JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

SCHOOL OF GRADUATE STUDIES

DEPARTMENT BIOLOGY (ECOLOGICAL AND SYSTEMATIC ZOOLOGY)

The emergence of resistant Anopheles and Culex Mosquitoes against PermaNet 3.0, Long Lasting Insecticidal Net, In Some selected districts of Jimma zone, South West Ethiopia

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A Thesis submitted to Department of biology, college of Natural Science, School of Graduate Studies, Jimma University, in partial fulfillment of the requirement For the Masters of Science Degree in Biology (Ecological and Systematic Zoology)

Sept 2015

Jimma Ethiopia

Approval Format

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Acknowledgements

First of all I would like to thanks God to which enabled me to reach this step. Secondly, I would like to express my deepest gratitude to Dr. Delenasaw Yewhalaw, who provided me a valuable professional guidance and consistent encouragement throughout without which this work would have not completed. My great thank also extended to Mr. to my Co-Adviser Mr. Abebe Asale who gave me un reserved support, friendly approach and critical comments starting from proposal up to the end of the thesis. I would like to thank Jimma University, Department of Biology for providing necessarily valuable professional assistance and offering me a chance to pursue my education. My genuine appreciation also goes to Omo-Nada and Tiro-Afeta district different administration workers, Malaria focal persons, health extension workers and the community members whom showed me their willingness to be involved in this study and provided me invaluable information.Finally I would like to pass my thanks to my respected families for their valuable moral and financial support.

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List of Acronyms and Abbreviation

ANOVA Analysis of variance

ITNs Insecticide treated nets

IRS Indoor residual spraying

KD Knock down

LLINs Long-lasting insecticide-treated nets

MDGs Millennium Development Goals

MT Mortality

NMCPs National Malaria Control Program

RBM Roll Back Malaria

PMI President's Malaria Initiative

PBO pipeeronylbutoxide

SPSS Statistical Package for Social Science

UNDP United Nation developmental program

WHO World Health Organization

Abstract

This study was carried in order to evaluate the emergence of resistant Anopheles and Culex Mosquitoes against PermaNet 3.0, Long Lasting Insecticidal Net, In Some selected districts of Jimma zone, South West Ethiopia. Taxonomically, mosquitoes belong to kingdom Animalia, phylum Arthropoda, class Insecta, order Diptera and family Culicidae. Many members of the genus Anopheles have the ability to transmit human malaria. Long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS) and environmental management are the most widely used tools for malaria vector control. Thus the objective of this study was to evaluate the Bio-Efficacy and wash resistance of PermaNet 3.0 LLIN using Anopheles gambiae and Culex quinquefasciatus mosquitoes in selected districts of Jimma zone, south west Ethiopia. This study was conducted from March, 2015 to August 2015.A community based survey was conducted using structured, questionnaire interview. In line with administration of interview, 150 old PermaNet 3.0 samples were collected by replacing with new PermaNet 3.0 on spot and the nets hole was measured. Anopheles and Culex mosquito larvae were collected from the outskirts of Jimma town and reared understandard conditions. Susceptible Anopheles arabiensis mosquitoes were obtained from Sekoru Vector biology and insectary unit, Jimma University. Vector mosquitoes were exposed to 30 cm x 30 cm pieces of impregnated bed nets for 3 min, Knockdown was measured after 60 minutes and the mortality was measured after 24 h recovery period. Among the socio-characteristic factors the association between Physical condition of the net and misuse of the net by children was highly significant (Df 1, P=0.000) whereas no significant relation was observed between factors net washing habit, soaking time, housing type and source of light. Reduced susceptibility of both Anopheles gambiae s.l. (79%) and Culex quinquefasciatus (85%) was recorded using unwashed PermaNet 3.0 samples. Comparison of mean percent mortalities and knockdown effects among different wash status of PermaNet 3.0 has shown significant variation (P=0.000) among different washes. The unwashed PBO-deltamethrin top netting induced mortality in Cx. Quinquefasciatus (100%) and An. gambiae s.l(97%) in both above WHO reference line indicating that PBO was synergizing the pyrethroid resistanceMean percent mortality of mosquito at the top panel of PermaNet 3.0 significantly higher than that of mosquito at the side part of the LLN(P<0.05).

Key words. Bioassay. Efficacy, An. gambiae s.l, Culex.

1 Introduction

Taxonomically, mosquitoes belong to kingdom Animalia, phylum Arthropoda, class Insecta, order Diptera and family Culicidae (Gillott, 2005). Family Culicidae consists of two subfamilies, the Anophelinae and Culicinae, included in 28 genera with more than 3000 species described so far (Harbach & Kitching, 2005;Gillott, 2005).Subfamily Anophelinae has three genera: *Anopheles* Meigen (cosmopolitan in distribution), *Bironella* Theobald (Australasian), and *Chagasia* Cruz (Neotropical). Two genera from sub-family Culicinae, Culex, Aedes and genus Anopheles from sub-family Anopheline are among the worst vectors causing more death of humans than all other species in kingdom Animalia combined. Many members of the genus *Anopheles* have the ability to transmit human malaria.

Long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS) and environmental management are the most widely used tools for malaria vector control (WHO, 2007). Similarly in Ethiopia LLINs & IRS are the two key vector control interventions while early diagnosis and treatment of cases using Artemisia and rapid diagnosis therapy (RDTs), early detection, prevention and control of epidemics are also used in Ethiopia(WHO, 2007).

Over the past decade, a major malaria control strategy has been the use of insecticide treated nets (ITNs), which are perhaps the evaluated and most cost-effective intervention for large-scale application. In recent years, a number of success stories have emerged and the incidence of malaria has begun to decline in many part of Africa (Ceesay *et al.*, 2008); Russell *et al.*, 2010).

The protective efficacy of ITNs results from both the physical barrier and the insecticidal action of the net. While it is intuitively clear that ITNs provide protection to individual users, what is less obvious is the impact of widespread ITN use at the community level. ITNs are able to reduce the density, feeding frequency and survival of mosquitoes and wide-scale use can mediate protection of all community members, including the vulnerable portion without a bed net. With moderate ITN coverage of the population, the 'mass effect' is at least as important as the personal protection provided to the user Gimnig (2003); Lindsay(1991).

In Ethiopia, there is widespread insecticide resistance has been reported in main malaria vector species, *Anopheles arabiensis,* Anopheles *funestus and An. pharoensis*. This resistance is due to both target site and metabolic mechanisms and cross-resistance between DDT and Pyrethroid (Yewhalaw *et al.*, 2012;2011;2010;Alemayehu and Mamuye, 2011).

The use of ITN in Ethiopia has started since 1997 and the process of scaling up begun in 2005 with the aim of obtaining a high coverage towards effective malaria control (Yewhalaw *et al.*, 2012) According to the National Malaria Control Program (NMCM) the distributed LLINs between the period 2005 and 2010 was about 36 million bed-nets, which considering about 52 million people at risk (WHO, 2012;2010). PermaNet 3.0 is an LLIN deltamethrin-treated combination net with the addition of synergist piperonylbutoxide (PBO) on the roof section of the net (Ahimed, 2006).Recently, the vector control advisory group of the world health organization supported vestergaard's claim that relative to Pyrethroid only LLINs, the combination net, prmaNet3.0 increased efficacy against malaria vectors with cytochrome P450-based metabolic Pyrethroid resistance, even if combined with kdr (WHO, 2014).
Although LLINs in general and PermaNet 3.0 in particular are recommended for malaria control purposes, their performance should be monitored in the field under various ecological settings to assess their durability and long-term effectiveness for malaria prevention and control. Thus, the aim of this study is to evaluate the Bio-efficacy of PermaNet 3.0 after 2-5 years of use, including the effects of the synergist, using Pyrethroid-susceptible and wild, Pyrethroid resistant *Anopheles arabinoses* in Burka-Asendabo and Kajello Kebele, Jimma zone, southwestern Ethiopia.

1.2 Statement of the problem

One of the keystones in malaria control strategy is tackling the vector, either by reducing the vector density or infectivity rate of the vector which will have an impact on malaria transmission and incidence (Ghebreyesus *et al*., 1999). However these intervention efforts could be endangered if other alternative vector control strategies are not harmonized. Based on previous research reports in Ethiopia, it appears that the mosquito population has developed resistant against most insecticides currently used for indoor residual spraying or to treat mosquito nets.

PermaNet 3.0 is one of the new brand LLINs that has been distributed for users around Jimma Zone in 2012 with the primary objective of tackling the newly emerging pyrethroid resistant vector mosquitoes and thereby decreasing malaria related morbidity and mortality. However, its durability and long-term effectiveness of the impregnated chemicals should be continuously monitored in the community weather the distributed LLIN is serving up to its intended target. Thus, in this study the following research questions were specifically assessed.

- Did the populations of *Anopheles gambiae* and culicine population in the study area developed resistance against WHOPES recommended LLINs, PermaNet 3.0?
- Did the households use properly PermaNet 3.0 for sleeping under to avoid mosquitos bite?
- Did PermaNet 3.0 prove effectiveness controlling Anopheline and culicine mosquito population in the study area?
- How much the washing and drying condition practiced by the community could affect the bio-efficacy of PermaNet 3.O against Anopheline and culicine mosquitoes?

