CHAPTER ONE

INTRODUCTION

1.1. Background

Despite vigorous efforts targeted against malaria, the disease remains a major world health problem. It affects people of all ages and races, however, pregnant mothers and children under the age of five years are the most susceptible groups in the population. Although there are four common parasite species causing malaria in humans, *Plasmodium falciparum* is the most dangerous and the major cause of death during an acute attack of malaria. Falciparum malaria is also the commonest type in sub-Saharan Africa (WHO, 2003, Snow *et al*, 2005). There are about 255 million cases reported worldwide and more than 781,000 people died in 2009 (WHO, 2010).

Malaria is transmitted by the infective bites of *Anopheles* mosquitoes during blood feeding from a human host. The most important African malaria vectors are members of the *Anopheles gambiae* Giles complex and the *An. funestus* Giles group (Gillies & Coetzee, 1987). Their patterns of abundance, behaviour and distribution over a range of habitats and ecotypes are complex and this contributes to difficulty in implementing appropriate control measures. However, the WHO (2006) recommends indoor residual spraying (IRS) and the use of long lasting insecticide nets (LLIN) for effective malaria vector control.

1.2. Malaria vectors in Zanzibar

The history of malaria vectors in Zanzibar was first recorded in 1934 by McCarthy who conducted a comprehensive survey that found both *Anopheles gambiae* s.l and *An. funestus* resting inside houses with high sporozoite rates (Odetoyinmbo and Davidson, 1968). In 1958 Iyengar (1962) also found *Anopheles gambiae* resting inside houses in Pemba and discovered another species of *Anopheles gambiae* s.l. breeding in salt water but was not able to distinguish them from those breeding in fresh water. These mosquitoes were found to be susceptible to dieldrin used for IRS. However, DDT resistance was recorded after several rounds of indoor residual spraying in Zanzibar (Curtis *et al,* 1983). When malaria eradication campaigns were launched in the 1950s, IRS was the main tool. Dieldrin was used on Pemba whereas DDT was used on Unguja island. After implementation of IRS *Anopheles gambiae* was not found resting inside houses. Instead, a large number of malaria vectors were collected through outdoor methods (Iyengar, 1962; Odetoyinmbo and Davidson, 1968; Zahar, 1985). By 1968, malaria was no longer a public health problem and the programme was abandoned due to political and other factors. As a result, in the 1970s malaria re-emerged as a major public health concern and by 1973, prevalence rates were 54% in Unguja and 10% in Pemba (AFM, 2008). Vector control activities resumed in 1981 when IRS was re-introduced complemented by larviciding, source reduction, and Ultra Low Volume Spraying (ULV). The project ended in the late 1980s and Zanzibar experienced the second malaria resurgence.

In 2005 an entomological survey (unpublished ZMCP report, 2005) was conducted with technical assistance from the University of Dar-Es-Salaam, and found the most

prevalent malaria vector in Zanzibar was *Anopheles gambiae* s.s (94.7%) with endophagic and endophilic behaviour. However, other members of the *An. gambiae* complex were present and *An. arabiensis* constituted 4% and *An. quadriannulatus* 1.3%. *Anopheles merus* was not detected during this survey. The human blood index was 100% in Pemba and 80% in Unguja and the annual entomological inoculation rate was 0.8 in Zanzibar. However, a person in Pemba would receive approximately 16.3 infectious bites per year compared with 8.6 bites per person per year in Unguja. Earlier in 2000, malaria vectors in Zanzibar were found to be almost fully susceptible against pyrethroids and DDT (ZMCP unpublished data). These data were collected before implementation of country-wide indoor residual spraying (IRS) and large scale coverage of long lasting insecticide nets (LLINs). Integrated vector management (IVM) that includes the use of long lasting insecticide nets and indoor residual spraying is the principal strategy for vector control in Zanzibar. Six rounds of IRS have been done so far using lambda-cyhalothrin (ICON 10WP/CS) with coverage of over 90% of the country since 2006. Considering the two main interventions targeting *An. gambiae* s.s., changes are expected over time in malaria species composition.

Integrated vector management (IVM) in combination with other interventions has resulted in the dramatic reduction of malaria prevalence in Zanzibar from 40% to 0.8% over the past five years. This outcome was obtained after several years of campaigning against the disease (Bhattarai *et al*, 2007). A big challenge ahead of the programme is to sustain that achievement and avoid malaria resurgence.

In addition, a major concern for many malaria vector control programmes is insecticide resistance that is increasing in places where IRS and LLINs are implemented,

especially when pyrethroids are used on both nets and walls as is the case in Zanzibar. A wide range of resistance to different insecticide classes has been reported from various parts of the world including India, Asia and Africa (Hemingway & Ranson, 2000, Lindsay *et al*, 2004). As a result, a robust programme for monitoring insecticide resistance in the local malaria vectors should be in place so that control efforts are not compromised if resistance emerges.

1.3. Role of long lasting insecticide nets (LLINs) in malaria control

WHO (2006) emphasizes the use of ITNs/LLINs in malaria control and regards this as a key intervention. This protects humans by preventing mosquito bites during those hours when people are sleeping. Historically, many people used untreated mosquito nets for protection against nuisance mosquitoes but the pyrethroids used to treat nets or modern LLINs (where net fibres are coated or impregnated with insecticides during manufacture) also help to repel and kill insects, even when the nets are damaged. This is proving to be an important way of controlling the spread of malaria (WHO, 2010).

When a large percentage of people in a community use ITNs/LLINs, a mass "community effect" is found that protects even those who do not use nets (Najera and Zaim, 2003). A mathematical model suggests that under the typical transmission conditions that prevail in rural Africa, when 35-65% of the entire population is covered by an LLIN, even those individuals and households that do not own or use a net are protected (Killeen *et al*, 2007).

WHO (2005) defined LLIN as “a factory-treated insecticidal mosquito net which is expected to retain its biological activity for a minimum number of standard WHO washes over a minimum period of time under field conditions”.

1.4. Long lasting insecticide nets scaling-up in Zanzibar

The benefits demonstrated by effective use of LLINs in reducing morbidity and mortality, proved that LLINs are a cost effective intervention for malaria control (Lengeler, 2009). In this regard, the Zanzibar Malaria Control Programme (ZMCP) started free LLINs distribution in 2006 targeting mainly pregnant mothers and children under the age of five years. However, as the prevalence of malaria decreased (0.8%) the entire population remained at risk against the disease and consequently, the nets are targeted at the general population. Although the WHO (2005) states that LLINs remain effective after 20 WHO standard washes and lasts for three years under field conditions, it should be understood that washing patterns, practices and drying methods as well as climatic conditions can vary significantly from one place to another. Consequently, these differences could have a great impact on net efficacy and malaria prevention in general (Smith *et al*, 2007). Therefore, a strong monitoring system is necessary so as to capture important information on the durability and efficacy of these nets for rational decision making.

1.5. Rationale

Based on the low malaria prevalence in Zanzibar, the question has been raised as to when the country-wide indoor residual spraying should be stopped. The decision would be based on both entomological and parasitological findings. If IRS is stopped, LLINs would be the only remaining strong tool to combat the disease at this critical stage when ZMCP is approaching the pre-elimination phase. Universal coverage of LLINs is most important to make sure that the entire population is protected. The community-wide LLIN coverage ensures that both vector density and longevity is affected. Again the excito-repellent effect emitted by pyrethroid compounds is useful in reducing the number of blood meals on humans. Consequently *Anopheles* mosquitoes are forced to find alternative host (animals) and ultimately this reduces malaria transmission (Killeen *et al*, 2007). Three years ago every household received two LLINs in Zanzibar. The Zanzibar Malaria Control Programme plans to replace the nets assuming that they are already showing less insecticidal effect and are torn allowing penetration by mosquitoes. This fact needs to be confirmed or supported by necessary field evidence so as to justify the replacement. Therefore, the aim of my study was to determine the efficacy of LLINs in the study area taking into consideration the species of malaria vectors found in the area and the susceptibility status of those mosquitoes against the pyrethroids used on LLINs. The information obtained is crucial to help make decisions on the periodicity of LLINs distribution as well as selection of the right brand of LLINs for our setting.

