

A Study on Bio-Efficacy and Acceptability of LLINs amongst Soldiers Posted in a Malarious Area

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Abstract

Malaria causes a lot of morbidity and mortality each year, esp. among troops operating in uncongenial malarial areas. Personal protection by use of bed nets is one of the main methods of protection against the Anopheles and other species of mosquitoes, besides the anti-larval and anti-adult sprays.

Insecticide-treated bed nets (ITNs) are already in use, but these involve periodic retreatment with costly chemicals. Long-lasting Insecticide-treated nets (LLINs) which do not require re-treatment, could therefore prove to be good substitutes for ITNs.

A study was thus planned and undertaken to compare the bio-efficacy of LLINs with ITNs, in an endemic area. The acceptability factors and collateral benefits if any were also analyzed.

The results showed that LLINs were more effective in knocking down/killing mosquitoes as compared to the ITNs. They were more comfortable and safe and hence more acceptable to the user and since collateral benefits were noted against some other insects like cockroaches, leeches; they were thought of as more advantageous.

The future of mosquito control largely depends on effective distribution, usage and retention of LLINs. More research on acceptability aspects besides the regular bio-efficacy evaluations, are required.

Keywords: Insecticide-treated bed nets (ITNs); Long-lasting insecticide-treated nets (LLINs); Bio-efficacy; Abbott's formula; Anopheles mosquito

Introduction

About 3.3 billion people - almost half of the world's population - are at risk of Malaria. Every year, there are about 216 million Malaria cases and an estimated 6, 55,000 deaths due to this scourge [1]. People living in endemic countries are most vulnerable. Approximately 80% of reported cases in the South-East Asia region occur in India [2]. Eighty-nine percent of India's population is still at risk of which 22% are in High transmission areas and 67% in Low transmission areas [1]. Around 1.5 million confirmed cases are reported annually by the National Vector Borne Disease Control Program (NVBDCP), of which, about 50% are due to *Plasmodium falciparum*.

One of the main strategies of Malaria control is personal protection by using bed nets, besides the anti-larval and anti-adult methods. Troops located in Malarial areas need to use this preventive measure as a routine. ITNs are in use [3]. Still the mosquitoes find entry into the nets through holes/tears and even bite through the meshes. Hence the physical barrier was supplemented by a chemical one consisting of long lasting deposits of modern synthetic 'pyrethroid', on the nets. Such "Insecticide treated bed nets" (ITNs) have been responsible for reduction in Malaria incidence, severe disease and deaths in endemic regions. In community-wide trials in several African settings, ITNs have been shown to reduce under-5 deaths from all causes by about 20% [4-7].

Nevertheless, even with advantages like low cost, low technology, longer durability, social acceptance, environment-friendliness and easy transportability, there exist few problems with ITNs. Rise in pyrethroid resistance, low and erratic re-treatment rates and erratic dosages during treatment, differential loading of the insecticide on the surface of the

nets, human errors and at times short supply; are some of the factors that weaken the efficacy of these nets. Piperonyl Butoxide (PBO) has been used along with Pyrethroids to help manage resistance, but has not been recommended by WHO [4,8].

To resolve most of these issues, the WHO advocates use of pre-treated, waterresistant, 'long lasting' insecticidal nets (LLINs) -ready-to-use pretreated mosquito nets, which require no further treatment during its expected life span of 3-5 years. The WHO Pesticide Evaluation Scheme (WHOPES) has given either full or interim approval to 13 LLINs [4]. Many different techniques exist - in one there is incorporation of permethrin in the fibers itself (Olyset and Olyset Plus), while in another deltamethrin is fixed on the fibers (PermaNet) and yet another modus operandi is of incorporating wash resistant deltamethrin (Dawa) in the net.

Several studies have been carried out on LLINs in various countries since few years now, e.g. Cambodia, Vietnam, Tanzania, Solomon Islands, Malaysia, Senegal, Cote d'Ivoire and Western and Central Africa. CDC too is currently testing some LLINs to assess their performance and durability in the field [8].