1.3 Significance of the study

The out puts of this study including the bio-efficacy of PermaNet 3.0, the resistance status of wild mosquitoes from the study area, peoples net handling practice, net washing and net drying conditions, net durability and net effectiveness will be used as a critical information in further vector control strategies and future directions in the contest of the disease as well in formulation of national Insecticide resistance management strategies.

1.4 Objective of the study

1. 4.1. General objective

To evaluate the Bio-Efficacy and wash resistance of PermaNet 3.0 LLIN using *Anopheles gambiae* and *Culex quinquefasciatus* mosquitos in selected districts of Jimma zone, south west Ethiopia.

1.4.2 Specific objective

- To determine the susceptibility of the populations of *Anopheles gambiae and culex quinquefasciatus population i*n the study area against WHOPES recommended LLIN, PermaNet 3.0 in Burqa-Asendabo and Qajello Kebele
- To determine PermaNet 3.0 possession, handling and usageby selected households in the study area
- To determine the impact of Net washing habit, Housing type, light source and daily handling habit on physical condition of the net in the study area
- To measure wash-resistance property of permanent 3.O against *Anopheles gambiae* in the study area.
- To measure wash-resistance property of permanent 3.O against *Culex quinquefasciatus* in the study area.
- To determine the washing and drying condition practiced by the community.

2 Literature Review

2.1 Biology and ecology of Anopheles and Culex mosquitoes

Like all mosquitoes, anophelines go through four stages in their life cycles: egg, larva, pupa, and imago. The first three stages are aquatic and last 5–14 days, depending on the species and the ambient temperature. The adult stage is when the female *Anopheles* mosquito acts as malariavector. The adult females can live up to a month (or more in captivity), but most probably do not live more than two weeks in nature.Adult mosquito lay egg in fresh or salt-water marshes,larva develop through 4 stages or instars, after which they metamorphose in to pupae. At the end of each instar, the larvae molt, shedding their exoskeleton, or skin, to allow for further growth. 1st stage larvae are1mm in length; 4th stage larvae are normally 5-8mm in length. The process from egg laying to emergence of the adult is temperature dependent, with a minimum time of 7 days (Vezenegho *et al.*, 2009).

The pupa is comma-shaped when viewed from the side. The head and thorax are merged in to a cephalothorax with the abdomen curving around underneath. As with the larvae, pupae must come to the surface frequently to breathe (Pates *et al.*, 2005), which they do through a pair of respiratory trumpets on the cephalothorax. After a few days as a pupa, the dorsal surface of the cephalothorax splits and the adult mosquito emerges (Bryan *et al.*, 1987).The duration from egg to adult varies considerably among species and is strongly influenced by ambient temperature. Mosquitoes can develop from egg to adult in as little as 5 days but usually take 10-14 days in tropical condition (Elisse *et al.*, 1994). Like all mosquitoes,adult *An*. has slender bodies with 3 sections: head, thorax and abdomen.

The head is specialized for acquiring sensory information and for feeding. The head contains the eyes and a pair of long, many-segmented antennae (Coetzee *et al.*, 2006). The antennae are important for detecting host odours as well as odours of breeding sites where females lay eggs. The head also has an elongated, forward-projecting proboscis used for feeding, and two sensory palps. The thorax is specialized for locomotion. Three pairs of legs and a pair of wings are attached to the thorax (Hargreaves *et al.*,2003).The abdomen is specialized for food digestion and egg development. This segmented body part expands considerably when a female takes a blood meal. The blood is digested over time serving as a source of protein for the production of eggs, which gradually fill the abdomen.

An. mosquitoes can be distinguished from other mosquitoes by the palps, which are as long as the proboscis, and by the presence of discrete blocks of black and white scales on the wing (Kristan *et al*., 2003). Adult Anopheles can also be identified by their typical resting position: males and females rest with their abdomens sticking up in the air rather than parallel to the surface on which they are resting (Pates *et al*., 2005).

Adult mosquitoes usually mate within a few days after emerging from the pupal stage. In most species, the males form large swarms, usually around dusk, and the females fly into the swarms to mate (Pates *et al*., 2005).

Males live for about a week, feeding on nectar and other sources of sugar. Females will a Hargreaves *et al*.,2003).The abdomen is specialized for food digestion and egg development. This segmented body part expands considerably when a female takes a blood meal. The blood is digested over time serving as a source of protein for the production of eggs, which gradually fill the abdomen.

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Males live for about a week, feeding on nectar and other sources of sugar. Females will also feed on sugar sources for energy but usually require a blood meal for the development of eggs. After obtaining a full blood meal, the female will rest for a few days while the blood is digested and eggs are developed. This process depends on the temperature but usually takes 2 – 3 days in tropical conditions (Sharp*et al*., 2007). Once the eggs are fully developed, the female lays them and resumes host seeking.

2.1. Taxonomy of Anopheline and Culicine mosquitoes

Taxonomically, mosquitoes are belongs to kingdom animalia, phylum arthropods, class Insecta, order Diptera and family Culicidae, family Culicidae consists of two subfamilies, the Anophelinae and Culicinae (Harbach & Kitching, 2005).Subfamily Anophelinae has three genera: *Anopheles* Meigen (cosmopolitan in distribution), *Bironella* Theobald (Australasian), and *Chagasia* Cruz (Neotropical). Subfamily Culicinae has 39 genera (Rueda, 2008).

23 Anopheline species and their distribution in Ethiopia

In southern Ethiopia, seven *Anopheles* species were identified, the predominant species was *A. arabiensis* followed by *An. coustani*, *An. pharoensisAn. funestus*, *An. niliAn. marshallii* and *An. Demeilloni(*Aseged *et al*., 2006). Again in south western part of Ethiopia, *An. gambiae s.l*. was the principal species, followed by very low densities of *An. funestus*, *An. nili* and *An. pharoensis*(Ribeio *et al*., 1996). According to BirhanTeklu*et al*. (2010), four *Anopheles* species were identified in area of central Ethiopia: *An. pharoensis*, *An. gambiae s.l.An. Coustani* and *An. squamous*. The major malaria vector known in Ethiopia is *An. arabiensis*. In some areas *An. pharoensis*, *An. funestus* and *An. nili* also transmit malaria (FDROEMOH, 2006). In addition to these, *An. Coustaini*comples*An. pauraludis*, *An. ziemanni* and *An. d`thalia*have been recorded to possess vector capacity (Ashenafi Woime, 2008).

2.4 CulucineMosquitoes

Species of *Culex* include a complex known as "house mosquitoes" and, more recently, "West Nile mosquitoes" because of their involvement in the transmission of West Nile Virus. Culex mosquitoes are involved in transmitting some arboviruses disease such as viral encephalitis and lymphatic filariasis. *Cx, pipies s*pecies is also vector of some disease including west Nile fever Riftvally fever and also some lymphatic filaries (Burnard, *etal*,2004 and Habrach *et al*, 1988).

Culex or house mosquitoes enters places in different ways and nuisance individual especially night.Besides culex biting causes problem like itching, burning and inflammation among sensitive person especially children and also involved in the transmission of West Nile Virus among birds and potentially to humans and horses. In addition to West Nile Virus, certain species of *Culex* are known to transmit St. Louis Encephalitis Virus and Western Equine

Encephalitis virus. *Cxpipies*bites human indoor aswell as outdoor mainly at sun set and night (savage, *etal*.1995).

2.5 Malaria vector Controlling methods

2.5.1 Chemical control (IRS)

IRS reduces malaria transmission by reducing the survival of mosquitoes that enter houses or sleeping units. It involves spraying an effective dose of insecticide, typically once or twice per year, on indoor surfaces where malaria vectors are likely to rest after biting. IRS is a method for community protection and, to achieve its full effect .WHO Pesticide Evaluation Scheme (WHOPES) currently recommends 12 insecticides belonging to 4 chemical classes for IRS. National malaria control programs need to select insecticides for a given area on the basis of the residual efficacy of the insecticide; costs, safety and the type of surface to be sprayed; and up-to-date insecticide resistance data. DDT has a comparatively long residual efficacy, lasting more than 6 months, and continues to be a widely used insecticide for IRS. (WHO, 2007)The efficiency of IRS and LLINs, whether deployed singly or in combination, depends on the continued susceptibility of the vectors to the insecticides delivered through these means. Resistance to the four classes of insecticides (pyrethroids, organophosphates, organo-chlorines and carbamates) approved for vector control has been found in a number of Anopheles gambiae populations (Asidi*et al.,* 2012)

2.5.2 Environmental modification

Environmental modificationis removal or permanent destruction of mosquito breeding sites. The larval habitats may be destroyed by filling depressions that collect water, by draining swamps or by ditching marshy areas to remove standing water. Container-breeding mosquitoes are particularly susceptible to source reduction as people can remove or cover standing water in cans, cups, and rain barrels around houses. Mosquitoes that breed in irrigation water can be controlled through careful water management.(WHO, 2007)