1.6. Broad objective

The main objective of this study was to assess the durability and efficacy of long lasting insecticide bed nets and determine the distribution of malaria vectors in Zanzibar.

1.7. Specific objectives

1. To identify malaria vector species through indoor and outdoor mosquito collections and species identification using PCR

2. To determine the susceptibility status of malaria vectors against the pyrethroids used on long lasting insecticide bed nets through insecticide resistance assays using impregnated papers

3. To assess the wear and tear of LLINs under regular use in the field environment

4. To determine the efficacy of LLINs using a modified cone bioassay.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Study sites

This cross-sectional descriptive study was carried out in the West District of Unguja and North Pemba, Zanzibar, between January-June, 2011. The West District covers an area of 209 sq km with about 262,676 people whereas North Pemba has a population of 221386 people estimated from the 2002 population and housing census.

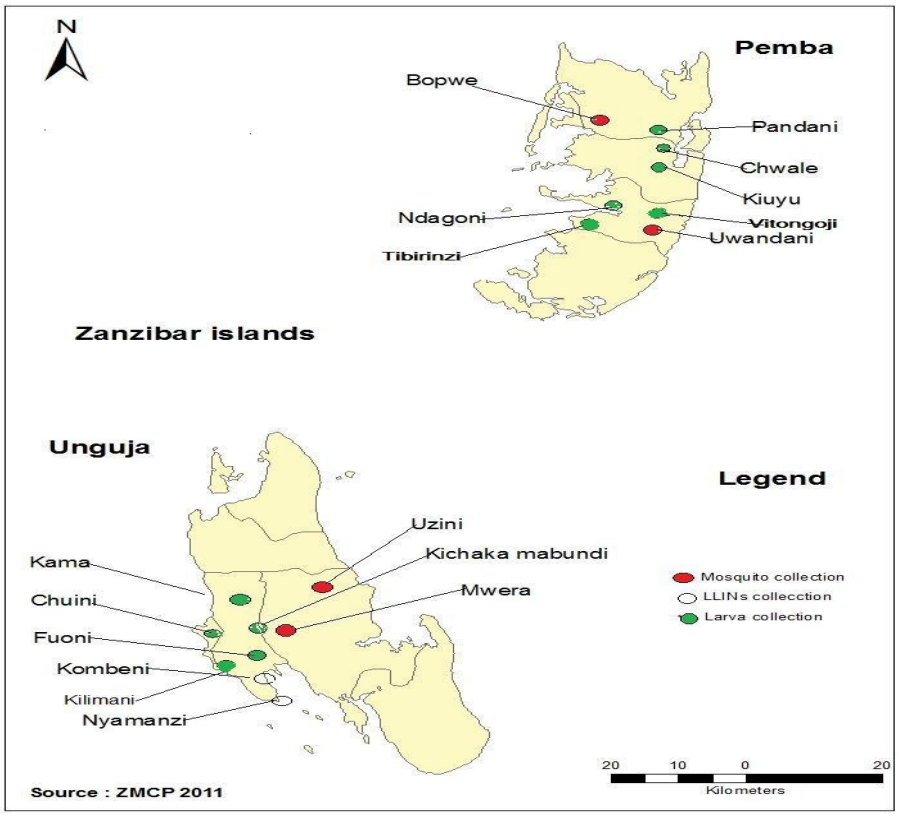


Fig. 2.1 Map of Zanzibar showing study sites

2.2. Mosquito sampling

This was done through indoor and outdoor mosquito collections, in which four sites were randomly selected. The selection criteria included the previous history of malaria, accessibility, and community participation. Mosquitoes were collected from Mwera and Uzini in Unguja, Bopwe and Uwandani in Pemba (Fig. 2.1). Collections were made fortnightly from each site making a total of six visits per site and twenty four visits for all sites between January and April, 2011. For the purpose of identifying malaria vector species in the study sites the following sampling methods were used as outlined by Service (1976) and WHO (1992).

2.2.1 Pyrethrum spray catch

Five houses were randomly selected from each site. Early in the morning, after spreading a white sheet on the floor of a single room per house, the room was sprayed with pyrethrum extract (25 %) diluted with kerosene to 0.3 %. A pair of spray-men, one indoor, and the other outdoor sprayed all round the room for approximately 3 minutes. The room was then closed and left for 10 minutes; the sheets were then carefully removed. Mosquitoes, that had been knocked down by the effect of pyrethrum were picked up using fine forceps (Fig. 2.2) and kept in a petri dish lined with moist cotton wool covered with white filter paper (Whatman No. 1). The petri dishes were then kept in a cool box for transporting back to ZMCP laboratory. This method usually collects blood fed indoor resting mosquitoes.

Fig. 2.2 Research assistants picking mosquitoes knocked down following PSC in a house at Uzini, Zanzibar.

2.2.2 Pit trap collection

Two pits of 5x4x3ft (length, depth, width) were dug at each site a short distance from human habitations preferably under a tree (Fig. 2.3). Small cavities, about 1.5 - 2ft from the bottom of the pit, were dug into each of the four sides, to provide dark sheltered areas for the mosquitoes to rest. Mosquitoes exhibiting exophillic behaviours are caught in this type of trap. Collections were made at dawn with a torch and a mouth aspirator. Mosquitoes were transferred to paper cups and stored in a picnic box with wet towels (to maintain humidity) before being transported to the ZMCP laboratory for further processing.

Fig. 2.3 Pit trap used for outdoor collection at Mwera, Zanzibar

2.2.3 Light trap collection

Two CDC light traps (Fig. 2.4) were set indoors per site between 18.00-06.00 hours. The traps were set up next to untreated bed nets to collect adult mosquitoes looking for a blood meal. A single trap was set per room and a total of two traps were set each sampling night. During the morning traps were removed for further sample processing.

When the field work was completed for a particular day, all paper cups, and petri-dishes containing mosquitoes were transported in a cool box lined with ice packs and covered with a damp towel so as to maintain a cool humid environment. In the laboratory, the mosquitoes were killed by putting them into a freezer and this was followed by sorting.

All culicines and male anophelines were discarded. The remaining anophelines were morphologically identified using the *Anopheles* identification key (Gillies and Coetzee, 1987). The mosquito specimens were then preserved individually on silica gel in a labeled eppendorf tube for PCR identification.