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With this background in mind, a study was planned and undertaken to establish the efficacy of LLINs and to compare their effectiveness with that of the in-use ITNs, in a malaria-endemic area of this country, amongst a disciplined clientele like troops, so as to maximize results.

Objectives

- To evaluate the bio-efficacy of LLINs, as compared to ITNs
- To assess the acceptability of such newer type of nets by the user
- To ascertain collateral benefits, if any, due to their use.

Epidemiology

Study area (environment)

The area of study where the troops' unit was located is a flat plain land beside the River Brahmaputra in Assam, India. The vegetation then was predominantly grassy with paddy fields abounding in surrounding areas. A few tea gardens existed in the vicinity. The altitude is around 105 m above mean sea level.

The climate was relatively cooler in these parts as compared to other parts of the state; the mean maximum temperatures (temp) being 30-33 degree Centigrade (C) and minimum temp 6-12° C. Monsoons lasted from June to Sep when the average rainfall was around 1500 mm. However, some rains occurred all through the year. Relative humidity was around 80%, with a dip of 10-15% in the drier periods [9].

Study population (Host) and anti-malaria precautions

The unit where the study was carried out had individuals from all over the country with no specific preponderance.

All personal precautions against the adult mosquito namely suppressive treatment with Chloroquine, uses of repellent oils/creams and of ITNs, was being followed, as per local orders. The ITNs were being dipped in cyfluthrin/ deltamethrin once every 6 months. Anti-larval and anti-adult procedures were however suspended during the period of study.

All the individuals taking part in the study were informed as regards the purpose of the study and the value of their co-operation. Necessary ethical clearance from concerned authorities was obtained, before proceeding.

Malaria vectors and parasites (agents)

Anopheles minimus and dirus, besides a few others like An. culicifacies and fluviatilis are the main malaria vectors in these areas [10]. All the species are mainly anthropophilic, endophilic and endophagic except a dirus, which is exophilic. None of these are very strong and enthusiastic fliers (range ½ km).

Both *Plasmodium falciparum* and *vivax* are common with the Pf percentage relatively low at 30% approx. [10].

Materials

Mosquito nets as trial and control

LLINs procured from M/s XYZ, with the under-mentioned specifications, were used as trial nets -

Material	Nylon
Weight	470 g
Mesh size	< 4 × 4 mm

Colour	Blue
Active ingredient	Permethrin 2% (w/w); 100 mg a.i. /m ²
Type	Single bed size

The dimensions of the nets, once hung over wooden / iron charpoys were as follows - length 69", breadth 47", height 59" (69" × 47" × 59"). There was no extra cloth stitched below.

ITNs treated with cyfluthrin 5% w/w, which were already in use by troops, with the following specifications, were used as control nets -

Material	Cotton
Weight	830 gms
Mesh size	1.5 × 1.5 mm
Colour	Khaki
Active ingredient	Cyfluthrin 5% (w/w); 25 mg a.i./m ²
Type	Single bed size

The dimensions of the nets once hung over charpoys are - 77" × 40" × 56", inclusive of the cloth stitched below.

Instruments

Flashlights and torches were used in the rooms and inside nets to locate the mosquitoes. Locally improvised suction tubes were used to trap the mosquitoes, which were subsequently put in different glass test tubes. Sometimes the mosquitoes were trapped, by using the test tubes themselves. Magnifying lenses were used to identify mosquitoes.

Animal bait and cages

For bio-efficacy tests, a rabbit was acquired and used as bait for the trapped female mosquitoes to have blood meal. The rabbit was housed in a cage for its stay when not being used for trials and when it was to be introduced in the experimental cage, it was removed and placed in a smaller wire mesh cage, which did not allow much movement.