2.5.3. Personal protection (ITN)

Personal protection measures include the use of window screens, ITNs, and repellents (such as DEET) and wearing light-colored clothes, long pants, and long-sleeved shirts. Well-constructed houses with window screens are effective for preventing biting by mosquitoes that bite indoors and may have contributed to the elimination of malaria, Lengeler and Meara showed that the use of LLINs constitutes the most advantageous intervention in terms of cost-efficacy at a large scale. These LLINs not only represent a physical barrier in reducing contact between human and vector but also a chemical barrier. The chemical barrier acts on the mosquitoes through the deterrent, lethal and repellent effects. Thus, LLINs reduce the density, the frequency of blood feeding, the success of blood feeding and the survival of Anopheles vector. Moreover, the assets of this tool are mainly rooted in the fact that those protected by LLINs are no more exposed to the bites of Anopheles vectors, and a strong coverage rate also provides protection to the rest of the community. Further, several results from studies carried out in Africa and in Papua New Guinea indicate the presence of an advantageous effect of LLINs at the community level. Indeed, LLINs have contributed to the reduction of the intensity of malaria transmission the number of severe malaria cases and infant mortality rates. (Lengeler *et al.*, 2013)

The technology of long-lasting insecticidal nets (LLIN) was developed in the late 1990 ties as a response to the poor re-treatment practices for conventionally-treated mosquito nets and the first evaluation reports for a polyethylene-based LLIN was published in 1999 followed only three years later by one for a polyester-based LLIN (Guillet *et al.,* 2001); Doannio *et al.,* 1995). Since then LLIN have become the recommended approach for malaria prevention with mosquito nets and in some countries the proportion of all nets that are LLIN is already exceeding 90%. There are a number products on the market that use the term “long-lasting” to advertise their insecticide- treated net product but not all of these are actually LLIN (Getachew*et al.,* 2010). Generally criteria’s for use of public funds on the purchase of LLIN as practiced by all major funders is the recommendation from the WHO Pesticide (Kilian *et al.*, 2011).

Malaria control efforts and elimination in Africa are being challenged by the development of resistance of parasites to anti-malarial drugs and vectors to insecticides. Alemayehu and Mamuye (2011). Insecticide-treated nets (ITNs) and long-lasting insecticidal nets (LLINs) are the primary interventions for preventing malaria in sub-Saharan Africa. Nets accumulate holes through wear and tear during the course of everyday use (Alex *et al*., 2012).

The effective malaria vector control using requires strict user compliance by adhering to daily proper deployment, maintenance and replacement of the torn or obsolete nets. The use of insecticide-treated nets (ITNs) has been adopted as a standard method for malaria vector control. Long-lasting insecticide-treated nets (LLINs) are recent innovations that have been proven to be more effective and bio-durable and are preferred to conventionally treated nets. Currently, there are several brands of LLINs on the market which have received approval by World Health Organization. Among them are four brands, includes: Olyset, Permanent net 2.0, BASF and Net Protect (TNT). Many more others are still under various stages of development. Before receiving WHOPES approval as LLINs, the above nets underwent a standardized testing procedure of undergoing up to more than 20 washes without losing their effectiveness (Atieli *et al*, 2010).
However, insecticide-treated bed-nets (ITNs) are being strongly promoted as a malaria control tools in Africa by World Health Organization and other international agencies. Their efficacy in reducing man-vector contact, malaria morbidity has been demonstrated in various cases (Chouaibou *et al*., 2006).

The development of insecticide resistance is probably the biggest threat to capacity to control malaria vectors. The chemical agents that make malaria vector control feasible are the pyrethroids. The best tools for delivering pyrethroids are long-lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) (WHO, 2008). Recent trends confirm that the scale up of these two tools is making inroads into the malaria problem in many African countries (Bhattarai *et al*., 2007).This has stimulated new discussion about malaria elimination which a few years ago seemed inconceivable (Tanner and Savigny, 2008). According to Tung coinciding with the increased coverage of LLIN and IRS is the development and spread of resistant mosquitoes that may ultimately undermine the effectiveness of the two tools (Tungu *et al*., 2010)

Unfortunately, a major problem with the use of LLINs currently is the appearance of the resistance of malaria vectors to insecticides, especially to pyrethroids. During the past few years, resistance to insecticides has become widespread in Western, Eastern, and Central and in Southern Africa. Therefore, it is important for NMCPs to know if they should continue to promote LLINs. Although resistance is perceived as a serious threat to the future of malaria control, the current distribution of resistance is occasional, and its severity seems to differ from location to another. (Lancet, 2012). Similarly in Ethiopia, pyrethroids resistance has been reported from southwest (Yewhalaw *etal*, 2010, 2012), west, central and southern Ethiopia (Balkew et al., 2012, Massebo *et al.*, 2013). However, there was no systematic study that has been conducted especially in southwestern Ethiopia with regard to the Bio-Efficacy of LLINs that are impregnated with Pyrethroid chemicals and distributed to community whether the intended efficacy is compromised or effective with reported resistance. Thus this study was designed to evaluate the bio-efficacy of LLINs which are locally available and distributed to the community using Pyrethroid resistant mosquitoes from Jimma area.

3. Methods and Materials

3.1 Study area and period

This study was conducted in Jimma zone, Omo nada and Tiroafeta districts from March, 2015 to August 2015. Jimma zone is located 346km from Addis Ababa, in Oromia regional state, southwestern Ethiopia. The region is located between latitudes $07^0$42'50"N and $07^0$53'50"N and longitudes 37011'22"E and 37020'36"E, at an altitude ranging from 1,672-1,864m above sea level. The region has a sub-humid, warm to hot climate, receives between 1,300 and 1,800 mm of rain annually and has a mean annual temperature of 19^oc. The rainfall is divided in the long rainy season starting from June and extending up to September, and the short rainy season beginning in March and extending to April/May.

3.2. Study design and sample size

Across-sectional study was conducted in 150 households purposefully selected from Burka-Asendabo *kebele* (Smallest Administrative unit in Ethiopia), Omo-Nada district and Kajello *kebele* Tiroafeta district, from March 2015 to August 2015. Seventy five Households were selected from each kebele. The purpose of selecting the above two *kebeles* was because these were the only sites in Jimma zone where PermaNet 3.0 (the brand net of our interest) was distributed before three years. Seventy five head of households were randomly selected from each kebele member's roster and considered for the study.

3.3 Data collection and Instrument

A community based survey was conducted from March 2015, to August 2015 using structured, questionnaire interview. In line with administration of interview, old PermaNet 3.0 samples were collected by replacing with new permaNet 3.0 on spot. During contact with household heads socio-demographic data such as educational status, housing condition, source of energy, source of pure water, latrine condition, family size, sleeping place, net condition, net usage and handling were asked. Observation was made to each house hold included in the study and net hanging place, sleeping place, flooring of the house, walling of the house, ceiling of the house, net storage condition and the number of holes (if present) per each net were recorded.

3.4 Evaluation of physical integrity of net

Net was individually deployed over a rectangular 180*180*180 frame. Hole, seam failure and repair were recorded. The size and location of each hole was recorded for each net and the hole size was measured as the long axis of the ellipse to the nearest cm .Only hole greater than greater than 0.5was counted. Hole location was recorded separately (Roof, Upper.Lower,seam) and hole size was done with ruler by categorizing size1(0.5- 2cm), size2(2-10cm), size3 (10-25cm),size4 (greater than 25cm)(who, 2011).

Plat .1 Measuring LLIN hole and size

3.5. Mosquito rearing

Both Anopheline and cuisine mosquito larvae were collected by dipping from a range of breeding habitats (road paddies, brick pits, pools, marshes, streams, surface water harvest, ditches, dam reservoir shore, and pits dug for plastering traditional tukuls) around Jimma town. Mosquito larvae was reared to adults in the field Vector Biology Laboratory, Jimma University under standard conditions (temperature 25 ± 2 ^{o}C, relative humidity 80 ± 4%). larvae was fed with dog biscuits and brewery yeast. Adults emerged from pupae were kept with 10% sucrose solution until experiment.

Plate. Mosquito rearing

3.6 LLIN sample preparation and WHO cone assays

Three rectangular nets of PermaNet 3.0, were randomly selected from 75 samples collected from Burka-Asendabo and another three rectangular nets of PermaNet 3.0 were selected from Kajello kebele. Concurrently Three unused PermaNet 3.0 nets of the same Bach were obtained from Asendabo health center. Untreated nets to be used as a negative control was be purchased from the local market in Jimma, Ethiopia. The production date and batch number of all nets was recorded. Three sub-samples per net (one from the roof and two from each long side of the net) was taken from each net and prepared for standard LLINs cone tests by cutting 30 cm x 30 cm pieces. Each sub-sample was rolled up in aluminum foil, labeled (by net type, net number and sample area) and kept individually in a refrigerator prior to the assay. For each individual sub-sample, four cone tests were conducted sequentially following the standard WHO procedure. Five non blood-fed, two to three days old, female mosquitoes were introduced into each cone and exposed to each bed net sample for 3 minutes before being transferred to paper cups and held with access to 10% sugar solution. Knockdown (KD) was recorded at 60 minutes and mortality (MT) was recorded 24 hours post-exposure. A total of 100 mosquitoes was tested for each net type (20 mosquitoes x 5 subsamples). Replicate of cone assays with sub-sample taken from untreated nets was also conducted concurrently as a negative control. Mortality was corrected using Abbott's formula when mortality in the control exceeded 5% (22). Bioassays was carried out at a temperature of 27±2°C and relative humidity of 80±4%

3.7 Wash resistance

The resistance of an LN to washing was determined through standard bioassays carried out on nets washed at intervals using the standard WHO wash, and dried and held at 30 °C. Bioassays was done after 0, 1, 5, 10, 15and 20 washes. Each bioassay was done just before the next wash. Regression curves should be drawn using respectively percentage mortality and knock down KD) versus number of washes (who, 2005)

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Plate 3.Washing Net and cone bioassay test

3.8 WHO washing procedure

Net samples (25 cm x25 cm) was individually introduced into 1-l beakers containing 0.5 l deionized water, with 2 g/l soap5pH 10–11) added just before and fully dissolved. Beakers was immediately introduced into a water bath at 30 °C and shaken for 10 minutes at155movements per minute. The samples are thenremovedand rinsed twice for 10 minutein clean, deionized water in the same shaking conditions as stated above. Nets are dried at room temperature and stored at 30 °C in the dark between washes.the soap that used towash was
Savon de Marseille" is recommended as the standard soap.

