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Research Article

Bio-efficacy of Long Lasting Insecticidal Mosquito Nets (LLINs) on Malaria Vector *Anopheles* Mosquitoes in Malaria-Endemic Areas of Myanmar

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Abstract: Sixty used Long Lasting Insecticidal Nets (LLINs) from six malaria endemic areas of States and Regions of Myanmar were assessed for their bio-efficacy against *Anopheles* mosquitoes. Total ten nets were collected from the randomly selected household of each area. Insecticide susceptibility and bio-efficacy test were done according to WHO testing method. Results revealed that a total of 4470 mosquitoes of 12 Anopheles species including main vector and secondary vectors were collected in high density in Beelin, Taungoo, Pyin Oo Lwin, Kamamaung and Hpa-an. All collected mosquitoes were found susceptible to insecticides as recommended by the WHO. Bio-efficacy of PermaNet 2.0, DAWA TANA, Yorkool, BASF, Net protect and Yahe LLINs nets were 12(100%), 11/12(91.67%), 4(100%), 8(100%), 5(100%) and 19(100%) respectively. The DAWA TANA net had 20% sensitivity after repetitive washing. The sensitivity of LLINs nets was higher for all selected areas of States and Regions in Myanmar. The study suggested that there is need to distribute a large number of effective LLINs nets in malaria endemic areas as well as the replacement of expired, less effective and very old LLINs nets with a new one is also necessary. LLINs nets are very effective *Anopheles* mosquitoes control tools and it can reduce the human-vector contact effectively. Moreover, research on bio-efficacy evaluations of the LLINs nets is required regularly to maintain and control the transmission of malaria through *Anopheles* mosquitoes.

INTRODUCTION

Long lasting insecticide treated nets (LLINs) have emerged as a potent tool in lowering morbidity and mortality of mosquito borne diseases. In Myanmar, previously malaria is a major public health problem with its burden and transmission patterns varying across the country. According to Health in Myanmar 2014, morbidity and mortality rate were 24.35/1000 population and 12.62/100000 population respectively in 1990 and after distribution of LLINs nets in malaria endemic areas by NMCP, government NGOs and Nongovernment NGOs and prompt treatment the morbidity and mortality decline to 6.44/1000population and 0.48/ 100,000 populations respectively in 2013 [1]. Malaria cases and death reduced by 62% and 91% in 2015 compared to 2012. The incidence of malaria is fall down by 49% from 2012 to 2015 and no reported malaria outbreaks since 2012 [2]. Malaria is still today a leading cause of morbidity and mortality in heart to reach areas and border areas in Myanmar. *An. dirus* and *An. minimus* is the main vector of malaria in Myanmar. *Anopheles minimus* adult are found in forested foothill areas and plain areas and larvae of *An. minimus* are found in irrigation areas of paddy field and slowly running water in other parts of the Myanmar [3, 4]. *Anopheles dirus* lives in dip forested hilly areas but now the mosquitoes are found in urban areas of coastal regions and larvae are found in water wells in coastal areas of Mon State and Taninthayi Region [5, 6]. *An. annularis* is a local vector of malaria in Rakhine State. Other malaria vectors as *An. sundaigus* and *An. subpictus* are abundantly found in coastal areas of Rakhine, Mon and Tanintharyi Regions. Secondary vector are *An. maculatus, An.vagus, An. culicifacies, An. varuna, An. stepensi,* and *An. phelippensis. An. annularis* found to be resistant to DDT4% in Rakhaine State. The transmission of malaria is largely influence by the abundance, survival and bionomics of the mosquito vectors [7].

Progress in the fight against the disease has been made yet malaria still poses a major burden for the society and the health system. The malaria burden is particularly high in the remote forested and hard to reach areas where

transport of supplies is difficult and collection of data as well as information is challenging. Additionally, there is growing the spread of antimalarial drug-resistance in Myanmar [8].