For feeding the mosquitoes on rabbit's blood, a cage of 'plain' net material of dimensions 21" × 21" × 21" was improvised. This cage had a wooden frame with net material as the walls. An opening (kept covered otherwise) was made on one of the sidewalls of this cage to introduce the rabbit and mosquitoes whenever feeding was required.

To study the efficacy of trial and control nets in knocking down live blood-fed female mosquitoes, two smaller cages (LLIN or trial cage, and ITN or control cage) of dimensions 11" × 6" × 6" each, were locally made by using wooden frames, and cut pieces of LLIN or ITN net material. A small opening was made on one side of both these cages, big enough for only the test tubes with trapped female mosquitoes to be introduced.

Barracks/ tents with charpoys

In the unit where the study was conducted, 2 small barracks of almost equal dimensions of approx. 20' × 18' × 10' and around ½ Km apart from one another, were identified. Each had 2 wire-meshed windows of dimensions 4' × 3' each and 1 door of dimension 7' × 4'. In each room, six iron charpoys were placed. One was labeled as the 'trial room' and other as the 'control room'.

In one room designated as the trial room, 6 LLINs were hung,

and in the other - control room - 6 new and freshly treated ITNs were put up. Five larger barracks of approximate dimensions 100' × 18' × 12' were also earmarked, to study the acceptability levels and collateral benefit aspects of the nets under trial. Each of these barracks had 20 wire-meshed windows and 2 doors of similar dimensions as in the smaller rooms. Each of the barracks housed 40 beds in 2 rows of 20, and an equal number of personnel, during the period of study. Wooden/iron charpays were used. One row of 20 beds had LLINs hung on them, while the opposite row of 20 had ITNs. Totally, 100 LLINs were distributed, so that there were 20 such in each room. The same numbers of ITNs were used.

Methods

Study design

The appraisal was conducted as an experimental or interventional type of field study in a single unit amongst troops. Randomization was not feasible. A type of parallel study design by means of comparison of one product with another keeping the older in-use product as control, was resorted to.

A study protocol was drawn up, keeping the objectives of the study in mind. Mosquito catchers, detailed from another unit, were trained and rotated during each collection. It was ensured that different personnel slept inside the nets each day in both the trial and control rooms. It was assumed that bias would be minimal as the users were from different backgrounds and communities having miscellaneous comprehension standards.

Study period

The LLINs were procured from the concerned firm in the II week of Sep 2010. The trials were then carried out with effect from 25 Sep till 14 Nov, i.e. for an approx. period of 2 months. Though these were post-monsoon months but past experience had shown low to moderate malaria transmission during these and winter months [10].

Mosquito collection

Mosquitoes were collected with the help of 8 helpers who had been imparted hands-on-training. Suction tubes and test tubes were used. A cotton wool soaked in glucose solution was plugged on the mouth of these test tubes. Other than female mosquitoes the rest (males) were allowed to escape later, by removing the requisite cotton plugs at the mouth of the tubes. A min. of 3-5 female mosquitoes were kept in one test tube. Adjustments were made by transferring from one tube to another by approximating the mouths of the tubes.

The following aspects of the trial nets (LLINs) and control nets (ITNs) were evaluated

Bio-efficacy: This was performed by exposing blood-fed female mosquitoes to the net material.

The rabbit's fur was carefully pruned and skin in some parts exposed. Then the rabbit was transferred to the smaller wire cage and this contraption introduced into the 'plain' net cage. Subsequently the trapped female mosquitoes were introduced in the plain net cage, to feed on the rabbit's blood.

