes were sexed and identified morphologically using morphological identification keys (Gillies and de Meillon 1968, Gillies and Coetzee 1987). Two to five day old mosquitoes were used for insecticide susceptibility tests.

2.3.4 Data collection on household coverage of ITNs

In the same site of adult mosquito collection and larvae sampling, the information of ITNs coverage were also collected. In each village questionnaires were administered to the heads of households. One field assistant worker was trained to assist on administering questionnaires to the households' head in relation to ITNs and IRS. By using this tool, the head of households were asked to answer questions concerning insecticide treated nets (See appendix 2). The questionnaires were filled and taken back to KEMRI-Kilifi center for analysis. The head of household in this study included father, mother, or any member of the family who is eighteen years or more. (See appendix 2)

2.4 Study design

The design of the study was done based on objectives as follows

2.4.1 Determining susceptibility of *Anopheles* mosquitoes to insecticides.

The larval and adult mosquitoes were reared in the insectaries to produce the first filial (F_1) generation. The F_1 generation was categorized into two groups: a test group (field collected mosquitoes subjected to insecticide) and a negative control group (field collected mosquitoes not subjected to insecticides). Meanwhile the laboratory colony *Anopheles gambiae* Kisumu strain constituted the positive control group.

2.4.1.1 Procedure and condition of susceptibility testing

Susceptibility test was done as per WHO standard guideline (WHO, 1998a). Twenty to twenty five female *Anopheles gambiae s.l.* mosquitoes aged 2 – 5 days and non fed female were exposed to the diagnostic dosages of standard WHO insecticide papers. The mosquitoes were exposed to a dosage of 4% DDT, 0.05% deltamethrin, 0.05% lamdacyhalothrin, 0.1% fenitrothion and 0.1% bendiocarb using the WHO susceptibility test kit to assess resistance level (Figure 6F).

Number of mosquitoes knocked down during exposure time was recorded at 10 minute intervals for 1 hour. The knocked down mosquitoes were then transferred to holding tubes where 10% glucose was provided and held for 24 hours then mortality recorded. Laboratory colony, that is, *An. gambiae* Kisumu strains and field collected mosquitoes were used as positive and negative control test respectively. This susceptibility test was conducted under 26 – 29°C and relative humidity of 74 – 82%. When mortality in the negative control group exceeded 20%, the experiment was repeated and if the mortality was between 5 – 20%, the Abbots formula was used to correct percentage mortality.

2.4.1.2 Survival of the mosquitoes

After recording mortality for 24 hours post exposure, all surviving and dead mosquitoes were kept in individual mosquito vials. The dead as well as the killed surviving mosquitoes were well

labeled then stored in desiccated silica gel for future processing such as mechanism of resistance including *kdr* genes and determination of sibling species.

2.5 Data management

Paper questionnaires for household survey and forms for laboratory work were used as acquisition or data capturing tools. Thorough counter check of the questionnaires and data entered in MS Excel database was done, and then hard copies and a back up were stored in a lock cabinet only accessed by a few people.

2.5.1 Data analysis

2.5.1.1 Susceptibility test

The mortality was recorded for the entire exposed field mosquitoes, negative and positive controls. The negative control was used to adjust both positive and the field mosquitoes using Abbots formula to correct percentage mortality when negative control mortality exceeded 5%. When there is a ninety eight to a hundred percent mosquito mortality this indicates the population is susceptible, 80 – 97% suggests potential resistance that needs to be confirmed while less than 80% mortality suggests resistance. Fifty and 95% knockdown time was estimated by the log-time probit model using the Ldp line^{R} software, while ANOVA was used to compare knockdown effect between different samples. Resistance ratios (RR) were calculated by dividing the KDT_{50} of the field population with KDT_{50} of the susceptible *Anopheles gambiae* Kisumu strains. To determine insecticide resistance, the level of insecticide was scaled by using resistance ratios (RR) which translated as: Susceptible (RR=1), Suspect of resistance (RR= 2) and Resistance (RR>3) (WHO 1998 and Hinzoumbe *et al.,* 2008).

2.5.1.2 Insecticide treated nets (ITNs) /Long lasting net (LLINs)

Chi-square of SAS version 9.2 was used to compare the LLIN coverage in different villages and districts in the study area.

2.6 Ethical considerations

Verbal consent was obtained from household head or their representative before commencing mosquito collection. These mosquito surveys were perfumed under human investigations protocol approved by Ethical Review Board of Kenya Medical Research Institute Nairobi Kenya. (Ethical clearance SSC # 1980). This study mainly focused on mosquito populations collected indoors/outdoors/larval stage. Human population involvement was limited to the collection of mosquitoes from their households/premises. No invasive form of human involvement was carried during the study i.e. blood smear for malaria parasites. Training of field workers who participated in data collection was conducted to ensure quality of data collection and to equip them with skills in community approach.