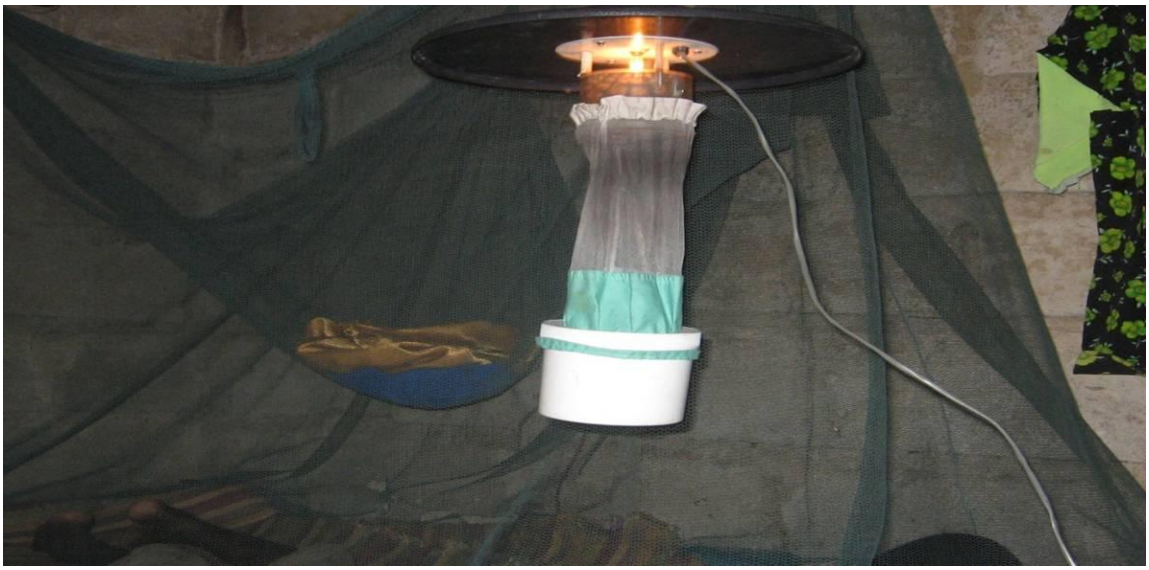


Fig. 2.4 Light trap set for mosquito collection at Mwera, Zanzibar.

2.3. Polymerase Chain Reaction (PCR) assays

Following morphological identification of collected mosquitoes, all members of the *Anopheles gambiae* complex were identified to species by PCR assay using the Scott *et al.* (1993) method.

The polymerase chain reaction (PCR) is an *in vitro* enzymatic synthesis and amplification of specific DNA sequences of interest using oligonucleotide primers.

The PCR assay has been developed to distinguish between members of the *Anopheles gambiae* complex. The assay uses diagnostic primers which bind to species specific nucleotide sequences in the ribosomal DNA intergenic spacer region.

2.4 Susceptibility test for pyrethroids

For the purpose of this study, three insecticides were tested i.e. deltamethrin (0.05%), lambda-cyhalothrin (0.05%) and permethrin (0.75%), as pyrethroids are the only insecticide class allowed for use on long lasting treated nets. WHO guidelines (1998) were used to conduct the tests. *Anopheles* mosquito larvae were collected from various breeding sites (Fig. 2.5) and reared to adults in the ZMCP insectary on Unguja and Mkoroshoni on Pemba. Tetramin® fish food was used to feed mosquito larvae. Pupa were picked daily using a pipette and transferred to numbered cages made up using 6mm iron bars and covered with untreated netting materials. Twenty five, 2-5 day old, non-blood fed *Anopheles* females were exposed to insecticide impregnated papers for 60 min using the standard WHO resistance assay. At the end of the 60 min exposure, the mosquitoes were transferred to untreated holding tubes and provided with a sugar solution (10%) (WHO, 1998). Knock down was recorded after 60 min post exposure (KD60 - number of mosquitoes knocked down at the end of sixty minutes exposure to insecticide) and % mortality was noted after 24 hours. Four replicates (100 mosquitoes) were tested for each insecticide. Assays were conducted at 27±2°C and 80±10% Relative Humidity. A single group of mosquitoes acted as the control. These were subjected to the same conditions as the treated mosquitoes, but without insecticide.

Fig. 2.5 Potential breeding sites for *Anopheles* at Kiuyu in Pemba island

2.5. Bed net studies

2.5.1 Bed nets collection from the field

One hundred and fifty (150) LLINs were collected from randomly selected households (Kombeni and Nyamanzi villages) as the ZMCP rolled out a net replacement campaign. Each collected net was packed individually in a clean plastic bag, labelled for identification and taken back to the ZMCP laboratory for further investigation.

2.5.2 Physical inspection of the nets

All LLINs were examined to identify the brands and manufacturer, other variables like number of washes and drying methods were recorded by the ZMCP. All 150 nets collected were evaluated at the ZMCP laboratory. To facilitate physical evaluation the

nets were draped over a cube-shaped frame (about the size of the net – 160x150x180cm) constructed using PVC pipes and fittings. A dark plastic sheet was wrapped around the frame to facilitate a clear contrast with the net (see Fig. 3.3). The net was examined carefully and all tears and holes were measured. Seam failures were also noted and recorded. Evidence of repairs to the net fabric, type of repair and in the case of sewn repairs, length of stitching on repair was also measured and recorded (Smith *et al*, 2007).

2.5.3 Bio-efficacy test of LLINs

Following the physical examination of the nets for their physical integrity, subsets of 50 nets were randomly selected from the collection of 150 for the bioassays tests. A double cone method for assessing mortality and knock down was used to evaluate the LLIN efficacy. In this method 4 pairs of cones, each pair sandwiching an area of netting, were supported by two pieces of cardboard (Figs. 2.6 & 2.7). The assemblage was held in place using clips. Upon releasing the mosquitoes (laboratory reared susceptible *Anopheles gambiae* s.s. R70) into the cones, mosquitoes that penetrated through the large mesh of the Olyset net were contained by another cone. This reflects the behaviour and insecticide exposure that occurs more realistically than single cone designs in which the large mesh size allows mosquitoes to rest without contacting the netting threads, or in which the mesh is doubled over and so decreases the mesh size but increases the dose per area. For every 10 replicates of 5 mosquitoes, four cones of 20 mosquitoes were also run as control groups. Fifty old nets and ten new LLINs were

tested against the susceptible strain. This allowed a comparison of insecticidal efficacy of the nets. Furthermore ten old and two new LLINs were tested on both susceptible and wild mosquitoes on Pemba. Bioassays using the wild strain allowed an assessment of the impact of resistance.

A 30cm x 30cm piece of netting was removed from the long side of each net and tested using a susceptible *Anopheles gambiae* mosquito colony from ZMCP insectary (originated from NIMR Muheza, Tanzania) and field mosquitoes from Pemba. Five female mosquitoes (2-5 days old non blood fed) were introduced into each cone for 3 minutes. Mosquitoes were then transferred to paper cups and provided with 10% glucose solution soaked in cotton wool (WHO, 2005). The test was repeated until a total of 50 mosquitoes had been exposed on each LLIN tested (10 replicates); therefore, each LLIN sample was tested three times on the same unit. Knockdown was scored at 60 minutes post exposure and % mortality recorded at the end of the 24 hours. Mosquitoes exposed to untreated nets were used as controls. The cone bioassay test was carried out in a humid environment at 27±2°C and 80±10% Relative Humidity (WHO, 2005). New LLINs were tested alongside the old ones so that the degree of insecticide deterioration amongst the nets could be compared.

Fig. 2.6 Double cone method of LLIN testing

Fig. 2.7 Double cone method of LLIN testing "photo: Pierre Guillet, WHO”.

2.6 Data management and analysis

All collected data were maintained in MS Excel. Descriptive statistics for the physical state of the nets were collated. These described the age of the nets, the numbers and percentages holed, and the numbers of repairs undertaken. Means of these measures and of insecticide efficacy (mortality / knockdown) were calculated. Abbott's formula was used to calculate corrected mortalities for all bio-efficacy tests where control mortality was between 5 and 20% (WHO, 1998; 2005). Results of susceptibility tests were analysed as: 98-100% mortality indicates susceptibility, 80-97% mortality suggests the possibility of resistance that needs to be confirmed and <80% mortality suggests resistance (WHO, 1998). Bio-efficacy result of LLINs was analyzed based on WHO (2005) that minimal effectiveness is KD60 ≥75% or mortality ≥ 50% whereas optimal effectiveness should be KD60 ≥ 95% or mortality ≥ 80%.