After allowing a feeding time of approx. 12 hours (1800 hrs. to 0600 hrs. approx.) the mosquitoes were collected back by the helpers, in test tubes, by following the same procedure (3 per tube) and subsequently introduced into the smaller cages. Only 10 blood-fed female mosquitoes were introduced in each of the smaller LLIN and

ITN cages. The knockdown/mortality was noted at intervals of 1, 2, 3, 5, 10, 20 and 30 minutes. Data was pooled together and percent-corrected mortality was calculated. Though there are several statistical methods in entomology for computing effectiveness of an insecticide, Abbott's formula as given below, was used, as it eliminates errors due to deaths in the control sample which are not due to the insecticide [11].

$$\% \text{ corrected mortality} = \frac{\% \text{ living in untreated sample} - \% \text{ living in treated sample}}{\% \text{ living in untreated sample}} \times 100$$

The test was repeated over a period of 10 consecutive days (25 Sep - 04 Oct) and then impact of washing was tested.

Acceptability by users: ITNs were issued to 100 individuals, as stated already. It was ensured that all these individuals had used ITNs for a minimum period of 2 months and had participated in at least one 6-monthly insecticide treatment drill, before being given the LLINs.

After about 2 months of use (20 Sep to 14 Nov) the individuals of both the units were asked the questions as per a standard questionnaire and the results tabulated. The following aspects were questioned: -

- ~ Side effects like irritation, allergy, disturbed sleep and so on
- ~ Expected durability, colour and look of nets, bigger mesh size, net size, lesser weight, nil requirement of repeated treatment with insecticide and comfort factor while using
- ~ Perception on effectiveness in killing/reducing mosquitoes, reduction in fever (malaria) cases, prevention of mosquito bites and effect of washing on efficacy
- ~ Suggestions, if any, for improvement and better usage

Collateral benefits

To find out about any added benefits of LLINs, the users of were asked whether they had noticed any knockdown/killing action against certain other insects namely- Cockroaches, House flies, Bed bugs, Centipedes, Body and head lice, Leeches, and any others.

Results and Discussion

Bio-efficacy

Results of bio-efficacy tests as regards knockdown/ mortality of mosquitoes are presented in (Table 1). It is evident that 100% knockdown/mortality was noted after 30 min of exposure time in case of LLINs. Of these, the maximum mosquitoes were knocked down within 20 min. The discriminatory dosage time for the next part of the trial was derived to be 30 min. In case of ITNs, maximum mortality was seen after 30 min of exposure but 100% mortality was noted only on 2 occasions. In the study by Messay et al. in 2 districts of Ethiopia, the mean knock-down varied from 94-100% as regards PermaNets and mean mortality rate after 24 hrs holding period varied from 67% to 72.2% [12]. Delenasaw et al. found a wider range of maximum mortality i.e. 13.9% - 81.1%, in their study on 6 WHO recommended LLINs [13].

On one occasion, 10 Anopheles mosquitoes were isolated, introduced in the trial cage and their knock down/mortality noted in the same time period as above. Hundred percent mortality was seen after 5 min of exposure.

Since different genus's of mosquitoes were not identified prior to introduction in the experimental cages, as this was beyond the scope of this study, the exposure time required to knock down Anopheles, Culex

Time	1 min			2 min			3 min			5 min			10 min			20 min			30 min		
	No. dead in			No. dead in			No. dead in			No. dead in			No. dead in			No. dead in			No. dead in		
Date	T	C	%	T	C	%	T	C	%	T	C	%	T	C	%	T	C	%	T	C	%
25 Sep	2	-	20	4	1	33.3	7	3	51.1	8	4	66.6	9	4	83.3	10	7	100	10	8	100
26 Sep	3	-	30	4	2	25.0	5	2	37.05	7	5	40	8	6	50	9	6	75	10	7	100
27 Sep	3	1	22.2	3	2	12.5	4	2	25.0	9	4	83.3	10	5	100	10	8	100	10	8	100
28 Sep	4	2	25	5	2	37.5	7	4	50.0	10	5	100	10	7	100	10	9	100	10	9	100
29 Sep	1	-	10	4	2	25.0	6	3	42.8	7	5	40	7	6	25	9	8	50	10	10	0
30 Sep	2	1	11.1	5	3	28.5	6	3	42.8	9	5	80	10	8	100	10	9	100	10	10	0
01 Oct	4	2	25	5	3	28.5	8	4	66.6	10	6	100	10	6	100	10	7	100	10	7	100
02 Oct	2	1	11.1	5	3	28.5	7	4	50.0	8	4	66.6	8	4	66.6	9	6	75	10	6	100
03 Oct	3	2	12.5	4	2	25.0	8	5	60.0	9	6	75	9	7	66.6	10	8	100	10	8	100
04 Oct	3	1	22.5	7	2	62.5	8	4	66.0	9	7	66.6	9	9	0	10	9	100	10	9	100