Figure 2.2 : Indoor adult mosquito collection using a mouth aspirator.

Figure 2.3 : A and B: Larvae sampling using the standard dipping method.

Figure 2.4. (a) Sorting out sampled larvae on a rearing tray and (b) sorted larvae from the field

Figure 2.5: WHO insecticide susceptibility test tubes

Fig 2.6: Children at Shibe village fishing using ITNs.

Fig 2.7: Mosquito nets used as fence for chicken at Jaribuni village.

3.0 CHAPTER THREE: RESULTS

3.1 Susceptibility test

3.1.1 Mortality of malaria vectors (*An. gambiae s.l*) in the three districts

A total of 4,484 *An. gambaie sl* were exposed in 42 susceptibility tests performed for the Deltamethrin (0.05%), Lambdacyhalothrin (0.05%), DDT (4%), Fenitrothion (0.1%) and Bendiocarb (0.1%). In all eight sentinel sites, 800 *An. gambiae s.l* tested with DDT were susceptible (100%) while 800 *An. gambiae sl* collected and tested for Fenitrothion mortality was 100% (Table 1). *An. gambiae s.l* populations were susceptible to Bendiocarb except in Kiwalwa (75.98%) and Kimundia (91.82%) villages in Taveta district. Deltamethrin scored low mortality rate at Burangi 93%, Shibe 83% and Mavuweni village 92%. These results indicate suspect of resistance which need further investigation to be confirmed. Likewise, Lambdacyhalothrin at Burangi showed mortality of 96%, Madunguni 89%, Shibe 87%, and Kimundia 95%. Based on mortality, the result of Lambdacyhalothrin shows resistance which also needs more investigation. The summary of the results per district for each insecticide is shown in Table 1 below.

Table 1: Susceptibility rates in *Anopheles gambiae sl* exposed to different insecticides in 8 villages of Kilifi, Malindi and Taveta districts (Abbotts corrected mortality).

District	Sentinel site	Deltamethrin	Lambdacyhalothrin	DDT	Fenitrothion	Bendiocarb
Malindi	Madunguni	100 (98%)	100 (89%)	100 (100%)	100 (100%)	100 (98%)
	Burangi	100 (93%)	100 (96%)	100(100%)	100 (100%)	100 (100%)
	Mbogolo	100 (100%)	100 (100%)	100 (100%)	100 (100%)	100 (100%)
	Subtotal	**300 (97%)**	**300 (97%)**	**300 (100%)**	**300 (100%)**	**300 (93.5%)**
Kilifi	Jaribuni	225 (99.6%)	125 (100%)	100 (100%)	125 (100%)	125 (100%)
	Shibe	100 (83%)	100 (87%)	100 (100%)	100 (100%)	100 (100%)
	Mavueni	100 (92%)	100 (100%)	100 (100%)	100 (99.09%)	100 (98%)
	Subtotal	**425 (93.5%)**	**325(95.6)**	**300 (100%)**	**325 (100%)**	**325(99.34%)**
Taveta	Kiwalwa	100 (100%)	100 (100%)	100(100%)	100 (100%)	100 (100%)
	Kimundia	100 (100%)	100(95%)	100 (100%)	100 (100%)	100 (91.8%)
	Subtotal	**200 (100%)**	**200 (97.5)**	**200 (100%)**	**200 (99.55%)**	**200(82.54%)**

3.1.2 Comparison of mean knockdown time in minutes between treatment and control (laboratory colony *Kisumu* strain) per district

DDT had 100% mortality after 24 hours in all the three districts, but in comparison to the mean knockdown time with positive control, there was a significant difference for Kilifi and Taveta tested mosquitoes ($p<0.05$). Fenitrothion also recorded 100% mortality in all the three districts but its mean knockdown time showed a significant difference compared to mean knockdown time of positive control in Malindi (29.56), Kilifi (26.8) and Taveta (31.84) (Table 2).

Deltamethrin recorded the lowest mortality in Kilifi at 93.5% as compared to Malindi (97%) and Taveta (100%), but when compared with positive control, Kilifi had significantly higher knockdown time of 13.82.

Bendiocarb had a mortality of 99.34% in Kilifi, 93.5% in Malindi and 92.54% in Taveta. A comparison of the mean knockdown time showed no statistical significant difference with the positive control in Kilifi knockdown time mean of 2.09 but there was a significant difference in Malindi mean knockdown time of 10.75 and Taveta at 31.84.

Mortality by Lambdacyhalothrin was highest in Taveta at 97.5%, followed by Kilifi (95.67%) then Malindi (95%). In addition, there was a significant difference in mean knockdown time between the positive control and the Malindi (18.14) and Taveta (23.34) tests (Table 2).