CHAPTER THREE

RESULTS

3.1. Mosquito sampling

Of all collected mosquitoes 9921 (94%) were nuisance biting *Culex;* these were mainly caught through pyrethrum spray catch and light trap methods. No *Culex* was collected from pit traps. A total of 596 (6%) *Anopheles* mosquitoes were collected from the four selected sites. All collections were identified using the *Anopheles* morphological identification key (Gillies and Coetzee, 1987). All those belonging to the *Anopheles gambiae* complex were stored for subsequent identification by PCR. The *Anopheles gambiae* group made up all malaria vector species in Zanzibar during this study.

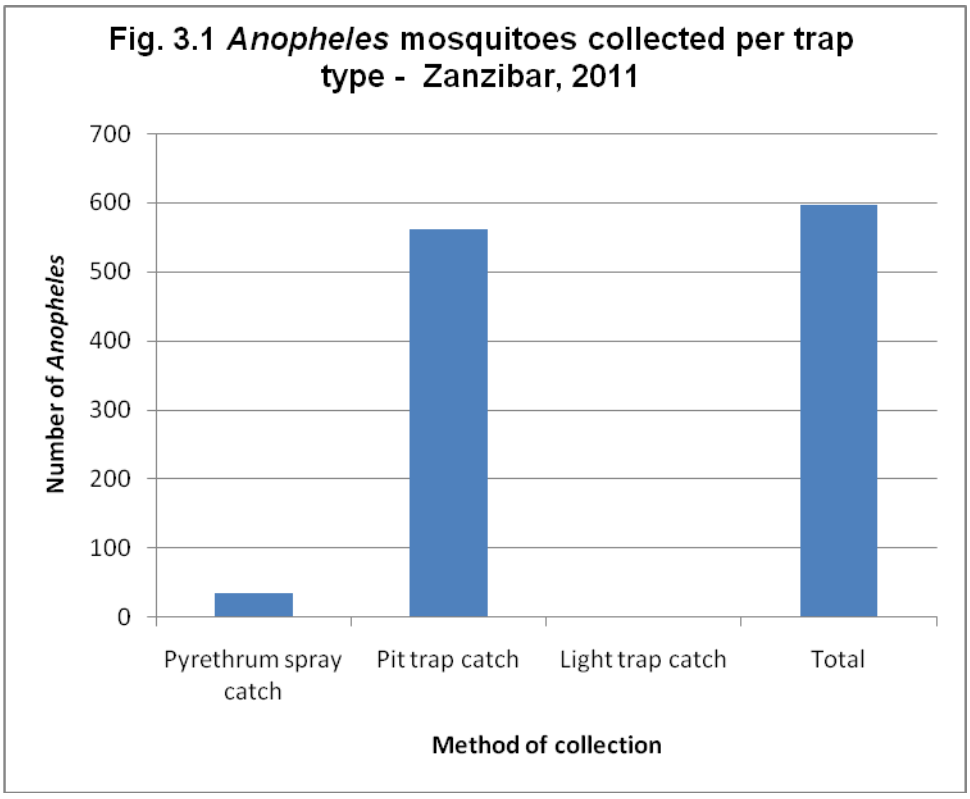
Pemba sites (Uwandani and Bopwe) had higher mosquito catches than the two sites on Unguja (Table 3.1 and 3.2). Over 94% of all *Anopheles* mosquitoes were collected from pit traps on both islands (Fig. 3.1), indicating a high level of outdoor resting behaviour. The pyrethrum spray catch method was successful in collecting the indoor resting mosquitoes while the CDC light trap collected none (Fig. 3.1). Instead, the light trap collected a huge number (9921) of *Culex* mosquitoes.

Table 3.1 *Anopheles* mosquitoes collected from two sites on Pemba, January - April, 2011

Site	***Anopheles* group collected**		Percent
	gambiae	***funestus***	
Uwandani	411	0	97.6
Bopwe	10	0	2.4
Total	**421**	**0**	**100**

Table 3.2 *Anopheles* mosquitoes collected from two sites on Unguja, January - April, 2011

Site	*Anopheles* group collected		Percent
	gambiae	***funestus***	
Mwera	175	0	100
Uzini	0	0	0
Total	**175**	**0**	**100**

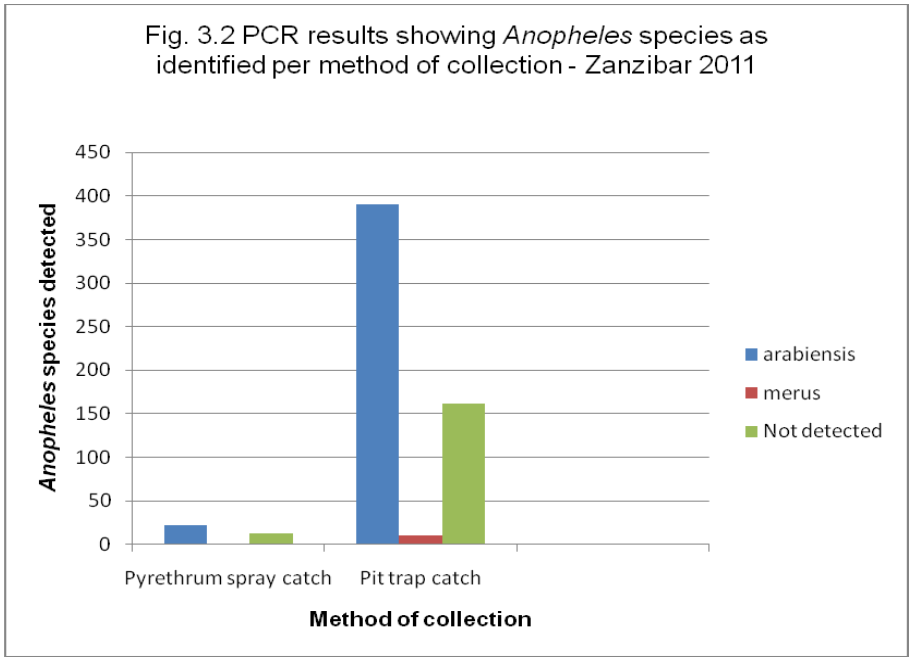


3.2. Polymerase Chain Reaction (PCR) assays

Identification of the sibling species of *Anopheles gambiae* s.l. was undertaken at Ifakara Health Institute, Ifakara, Tanzania. The results are summarized in Table 3.3. In total, 412 *An. arabiensis,* and 10 *An. merus* were identified of the total 596 *Anopheles* collected. The PCR assay did not identify 174 (29%) specimens which could be the result of wrong morphological identification or decomposed samples. All *An. merus* (10) and over 94% (390) of all *An. arabiensis* were collected in pit traps. This indicates outdoor resting behaviour of these species (Fig. 3.2).

Table 3.3 Summary of species identification

Site	*Anopheles gambiae* group collected			
	merus	***arabiensis***	**Unidentified**	**Total**
Pemba	0	315	106	421
Unguja	10	97	68	175
Total	**10**	**412**	**174**	**596**



3.3. Susceptibility test for pyrethroids

Results given in Table 3.4 show strong resistance in Pemba adult anophelines reared from wild larvae against pyrethroids used on both indoor residual spraying and long lasting nets. All three pyrethroids tested on Pemba island revealed less than 80% mortality, lambda-cyhalothrin 9% mortality, deltamethrin 36% and permethrin 51%. On the island of Unguja (Table 3.5) however, cohort were fully susceptible to deltamethrin and permethrin but more investigations are needed on lambda-cyhalothrin. PCR results on tested mosquitoes (both dead and survivors) have shown 100% *An. arabiensis* (n= 600), however, 7/150 from (Tibirinzi, Pemba) control group were identified as *An. merus*.

