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SHORT COMMUNICATION

EFFECTIVE UTILIZATION PERIOD OF LONG-LASTING INSECTICIDE TREATED NETS AGAINST MALARIA VECTOR MOSQUITOES AND THE SPECIES COMPOSITION IN NORTH WEST ETHIOPIA

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ABSTRACT: The study was conducted to evaluate the bioefficacy of long-lasting insecticide treated nets (LLITNs) (PermaNet®2.0) over time and the species composition of *Anopheles* mosquitoes around Bahir Dar. The space spray collection method was used to determine the species composition of indoor resting *Anopheles* mosquitoes in the study area. Field collected samples of household used PermaNet® 2.0 were tested for their bioefficacy against laboratory reared *An. arabiensis* following the World Health Organisation standard cone test protocol. The study revealed that 75% of *Anopheles* mosquitoes collected from indoor location in the study area was *An. arabiensis* indicating that this species was the primary potential vector of malaria in the study area. The mean percentage knockdown effect of PermaNet® 2.0 up to two years of household usage against females *An. arabiensis* was 100%. However, this effect decreased to 44.5% after three and half years of household use. There was no significant difference ($P > 0.5$) in the mean percent mortality caused by PermaNet® 2.0 after six months and two years household usage (92.5% and 84%, respectively). However, under laboratory conditions there was recorded a markedly significant reduction ($P < 0.01$) in the mean percent mortality of females *An. arabiensis* exposed to PermaNet® 2.0 that has been used for three and half years (27%). Close monitoring on the feeding and resting behavior of malaria vector mosquitoes and awareness creation on the proper utilization of the bed net need to be conducted to check and maintain its feasibility as part of integrated malaria vector management.

Key words/phrases: *Anopheles arabiensis*, Bioefficacy, Long-lasting insecticide treated nets, PermaNet.

INTRODUCTION

Globally, malaria affects about 300 million people annually and causes about one million deaths, mostly among children in sub-Saharan Africa. In this region, mosquitoes, in the *Anopheles gambiae* species complex, are the

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most efficient vector of malaria (WHO, 2010). Ethiopia is one of the countries in sub-Saharan Africa where the prevalence of malaria is at its most intense (MOH, 2004). The mortality of people who become infected with malaria is the highest of all diseases in the country, especially for children under five year's age.

Due to the socio-economical importance of malaria, several countries have implemented vector control activities in an attempt to curtail the epidemic. Vector control interventions, combining insecticide treated bed nets (ITNs) and indoor residual spraying (IRS) have significantly suppressed *An. gambiae* populations in sub-Saharan Africa, including Ethiopia (WHO, 2010; WHO, 2011). However, reports have appeared on the behavioral resistance of *An. arabiensis*, i.e their tendency to rest and bite outdoors. As a result, malaria is still a major health concern in sub-Saharan Africa (WHO, 2011).

The malaria prevention and control strategy in Ethiopia includes patient treatment and vector control (MOH, 2004). Ethiopia launched the National Malaria Eradication Service in 1967. The strategy initiated at this time was to break the chain of transmission by spraying pesticides that kill the mosquito vector (Gish, 1992). The strategy was not successful partly because of some technical and financial constraints in the country and institutions that were supporting the eradication effort (MOH, 2000).

The current malaria vector control strategy in Ethiopia relies on the use of different vector control techniques applied in an integrated manner as suited to the local malaria transmission conditions (MOH, 2004). Although there is considerable experience in malaria control through indoor residual spraying (IRS), historically the use of insecticide treated nets (ITNs) in Ethiopia has been limited and implementation of ITNs for malaria prevention is still at an early stage (MOH, 2002). Recently, the malaria control program in Ethiopia is scaling up the distribution of ITNs (MOH, 2007).

Recently, long-lasting insecticide treated nets (LLITNs) have become the main component of integrated vector management to control malaria in Ethiopia. LLITNs differ from previous ITNs that need to be retreated with chemicals every six months. The WHO recommended LLITNs (PermaNet®) chemical impregnated bed nets at the factory level as personal protective measure of malaria for more than three years. Since 2005, 20 million long lasting insecticide treated nets have been distributed to 10 million households nationwide (MOH, 2007).