Table 2: Comparison on knockdown (KD) time between treatment group and *Kisumu strain* per district

	Malindi		Kilifi		Taveta	
Treatment	24 hrs. Mortality %	Mean KD time.	24 hrs. Mortality %	Mean KD time	24 hrs. Mortality %	Mean KD time
Deltamethrin	97	6.69(-4.52- 17.90)	93.5	13.82(3.64-24.01)***	100	6.21(-7.31- 19.74)
Lambdacyhalothrin	97	18.14(6.93- 29.35) ***	95.67	5.68(-5.76- 17.11)	97.5	23.34(9.82- 36.86) ***
DDT	100	6.46(-4.76- 17.67)	100	11.52(0.09- 22.95)***	100	26.03(12.50- 39.55) ***
Fenitrothion	100	29.56(16.27- 42.86)***	100	26.80(15.37- 38.23)***	99.55	35.00(21.47- 48.52) ***
Bendiocarb	93.5	10.75(0.75- 20.76) ***	99.34	2.09(-9.34- 13.53)	82.54	31.84(20.34- 43.35) ***

Comparisons significant at the 0.05 level are indicated by ***

3.1.2 Knockdown time Ratio (KDT50 R) and KDT95R at 95% CL

Based on the knockdown time ratio ($KDT_{50}R$), the Kilifi mosquito population exhibited suspected resistance to Deltamethrin at $KDT_{50}R$ - 2.13, DDT at $KDT_{50}R$ - 2.04 and Fenitrothion at $KDT_{50}R$ - 2.73. Furthermore, the population of mosquitoes was susceptible to Lamdacyhalothrin at $KDR_{50}R$ - 1.31 and to Bendiocarb at $KDR_{50}R$ - 1.36 (Table 3).

In Malindi district the mosquito population showed suspected resistance to Deltamethrin at $KDT_{50}R$ - 1.46, DDT at $KDT_{50}R$ - 1.66, Bendiocarb at $KDT_{50}R$ - 1.55 and Lambdacyhalothrin $KDT_{50}R$ - 1.92. However, they were resistant to Fenitrothion at $KDT_{50}R$ - 3.35.

In Taveta district, Lambdacyhalothrin had $KDT_{50}R$ - 3.13, DDT at $KDT_{50}R$ - 5.03, Fenitrothion at $KDT_{50}R$ - 1.3.94, Bendiocarb at $KDT_{50}R$ - 2.84 and Deltamethrin at $KDT_{50}R$ - 1.3 (Table 3).

Table 3; Knockdown times (kdt) and knockdown time ratio ($kdt_{50}R$ &$kdt_{95}R$) of *An. gambiae sl* exposed in the five treatments

District	Treatment	% KD after 60 min	KDT_{50} (95% CI) in minutes	KDT_{95} (95% CI) in minutes	*KDT_{50} R	KDT_{95} R
Kilifi	Deltamethrin	98.75	26.2(9.39 -43.01)	92.05(83.89- 100.21)	2.13	1.04
	Lambdacyhalothrin	96.33	42.67(10.73- 74.61)	93.20 (77.58- 108.82)	1.31	1.03
	DDT	98.0	27.33(4.32- 58.98)	93.33 (88.16- 98.51)	2.04	2.73
	Fenitrothion	88.6	20.4(5.95- 46.75)	65.87 (32.83- 98.90)	2.73	1.46
	Bendiocarb	99.4	41.06(8.75 - 73.38)	96.67 (89.8- 104.26)	1.36	0.99
Malindi	Deltamethrin	98.67	41.45(12.54 - 95.47)	87.60 (62.82 - 112.38)	1.46	1.09
	Lambdacyhalothrin	93.33	31.53(24.89 -87.95)	73.47 (17.08 - 129.86)	1.92	1.3
	DDT	100	36.4(4.18 - 68.62)	98.67 (92.93- 104.40)	1.66	0.97
	Fenitrothion	97.0	18(20.12 - 56.12)	48.50 (4.03 - 92.97)	3.35	1.97
	Bendiocarb	99.5	39(21.67- 56.33)	83.00 (73.45- 92.55)	1.55	1.15
Taveta	Deltamethrin	98.5	54(174.71- 282.71)	92.00 (9.65- 193.65)	1.30	1.05
	Lambdacyhalothrin	95.0	22.5(16.15 - 28.85)	76.50 (18.50- 171.80)	3.13	1.26
	DDT	97.5	14(36.83 - 64.82)	77.50 (55.92- 210.92)	5.03	1.24
	Fenitrothion	88.05	17.87(2.48 - 33.28	47.57 (133.39- 228.54)	3.94	2.03
	Bendiocarb	84.3	30.91(8.20 -53.61)	58.75 (15.44- 102.06)	2.84	1.64

KDT ratio= KDT_{50} of the exposed population per KDT_{50} of the control susceptible *Kisumu strain*.