3.9 .Data analysis

Data was entered in to a computer and then was be checked for consistency and completeness. The data (mortality rate and kdr) aswell as questioner data analyzed using SPSS version20.0 software package and count data was long transformed before analysis. In addition to that the outcome of the study was expressed using tables, figures or as mean standard deviation and all testes was considered significant at ($p<0.05$) of confidence interval 95%.
The total number of hole per net (total and size category) and the hole size was determined by the median and inter quartile range and interquartile range and the overall range by net comparisons over time group of collection used Wilcoxon sign rank proportions net with whole was compared over time using chi-square test. Hole index was stated following the method described by Kilian *et al*2010)and Will s*et al*2014)

4 Results.

4.1 Socio Demographic Status of the study area.

Out of 150 respondents, 63 (42%) of tem attended religious school, 58(38.7%) were illiterate and very few number of respondents attended secondary school 3(2%). Regarding the possession of Electricity from the total of 150 households 113(75.3%) of them did not possess electricity and the remaining 37(24.7%) possess electricity in their residences. majority of the respondents use spring water 83(55.3%) as source of drinking water followed by 45(30%) protected water. Seven (4.7%) use unprotected public well and surface water. Private pit latrine is the predominantly used type of toilet possessed by most households 142.(94.7%) in the study area. Regarding the family size majority of the households 76(50.7%) possess 5-7 family size, 62(41.3%) possess 1-4 family size and 12(8%) of them possess family size above eight (Table-1).

Table 1 Socio Demographic characteristics of Omo-Nada and Tiro-Afeta districts, Jimma Zone, South West Ethiopia (March 2015-August 2015)

Variables	Respondents	N(%)
Education	None	58(38.7%)
	Religious	63(42%)
	Primary	26(17.3%)
	Secondary	3(2%)
Electric possession	Exist	37(24.7%)
	Not exist	113(75.3%)
Drinking water	Protected Public	45(30%)
	Unprotected	15(10%)
	Surface water	7(4.7%)
	Spring water	83(55.3%)
Latrine	Own pit latrine	142(94.7%)
	Bush or Field	5(3.3%)
	Other	3(2%)
Family size	1-4	62(41.3%)
	5-7	76(50.7%)
	Above 8	12(8%)

4.2 Net possession, handling and usage

All 150 sample Nets collected from the study area were found to be PermaNet 3.0, brand new generation of Long-Lasting Insecticidal Net (LLIN) distributed in the area in 2013 with batch number PermaNet 3.0 101213. 147 (98%) of the LLINs collected from each house hold were used for sleeping purpose prior to the collection day. Moreover, 126(84%) of the respondents use the nets most often. In houses where nets are scarce priority was given to children and all children in the study area were protected. Most respondents more than 120(80%) have combination of different sleeping placeWooden bed frame, Wooden bed stick and mattress placed on carpet on bare floor all three combined. Most respondents 103(68.6%) practice net washing with 84(56%) of them washed in the last 6 months prior to net collection and very few respondents washed before 6months. Most Respondents 92(61%) wash their net with local bare soap and dried their net outside in shade 79 (53%) where as very few 24(9) wash and dry their net in side in the house (Annex 1)

4.3 Net condition

Out of 150 nets collected from both districts almost all of them found hanged over sleeping places either hanging folded 57 (38%), hanging tied 56(37.33) or hanging lose 34(22.7%). Very few nets were found 3(2%) stored. 144(96%) of nets found inside the home and only 6 (4%) were found outside the home. Housing conditions were of typical traditional set up with 147 (98%) of the houses with Soil floor, 143(95.3%) walling made from mud with wooden frame and 95(63%) of the houses covered by grass thatch with the rest 55(36.7%) of the houses covered with corrugated Iron sheet. 148 (98.5%) of the respondents agreed that open flame from fire wood is the predominant source of power for cooking and the same percent of respondents agreed that oil lump is used during the night as source of light (Annex 2). The association between net condition and the impact of housing, cooking, washing and net drying is presented in Table 2.

Table 2. **The impact of Net washing habit, Housing type, light source and daily handling habit on physical condition of the net in the study area**

Observations	Response	Physical status of net		X^2-square	p-value
		With Hole (%)	Without hole (%)		
Net ever been washed	Yes	50	41	0.886	0.346
	No	50	59		
Time lap taken for soaking net	Never soaked	35	28	0.497	0.780
	Less than 1hr	23	25		
	Do not know	42	47		
House type	Tukul	67	81	2.452	0.117
	Corrugated Iron	33	19		
Oil-lump used as source of light	Yes	64	56	0.571	0.450
	No	36	44		
Misuse by children	Yes	40	0	18.562	0.000*
	No	60	100		

The association between Physical condition of the net and misuse of the net by children was highly significant (Df 1, P=0.000) whereas no significant relation was observed between factors net washing habit, soaking time, housing type and source of light (Table 2.)

4. 4. Determination of Net hole and size

Out of 150 nets collected from the community, 116 (77%) of them were with different number and size of holes whereas the rest 34(22.7) were in good condition. 61(40.7) of the nets were with horizontal tear to bottom, 93(62%), of the nets had tear at hanging points, 81(54%) of the nets with burn holes. 127 (85%) of the nets had holes from rodents and 51(34%) of the nets were with open seam with the whole section missing (Annex 3).

Out of 116 PermaNet 3.0 samples collected, there were 293 holes recorded with hole size one (0.5-2cm), 200 holes recorded of hole size two (2-10cm), 69 holes recorded of hole size three (10-25cm) and 38 holes of hole size four (>25cm) (Table3).

Table 3. Net whole number and size of net collected from Burqa-Asendabo and Qajelo villages, Jimma Zone, southwestern Ethiopia.

Hole size	Number	Net section			
Hole size one(0.5-2cm)	Gap	Roof	Upper	Lower	Seam
	0	58(38.7%)	47(31.3%)	78(52%)	126(%
	1-20	74(49.3%)	84(58%)	64(42.6%	24(16%)
	21-40	12(8%)	11(7.3%)	7(6.6%)	-
	>41	6(4%)	8(5.3%)	3(2%)	-
	Total	150(100	150(100)	150(100)	150(100)
Hole size two (2-10cm)	011	88(58%)	71(47.3%	102(62%)	139(92.7
	1-10	61(40.6%)	71(47.3	45(30%)	11(7.33)
	11-20	1(0.7)	5(3.3)	-	-
	>20	-	3(2%)	3(2%)	-
	Total	150(100)	150(100)	150(100)	150(100)
Hole size three (10-25cm)	0	121(80.7%	129(86%)	136(90%)	145(96.7)
	1-10	29(19.3%)	21(14%)	14(9.3)	5(3.3)
	Total	150(100)	150(100)	150(100)	150(100)
Hole size four (>25cm)	0	141(94%	139(92.7	138	144
	1-10	9(6%)	11(7.3%)	12(8%)	6(4%)
	Total	150(100	150(100)	150(100)	150(100)

4.5 Bioassay tests on Unused PermaNet 3.0 samples using wild collected populations of *Anopheles gambiae* s.l. and *Culex quinquefasciatus*

Mortality and Knock down Effect

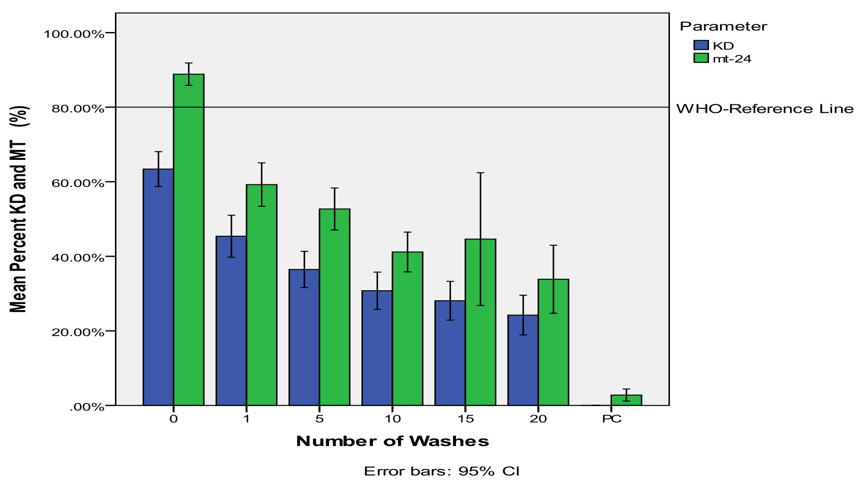

Fig1 Efficacy of PermaNet 3.0 after washing as determined by WHO cone bioassay tests with *Culex quinquefasciatus*

PermaNet 3.0 performance was slightly good against wild population of Culex quinquefasciatus causing more than 80% mortality and more than 60% knockdown. However, its efficacy and wash resistance rapidly downgraded as the number of washes increase from 0 to 20 times (figure1).The untreated nets (Positive control, PC) recorded 2.0 % mortality and 0.00% knockdown. Comparison of mean percent mortalities and knockdown effects among different wash status has shown significant variation (P=0.000) among different washes.