T – Trial cage

C – Control cage

% - Percent-corrected Mortality

Table 1: Results of bio-efficacy tests on knock-down/mortality & discriminatory dosage.

or Aedes cannot be commented upon.

The results therefore show that the LLINs have enhanced efficacy against mosquitoes compared to the ITNs, in the study area, very similar to other studies conducted in various parts of the world.

From the isolated observation one can presuppose that the LLINs have a quicker knock down capability as regards Anopheles as compared to ITNs but more elaborate trials are required in this direction.

Since this part of the trial was conducted for 10 days only, one cannot comment on the efficacy of 'unwashed' LLINs, for much longer durations.

Acceptability by users: The results about various aspects pertaining to acceptability of the LLINs are tabulated in (Table 2). All the users (100%) were of the opinion that the new LLINs were more comfortable to sleep in, with better air entry and therefore ventilation. They also felt that the LLINs would reduce fever (Malaria) cases as they found no live mosquito inside the nets on waking up as well as no mosquito bites on their person during sleep even when sometimes their limbs came in contact with the nets. Since there was no requirement of periodic chemical treatment (this was disclosed to them before seeking the answers), all were of the opinion that the nets were certainly more suitable. All were also of the opinion that the new nets were lighter and therefore easier to pack and carry from place to place.

Complaints of nausea/vomiting, headache, insomnia/irritability or skin irritation were inquired into. No one (99%) complained of any side effects like headache, nausea, etc. Only 1 individual did not answer. Majority also felt that the new nets would prove to be more robust and durable (61%), were better in appearance and colour (72%), had better visibility inside due to bigger mesh size (59%) and were adequate in size (54%). Similar results have been observed in the study conducted by Sood et al. in Gautam Budh Nagar, UP, India where all the respondents were satisfied about the performance of the LLINs in reducing the mosquito nuisance, safety of use and collateral benefits in LLIN villages [14]. In another study in Solomon Islands, it was observed that the main criterion for LLIN brand acceptability was effectiveness in preventing mosquito bites and malaria [15].

The principal reasons why some users felt that certain aspects of the new nets were not very superior compared to the ITBNs were: -

~ Length of the nets was not adequate for taller individuals

~ The nylon/polyester type material could catch fire more easily and also get damaged more easily when in contact with sharp objects like nails

~ The bigger mesh size might allow entry of some other insects.

Collateral benefits: The results pertaining to additional benefits of the LLINs in killing or incapacitating any other insects as brought out by the users is tabulated in (Table 3). Few of the individuals had been attentive enough to note that cockroaches, bed bugs and some other insects were knocked down/killed either while using ITNs in the same barrack where LLINs were hung, or after being exposed to LLINs. However, majority of the individuals had not observed any effect on - houseflies, centipedes, lice or leeches. In a study carried out almost for a period of 3 years at the Indo-Nepal border areas, it was noted that the LLINs had an effect on sand-flies and therefore in reduced incidence of Kala-Azar [16]. This is one important collateral benefit of LLINs as compared to ITNs. In the study in UP, 98.3% of the respondents asserted the use of LLINs, as their use reduced the number of mosquitoes as well as other non-target insects [14].

Future prospects/ Recommendations

LLINs have come to stay. They are more effective and hygienic as compared to ITNs. They reduce the mosquito-density. They could either complement or replace some of the preventive and protective measures against the mosquito. In future they may eliminate the need for insecticide retreatment of ITNs [17].