The risks to public health by deployment of DDT or other insecticides must be carefully weighed against the benefits, in this case the prevention of vector-borne diseases. Moreover, there are strong evidences that more insect vectors species are becoming resistant to the toxic action of these insecticides and through different resistance mechanisms, especially knock-down resistance (kdr) mechanism to DDT and Pyrethroids [9,10]. The spread of insecticide resistance vertically to new species and horizontally to new countries poses a great danger likely to undermine the contribution of vector control efforts to control of diseases. Data from 1997 showed that vectors are sensitive to pyrethroids and organophosphates whilst resistance of *An. annularis* to DDT has been documented in the Rakhine State [8]. The insecticides recommended by the WHO pesticide evaluation scheme (WHOPES) are alpha-cypermethrin(10%), cyfluthrin(5%), deltamethrin (1-25%), etofanprox (10%), lambda cyhalothrin (2.5%) and permethrin (10%) [11,12]. A number of novel technologies have been developed to combat malaria including insecticide treated nets (ITNs) and malaria vaccine but the vaccine have shown limited efficacy and need further development [13]. Pyrethroid insecticides being cost–effective, highly insecticidal at low dosage and highly biodegradable with low mammalian toxicity were recommended by WHO Pesticides Evaluation Scheme (WHO-PES) for large–scale use on mosquito bed nets for malaria prevention [14]. Pyrethroids treated LLINs and ITNs lead to a reduction of malaria transmission and mosquito population. Three LLINs have been fully recommended by the WHO and are now commercially available: Olyset, PermaNet2.0 and Yorkool LLINs [15].

Personal protection against mosquito bites using long lasting insecticide treated nets (LLINs) is cost-effective and specifically reduces the contact between target vectors and human hosts [16]. The use of LLINs is one of the main strategies for malaria control in endemic areas [17] as well as the most prominent malaria preventive measures for vector control [18]. It has been reported that Olyset® nets are highly effective against malaria vectors and moderately against other non-target household insects [19]. The use of LLINs is more effective than the application of indoor residual spraying with the possibility of high public participation as well as integrating primary health care which shows reduction of overall morbidity and mortality in children aged 1–4 years by 53% and 70% respectively [20].

In Myanmar different brands of LLINs nets, WHO recommended Olyset net and especially PermaNet 2.0 (LLINs) have been widely distributed by Government society, NGOs and INGOs to reduce malaria transmission in endemic areas. PermaNet 2.0 with deltamethrin at 55mg/m^2 incorporated into a resin coating the fibers at factory were evaluated for bio–efficacy in Pakistan, Tanzania, India, Uganda, and a few other countries and reported to be performing well even after repeated washing [21-23].

Main mosquito borne diseases are malaria transmitted by *Anopheles mosquitoes*, Dengue fever transmitted by *Aedes* mosquitoes and Japanese encephalitis and Filariasis transmitted by *Culex* mosquitoes. Insecticide treated Nets (ITNs) and LLINs lead to a reduction of human-vector contact and diminish mosquito population [24-26] and also provide a physical barrier with high coverage levels that benefit the whole community [27]. However, efficacy of long term insecticidal action of LLINs depends on the washing frequencies, washing detergents and duration of using time of the unwash LLINs nets during the recommended period. Based on previous studies, it has been shown that exposure of climate, repeated washing and long term using of LLINs can result in reduction of insecticidal efficacy [21, 28, 29]. Deltamethrin insecticide treated Net (ITN) were found 100% efficacy within 5 washed [4]. Moreover there is a lack of information about the insecticidal action of repeated washed LLINs and long term used unwashed LLINs nets on *Anopheles* mosquitoes in malaria endemic areas in Myanmar. Therefore, bio-efficacy of, with and without washed LLINs and in relation to washing frequency as well as the period of used LLINs nets in field against *Anopheles,* mosquitoes should be investigated to provide information gap concerning on retention of LLINs.