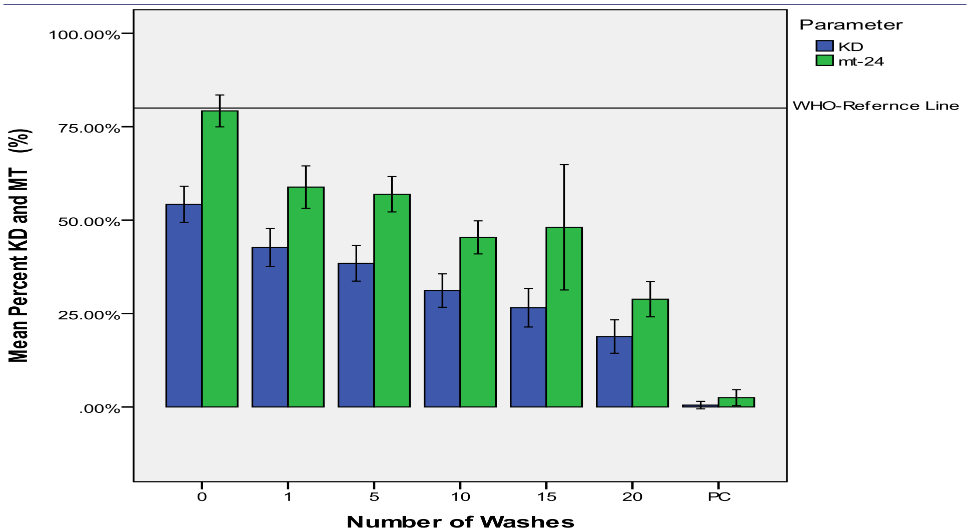

Fig2 Efficacy of PermaNet 3.0 after washing as determined by WHO cone bioassay tests with *An. gambiae s.l.*

Bioassay of similar net samples using wild collections of *An. gambiae* s.l. has provoked mortality of 79% and knockdown effect of 54%. Similarly its bioactivity rapidly decreased to 28% mortality and 18% knockdown when the numbers of washes increase from 0 to 20 (Figure 2). The untreated nets (Positive control, PC) recorded 2.5 % mortality and 0.00% knockdown. Comparison of mean percent mortalities and knockdown effects among different wash status has shown significant variation (P=0.000) among different washes.

The effect of synergist on un unwashed PBO-deltamethrin top netting induced an almost identical level of mortality in *Cx. quinquefasciatus*(100%)and *An. gambiae s.l*(97%) indicating that PBO was synergizing the Pyrethroid resistance. Over the course of 20 washes of the PBO-deltamethrin netting there was a partial loss of activity against both mosquito populations and a near complete loss towards the end (Figure 3&4). This indicates that the surface concentration of PBO was largely removed by washing so no further synergy was evident against the resistant population and any PBO replenishment from the core of the fibers after washing was insufficient to regain toxic activity. Comparison of mean mortality induced by top and side sections significantly different (p=0.001) for both populations *Cx. quinquefasciatus* and *An. gambiae s.l.*

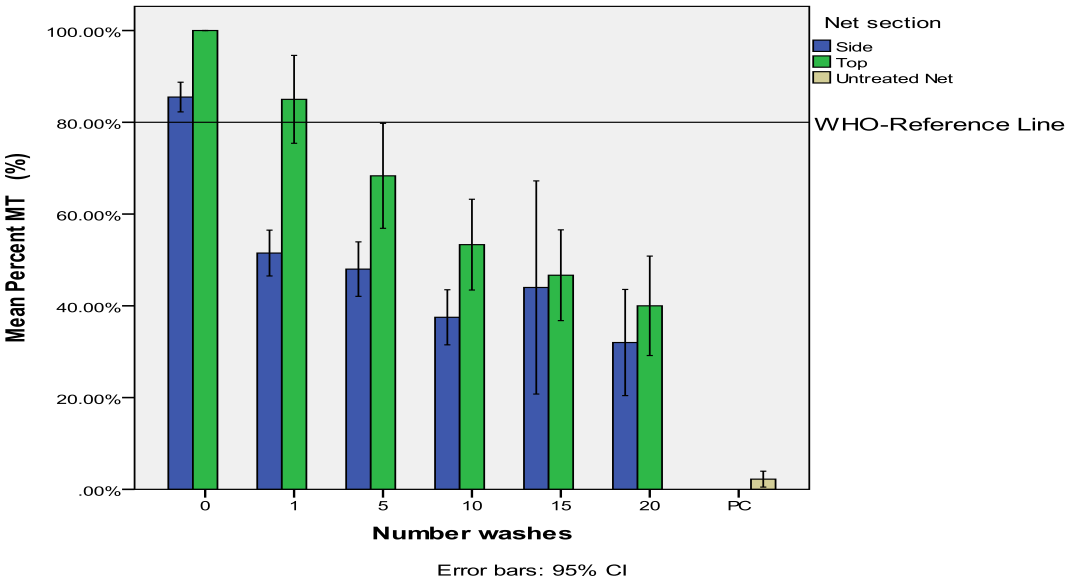

Fig.3 Efficacy of PermaNet 3.0 top and side panels against pyrethroid resistant *Culex quinquefasciatus* determined by WHO cone bioassay tests

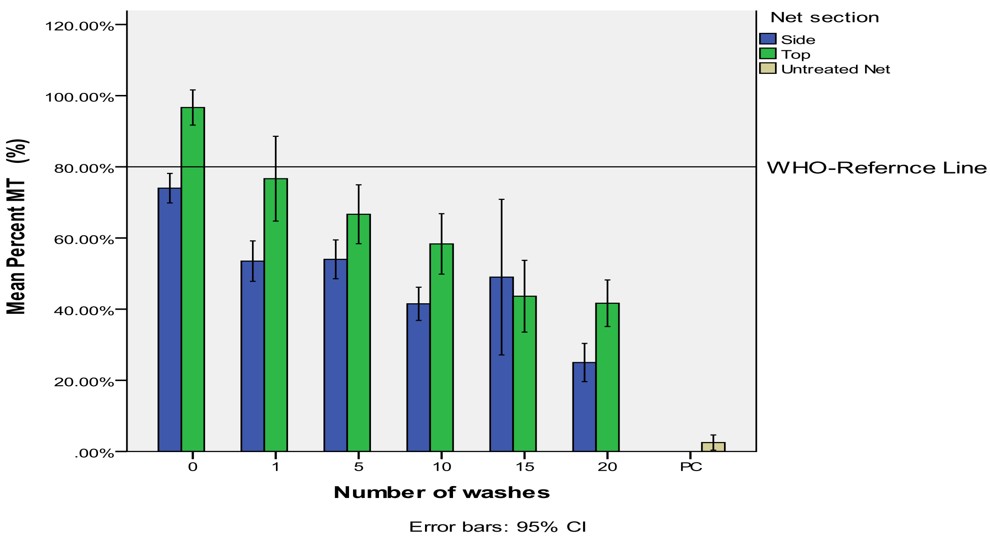

Fig.4 Efficacy of PermaNet 3.0 top and side panels against Pyrethroid resistant An. *gambiae* S.l. Determined by WHO cone bioassay test

4.6. Efficacy of permanent 3.0 samples Nets collected from field determined by WHO cone Bioassay test

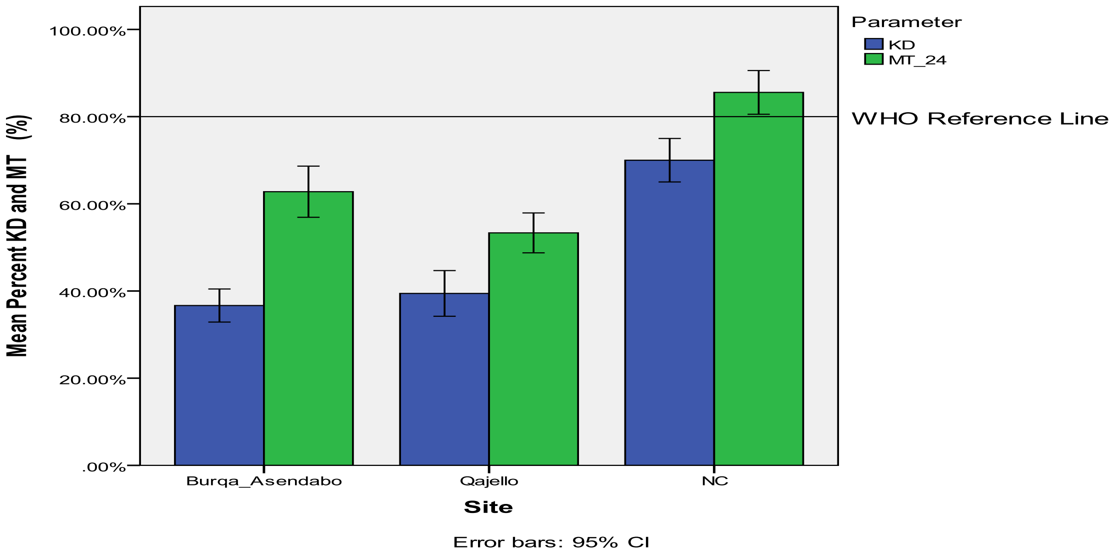

Figure 5. Efficacy of Field collected PermaNet 3.0 samples from Burqa-Asendabo and Qajello villages against wild *An. gambiae* s.l. and susceptible strains of *An. arabiensis* in Jimma zone, southwestern Ethiopia

PermaNet 3.0 samples collected from Burqa-Asendabo has resulted in knockdown and mortality of 34% KD and 63% respectively using field collected *An. gambiae* s.l. Similarly PermaNet 3.0 samples collected from Qajello village resulted knockdown and mortality of 39% and 53% respectively using field collected An. gambiae s.l. concurrently, exposure of same net samples from both villages to susceptible strains of *An. arabiensis* (Negative control, NC) has resulted in knockdown and mortality of 70% and 86% respectively (Figure 5).