Table 3.4 Susceptibility test results of *Anopheles gambiae s.l.* against pyrethroid - Pemba

Insecticide	Mosquito tested	% KD at 60 Min	24hrs % Mortality
Lambda-cyhalothrin	100	22	9
Deltamethrin	100	69	36
Permethrin	100	64	51
Control	75	0	0

Table 3.5 Susceptibility test results of *Anopheles gambiae s.l.* against pyrethroid - Unguja

Insecticide	Mosquito tested	% KD at 60 Min	24hrs % Mortality
Lambda-cyhalothrin	100	90	95
Deltamethrin	100	99	99
Permethrin	100	99	99
Control	75	0	0

3.4. Bed nets collection from the field

Of 150 nets collected, 149 were Olyset^{TM} Nets and the remaining 1 was a PermaNet that was too old to identify its version. One Olyset net was found in its original package, i.e. the net had never been used since it was distributed three years previously. LLIN data presented in Table 3.6 show the number and types of nets collected for this study.

Table 3.6 Long lasting nets collected from two villages of West District in Zanzibar, January, 2011

Village	**LLIN collected**				**Total**
	Olyest Net	**%**	**PermaNet**	**%**	
Nyamanzi	99	66	1	0.7	100
Kombeni	50	33.3	0	0	50
Total	**149**	**99.3**	**1**	**0.7**	**150**

3.5. Physical inspection of the nets

Based on the information from the ZMCP it was found that some people feel ashamed to admit to the actual washing frequency of the net. Some nets were very dirty and some were found covered in soot from wood fires (wood is the most common source of fuel in rural areas). Despite this some owners insisted that the nets were washed monthly. The study found the mean number of washes was 23 ranging from 3 to 36. Again the ZMCP reported that hanging a washed LLIN to dry either on shaded places or inside houses was found to be the usual method for net drying in the study area (89/150 or 59.3%). A physical examination of the nets revealed 1298 holes of >0.5cm with an average of 10.3 holes per net.

More than 61% of these holes were located on the lower or bottom part of the nets and 4.5% of holes (1.3 per net) were found on the LLINs roof. Nets had an average of 5.3 larger holes of >5cm (Fig. 3.3) and there were 41 seam failures identified from 18 LLINs with a mean of 2.3 per net. The most common methods for net repairs were hand stitching (204 or 63% of repairs), knots (108 or 33%) and patches (11 or 3%).

Fig. 3.3 Damaged LLINs being examined on a plastic frame showing distribution of large holes (>5cm) and knots as a method of net repair.

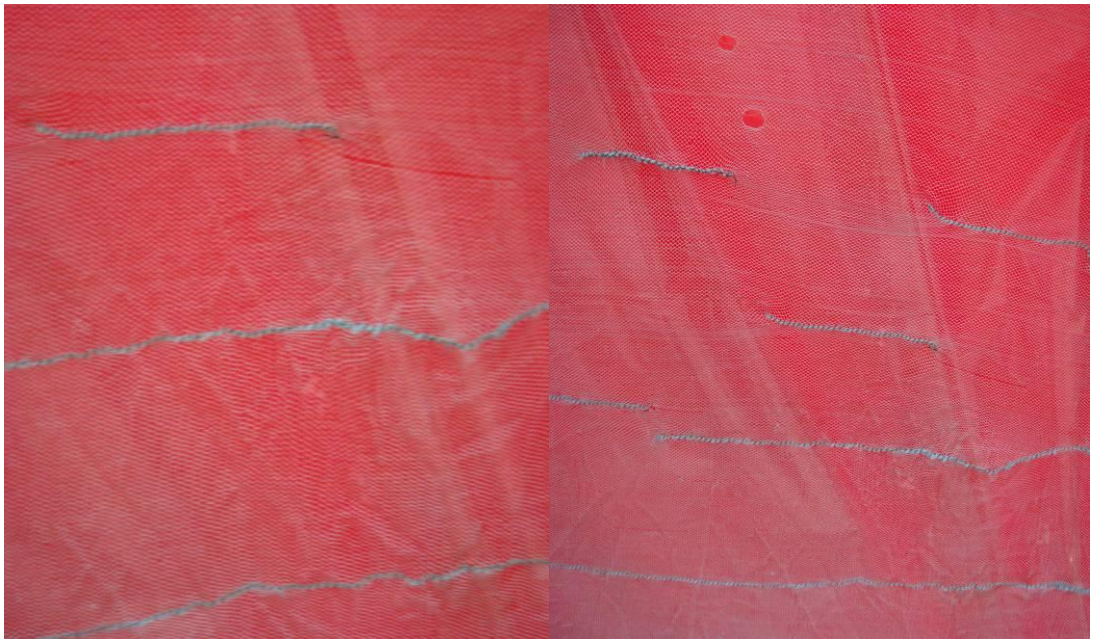


Fig. 3.4 Stitching as a common method for LLIN repair

3.6. Bio-efficacy test of LLINs

The responses of susceptible mosquitoes against new nets showed that both knock down at 60 minutes and mortality at 24hrs was 100%. When the nets were tested using the wild Pemba strain the result was similar to that of susceptible mosquitoes (100%, KD60 and 99.2% 24hrs mortality (Figs. 3.5 and 3.6). On the other hand, with the used nets (estimated to have been washed between 3 and 36 times), the results using susceptible *An. gambiae* s.s gave a KD60 of 53.3% and 24hrs mortality of 28.7%. The wild Pemba strain displayed far less mortality giving only 2.4% KD60 and 8% mortality at 24hrs. The results shown in Fig. 3.7 indicate that there was no relationship between the number of washes and the mortality response. Maxwell *et al.* (2006) reported similar results that no significant impact of washing or heating the nets was detected.