3.2 The coverage and usage of Long lasting insecticide nets and Indoor residual spraying.

3.2.1 Different categories of people using long lasting mosquito nets

A total of 800 respondents were interviewed. It was established in the study population that a total of 1,152 long lasting insecticide nets were present. The number of children under five years in surveyed population was 765 while the total number of people above five years of age was 2207. The coverage of LLINs for under fives who slept under the net the previous night was 78% in Taveta, 72.3% in Malindi and 41.6% in Kilifi. Of the 2207 aged 5 years and above, only 1,175 (53%) slept under long lasting nets. Taveta had a coverage of 62.1%, followed by Malindi and Kilifi at 58.2% and 36.8% respectively. The average LLINs per household in Kilifi and Malindi was 1, while in Taveta district it was 2 long LLINs (Table 4.1). The lowest coverage of nets for children under 5 was 32.4% in Mavueni of Kilifi district. There was a significant difference between children under 5 and above 5 years using long lasting nets in the three districts (χ^2 = 20.10557, df(2)= 5.99, $p<0.05$).

The study also revealed that the coverage of long lasting nets in Taveta (99.5%)(range 99.5% to 100%) and Malindi (77.2%)(range 75.8 to 77.9%) is higher than in Kilifi district (58.1%) (range 43.5 to 64.6) (Table 4.2). There was a significant difference in the three districts between those who owned at least one net (LLINs) and those who did not have a net (χ^2 = 100.9, df (2)= 5.99, $p< 0.05$).

Table 4.1: Proportion of groups using net within age categories in the sampled districts

	Village site	Respondent	Total number of LLINs	Total of <5 age in surveyed area	Total of <5 age using LLINs	Total of >5 age in surveyed area	Total of >5 age using LLINs	Average per house hold
Kilifi	Jaribuni	112	109	105	54(51.4%)	247	100(40.5%)	1
	Mavueni	99	118	142	46(32.4%)	216	75(34.7%)	1
	Shibe	62	43	51	24(47.1%)	149	50(33.6%)	1
	Subtotal	**273**	**270**	**298**	**124(41.6%)**	**612**	**225(36.8%)**	**1**
Malindi	Burangi	119	182	155	103(66.7%)	379	209(55.1%)	2
	Madunguni	104	161	113	78(69.0%)	339	192(56.6%)	2
	Mbogolo	118	152	108	91(84.0%)	318	202(63.5 %)	1
	Subtotal	**341**	**495**	**376**	**272(72.3%)**	**1036**	**603(58.2%)**	**1**
Taveta	Kimundia	51	99	23	19(82.6%)	175	112(64%)	2
	Kiwalwa	135	288	68	52(76.5%)	384	235(61.2%)	2
	Subtotal	**186**	**387**	**91**	**71(78.0%)**	**559**	**347(62.1%)**	**2**
	Grand Total	**800**	**1152**	**765**	**467(61.0%)**	**2207**	**1175(53%)**	**1**

Table 4.2: Households owning at least one long lasting insecticide nets/ Insecticide treated nets.

District	Sentinel site	No net in hh	Owned at least one net	Total respondents
	Burangi	23 (22.1%)	81 (77.9%)	104
	Madunguni	21 (22.1%)	74 (77.9%)	95
	Mbogolo	23(24.2%)	72 (75.8%)	95
Malindi	**Total per district**	**67 (22.8%)**	**227 (77.2%)**	**294**
	Jaribuni	39 (39.4%)	60 (60.6%)	99
	Mavueni	35 (35.4%)	64 (64.6%)	99
	Shibe	35 (56.5%)	27 (43.5%)	62
Kilifi	**Total per district**	**109 (41.9%)**	**151 (58.1%)**	**260**
	Kimundia	0 (0.0%)	51(100%)	51
	Kiwalwa	1 (0.8%)	130 (99.5%)	131
Taveta	**Total per district**	**1 (0.5%)**	**181 (99.5%)**	**182**
Total per study area		**177 (24.0%)**	**559 (75.9%)**	**736**

House hold (HH), owned at least one ITN/LLINs in the eight sentinel sites of three districts.

3.2.2 Source of mosquito nets in the community

A total of 1,152 mosquito nets were recorded, of which 533 nets had a known source while 619 nets had no known source. The Kenya Government provided 64.35% of the mosquito nets in the community. Burangi, Mbogolo and Madunguni villages (Malindi district) had 90.79%, 62.5% and 71.79% nets coverage respectively distributed by the Government of Kenya through Malaria prevention program for the under 5 and pregnant mothers. In Mavueni and Shibe villages (Kilifi district) all nets were distributed by the government while in Jaribuni most of the nets were available from the local market (86.3%). In Kimundia village (Taveta district) 75% of nets were distributed by the government with the remaining coming from local markets while at Kiwalwa, nets were provided by the government (51.43%) and from local market (48.57%) (Table 5). In general, the results show that there is significant difference in distribution of long lasting insecticide nets between Government of Kenya and local markets ($p<0.05$).