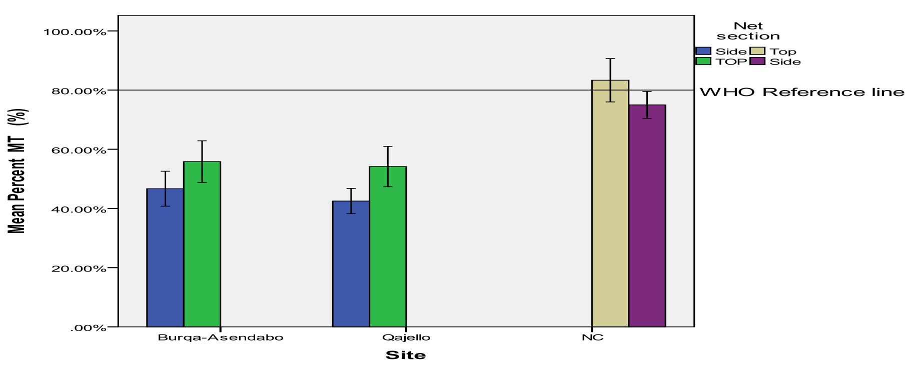

Fig. 6 Efficacy of PermaNet 3.0 collected from field after three years' service against pyrethroid resistant *An. gambiae* S.l. Determined by WHO cone bioassay tests

Evaluation of the synergetic effect of PBO in pyrethroid resistance using field collected nets has also proven the synergy with in both fields top side produced greater mortality compared to side panel of the net. However, the bioactivity of field collected nets has resulted very low mortality against in both wild *An. gambiae* s.l. and laboratory reared susceptible strains of *An.arabiensis* compared to WHO reference line (80%) (Figure 6). This indicates that the surface concentration of PBO was largely removed due to washing and other anthropogenic activities.

5 .Discussions

According to a study by the UNDP (United Nations, 2009), the success in achieving the objective of the sixth MDGs (Objective of the Millennium Goals) and other global targets for malaria depends on public awareness about the value of human health, the use of treated mosquito nets and the provision of effective access to nets. In line with this, the national malaria control program (NMCP) in Ethiopia currently relies on strategies targeting mosquitoes vector control, which involves the use of long-lasting insecticidal nets (LLINS), Indoor Residual Spray and Environmental modification of larval breeding habitats(NMCP,2010). However, access to universal coverage of LLINs, proper use of these nets and the emergence of resistant vector population are a challenge for an effective control of the disease. Thus, in the present study, we evaluated, net possession, handling and usage by selected members of Burqa-Asendabo and Qajello *Kebele*, Southwestern Ethiopia. We also evaluated the Bio-Efficacy and wash resistance of PermaNet 3.0 LLIN (both used and unused) using wild *Anopheles gambiae* s.l. and *Culex quinquefasciatus* mosquitoes in the study area.

Current study shows that most of the respondents in the study area were either illiterates or only went to religious schools. Spring water and protected public wells were primary source of drinking water and most of the respondents own pit latrine. Half of the respondents have family size between 5 and 7 (Table1). Similar findings were reported from Amhara regional state Ethiopia in 2013 stated that most of the rural participants (68%) were illiterate (Aderaw and Gedefaw, 2013)

Current study shows that LLIN coverage in the study area reached 100% with majority of the LLINs were being used for sleeping purpose prior to the collection day. Moreover, majority of the respondents use the nets most often and in houses where nets are scarce priority was given to children. Most respondents practice net washing habit with more than half of them reported as they have washed in the last 6 months prior to net collection day. Most Respondents wash their net with local bare soap and dried their net outside in shade where as very few wash and dry their net in side in the house. However, hanging problems, hanging points, misuse by the children, housing conditions, open flame fire used for cooking and other factors still could challenge the intended preventive capacity of the LLIN in the study area (Table 2). Similar finding were reported from Amhara region, Ethiopia by Aderaw and Gedefaw (2013), whom stated that 84.67% of the households possessed functional bed nets, and 71.4% of them have been slept under bed net a day before the interview took place. Likewise, high LLIN ownership and usage rate was reported from Amhara and Oromia regional states, Ethiopia with 91% of the respondents own at least one ITN prior to the survey date (Carlo *et al.*, 2009). In contrast, to the current study, low ITN ownership and usage rate (62 % and 65%) was reported from Eastern Ethiopia (Biadgilign*et al.*, 2012; Gobena *et al.* (2012).

In the present study, most nets collected from the community had high frequency of holes per net and varying sizes of holes with only (22.7%) of LLIN nets in good condition. The damages recorded include horizontal tear to bottom, tear at hanging points, burn holes, holes from rodents and the nets with open seam with the whole section missing. The size and number of holes also vary from hole size one to hole size 4 (Table3).

In contrast to high wear and tear rate reported in our current study i.e., 78%, similar studies conducted in Amhara, Oromia and eastern Ethiopia has reported low damage level with 10% and 36% respectively (Carlo et al., 2009;Gobena et al. (2012). In addition to this, low net damage rate was also reported from Kenya and Benin with 48% and 24% respectively (Ochomo*et al.*, 2013;Osse *et al*., 2013).

In the present study, more than 80% mortality was observed on unwashed PermaNet 3.0 against wild population of *Culex quinquefasciatus* and 79% mortality was recorded against *An. gambiae* s.l. Similarly bioassay of unwashed PermaNet 3.0 against both Culex and Anopheles population has resulted lower knockdown effect of 60% and 54% respectively. Moreover, the efficacy and wash resistance rapidly downgraded as the number of washes increase from 0 to 20 times (figure1 and 2). Both unwashed and washed 20 time Nets failed to qualify for WHO cut point of provoking ≥ 80% mortality and **≥** 95% knock down except unwashed PermaNet 3.0 against *Cx. quinquefasciatus*. However, The unwashed PBO-deltamethrin top netting induced an almost identical level of mortality in *Cx. quinquefasciatus*(100%) and *An. gambiae s.l*(97%) indicating that PBO was synergizing the pyrethroid resistance (Figure 3 &4). Similarly, Yewhalaw et al., 2012, from Jimma zone, southwestern Ethiopia reported that, optimal bio-efficacy was observed for the deltamethrin + PBO roof of PermaNet 3.0 against all four populations of *An. arabiensis*. Likewise (Tungu *et al*., 2010) from Tanzania reported that pyrethroid resistance is synergized by oxidases and Kdr mutation as it was observed by significantly different mortalities between top and side panels of PermaNet 3.0 against both *An. gambiae* s.l. and *Cx. quinquefasciatus*.

However, reduced susceptibility of both KD and MT in current study when the side and roof records combine is first of its type with PermaNet 3.0 against the local populations *An. gambiae* s.l. and *Cx. Quinquefasciatus*.

PermaNet 3.0 samples collected from *Burqa-Asendabo and Qajellokebeles* has resulted in knockdown of 34% and 39%. Similarly same sample nets induced mortality of 63% and 53% respectively using field collected *An. gambiae* s.l. concurrently, exposure of same net samples from both villages to susceptible strains of *An. arabiensis* (Negative control, NC) has resulted in knockdown and mortality of 70% and 86% respectively, field collected samples performing poor against both wild and susceptible populations (Figure 5). Nevertheless, Evaluation of the synergetic effect of PBO in pyrethroid resistance using field collected nets has proven the synergy with in both fields top side produced greater mortality compared to side panel of the net despite very low mortality against both wild *An. gambiae* s.l. and laboratory reared susceptible strains of *An.arabiensis* (Figure 6). This indicates that the surface concentration of PBO was largely removed due to washing and other anthropogenic activities. Similar findings were reported from Kenya by Ochomo et al., 2013, which stated that Nets collected from field retained strong activity against a susceptible laboratory strain, but not against f1 offspring of field-collected *An. gambiae* s. l.

6. Conclusion

Current study shows that LLIN coverage in the study area reached 100% with majority of the LLINs were being used for sleeping purpose prior to the collection day. However, hanging problems, hanging points, misuse by the children, housing conditions, open flame fire used for cooking and other factors still could challenge the intended preventive capacity of the LLIN in the study area.

