

Field Residual Efficacy of PermaNet® 2.0 against *gambiae* s.l in different rural communities of Ethiopia

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Abstract

Background: Long lasting insecticide treated mosquito nets (LLINs) could retain their effective biological activity for at least twenty WHO standard washes under laboratory conditions and three years effective life under field conditions. However, the dynamics of insecticide loss could vary with users' practices in handling and wearing of LLINs. Therefore, bio-efficacy test is recommended to detect net status before it gets lost its lethal dose.

Method: Long lasting treated mosquito nets were sampled randomly from three different localities found in different districts and the performances of these nets were evaluated using WHO cone test procedures. Efficacy was measured in terms of mortality and knockdown effects on susceptible *Anopheles gambiae* s.l.

Results: Except two nets all LLINs showed high level of mortality and knockdown rates implying that the nets could retain their biological activity under field conditions as recommended.

Conclusion: The present study showed both success and failures showing that LLINs could retain the lethal effect for three years as recommended if used properly while failures suggest the need to conduct a follow-up efficacy tests in order to identify the limitations that could contribute for net failure or shrink the effective life of the nets.

Key words: residual efficacy, long lasting mosquito nets, *An. gambiae* s.l, Ethiopia

Introduction

Insecticide treated mosquito nets are one of the key components of the existing malaria vector control strategies both at national and international level. Insecticide treated mosquito nets could be either conventionally treated mosquito nets (ITNs) or long lasting insecticidal mosquito nets (LLINs). Conventional treated nets are mosquito nets that have been treated by dipping in a WHO-recommended insecticide that requires re-treatment after three washes, or at least once a year for its continual insecticidal effect while LLIN are factory-treated mosquito nets made with netting material that has insecticide incorporated within or bound around the fibers. Long lasting insecticidal mosquito net does not require retreatment because LLINs could retain effective biological activity for at least twenty WHO standard washes under laboratory conditions and three years effective life under field conditions. Thus, LLINs are preferred to conventional treated nets because LLINs avoid the problems associated with re-treatment of conventional nets. PermaNet® 2.0 is one of LLINs approved by World Health Organization Pesticide Evaluation Scheme (WHOPES) which could provide 80% mortality for up to twenty laboratory washes (WHO, 2005).

The effective life of LLINs could be influenced by misuse of nets such as spreading LLINs under mattress to kill household pests like bedbugs, fleas, lice, as bed sheets or bedspreads, boundary marker for pit latrines,

washing and drying them in direct sun light and staining, could cause loss of residual insecticide more quickly and compromise LLINs efficacy (Laura and Douglas 2011). Development of insecticide resistance by the vector could also reduce the efficacy of LLINs. Therefore, a follow-up efficacy assessment is recommended along with the distribution of these nets to identify the factors that could contribute for loss of nets efficacy and take corrective measures before the occurrence of any control failure.

The use of ITNs was initiated in Ethiopia in 1997/98 and the country has had great success in rapidly scaling up the national ITNs distribution and coverage since then although a slight decrease has been observed in 2011 (ENMIS 2011). The national ITNs coverage in 2000, 2005, 2007 and 2011 was 0.2%, 6.5%, 68.9%, and 55.2%, respectively, (Central Statistics Authority 2001 and 2006; Jima *et al.* 2010; ENMIS 2011). According to Ethiopia national malaria indicator survey (ENMIS 2011), the use of ITN among malaria vulnerable groups was different, i.e., ITNs usage among children under five years of age increased from 60.2% in 2007 to 64.5% in 2011 while the statistics was the same among pregnant women. According to Jima *et al.* (2010) and ENMIS (2011) 95% of the nets that have been distributed and used by households in all parts of the country were/are LLINs. Field efficacy trials on PermaNet® showed that this LLIN was effective at least for three years in different countries (Graham *et al.* 2005 and Kroger *et al.* 2004).

A similar study from Ethiopia showed below 80% mortality within three years of use in real life condition (Fetene *et al.* 2009) which is less than the cut of pint recommended by WHO. Valerie and his colleagues also (2012) reported 73% mortality from field used insecticide treated mosquito nets in Burkina Faso. The differences observed from these studies demonstrate that effective life of insecticide treated mosquito nets could vary with eco-cultural and eco-epidemiological conditions. Therefore, the performances of these frontline malaria preventive intervention vector control tools should be determined under local ecological settings.