Table 5: Source of mosquito net distribution in the study area

Village	GoK	Local Market	Total
Burangi	69 (90.79)	7 (9.21)	76
Jaribuni	13 (13.68)	82 (86.32)	95
Kimundia	18 (75.00)	6 (25.00)	24
Kiwalwa	36 (51.43)	34 (48.57)	70
Madunguni	56 (71.79)	22 (28.21)	78
Mavueni	60 (100.00)	0 (0.00)	60
Mbogolo	65 (62.50)	39 (37.50)	104
Shibe	26 (100.00)	0 (0.00)	26
Total	**343 (64.35)**	**190 (35.65)**	**533**

3.2.3. Condition of net in each village

A total of 1152 nets (82.6%) were seen during the study for verification of their condition (Table 6). Most of mosquito nets in Kiwalwa were in good condition compared to other sites. Malindi and Kilifi districts had between 41 to 47.6% defective nets while Taveta district had the least defective nets at between 25.7 to 27.7% (Table 6).

Table 6: Nets condition

District	Village	Net in Good Condition	Defective Nets	Total
Malindi	Burangi	86 (52.4%)	78 (47.6%)	164
	Madunguni	55 (53.4%)	48 (46.6%)	103
	Mbogolo	73 (58.9%)	51 (41.1%)	124
Kilifi	Jaribuni	72 (66.1%)	37 (47.6%)	109
	Shibe	33 (58.9%)	23(41.1%)	56
	Mavueni	88 (56.4%)	68 (43.6%)	156
Taveta	Kiwalwa	130 (74.3%)	45 (25.7%)	175
	Kimundia	47 (72.3%)	18(27.7%)	65
	Total	**584(61.3%)**	**368 (38.7%)**	**952**

3.2.3 Intervention on mosquito control activities

A total of 742 (92.75%) out of 800 interviewed heads of household in eight sentinel sites responded to the question on mosquito control measures. The results showed that Kimundia and Kiwalwa villages in Taveta district were more active in mosquito control at 100% and 97.7% respectively. The mosquito control strategies in Taveta were started from colonial rule and during the first East African Community. This influenced awareness of the community on mosquito control. In Shibe village (Kilifi) only 47.2% had awareness in implementing mosquito control (Table 7). In Malindi district the level of awareness on mosquito control ranged between 78-84.5%. The high level of awareness in the district may be due to the current larval intervention on malaria control activities.

Table 7: Status of mosquito control in each village site within the three districts

District	Village	Households with intervention	Households without intervention	Total
Malindi	Burangi	98 (84.5%)	18 (15.5%)	116
	Madunguni	78 (79.6%)	20 (20.4%)	98
	Mbogolo	73 (78.5)	20 (21.5)	93
Kilifi	Jaribuni	97 (92.4%)	8 (7.6%)	105
	Shibe	25 (47.2%)	28 (52.8%)	53
	Mavueni	69 (67.6%)	33 (32.4)	102
Taveta	Kiwalwa	126 (97.7%)	3 (2.4%)	123
	Kimundia	46 (100%)	0 (0%)	46
	Total	**612 (82.5%)**	**130 (17.5%)**	**742**

3.2.4 Different measures taken by community in Malindi, Kilifi and Taveta.

A total of 704 (87.75%) out of 800 households used different methods for mosquito control. In Taveta district 27.43% used mosquito nets, 3.1% used repellants, 2.21% wore long clothing to protect against biting, 15.04% drained stagnant water, 18.14% had mosquito coils in the house, and 16.37% burnt organic matter to keep away mosquitoes while 8.41% screened their windows. These results illustrate the awareness of mosquito control by the communities of Taveta district compared to the other districts. Considering domestic application of insecticides, 9.29% of households in Taveta district used insecticide sprays whereas none (0%) was used in Kilifi and Malindi districts.

Table 8: Different methods used to control mosquitoes in three districts

District	Mosquito nets	Repellants	Clothing	Draining stagnant water	Mosquito coil	Insecticide sprays	Burning organic matter	Screening windows	Total
Kilifi	158(69%)	1(0.44%)	18(7.86%)	0(0%)	4(1.75%)	0(0%)	3(1.31%)	45(19.65%)	229
Malindi	240(96.39)	0(%)	1 (0.4%)	0(0%)	5(2.01%)	0(0%)	3(1.2%)	0(0%)	249
Taveta	62(27.43%)	7(3.1%)	5 (2.21%)	34(15.04%)	41(18.14%)	21(9.29%)	37(16.37%)	19(8.41%)	226
Total	460(65.34%)	8(1.14%)	24(3.41%)	34(4.83%)	50(7.1%)	21(2.98%)	43(6.11%)	64(9.09%)	704

3.2.5 Indoor Residual Spraying coverage in the eight villages

Generally, 0 to 2.94% of households use indoor residual spraying in the eight surveyed villages. These results indicate that the Indoor Residual Spraying (IRS) as a weapon of vector control in the study area is not implemented, except for few houses where it is done by individuals and not the Government.