Current study also shows that the wild populations of both *Culex quinquefasciatus* and *An. gambiae* s.l. around Jimma were resistant against both unwashed and washed samples of PermaNet 3.0. Moreover, the efficacy and wash resistance rapidly downgraded as the number of washes increase from 0 to 20 times. However, the unwashed PBO-deltamethrin top netting induced mortality in *Cx. Quinquefasciatus* and *An. gambiae* s.l in both above WHO reference line indicating that PBO was synergizing the pyrethroid resistance.

Relatively low mortalities and knock down were observed from one wash to twenty Evaluation of the efficacy of PermaNet 3.0 against *An. gambiae* and culex quinquefasciatus populations from malaria transmission area has provided valuable information on wide variations depending on the population and LLIN being tested.

PermaNet 3.0 samples collected from *Burqa-Asendabo and* Qajello *kebeles* has resulted in knockdown and mortality extremely low record confirming the emergence of resistance mosquitoes to the new recruited LLIN.

Recommendation

Based on the results of this study, the following recommendation was forwarded.

- Communities live around malarias area should have LLIN and use properly to control or protect the vector of malaria.

- Due to performance durability and long-term effectiveness of physical and chemical control of vector, the local communityshould be aware of usage, handling and washing proecedures of PermaNet3.0.
- Monitoring the efficacy of LLINs should be undertaken regularly in order to guide policy selection and distribution of LLINsPermaNet3.0.
- Government, International and local NGOS work in collaboration so as to control Insecticide resistance.

Reference

Abate, A. and Hadis,H. (2011) Susceptibility of *Anopheles gambiae* s.l. to DDT, malathion, permethrin and deltamethrin in Ethiopia **6 (**4): 486-491

Aderaw Z, Gedefaw M (2013) Knowledge, Attitude and Practice of the Community towards Malaria Prevention and Control Options in Anti-Malaria Association Intervention Zones of Amahara National Regional State, Ethiopia. *J Trop Dis***1**(3):118. doi:10.4172/2329-891X.1000118

Aseged, T., Mamuye, H., Nesibu, A., Dejene, T. and Wirtz, A. R. (2006). Biting behavior and *Plasmodium* infection rates of *Anopheles arabiensis*from Sille, Ethiopia. *ActaTropica***97**: 50-54.

Asidi A, N'Guessan R, Akogbeto M, Curtis C, Rowland M(2012. Loss of household Protection from use of insecticide-treated nets against Pyrethroid-resistant mosquitoes, Benin. Em erg Infect Dis **18**:1101–1106.

Balkew, M., Getachew, A., Chibsa, S., Olana, D., Reithinger, R. and Brogdon, W.(2012). Insecticide resistance: a challenge to malaria vector control in Ethiopia. *Malaria Journal* **11**:139.

Bhattarai, A., Ali ,S., Kachur, S.P., Martensson, A., Abbas, A.K., Khatib, R., Al-mafazy, A., Ramsan, M., Rotllant, G., Gerstenmaier, J.F., Molteni, F., Adbulla, S., Montgomery, S.M., Kaneko, A. and Bjorkman, A. (2007). Impact of artemisin in based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS Medicine***4**:30

Biadgilign, Ayalu Reda and Haji Kedir (2012). Determinants of Ownership and Utilization of Insecticide-Treated Bed Nets for Malaria Control in Eastern Ethiopia. *Journal of Tropical Medicine*.doi:10.1155/2012/235015

Teklu, B., Habte, T., McCartney, M. and Solomon, K. (2010). The effect of water physical quality and water level changes on the occurrence and density of larvae of *Anopheles* mosquitoes around the shoreline of the Koka reservoir, Central Ethiopia. *Hydro Earth System Science Discussion* **7:** 6025–6055

Carol A Baume, Richard Reithinger and Sara Woldehanna (2009). Factors associated with use and non-use of mosquito nets owned in Oromia and Amhara Regional States, Ethiopia. *Malaria Journal***8**:264 doi:10.1186/1475-2875-8-264

Ceesay, S.J., Casals-Pascual, C., Erskine, J., Anya, S.E., Duah, N.O., Fulford, A.J., Sesay,S.S., Abubakar, I., Dunyo ,S. and Sey, O. (2008). Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis. *Lancet***372** (9649):1545-1554.

Coetzee, M., Van Wyk, P., Booman, M., Koekemoer, L.L. and Hunt, R.h. (2006).Insecticide resistance in malaria vector mosquitoes in a gold mining town in Ghana and implications for malaria control. Bulletin of the Exotic Pathology Society**99:** 400-403.
DPP/2005.11, 18 p

Elissa**,** N., Mouchet, J., Riviere, F. and Meunier, J.Y. and Yao, K.(1994).Susceptibility of *An.gambiae* to insecticides in the Ivory Coast. *Sante***4:** 95-99.

Gimnig JE, Vulule JM, Lo TQ, Kamau L, Kolchak MS, Phillips-Howard PA, Mathenge EM, Ter Kuile FO, Nahlen BL, Hightower, A.W(2003) Impact of permethrin-treated bed nets on the entomologic indices in an area of intense year-round malaria transmission**.** *Am J Trop Med Hyg* 2003, 68(90040):1 6-22.

Gobena, T, Yemane Berhane2† and Alemayehu Worku. (2012). Low long-lasting insecticide nets (LLINs) use among household members for protection against mosquito bite in kersa, Eastern Ethiopia. *BMC Public Health***, 12**:914.

Harbach, R.E and Kitching, I.J. (2005). Reconsideration of Anopheline mosquito phylogeny (Diptera: Culicidae: Anophelinae) based on morphological data. *Systematic and Biodiversity***3**: 345–374.

Harbach,R.E(1998) ,The mosqitoes of the subgenus culex in southwest Aesia and Eygpt Diptra.Culicidae) Contribution the American Entomological institute **24**(1).1-2.

Hargreaves, K., Hunt, R.H., Brooke, B.D., Mthembu, J., Weeto, M.M., Awolola, T.S. and Coetzee, M. (2003). *An. arabiensis* and *An. quadriannulatus* resistance to DDT in South Africa**.** *Medical and Veterinary Entomology* **17:**417-422.

Hill J, Lines J, Rowland M (2006). Insecticidetreated nets. Adv. Parasitol., 61:77-128. http://dx.doi.org/10.1016/S0065308X (05)61003-2

Jima, D., Getachew, A., Bilak, H., Stekeete, R.W., Emerson, P.M., Graves, P.M., Gebre, T., Reithinger, R. and

Hwang. (2010). Malaria indicator survey 2007, *Ethiopia: coverage and use of major malaria prevention and control interventions* **9**:58.

Kilian,A., Wilso, B., Olivier, P., John, G., Francis, A., Lizette, K and Natacha, P. (2011), Evidence for a useful life of more than three years for a polyester-based long-lasting insecticidal mosquito net in Western Uganda. *MalariaJournal***10**: 299.

Kristan, M., Fleischman, H., Della Torre, A., Stich, A. and Curtis, C.F. (2003).Pyrethroid resistance/Susceptibility and differential urban/rural distribution of *An.arabiensis* and *An. gambiae s. s.* malaria vectors in Nigeria and Ghana. *Medical and Veterinary Entomology***17:**326 –332.

Lengeler, C. (2004) Insecticide nets and curtains for preventing. Malaria Cochrane Database. *Systematic Review***2**:363.

Lindsay SW, Adiamah JH, Miller JE, and Armstrong JRM (1991) Pyrethroid-treated bed net effects on mosquitoes of the *Anopheles gambiae* complex in The Gambiae**.** *Med Vet Entomol* , 5:477-483.

Massebo, F. Balkew, M., Gebre-Michael, T. and Lindtjørn, B.(2013). Blood meal origins and insecticide susceptibility of Anopheles arabiensis from Chano in South-West Ethiopia. *Parasite Vectors***6**:44.

Ochomo E.O., Nabie M Bayoh , Edward D Walker , Bernard O Abongo , Maurice O Ombok , Collins Ouma , Andrew K Githeko , John Vulule , Guiyun Yan and John E Gimnig (2013). The efficacy of long-lasting nets with declining physical integrity may be compromised in areas with high levels of pyrethroid resistance. *Malaria Journal*,**12**:368.

Okia M., Richard Ndyomugyenyi, James Kirunda , Anatol Byaruhanga , Seraphine Adibaku , Denis K Lwamafa and Fred Kironde (2013). Bioefficacy of long-lasting insecticidal nets against pyrethroid-resistant populations of *Anopheles gambiae* s.s. from different malaria transmission zones in Uganda. *Parasites & Vectors***6**:130.

Ossè, R.A., Aïkpon, R., Sovi, A., Padonou, G.G., Oké –Agbo, F., Gnanguenon, V. and Martin Akogbéto, M. (2013). Long lasting insectcidal nets use efficacy and physical integrity in a vector resistance area after a nationwide campaign in southern Benin, West Africa. *Journal of Public Health and Epidemiology* 5(8)325-335

Pates, H. and Curtis. C.(2005). Mosquito behavior and Vector control. *Annual Review of Entomology* **50**:53-70.

Protection from use of insecticide-treated nets against Pyrethroid-resistant mosquitoes, Benin. Em erg Infect Dis 18:1101–1106.

Protopopoff, N., Verhaeghen, K., Van Bortel, W., Roelants, P. and Marcotty, T.(2008). A high increase in kdr in *An. gambiae* is associated with an intensive vector control intervention in Burundi highlands. *Tropical Medicine and International Health***13**: 1479–1487.