MATERIALS AND METHODS

Activities undertaken:

Entomological survey, Susceptibility test, LLINs nets collection, Bio-efficacy of LLINs test were conducted during the study periods of June 2016 to Aug 2017.

Study areas:

Six study areas were selected from four State and Region in Myanmar as Than Byu Zayat and Beelin Township in Mon State, Taungoo Township in Bago Region, Pyin Oo Lwin Township in Mandalay Region and Kamamaung and Hpa-an Townships in Kayin State were selected for testing the bio-efficacy of used Long Lasting Insecticidal Nets (LLINs).

Detailed activity schedule

1. **Study design and study period:** Field base cross sectional descriptive study design was done with laboratory reared *Anopheles dirus* and wild caught *Anopheles* mosquitoes against different brands of distributed used LLINs from June 2016 to August 2017 (one year study).
2. **Study site and study population:** The study was conducted in 6 areas of four States and Regions in Myanmar:

   (1) Mon State -2 areas (Hnit Kine village, Than Byu Zayat and Khae Mouk village, Beelin Townships)
   (2) Bago Region - 1 area (Kyauk Lone Gyi village, Taungoo Township)
   (3) Mandalay Region - 1 area (Pho Nay Htun village, Pyin Oo Lwin Township)
   (4) Kayin State 2 areas (Nyinyar Ayechan village Kamamaung Sub-Township and Ywar Thit Kone village Aindut District, Hpa-an Township) were selected for determining bio-efficacy of distributed used long lasting insecticidal nets (LLINs) against field collected *Anopheles* mosquitoes.

Sample size determination and sampling procedure
Considering for difference is bio-efficacy of LLINs between those nets used for one year and more than one year being 20% (P_1=90% with one year; P_2= 70% >1 year) at 95% confidence level and 80% power, the sample size real used was 62.

Ten houses from each site were selected randomly. One LLIN each was collected from all randomly selected houses. Therefore a total of 60 LLINs were collected from 6 sites of the four State and Regions were covered the present study.

Data collection

Susceptible strain collection
Susceptible strain of *Anopheles dirus* mosquitoes from Mudon Township Mon State were collected and reared for mass production in Laboratory of Medical Entomology Research Division, Department of Medical Research was used as control mosquito in field areas.

Field mosquito collection
Field *Anopheles* mosquitoes were collected from selected areas using animal bait big mosquito catches net and indoor and outdoor collection by light trap method at 18:00 to 06:00 hour. The susceptibility status of collected mosquitoes was tested with WHO test kit [14]. Susceptibility test, bio-efficacy of randomly collected LLINs nets from different villages were tested against field collected mosquitoes, simultaneously laboratory reared *An. dirus* was used as control. .

Mosquito species identification
Species identification of field collected adult mosquitoes, susceptibility and bio-efficacy tested mosquitoes and adult emerged from larval survey were done by morphological methods according to different identification keys [30,31,32].

Preparation of mosquito net samples
One to two years old washed and unwashed 10 LLINs per village were collected randomly from different selected areas. Untreated standard polyester net was used as control. Susceptible strain of laboratory reared 3-5 days old 5 *Anopheles dirus* mosquitoes each/cone was used as control test on each collected LLINs mosquito net. Field collected mosquitoes were exposed at two sites as top and lateral side of each mosquito net in cone for 3 minutes for bioassay test.

Procedure of insecticide susceptibility test
Susceptibility test was performed using impregnated paper assay on wild caught *Anopheles* mosquitoes with WHO test kit [14] to confirm the susceptible strains. Laboratory reared 5 *An. dirus* each was used as control. Ten each field collected adult female *Anopheles* mosquitoes (pool sample) were exposed to each impregnated papers exposed tube for one hour. After one hour mosquitoes were transferred to attach clean tubes from the testing tubes with assess to 10% glucose and humidity was maintained with water soaked gauze covered with on it. The percentage mortality was assessed 24hr after exposure.