Table 9: Indoor Residual Spraying (IRS) use in study area

District	**Sentinel site**	**Sprayed**	**Not sprayed**	**Total**
Malindi	Burangi	2(1.75%)	107(93.86%)	114
	Madunguni	0(0.00%)	100(100%)	100
	Mbogolo	3(2.94%)	94(92.16%)	102
Kilifi	Jaribuni	3(2.73%)	47(42.73%)	110
	Shibe	1(1.59%)	17(26.98%)	63
	Mavueni	0(0.00%)	89(87.25)	102
Taveta	Kiwalwa	3(2.19%)	120(87.59%)	137
	Kimundia	0(0.00%)	39(75%)	52
	Total	**12(1.5%)**	**613(76.63%)**	**800**

4.0 CHAPTER FOUR: DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 Discussion

Malaria vector resistance to pyrethroid and other insecticides is a major threat to the gains achieved by use of LLINs and IRS malaria control campaigns in Africa. Knowledge of insecticide resistance levels is important to policy makers within the Ministry of Health in Kenya. This knowledge has an advantage for early planning and development of resistance management strategies in order to safeguard the already existing chemical based vector control tools. In the present study, investigations of *Anopheles gambiae s.l.* susceptibility against Pyrethroids (Deltamethrin (0.05%), Lambdacyhalothrin (0.05%), Organochlorine (DDT 4%), Organophosphates (Fenitrothion 0.1%) and Carbamate (Bendiocarb 0.1%) was conducted along Coastal Kenya based on WHO protocol (WHO 1998a, Matowo *et al.,* 2010). A conventional criterion of separating susceptible and non susceptible mosquito population was defined as mortality rate between 98 to 100%, 24 hours after exposure. A mortality rate of 80-97% shows suspect of resistance while a mortality rate of less than 80% shows resistance to the insecticide.

Suspected resistance was evidenced in eight different sentinel sites of Malindi, Kilifi and Taveta districts to five insecticides. Mosquito mortality against Deltamethrin, showed suspected resistance in Kilifi and Malindi districts, while in Taveta district there was no resistance. Significant difference in mean KDT was only shown in Kilifi district mosquitoes exposed to deltermethrin, whereas those in Malindi and Taveta shows a close association in mean KDT.

Based on KDT_{50} ratios, mosquitoes in Kilifi district showed suspected resistance to Deltamethrin while those in Malindi and Taveta showed no resistance at all. This condition generally is unknown but might be due to uncontrolled use of the insecticide in small scale farming, and misuse of ITNs such as fishing. The findings similar to this have been reported in West Africa

such as Ivory Coast and Burkina Faso where the mortality by deltamethrin was less than 40% while in southern Benin the mortality was between 30 - 40% (N'Guessan *et al.,* 2007, 2010, Tungu *et al.,* 2010, Koudou *et al.,* 2011, Yadouleton *et al.,* 2009, Diabate *et al.,* 2002a,b).

Bed nets and indoor residual house spraying remain the major control strategy against malaria vectors. Deltamethrin is the most used pyrethroid in agriculture and public health, thus resistance suspects of this compound should be taken into serious consideration before widespread (Etang *et al.,* 2003b). Mortality by the Lambdacyhalothrin insecticide showed suspected resistance in the three districts under study. There was a significant difference in mean KDT in Malindi and Taveta districts. The KDT_{50} ratio showed suspected resistance in Malindi and Taveta districts while there was no resistance to Lambdacyhalothrin in Kilifi district. The resistance to mortality by Lambdacyhalothrin in Kilifi district might be due to the mosquito population having the resistance dominant. Observation made by Matowo and others (2010) reported that the population with a recessive gene or at a low frequency dose, the use of mortality as an indicator of resistance may lack necessary precision unless the population of mosquito is dominant.

A hundred percent mortality of mosquitoes against DDT was observed in all three districts. However, there was a significant difference in mean KDT in Kilifi and Taveta districts. Based on the KDT_{50} ratio, there was suspected resistance to DDT in Kilifi and Malindi districts while in Taveta there was resistance to DDT. This observation could be due to the presence of recessive resistance in the mosquito population of the three districts.