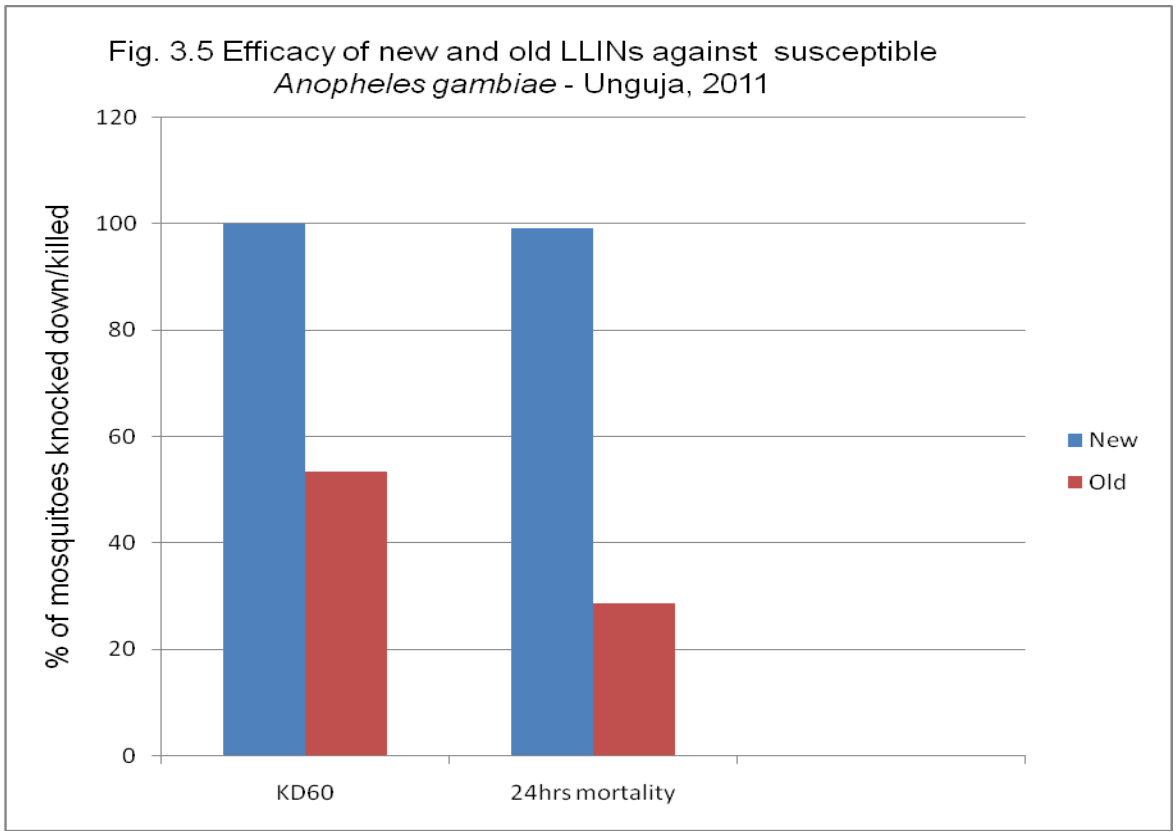


Fig. 3.5 Efficacy of new and old LLINs against susceptible *Anopheles gambiae* - Unguja, 2011
% of mosquitoes knocked down/killed
120
100
80
60
40
20
0
KD60
24hrs mortality
New
Old

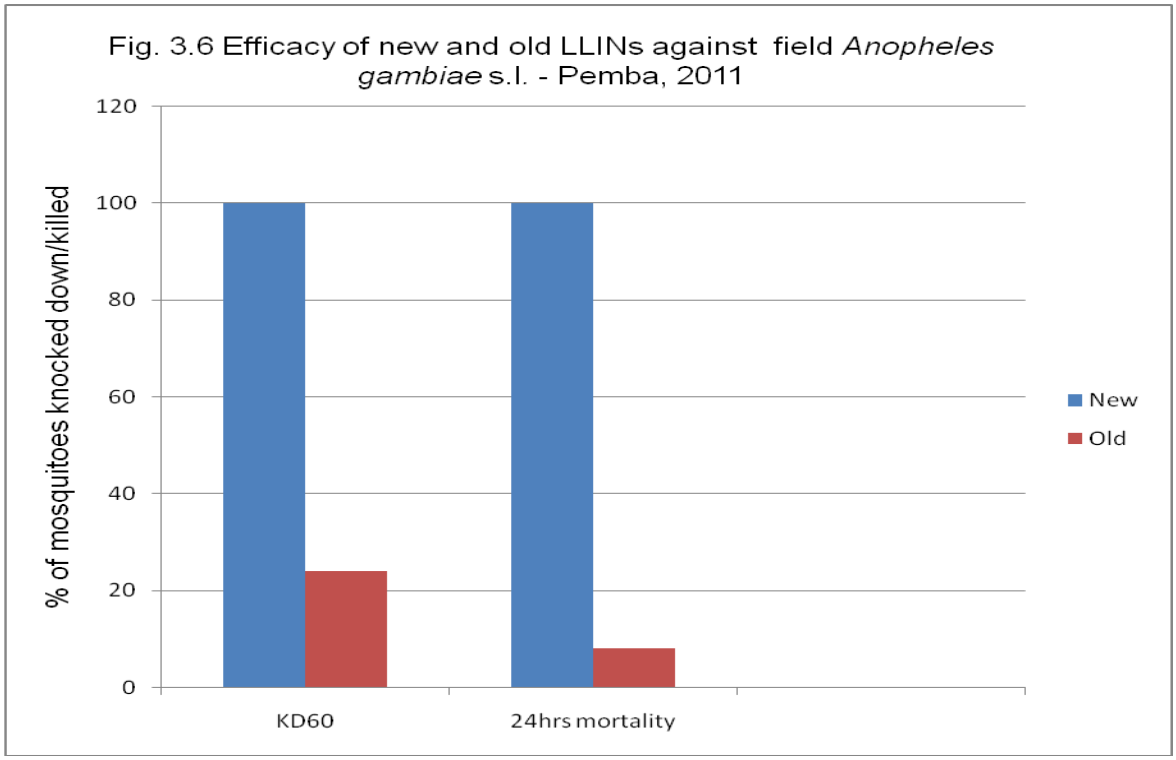


Fig. 3.6 Efficacy of new and old LLINs against field *Anopheles gambiae* s.l. - Pemba, 2011
% of mosquitoes knocked down/killed
120
100
80
60
40
20
0
KD60
24hrs mortality
New
Old

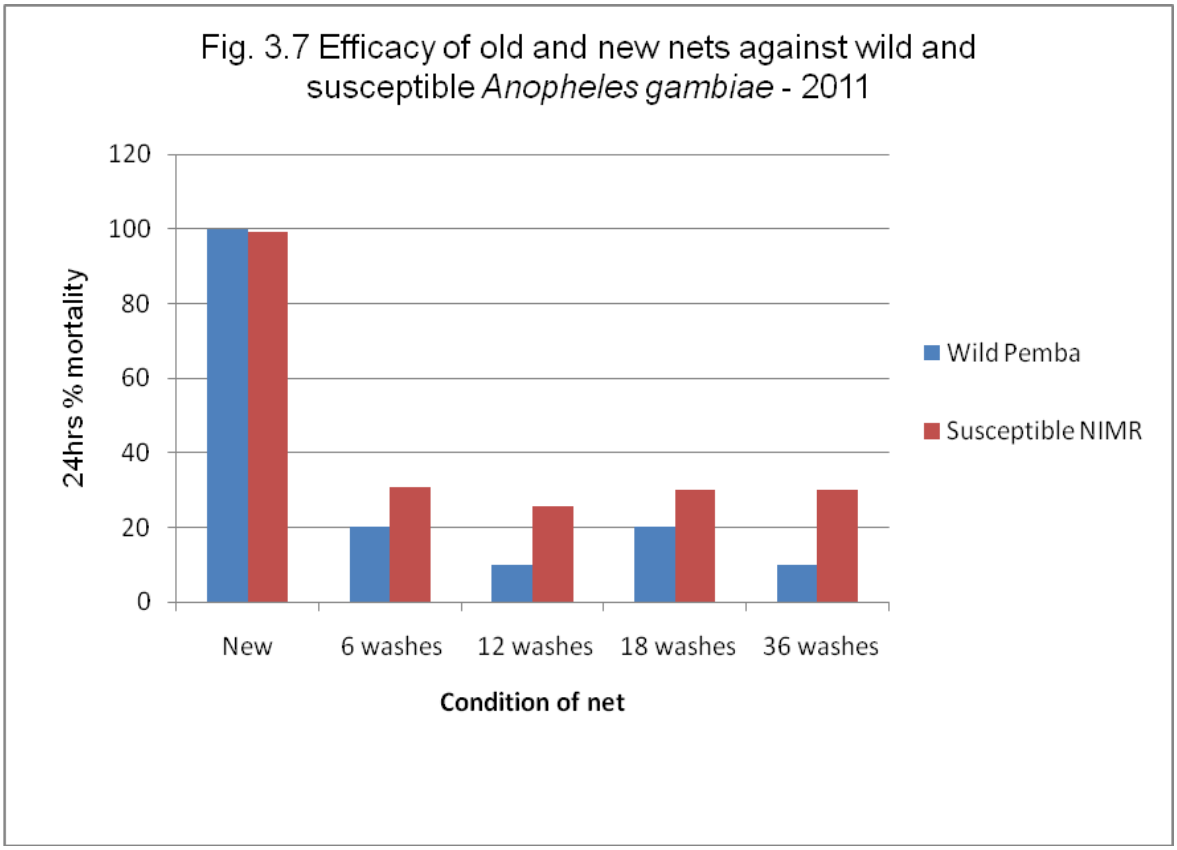


Fig. 3.7 Efficacy of old and new nets against wild and susceptible *Anopheles gambiae* - 2011
120
100
80
60
40
20
0
24hrs % mortality
New
6 washes
12 washes
18 washes
36 washes
Condition of net
Wild Pemba
Susceptible NIMR