Bioassay test
Bio-efficacy of long lasting insecticidal nets was evaluated according to standard method of WHO cone technique [26] with wild caught susceptible strains. Simultaneously insecticide susceptible strain of *An. dirus* (colony from Medical Entomology Research Division) was used as control. Two cones (one top, and one lateral) were placed horizontally on the nets and 5 each field collected adult female *Anopheles* mosquitoes (pool sample) and 5 laboratory bread *An. dirus* were exposed to each cones for 3 min. After 3 min mosquitoes were removed from the cones and placed in individual paper cups with assess to 10% glucose and humidity were maintained with water soaked gauze covered with on it.

The percentage mortality was assessed 24hr after exposure period. Insecticide efficacy curve was established and bio-efficacy was compared for nets washing frequency and duration of used LLINs nets.

Data analysis
Percentage mortality against number of washes and duration of used LLINs nets curve were drown. If the percentage of mortality in the negative control was above 5%, then a correction was made using Abbots formula [33]. Bio-efficacy of LLINs nets against maximum number of washes and duration of used LLINs net providing mortality below or above of the cut of point (80% mortality after 24 hours) was reported.

Ethical consideration: Ethical clearance was obtained from DMR ethic review committee.

RESULTS

Collection of *Anopheles* mosquitoes

Mon state: Result revealed that a total of 374 and 767 *Anopheles* mosquitoes were collected from Than Byu Zayat and Beelin Townships, Mon State within 5 days. Of this *An. tessellatus* 117, *An. vagus* 76, *An. subpictus* 113 , *An. dirus* 3 and *An. barbirostris* 65 were collected from Than Byu Zayat and *An. minimus* 72, *An. aconitus* 115, *An. philippinensis* 114, *An. annularis* 124, *An. jamesii* 153, *An. vagus* 123 and *An. barbirostris* 66 were collected from Beelin Townships respectively.

Bago Region: In Kyauk Lone Gyi village, Taungoo Township, Bago Region, a total of 877 *Anopheles* mosquitoes were collected. Of this *An. minimus* 118, *An. jamesi* 113, *An. maculatus* 148, *An. culicifacies* 141, *An. philippinensis* 128, *An. vagus* 141 and *An. barbirostris* 88 were collected respectively.

Mandalay Region: A total of 611 *Anopheles* mosquitoes were collected from Pho Nay Htun village, Pyin Oo Lwin Township Mandalay Region within 7 days. Of this *An. minimus* 141, *An. maculatus* 137, *An. vagus* 103, *An. culicifacies* 77, *An. annularis* 96 and *An. aconitus* 57 were collected respectively.

Kayin State: A total of 829 and 1012 *Anopheles* mosquitoes were collected from Nyinyar Ayechan village Kamamaung Sub-Township and Ywar Thit Kone village Aindut District, Hpa-an Township Kayin State within 7 days. Of this *An. vagus*

186, *An. jamesii* 146, *An. phillipinensis* 165, *An. annularis* 203 and *An. barbirostris* 65 were collected from Kamamaung Sub-Township and *An. vagus* 201, *An. jamesii* 169, *An. phillipinensis* 143, *An. annularis* 237. *An. minimus* 166 and *An. barbirostris* 96 were collected from Aindut District Hpa-an Township respectively.

Insecticide susceptibility test

All collected *Anopheles* mosquitoes from the study areas (Than Byu Zayat and Beelin Townships, Mon State, Kyauk Lone Gyi village, Taungoo Township, Bago Region, Pho Nay Htun village, Pyin Oo Lwin Township Mandalay Region and Nyinyar Ayechan village Kamamaung Sub-Township and Ywar Thit Kone village Aindut District, Hpa-an Township Kayin State) were found susceptible to WHO impregnated papers of DDT 4%, Deltamethrin 0.05%, Permethrin 0.75% and Cyfluthrin 0.15%.