This result is consistent with mortality rate against DDT in Kilifi while in Malindi KDT_{50} ratio indicated suspected resistance whereas Taveta showed more evidence of resistance with high

KDT_{50} for DDT. These results are similar to the study conducted by Davidson (1951) at Taveta Southern Kenya which investigated the use of DDT and BHC against *An. gambiae s.l.* He reported that, only 80% of exposed mosquitoes to DDT treated huts died within 24 hrs which proved to be less susceptible strain. The history shows that DDT was used by Pare Taveta IRS project under the African Fighting Malaria initiative implemented between 1955 and 1959 resulted in a complete disappearance of *Anopheles funestus*, but when the project ceased both vector abundance and malaria transmission rate increased. Although Kenya had officially stopped the use of DDT in 1986, these chemical substances have long residual effect in the environment therefore can contaminate mosquito breeding sites. The persistence of DDT in Kenyan environment has not been studied separately from other pesticides, but most published data are comparative analysis of DDT with Organophosphate and Carbamate (Saoke 1985). However, evidence on the use of DDT has been detected mostly in water and soil samples from the Indian Ocean Coast of Kenya along river Sabaki and Kiwaya bay (Lalah 1993, Everaats *et al.,* 1996).

The mosquito population in all three districts, based on mortality rate was susceptible to Fenitrothion. Kilifi district, mosquito population was susceptible to Bendiocarb whereas Malindi and Taveta showed suspected resistance. These results revive hope on the alternative insecticides in malaria vector control. The resistance of *Anopheles gambiae sl* against pyrethroid insecticides was first discovered *in* Côte d'Ivoire and Cameroon (Elisa *et al.,* 1993). Later on, many other cases of pyrethroid resistance in *Anopheles* vectors were detected in Central, Eastern and Southern Africa (Vulule *et al.,* 1994,1999, Hargreaves *et al.,* 2000 , Diabate *et al.,* 2002, Etang *et al.,* 2003, Erlanger *et al.,* 2004, N'Guessan *et al.,* 2007,2010).

In western Kenya, a study conducted by Kamau and others (2007) showed mortality rate after 24 hour exposure to be 100% with Permethrin, Bendiocarb, and Fenitrothion but were slightly reduced with Lambdacyhalothrin and DDT (Chandre *et al.,* 1999). Permethrin is a good indicator when using knockdown time to monitor resistance (Pivora 1975, Elissa 1993, Kang *et al.,* 1995, Chandre *et al.,* 1999) but in the present study Deltamethrin and Lambdacyhalothrin were used as an alternative. Furthermore, KDT_{50} and KDT_{95} found in this study were similar to those observed in *Anopheles gambiae s.l.* population categorized as non susceptible in Multi-Country study, whereby the pyrethroid insecticide was shown to be a suspect of resistance to tested mosquito population (Ranson *et al.,* 2009). This is the first investigation of its kind done along the Coastal Kenya where the results indicate development of early stages resistance to pyrethroid insecticides.

Despite of assessing insecticide resistance, this study also collected information on the coverage and use of long lasting net, source of nets and different methods used by the community to protect themselves against mosquito bites. The use of long lasting insecticide nets in this study was defined as households which reported to have slept under the LLINs during the night preceding the survey. The use of long lasting insecticide net for children below 5 years in Taveta and Malindi was higher than in Kilifi district. This implies that the children of Taveta and Malindi are more protected against effective bites of mosquitoes compared to their counterparts in Kilifi. Mavueni village had the lowest usage followed by Shibe and Jaribuni villages. The low coverage of usage of long lasting insecticide nets in Kilifi district compared to other districts may possibly be due to lack of knowledge, insufficient community sensitization, low socio-economic status, belief and altitude and availability. The results support those of Chuma and

others (2010) who sought to identify and address the barrier to access and use of ITNs in the poorest populations of Kenyan. These findings suggest scaling up of long lasting nets use in Kilifi in order to control malaria transmission effectively. Generally 61% of the ITNs use to under five children is much lower than the National target of 80% coverage. Nonetheless, these findings suggest more efforts on community sensitization towards rural communities.

The study revealed that most of LLINs were distributed through Kenya government programmes (GoK), the cost of which was subsidized. There were some few nets from local markets. Similar results were found in a study done in poor population Kenyan to identify the barrier of ITNs distribution which observed that, the main source of ITNs/LLINs were from Government health facilities at 65.9% and the retail sector (local sources) at 16.9% (Chuma *et al.*, 2010). Jaribuni village in Kilifi district had the lowest coverage of long lasting insecticide nets from the Government program most of mosquito nets were from the local market. In Jaribuni village the distribution of net through the Government program possibly was not done sufficiently.