CHAPTER FOUR

DISCUSSION

4.1. Malaria vector species and insecticide susceptibility

Both indoor and outdoor collections of *Anopheles* mosquitoes were conducted using three sampling methods. *Anopheles gambiae* s.l. was found to be the only vector group on both islands. Of the members of the *An. gambiae* s.l. collected on Pemba, *An. arabiensis* was the only species collected. Over 94% of the *An. arabiensis* was collected through outdoor trapping method (artificial pit trap) indicating outdoor resting behaviour. Baseline study results (unpublished ZMCP report, 2005) showed *An. gambiae* s.s (94.7%) was the predominant malaria vector on both islands of Zanzibar followed by *An. arabiensis* 4%. *Anopheles gambiae* was found to be endophilic and endophagic with 100% human blood index in Pemba and 80% in Unguja. This result was used to justify the re-introduction of IRS and scaling-up the distribution and use of LLINs in 2006.

On the other hand the result from the adult catch is consistent with larval collections that recorded 98% of samples used in the resistance study was *An. arabiensis* and a few *An. merus* that were collected from Tibirinzi in Pemba. Further investigations with wider coverage are needed to monitor changes in malaria vector species. The presence of *An. merus* in Tibirinzi is not strange as this site is close to the sea whereby at high tides, sea water flows in to the area and consequently increases water salinity, forming the potential breeding environment for *An. merus.* Mosha and Matero (in Gillies & Coetzee, 1987) found *An. merus* breeding in brackish water ponds in coastal area of Kenya,

furthermore they also reported that density picked up when the salinity rose up to the equivalent of 50% sea water.

This study found strong pyrethroid resistance in *An. arabiensis* on Pemba island. Resistance has been documented before, during other vector control campaigns. The first record of DDT resistance in Zanzibar was reported from Pemba island (Curtis *et al*, 1983, Gillies & Coetzee, 1987), while Lines and Nassor (1991) reported the spread of DDT resistance to Unguja island. Like many other African countries pyrethroids have been extensively used for both LLINs and IRS in Zanzibar for over six years now (ZMCP unpublished report, 2010). Adasi and Hemingway (2008), Corbel *et al*. (2007) and many others have noted that agricultural activities may contribute to the selection of insecticide resistant mosquitoes but the current study did not find any official record of pyrethroid use in the agricultural sector. Nevertheless, livestock farmers use pyrethroids for the control of ticks and other veterinary pests. This may contribute to the greater exposure of cattle feeding mosquitoes such as *An. arabiensis*. Lines (in WHO, 2010) suggests that public health insecticides are a source of selection for resistance in malaria vectors and reported that in some cases IRS was found to play a role in selection for resistance. This may happen if vectors are exposed on low insecticide dose during spraying operations as a consequence of both in adequate supervision and training and long term use of the same class of insecticide on both IRS and LLINs, as in the case of Zanzibar. But the question still remains uncertain as to why the emergence of resistance in Zanzibar is most of the time recorded from Pemba and not Unguja island. ZMCP implements vector control interventions on both islands of Zanzibar, so it

is very strange to see Pemba malaria vectors are tolerant to pyrethroids while anopheline vectors on Unguja remain sensitive.

On Unguja island, mosquito collection results showed a bit different picture from that found on Pemba. *Anopheles arabiensis* (90.7%) was the more prevalent malaria vector followed by *An. merus* that constituted only 9.3%. Collection of *An. merus* from Mwera is very strange as this species prefers breeding site with high salt content Mosha and Matero (in Gillies & Coetzee, 1987). In contrary Mwera is an inland site used for rice irrigation located about 12 km away from the Indian Ocean. Nevertheless the current result is consistent to other studies that found the salt water breeder in coastal areas of Kenya and Tanzania, but also far inland in Zimbabwe and South Africa (Gillies & Coetzee, 1987). On the other hand no *Anopheles* mosquito collected from Uzini (the second site of Unguja) this could be attributed to the fact that the area was too dry at the time of this study.

The fact that *An. gambiae* s.s. is not found on Zanzibar could be taken as the effect of indoor residual spraying using lambda-cyhalothrin 10WP/CS and universal coverage of long lasting nets around the islands. Earlier Odetoyinmbo and Davidson, (1968), Iyengar (1962) and others documented that *An. gambiae* was no longer found inside sprayed housed in Zanzibar. Other studies suggested a shift or change of *An. gambiae* s.s behavior to avoid contact with IRS treated surfaces or LLINs (Govella *et al*, 2010). Bayoh *et al.* (2010) found *An. gambiae* declined from indoor collections in relation to *An. arabiensis* following increased ownership of long lasting nets in western Nyanza Province, Kenya. Other studies conducted elsewhere have also shown that using a combination of IRS and LLINs in endemic malaria areas has resulted in incremental

effects in reducing the density of endophilic *Anopheles* mosquitoes (Kleinschmidt *et al*, 2009).

The current result is also consistent to what was found from the study conducted in Kenya that suggested in areas where IRS is implemented together with LLINs, the importance of indoor resting, indoor biting mosquitoes such as *An. gambiae* s.s is diminished (Bayoh *et al*, 2010). The outdoor resting behavior poses a challenge for vector control as the most effective interventions (IRS & LLINs) are mainly targeted against indoor biting and resting mosquitoes. In this situation, the effect of both IRS and LLIN is doubtful since the largest numbers of malaria vectors have adapted exophilic behaviour. Nevertheless, Govella *et al.* (2010) suggested a way how these interventions can still be effective subject to human behavior. With the increase in *An. arabiensis,* outdoor malaria transmission is anticipated, though this also depends on human behavior as regards to outdoor activities during night time. This scenario justifies the need of finding alternative control measures that will be appropriate against the outdoor malaria transmitting vectors (Bayoh *et al*, 2010).

A huge number of *Culex* mosquitoes (9921 - 94% of all collections) were found indoors during the mosquito search on both islands of Zanzibar. These were mostly collected indoors with the CDC light traps, although pyrethrum spray catches also collected some *Culex*. The artificial pit traps did not catch any culicine mosquitoes. This observation could be attributed to the fact that culicine breeding sites and habits are closely related to sewage systems, polluted water bodies, and wet pit latrines, in contrast to that of anophelines breeding linked to rainfall. This could be taken as the main reason for low *Anopheles* collection during the study period taking into consideration that January –

March is the dry season in Zanzibar. This is also proved by the fact that over 75% of all *Anopheles* collection was done on the month of April which is the beginning of rainy season in Zanzibar.