Ribeiro, J.M., Seulu, F., Abose, T., Gebramariam, K. and Addis, T. (1996). Bulletin of the World Health Organization (WHO).**74**: 299-305

Savage, H. and Miller,B. (1995). House mosquitoes of the U.S.A Culexpipiens,*Wing Beast***6**(2).8 -9.

Sharp, A.J, Selzer, R.R., Veltman, J.A., Gimelli, S., Bimelli, G., Strino, P., Coppola, A., Geran, R., Price, S.M., Knoers, N.V., Eis, P.S., Brunner, H.G., Hennekam, R.C., knight, S.J., de Vries, B.B., Zuffardi, O. and Eicheler, E.E. (2007)Characterization of a recurrent 15q24 microdeletion syndrome**.***Mar* **116**(5):567-72.

Thwing J, Hochberg N, Vanden Eng J, Issifi S, Eliades MJ, Minkoulou E, Wolkon A, Gado H, Ibrahim O, Newman RD, Lama M (2008). Insecticide-treated net ownership and usage in Niger after a nationwide integrated campaign. Trop. Med. Int. Health 13(6):827-834

Tungu,p., Magesa,S., Maxwell, C. Malima.R, Masue, D, Sudi W, Myamba, J., Pigeon O., Rowland, M. Evaluation of PermaNet 3.0 a deltamethrin-PBOcombination netagainst Anopheles gambiae andpyrethroid resistant Culex quinquefasciatus mosquitoes: an experimental hut trial in Tanzania. *MalariaJouurnal* 9.21.

United Nations (2009). The Millennium Development Goals Report 2008

Vezenegho, S.B., Brooke, B.D., Hunt, R.H., Coetzee, M. and Koekemoer, L.L. (2009). Malaria vector mosquito composition and insecticide susceptibility status in Guinea Conakry, West Africa. *Medical and Veterinary Entomology* **23**:326 – 334.

WHO(2011).Guide lines for monitoring the durability of long lasting insectcidial Mosquito nets undwr opretional condition .Control of neglected tropical disease world pesticide evaluation schame and global malareia vector unit Geneva Suizerland ISBN9789241501705.

WHO (2010). World Malaria Report Geneva: World Health Organization: 136.

WHO (2008). World Malaria Report http://www.whoint/malaria/ wmr, access 28, March, 2014,

WHO (2005). Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets [http://whqlibdoc.who.int/hq/2005/ access 28, March, 2014.

WHO (2005). Malaria control in complex emergencies: An inter-agency field handbook, WHO/HTM/MAL/05, 1107.

WHO (2004). Guiding principles for malaria control in acute and chronic phase emergencies in Africa. DIP/MAI/05, 08.

Woime, A. (2008). *Changes in the Spread of Malaria in Ethiopia: Case Study from Awassa and Hossana Area* 2006-2007. M.Sc. thesis, Telemark University College, Telemark, Norway.

Yadouleton, w., padonou, G.,Asidi, A., Moiroux, N. and Bio-Bandannas, S etal (2010).Insectcide resistance in An.gambaie in southern Benin.*Malaria Journal* **9**.83.

Yewhalaw, D., Wassie, F., Steurbaut, W., Spanoghe, P., Van Bortel, W., Denis, L., Tessema, D.A., Getachew, Y., Coosemans., M., Duchateau, L. and Speybroeck, N:(2011).Multiple insecticide resistance: an impediment to insectside based malaria vector control. *Program public library science one* 6(1) : e16066.doi:1o.1371/journal.pone.0016066.

Yewhalaw. D, Asale. A, Tushune. K, Getachew Y, Duchateau Speybroeck(2012)Bio-efficacy of selected long-lasting insecticidal nets against pyrethroid resistant *Anopheles arabiensis* from South-Western Ethiopia.*ParasiteVectors*,**5**:15 Alex, A., Raphael, N. & Martin, A. (2012). Loss of Household Protection from Use of Insecticide-Treated Nets against Parathyroid-Resistant Mosquitoes, Benin *emerginginfectious Diseases***18** **(7**)1101-11061.

Annex 1 LN use and handling in Omo-Nada and Tiro-Afeta, districts Jimma Zone, South West Ethiopia. (September 2014 -August 2015).

Variable	Response	Respondents (%)
Net was found in the house and used for sleeping	Yes	147(98%)
	No	3(2%)
	Total	150(100)
Net ever Been used for sleeping	Yes	148 (98.7%)
	No	2(1.3%)
	Total	150(100)
Net used last night to sleeping	Yes	148(98.7%)
	No	2(1.3%)
	Total	150(100)
How_often_was_the_net_used	Every night	126(84%)
	Most night	20(13%)
	Same night	2(1.3%)
	Not used	2 (1.3%)
	Total	150(100)
Number_of_adults_Slept_lastnight_undernet greater than 15yrs _	0	4(2.66%)
	1-3	121(80.66%)
	4-7	20(13.33)
	above 7	5(3.33%)
	Total	150(100)
Number of children 5-15 Slept last night Undernet	No person in the	27(18%)
	1-3	115(76.6%)
	4-7	8(5.3%)
	Total	150(100)
Number of children less than five Slept last night under net	0	40(26.7%)
	1	72(48%)
	2	35(23.3%)
	3	3(2%)
	Total	150(100)
At which period of the year people use nets	Through Out the year	142(94.6%)
	During Rainy Season	8(5.3)
	Total	150(100)
Type of sleeping place used Wooden bed frame	Yes	132(88%
	No	18(12%)
	Total	150(100)
Type of sleeping_placeused	Yes	120(80%)
	No	30(20%)

Wooden_bed	Total	150(100)
Type_of_sleeping_place bare floor	Yes	130(90%)
	No	10(10%)
	Total	150(100)
Type_of_sleeping_place other	Yes	92(61.33)
	No	58(38.7
	Total	150(100)
Net_ever been_washed	Yes	103(68.6)
	No	47(31.4%)
	Total	150(100)
The last_time_net_was_washed	1week ago	47(31.4%)
	1-3 month	35(23.33%)
	3-6 month ago	49(32.66%)
	>3month	19(12.7)
	Total	150(100)
Type of soap	None	47 (31.4%)
	Local bar soap	92(61.33)
	Other	11(7.3)
	Total	150(100)
Where_was_the_net dried Place of drying	Outside in the sun	47(31.33)
	Outside in shade	79(52.66)
	In side	) 24(16
	In side	24(16)
	Total	150(100)

Annex 2 Net condition (for selecting net identification Omo-Nada and Tiro-Afeta,districts Jimma Zone, South West Ethiopia. (September 2014 -August 2015).

Variables	Response	N(%)
How_is_thenet_found	Hanging loose	34(22.7)
	Hanging tied	56(37.33)
	Hanging folded	57(38)
	Stored away	3(2)
	Total	150(100)
Thenet_found	Inside	144(96)
	Outside	6(4%)
	Total	150(100)
Type_of_flooring	Soil/sand	147(98)
	Cement	3(2)
	Total	150(100)
Type of_Roofing_or_ceiling	Grass thatch	95(63.3)
	Corrugated iron	55(36.7)
	Total	150(100

Type of walling	Mud with wood frame	143(95.3)
	Mud brick	7(4.7)
		150(100)
Open Flame_used_for_cooking_ heatingandlighting_firewood	Yes	150(100)
	No	0
	Total	150(100)
Flame_used_for_cooking_heatingandlighting_Charcoal	Yes	148(98.6)
	No	2(1.4)
	Total	150(100)
Flame used for lighting wax candle	Yes	19(12.66)
	No	131(7.4)
	Total	150(100)
Flame used for lighting using gas with glass	Yes	150(100%
	No	
	Total	150(100)

Annex 3 Type of holes observed from the net collected

Response	Yes/no	Respondents N (%)
Apperance_of_newhole_in_thepast_months	Yes	116(77.3)
	No	34(22.7)
	Total	150(100)
Type of hole horizontal tear to bottom	Yes	61(40.7)
	No	89(59.3)
	Total	150(100)
Type of hole at hanging points	Yes	93 (62%)
	No	57(38%)
	Total	150(100)
Type of holes _open seam	Yes	51(91)
	No	99(66%)
	Total	150(100)
Type_of_holes_Burnholes	Yes	81(54%)
	No	69(46%)
	Total	150(100)
Type_of_holes_from rodents	Yes	127(84.6%)
	No	23(15.34%)
	Total	150(100)
Type of holes Whole section missing	Yes	18(12%)
	No	132(88%)
	Total	150(100)

Annex 4

WHO cone data Recording Form

Date--------------------------- Mosquito species-------------------

LLIN PN 2.0 PN 3.0 Net protect Olyset Interceptor Yorcool

mosquito

Collection Asendabo Secoru Tiro afeta Kersa Jimma area.

Exposure time. 3minnutes

Sample code	Cone number	Exposure time	No of Mosquio	No of KD 60	No of MT24
	1				
	2				
	3				
	4				
		Total			

Sample code	Cone number	Exposure time	No of Mosquio	No of KD 60	No of MT24
	1				
	2				
	3				
	4				
		Total			

Annex 5-Different,plates

Different Plates

When larva collected and reard in cages.

When cone bioassay was takes place

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when that the net that distributed before2-3yrs collected from the communities measure the hole of each net

when who standerd washing procedure was done with water bath and standerd soap in the laboratory.