Although Ethiopia is scaling up ITNs distribution and usage rapidly, LLINs efficacy field data were/are limited or not available so that this study was initiated to generate evidence based information for policy makers and malaria control programmers. Thus, the purpose of this study was to measure the effective life of LLIN against the major malaria vector in different rural communities (communities having different cultures and environmental conditions) of Ethiopia.

Materials and methods

Descriptions of the study area: The study was conducted in three different communities found in three different districts of three different regions (Amhara, SNNP, Afar) having different local ecological settings between November 2006 and January 2007 (Table 1).

Study design: The three districts were selected based on history and intensity of malaria transmission, LLINs distribution, difference in attitudes and practices of communities because all are anticipated to influence the effective life of mosquito nets. A study site in each district was also selected based on history and intensity of malaria transmission, LLINs distributions in consultation with district health offices and accessibility and proximity to the district capital. LLINs were also sampled randomly from houses having close proximity to mosquito breeding sites where the probability of mosquito biting would be high so that people could make use of their mosquito nets intensively to protect themselves from mosquito biting and other nuisance insects.

Table 1: Description of the study sites, distribution and survey of period of LLINs assessed (December 2006 - January 2007)

Locality/district	Major economic activities	Altitude(in meters)	Coordinates	Period of LLNs distribution	Study period (Month &year)
Andassa/ Bahirdar zuria	Irrigated & rain fed farms Farms, animal keeping	1698	N11°37'.954" E37°29'.274"	July,2005	December,2006
Sabure/Awash- Fentale	State cotton & orange plantation, pastoralist	802	N 09°07'.879" E 039°57'.677"	August,2005	January,2007
Ado/Wondogenet	Small scale irrigated & rain fed farms, cattle keeping	1178	N06°5'9.789" E038°34'.738"	July,2005	December,2006

Coding of LLINs: All the nets used for the test were PermaNet® 2.0 treated with deltamethrin. A total of 12 LLINs (four nets/study site) were sampled at random and coded as for Andassa AB, K, G and Y, for Sabure C, ES, D and P, and for Ado EW, W, AW and T nets (Table 2). Mosquito nets were in use for about eighteen months and received between 0 and 4 washes during this time (Table 2).

WHO test tube insecticide susceptibility test: Indoor resting blood fed female *An. gambiae* s.l that had been used for cone bio-efficacy test were collected early in the morning manually using mouth aspirators and identified using morphological keys developed by Veron (1962) and Gilles and Coetzee (1987). Susceptibility of female *An. gambiae* s.l against 0.05%

deltamethrin was confirmed using WHO test procedures (WHO, 1998) before using them for the cone tests. A minimum of four treatments and two controls replicates (25 female *An. gambiae* s.l / test tube) were used at each study site. Knockdown scored after 60 minutes and mortality after 24 hours holding period. All the experiments were undertaken under field conditions but within insecticide free premises maintained at 62% relative humidity and temperature of 26°C.

WHO cone bio-efficacy test: Five susceptible blood fed female *An. gambiae* s.l were exposed to LLIN nettings for 3 minutes under WHO plastic cones (WHO, 2005). Mortality and knockdown rates (kdr) were measured after 24 hours and 60 minutes,

respectively. Knock down (collapsing and falling down of mosquitoes at the bottom of the test tube)

rates are indicative of susceptibility status of *Anopheline* under examination.

Table 2: Locality/ district, code given to LLINs and number of washes each LLIN received

Locality/ district	Code	Number of washes
Andassa/ Bahirdar zuria	AB	2X
	K	2X
	G	0
	Y	1X
Sabure/Awash-Fenetale	C	2X
	ES	2X
	D	4X
	P	4X
Ado/Wondogenet	EW	2X
	W	2X
	AW	2X
	T	0

Card boards were used as control for the cone tests because obtaining untreated mosquito nets was impossible during the study period. A total of 50 mosquitoes/net and 200 mosquitoes/4 LLINs were used at each study sites. All the experiments were carried out under field conditions but within insecticide free premises maintained at 62% relative humidity and temperature of 26 degree centigrade.

Data analyses: Abbotts (1925) formula was used to correct percent mortality when control mortality rates were between 5% and 20%.