Mosquito nets distributed in high malaria prevalence areas were reported to lose their quality after one year because of getting worn out (Protopopoff *et al.*, 2007). In the present study Malindi and Kilifi sentinel sites showed high coverage of worn out long lasting insecticide nets. This condition could be influenced or related to socio-economic status. The wearing out of nets could also be due to the use of wooden sticks for supporting the nets, open tin lamps which can burn the nets and rats gnawing through net when eating mattresses. A study done in Tanzania by Maxwell and others (2006) revealed that 44.9% of nets were worn out, whereas Erlager and others (2004) also reported that 40% of observed nets were in poor condition. In addition, some

households abandoned mosquito nets outside the houses, some people used them for fishing while others fenced their domestic animals such as chicken (Figure 2.6 and 2.7).These findings along coastal Kenya districts might be influenced by accumulation of old nets after reception of new mosquito nets during the free net campaign, or the affected communities do not value things which are provided free of charge. It has also been reported that 84.5% of people around Lake Victoria use freely distributed and subsidized mosquito nets for fishing. In Ethiopia, communities preferred to use their own purchased mosquito nets compared to freely distributed nets (Baume *et al.,* 2009). The accumulation of unused mosquito nets in the household might be one of the causes of abuse of this important weapon in malaria control.

The study also investigated the knowledge, attitude and practice of the residents in malaria intervention. Residents of Taveta district were shown to be more active in implementation of malaria control activities compared to other districts. The efforts of controlling malaria in Taveta have been on-going since 1955 when the Pare Taveta project was implemented to eradicate *An. funestus*. In addition, the high population of mosquitoes in Taveta district could have also made residents therein to have multiple intervention strategies. Malindi district follows closely behind Taveta in malaria control intervention strategies due to the ongoing larvicidal applications, while Kilifi residents had the lowest knowledge and attitudes on malaria control intervention.

Children in Taveta and Malindi district looked well protected against mosquito bites compared to Kilifi due to use of long lasting insecticide nets that protect them from nuisance biting and act as repellant to the mosquitoes. Thus the exposure to mosquito biting children in Kilifi district may lead to transmission of malaria and other vector borne diseases.

In combating malaria disease, the community had employed different ways of reducing transmission. The study revealed that Taveta district residents used multiple strategies such as

use of nets, burning organic matter, mosquito coils, use of long clothing, cleaning the drains, use of insecticide and screening windows in the houses. Malaria as a disease cannot be controlled by one approach as such multiple approaches are needed for elimination or control so that the disease does not cause any public health problems. The use of repellants, mosquito coils and aerosol insecticides lower the risk of developing severe malaria (Snow *et al.*, 1998). This implies that Taveta district residents have the lowest risk of developing malaria compared to Kilifi and Malindi residents.

The use of indoor residual spraying (IRS) is important against mosquitoes that land on the walls and materials in the house. This study revealed that currently no IRS program is active in all three districts. IRS when used together with LLINs has a high risk of enhancing resistance development especially when same classes of insecticide are used. In the 3 districts, LLINs efficacy could be safeguarded and improved by targeting blood seeking *Anopheles* mosquitoes both indoor and outdoor. Hence, a variety of vector control tools packaged together in an integrated vector management (IVM) strategy would be ideal in suppressing mosquito population (WHO 2004). The IVM strategy in the 3 districts would utilize vector control tools such as LLINs and habitat based management strategies like larviciding and environmental management (Gu *et al*., 2006, Killen *et al*., 2000). Habitat management aims at achieving larval source reduction which would be ideal, when coupled with LLINs in reducing both vector populations and biting nuisance mosquitoes. Only LLINs is the primary malaria vector intervention along coastal Kenya. Applicability of IRS in the 3 districts is a major challenge due to the type of building materials and the house designs in the sentinel sites of this study.

4.2 Conclusion

I. The finding from this study, being the first of its kind for the region has greatly contributed to the knowledge of mosquito resistance to insecticide. Such knowledge is a key to the future management of mosquito resistance to insecticides, not only for the coastal region but for other region in Kenya.

II. Different levels of resistance from different insecticides were detected in the study area. Based on mortality, *Anopheles gambiae s.l.* were susceptible to DDT4% and Fenitrothion 0.1% in all three districts. Suspected resistance to Deltamethrin in Kilifi and Malindi has detected; Lambdacyhalothrin in all three districts, while Bendiocarb had a resistance suspect in Malindi and Taveta.

III. The coverage of long lasting insecticide nets was 75.9%, for all three districts which is below the WHO/UNICEF target of 80%. The children below 5years of age who used LLINs in the night preceding were 41.6% in Kilifi, 72.3% in Malindi and 61% in Taveta.

IV. Furthermore the coverage of LLINs usage for children under five years is higher in Taveta and Malindi than in Kilifi.

4.3 Recommendations

- It is therefore strongly recommended that the impact of this development on malaria control efforts be closely monitored before this problem becomes widespread in Coastal Kenya.
- In the future, there is need to determine the mechanism of resistance by conducting PCR for kdr analysis and biochemical assays for detection of metabolic enzymes.

- Basing on this baseline data, there is need to continue monitoring mosquitoes at least twice in a year to determine resistance levels, and Ministry of Health and other interested stakeholders should develop keen interest in resistance monitoring.
- The coverage usage of LLINs for below five years children is low, therefore the Ministry of Health should increase effort on sensitization, mobilization and distribution in order to reach the WHO set target of 80%

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