The WHO Global Malaria Program (WHO/GMP) recommends "usage" of LLINs as one of the 3 primary interventions for effective malaria control. Usage should be scaled up if endemic countries like ours have to move towards achieving the United Nations' Millennium Development Goals, by 2015 [18].

Malaria incidence has dipped in many countries where Malaria programs have incorporated and ensured LLIN "distribution" to the masses. WHO recommends "universal coverage" in endemic areas? Their effectiveness for a period of 3 years or more, is an added advantage [4,8]. The world spends more than \$500m on LLINs every year. It is the largest single item in the global malaria control budget.

Aspects Checked	Yes	No	No answer	Remarks
Side effects	Nil	99	01	-
Durability & better net material	61	39	-	Individuals who answered no, felt that the material was more flammable and easily tear able.
Preferred color & look	72	10	18	10 users, who answered no, preferred white & disruptive pattern.
Adequate net size	54	46	-	46 individuals who answered no, found length of LLINs inadequate.
Bigger mesh size and better visibility	59	20	21	20 users, who said no, felt that mosquitoes & other insects might enter the net.
Lighter weight	100	-	-	None
No requirement of chemical treatment	100	-	-	-do-
Comfort factor during sleep	100	-	-	-do-
More effective reduction of mosquitoes	80	-	20	-do-
Greater reduction in fever cases	80	-	20	-do-
Better prevention against mosquito bites	100	-	-	-do-

Table 2: Aspects of acceptability of LLINs by users (n=100).

Killing / knock down action against	Yes	No	No answer	Remarks
Cockroaches	20	-	80	Individuals who did not give any answer had not observed the specific action.
House flies	-	-	100	-do-
Bed bugs	60	22	18	Nil
Centipedes	-	-	100	Individuals who did not give any answer had not observed the specific action.
Head/body lice	-	-	100	-do-
Leeches	-	-	100	-do-
Any other insects	54	30	16	Nil

Table 3: Collateral benefits of LLINs (n=100).

The WHO Global Malaria Program has now developed a system for reducing the cost-per-year of LLIN coverage, by allowing country programs to select, from the existing range of products, the one that is most durable in the local setting [19]. However, still many fall short of

targets contained in the epoch 2005 World Health assembly resolution [20]. LLIN distribution should be implemented in our country too, at the earliest, esp. in high endemic areas to start with.

Distribution is important, but “utilisation and retention” of these nets is also of utmost significance. Messay et al found very high retention (72%) and usage rates (62.2%) amongst villages in Ethiopia [12]. In Sierra Leone retention rates were found to be as high as 86% by a study conducted by Adam Bennett et al, after 6 months of a national Mass-Distribution campaign [21]. This aspect also needs to be researched and reasons of non-retention found out and eliminated.

Research to “increase lifespan” of the LLINs is on. A recent study estimated that upto \$ 3.8 billion can be saved over 10 years by increasing lifespan of the nets from the present 3 years to 5 years [8]. Future times may see widespread use of such LLINs with longer lifespan.

Presently, in many settings ITNs/LLINs are in use along with Indoor Residual Sprays (IRS) compromising control initiatives, thus threatening global malaria elimination strategy [13]. However, neither LLINs nor IRS alone, are sufficient to achieve and maintain interruption of transmission in holo-endemic areas of Africa or in hyper endemic areas in other regions [18]. More evidence is needed on the efficacy of combining other vector-control methods and LLINs - epidemiological impact, resistance management, feasibility of combination, and social acceptability, compliance and costs.

Bio-efficacy evaluations using local mosquito populations should be conducted where possible to make evidence-based decisions on most suitable control products [13]. Resistance to pyrethroids used in LLINs is also an emerging area of concern as has been established in studies conducted in Africa [22-24]. This aspect should also be borne in mind and future researches directed towards solving such issues too.

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