The presence of large numbers of nuisance biting *Culex* indoors has implications for malaria vector control since these mosquitoes are commonly resistant to the insecticides used for malaria control (Malima *et al,* 2008; Corbel *et al*, 2007). Consequently, communities may become reluctant to accept interventions like indoor residual spraying or LLINs arguing that these tools are not effective and that they see mosquitoes flying inside their houses soon after spraying or buzzing around the LLINs (Corbel *et al*, 2007). The ability to identify *Anopheles* from *Culex* is not common among community members, but this could be taken as a challenge and form part of the advocacy/information, education, and communication component of the malaria control programme.

As regards to susceptibility status in Unguja, malaria vectors were found fully susceptible to deltamethrin and permethrin however, more investigations are required on lambda-cyhalothrin (95% 24hrs mortality). This finding is contrary to those reported earlier in this report that most of malaria transmitting mosquitoes on Pemba are resistant to pyrethroids used on LLINs and IRS. It is recommended that the ZMCP should strengthen efforts on implementation of resistance management strategies.

4.2. The wear and tear of LLINs under regular use in the field environment

Physical inspection of LLINs distributed in early 2008 by the Zanzibar Malaria Control Programme in two villages in the West district has shown that over 66% of the nets

were in poor physical condition. This could be attributed to the type of beds used in the villages, the common type being that of wood connected by metal clamps with protruding bolts from all four corners of the bed. Another type of bed that is locally made uses wood sticks connected with rope. These kinds of beds can damage the nets, particularly on the lower portion where the nets are touching the bed frame or are tucked under the mattress. Over 61% of all holes were found on the lower part of the LLIN. Smith *et al*. (2007) and Kilian *et al*. (2008) found similar results in Ghana and rural Uganda where the number of holes increased towards the lower part of the nets.

On the other hand holes on the upper portion of the nets were mostly caused by tearing with sharp pointed sticks and other materials found in the village environment. On some occasions, burning was another cause of net damage as a result of naked flames from wood fires and kerosene lamps. In other instances seam failure was responsible for damages found on the upper portion of the nets. Smith *et al.* (2007) suggested that the extent and size of holes and the frequency of seam failures observed in his study demonstrated the need to raise the performance requirements for LLINs in terms of both fabric quality and seam strength. But still, the LLIN durability is highly dependent on where the nets are being used. Skovmand and Bosselmann (2011) found that while higher denier provided higher bursting strength and tension strengths, texturizing weakened the yarn taking into consideration that Olyset Net and Duranet have the thickest yarns and highest square metre weights.

Based on the criteria set by Maxwell and others (in Smith *et al*, 2007), nets were “undamaged” if they fulfilled the following criteria: less than 20 holes, of less than 2 cm in diameter; < 5 holes of 2–5 cm in diameter; and < 2 holes, more than 5cm in

diameter. Using the same criteria, more than 66% of LLINs distributed 3 years ago in Zanzibar were damaged. This finding is contrary to other report (unpublished data) that states that most nets (N=18) remained in a good physical condition after 2 years of field use. Similarly, Smith *et al*. (2007) found 84.8% of nets distributed in the highlands of Ghana were intact after 4-5 years of field use but 56.6% of nets that were distributed in lowland villages were only intact for 3–4 years after distribution, suggesting that net longevity is highly dependent on where they are being used.

4.2.1 The efficacy of LLINs as measured by cone bioassay

Long lasting insecticide nets are one of the most powerful weapons in the fight against malaria. These nets inflict a lethal (insecticidal) effect as well as forming a physical barrier against malaria transmitting mosquitoes. Unless a strong system for LLIN monitoring is implemented, control efforts would be compromised. This study, reports the findings from cone bioassay tests conducted on long lasting nets after 3 years of field use in Zanzibar. New LLINs were tested in parallel with the old ones so as to be able to compare the degree of effectiveness of old nets. Both were tested against a susceptible colony and resistant, wild *Anopheles gambiae* s.l. from Pemba. This gave information on the impact of insecticide resistance on the efficacy of the nets.

Maxwell *et al.* (2006) and Magoma (2005) suggested that using WHO cone method, some mosquitoes may place their tarsae through the broad mesh on to the cardboard, thus avoiding insecticide contact, and for this reason some studies have suggested doubling the net over to reduce the mesh size (Maxwell *et al,* 2006). Unfortunately, this

also doubles the dose in a given area which is why the double cone test was adopted for this project.

In the present study, the cone bioassays showed that new unwashed Olyset^{TM} nets are completely effective against field and susceptible *Anopheles gambiae* (100 and 99.2% mortality respectively). Washed LLINs however, gave only 8 and 28.7% mortality against field and susceptible mosquitoes. Susceptible mosquitoes and field mosquitoes showed a KD60 of 53.3 and 2.4% against nets that had been in use for a 3 year period.

These mortalities are far less than the minimum effectiveness recommended by WHO (2005) of KD60 ≥75% or 24hrs mortality ≥ 50%. The present result is in contrast to data from other studies using the same WHO cone method. Kilian *et al*. (2008) found LLINs had a knockdown rate of 95% and mortality rate of 80% after 36 months of field use in Uganda, again Fettene *et al*. (2009) had more or less similar results in Buie and Fentalie districts of Ethiopia. Malima *et al*. (2008) documented similar findings on OlysetNet® after seven years use in Tanzanian village. The current results also contradict the findings documented by Maxwell *et al*. (2006) and Tami *et al*. (2004) who found that old Olyset nets which had been in domestic use for four years were as good as new nets. Nevertheless, WHO (2009) reported in an overview of the variation of mortality and knockdown as a result of cone tests, that only 33% of the 120 nets fulfilled the bioassays criteria, i.e. 40 nets for the KD and 3 for the mortality. Furthermore when the 80 nets that failed on the bioassay cone tests were subjected to a tunnel tests, 61% fulfilled the WHOPES criteria. This finding underlines the need for conducting the tunnel test following cone bioassays to find out if the nets are still

capable of reducing the numbers of bites received by humans (i.e. still have a repellent effect).

This study documented 100% 24hrs mortality on both field and susceptible *Anopheles* mosquitoes tested on new LLINs. The low (28.7%) bioassay results of old nets tested on laboratory reared *Anopheles gambiae* could be interpreted that the Olyset LLINs are no longer effective against malaria vectors in Zanzibar and therefore justify the need for replacement.

4.3. Conclusion

Mosquitoes collected in Pemba and Unguja from January – April, 2011, showed that *Anopheles arabiensis* was the most prevalent sibling species followed by *An. merus*. Longitudinal studies with a wide geographical coverage are recommended to monitor changes over time on malaria species composition.

Pyrethroid resistance was confirmed from Pemba (the sister island of Zanzibar) for the first time in the region following ZMCP assays. However, this was not the case in Unguja island where malaria vectors were found to be sensitive against the pyrethroids used for malaria control in Zanzibar. The results demand that a routine, systematic monitoring campaign for insecticide resistance is implemented so that the impact of control efforts across the islands can be predicted and understood. As a part of resistance management, the Zanzibar Malaria Control Programme has recommended a shift to bendiocarb (carbamate) for IRS in a rotation system. Malaria vectors in Zanzibar

were found to be 100% susceptible to bendiocarb (unpublished data). Again the rotation of the insecticides may allow a reduction of selection pressure on pyrethroids.

Following an evaluation of LLINs in the field, > 66% was found in poor physical condition after 3 years. Moreover, bioassays on these nets showed far less mortality than that recommended by WHO. More studies are required to determine whether these nets are still effective at reducing the numbers of bites received by humans. Finally, these results suggest that the LLINs distributed in 2008 to the West district are no longer effective against malaria vectors in Zanzibar and therefore replacement is of paramount importance for the community to remain protected.

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