Results

WHO test tube insecticide susceptibility test: Insecticide susceptibility status of *An. gambiae* s.l to the diagnostic dose of deltamethrin is given (Table 3). The percentage knock down rates after 60 minutes were 97.6%, 100% and 97.6% while mortality rates after 24 holding hours were 98%, 100% and 98% in Ado, Sabure and Andassa, respectively. Both percent mortality and knockdown rates indicated that the vector was susceptible to deltamethrin at all study sites.

Table 3: WHO test tube bioassay test results with percentage mortality, total number of mosquitoes tested (n) and percent knock down of *An. gambiae* s.l used for cone bioassay

Insecticide	Locality	Mean % Mortality(n)	Mean % knock down	Resistance status
Deltamethrin (0.05%)	Ado	98 (125)	97.6	Susceptible
	Sabure	100 (100)	100	Susceptible
	Andassa	98 (122)	97.6	Susceptible

WHO cone bio efficacy test: The cut of points for LLINs efficacy are $\geq 80\%$ mortality and knock down $\geq 95\%$ (WHO, 2005). Mortality and knock down results of cone tests for all LLINs are shown on Table about eighteen months. Mortality and knockdown rates for P and D nets were $\leq 4\%$ and $\leq 6\%$, respectively, indicating that the performances of these

4. Except P and D mosquito nets from Sabure, all LLINs showed $> 80\%$ mortality and $> 95\%$ knockdown demonstrating that the nets were effective against susceptible *An. gambiae* s.l after being used nets were declined before their recommended three years effective life.

Table 4: WHO cone bioassay test results of percent mortality with the total number of mosquitoes tested (n) and percent knockdown

Locality/ district	Code of LLIN	% mortality(n) after 24 hours	% knock down at 60 minutes
Ado/Wondogenet	EW	100 (50)	100
	W	100 (50)	100
	Aw	100 (50)	100
	T	100 (50)	100
Sabure/Awash- Fentale	C	88 (50)	100
	ES	96 (50)	100
	D	4 (50)	6
	P	2 (50)	2
Andassa/Bahirdar zuria	AB	100 (50)	100
	K	100 (50)	100
	G	100 (50)	100
	Y	100 (50)	100

Discussion

The main purpose of this study was to assess whether the efficacy of LLINs that have been used in real life condition under local ecological settings of Ethiopia meets the criteria (> 80% mortality and 95 % knock down) set by WHO. Based on these criteria 83.3% of the LLINs tested demonstrated high level of mortality and knock down rates while 16.7 % of them (D and P LLINs nets) showed < 4% and 6% rate of mortality and knockdown, respectively. D and P nets were washed 4 times while other nets were washed $\leq$ 2 times so that this would be the potential reason for the differences observed in bio-efficacy results of the nets tested. Other malpractices due to knowledge gaps in handling of LLINs while using and wearing them may also diminish nets' effective life (Laura and Douglas 2011). Some mosquito net users use LLINs as bed sheets or bed spreads to kill household pests such as lice, bed bug, fleas , to collect animal feed, those who are living in warmer areas use nets as refuges and barrier for mosquito bites out of but nearby their houses to stay away from hotter room temperatures, others wash and expose nets to direct sun light.

Similarly, Fetene and his colleagues (2010) reported 72.15 % mortality and 94% knockdown and 67% mortality and 100% knockdown rates from PermaNet® LLINs that have been used for three years under two different ecological settings. In a multi-county efficacy trial PermaNet® 2.0 indicated that the nets retained lethal dose of the insecticides that showed 80% mortality in *Anopheles* mosquitoes after 20 washes (Graham *et al.* 2005; Kroegere *et al.* 2004). The differences observed in bio-efficacy results

between the present and the previous studies could be justified by time differences that LLINs were in use; i.e. the present study did the test on LLINs that have been used about eighteen months while the previous studies were done on LLINs that have been used for about three years. Furthermore, the role of eco-cultural and eco-epidemiological factors should be taken into consideration.

Conclusion

The present study revealed that most of LLINs show high level of efficacy in terms of killing and knocking down mosquitoes after being used for about eighteen months in real life condition while two of them lost their efficacy before the expected three years of effective life. This signifies that a follow-up assessment on field performances of LLINs under ecological settings is vital to determine when LLINs should be replaced and plan should be in place for how best to prepare for net failure.

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Conflict of interest

The authors declare that they do not have any conflict of interest.

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