The goal is to provide 2 LLITNs per household in the rural target populations (PMI, 2007). The vast majority of nets distributed are Vestergaard-Frandsen white rectangular polyester LLITNs (PermaNet®) (PMI, 2007). PermaNet® is a polyester net treated with 55 mg/m^2 deltamethrin. The insecticide is diluted in a wash resistant resin, which coats the fibres (Graham *et al*., 2008). The objectives of this study, therefore, were to assess the longevity of PermaNet® mosquito net in use by farm households around Bahir Dar and determine the dominant malaria vector species.

MATERIALS AND METHODS

The study area and duration

This study was conducted in rural villages' around Bahir Dar City, Amhara Regional State from November 2008 to January 2009. The study area is situated at an average altitude of 1800 meters above sea level, latitude 11º 36'N, longitude: 37º 24'E. The area receives an annual mean rainfall 1455.8mm and mean maximum temperature and mean minimum temperature are 26.8ºC and 11.7ºC, respectively (NMA, 2008).

Collection of samples of PermaNet®2.0 for the bioassays

Four samples of the same batch MOH distributed PermaNet®2.0 LLITNs were collected for bioassay test following the WHO guidelines, (WHO, 2005) and thus a total of 12 nets were collected from the three batches, distributed in May 2005, November 2006, and May 2008. The ages of nets was identified using information from the leader of the households and confirmed by records from the local health center.

Collection of indoor resting Anopheles mosquitoes

The method used was space spray collection. Each month mosquitoes were collected once a week, early in the morning from 5:00 am to 6:30 am, from each location. The aerosol insecticide used was PifPaf™ (0.055% Prallethrin, 0.216% Tetramethrin, 0.095% d-Phenothrin and 99.6% inert ingredients) manufactured by Jordan Petra Trading and Investment Company.

All human occupants, domestic animals, utensils, exposed food and water were voluntarily moved out of human dwellings and the floor was covered with white cloth sheet (1.20m x 2.20m in size). Each house was sprayed with the aerosol for 15 seconds and waited for 10 minutes until the aerosol produced the knockdown effect. Knockdown female *Anopheles* mosquitoes

were collected from the white cloth sheets using forceps and paper cups. The specimens were pinned using entomological pins and stored in insect boxes prior to transportation to the Department of Biology, AAU, for species identification using the morphological keys of Verrone (1962).

Bioassays

The field collected samples of household used PermaNet®2.0 were tested for their bioefficacy using laboratory reared *An. Arabiensis*,and standard WHO cone bioassay techniques (WHO, 2005).

Each net was cut in to 25 cm x 25 cm sizes from the roof and four sides and ten unfed female *An. Arabiensis,* 2-5 days after emergence, were transferred to a cone and exposed to the pieces of netting material for three minutes. The exposed female *Anopheles* mosquitoes were transferred to an insecticide-free cage, supplied with 10% sugar solution soaked in cotton and covered with polyethylene bag to observe and count knocked down mosquitoes after 60 minutes (KD_{60}) and dead mosquitoes after 24 hours. Each bed net was tested 5 times using 10 mosquitoes each, and thus a total of 200 mosquitoes were assayed. The service periods of the bed nets were ½ year, 2 years, or 3½ years. Four bed nets from each service period were utilized for the bioassays.

Data analysis

The mean percent knockdown effect and mean percent mortality caused by PermaNet®2.0 samples of different utilization periods were subjected to analysis of variance using SPSS version 17.0 statistical software. When significant differences exist, means were separated using Tukey's HSD test at $\alpha = 0.05$.

RESULTS

Density and species composition of indoor resting Anopheles mosquitoes

An average number of *Anopheles* mosquitoes /household were 150 in the study area. Out of these 113 (75%) were *An. gambiae s.l.* (presumably *An. arabiensis*) based on previously described distribution of this mosquito in Ethiopia.

This was followed by 16 (10%) *An. pharoensis,* 11 (8%) *An. d'thali* and 10 (7%) *An. rhodesiensis.* The number of female *Anopheles* mosquitoes collected during the sampling period declined from November 2008 to January 2009 (Table1).

Table1. Species composition and number of indoor resting female Anopheles mosquito collected in rural villages around Bahir Dar (Andassa, Tisabay, Yibab).

Anopheles species	No. of mosquitoes collected/household			Total	
	Nov	Dec	Jan	No.	%
An. gambiae s.l	56	30	27	113	75
An. pharoensis	6	6	4	16	10
An. d'thali	5	3	3	11	8
An. rhodesiensis	3	3	4	10	7
Total	70	42	38	150	100

Bioefficacy of PermaNet®2.0

Analysis of variance indicated that there were significant differences in mean percent knockdown effect and in mean percent mortality among PermaNet®2.0 bed nets with different service period and the untreated control ($P < 0.01$). The knockdown effect of PermaNet®2.0 both after ½ a year and 2 yrs. household usage was 100%. However, this knockdown effect was significantly reduced to 45.5% in PermaNet®2.0 bed nets after 3½ yrs household usage (Table 2). Analysis of variance also showed that there were significant differences in mean percent mosquito mortality among the PermaNet®2.0 and untreated control bed nets ($P < 0.01$). Mean percent mosquito mortality recorded after exposure to PermaNet®2.0 bed nets used for ½ a year and 2 years were similar (92.5% and 84%, respectively), while this mortality effect was markedly reduced to 27% after 3½ yrs household usage of PermaNet®2.0 (Table 2). Mean percent knockdown effect and mean percent mosquito mortalities observed by exposure to PermaNet®2.0 after the three usage periods were higher than after exposure to the untreated control.

Table 2. Bioefficacy of PermaNet®2.0 after different household utilization periods against laboratory reared An. arabiensis in cone bioassays with 3 minutes exposure.

Bed net utilization period	Mean % Knockdown (KD_{60})	Mean % mortality after 24hrs
½ year	100 ± 0.00a	92.5 ± 2.06a
2 years	100 ± 0.00a	84.0 ± 2.36a
3½ years	44.5 ± 0.50b	27.0 ± 2.64b
Untreated control	0.00 ± 0.00c	0.00 ± 0.00c

DISCUSSION AND CONCLUSIONS

The present study revealed that *An. gambiae* s.l. (presumably *An. arabiensis*) were found to be the dominant species collected from indoor,

followed by *An. pharoensis.* This is in line with findings of earlier studies where *An. arabiensis* followed by *An. pharoensis* were found to be responsible for the majority of malaria transmission in Ethiopia (Wondatir Nigatu *et al*., 1994; Birknesh Ameneshewa, 1995; Tarekegn Abose *et al*., 1998).

Both the knockdown effect and mortality rate observed in the present study were highly satisfactory for PermaNet® 2.0 up to 2 years of household utilization and meet the bioefficacy standards for LLITNs set by WHO (95% knock down effect and > 80% morality rate as described by Curtis *et al.* (1990, 1996). These results were similar to those reported by Messay Fettene *et al.* (2009) in Ethiopia. In agreement with the present study, Kroeger *et al.* (2004) concluded that PermaNet® bed nets are effective for at least 3 years based on the bioassay results they carried out on *An. nuneztovari* and *An. rangeli*. Ansari *et al.* (2006) reported that PermaNet® LLITNs are resistant to multiple washes, and have biological activity that lasted as long as the net itself (up to 3 years).

However, the observed knockdown effect (44.5%) and mortality rate (27%) of PermaNet® 2.0 after 3½ years household utilization period was better than untreated control nets but was far below the bioefficacy standards set by WHO. Similarly, low bioefficacy results for PermaNet® 2.0 were reported by Messay Fettene *et al.* (2009) in Ethiopia following an evaluation of the nets used for more than 3 years. This decrease in efficacy could be a result of the decline in the amount of deltamethrin available on the surface of the nets due to repeated washing and the deposition of dust and soot over time (Mahama *et al*., 2007; N'Guessen *et al*., 2001). Supporting this, Rozendaal and Curtis (1989) reported that chemical degradation over time, exposure to direct sunlight, handling and folding for day time storage and washing are among the most common factors influencing the residual insecticidal activities of impregnated mosquito nets. According to the Project report in London (Project Report, 2005), there was indication of loss of insecticidal power of LLITNs due to vigorous hand washing in contrast to shaker washing.

In conclusion LLITNs mosquito bed nets replenishment activities should be accompanied by close monitoring on the feeding and resting behavior of malaria vector mosquitoes and awareness creation on the proper utilization of the bed net need to be conducted to check and maintain its feasibility as part of integrated malaria vector management.

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