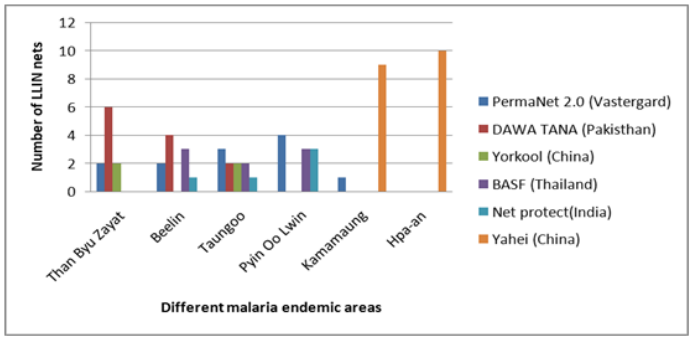

Fig 1. Different brands of LLINs nets collected from six studied areas

Fig.1. shows that a total of 60 LLIN nets (10 each) were collected from Than Byu Zayat and Beelin Township in Mon State, Taungoo Township in Bago Region, Pyin Oo Lwin Township in Mandalay Region and Kamamaung Sub Township and Hpa-an Townships in Kayin State for testing bio-efficacy of LLIN nets. Of this 2 PermaNet 2.0 (Vestergard) nets, 6 DAWA TANA (Pakistan) nets and 2 Yorkool (China) nets from Than Byu Zayat and 2 PermaNet 2.0 nets, 4 DAWA TANA nets, 3 BASF (Thailand) nets and one Net Protect (India) (3DF) net were collected from Beelin Townships, and 3 PermaNet 2.0, 2 DAWA TANA, 2 BASF, One net protect and 2 Yorkool nets were collected from Kyauk Lone Gyi village Taungoo Township. 4 PermaNet 2.0, 3 BASF, 3 Net protect nets were collected from Pyin Oo Lwin Township. In Kayin State, 1 PermaNet 2.0, 9 Yahe (Yahei China) nets were collected from Kamamaung Sub Township and 10 Yahe LLINs nets were collected from Hpa-an Township. All collected nets were washed 0 to>5 times with soap powder.

Table 1 shows that Bio-efficacy of LLINs nets were found to be PermaNet 2.0 100%, DAWA TANA nets 83.33%, Yorkool nets 100% mortality of *Anopheles* mosquitoes in Than Byu Zayat Township and PermaNet 2.0, DAWA TANA, BASF, and Net Protect nets were found to be 100% bio-efficacy (100% mortality) in Beelin and in Taungoo PermaNet 2.0, DAWA TANA, BASF, Net protect and Yorkool nets were found 100% bio-efficacy (100% mortality of *Anopheles* mosquitoes). In Pyin Oo Lwin Township PermaNet 2.0, BASF and Net protect were found 100% bio-efficacy (100% mortality). In Kayin State PermaNet 2.0 and Yahe in Kamamaung and Yahe in Hpa-an Township were found 100% bio-efficacy.

Table 1. The bio-efficacy of different brands of long lasting insecticidal nets (LLINs) collected from 6 different areas

Different brands of LLINs nets	Than Byu Zayat	Beelin	Taunggu	Pyin Oo Lwin	Kama maung	Hpa-an	Laboratory *An.dirus* (Control)	Total LLINs
	20 mosquito /net	20 mosquito /net	20 mosquito /net	20 mosquito /net	20 mosquito /net	20 mosquito /net	10 mosquito /net	
	LLINs (% efficacy)	LLINs (% efficacy)	LLINs (% efficacy)	LLINs (% efficacy)	LLINs (% efficacy)	LLINs (% efficacy)	(% efficacy)	(% efficacy)
PermaNet.2 (Vastergard)	2 (100%)	2 (100%)	3 (100%)	4 (100%)	1 (100%)	0	12 (100%)	12 (100%)
DAWA TANA (Pakistan)	5/6 (83.33%)	4 (100%)	2 (100%)	0	0	0	11/12 (91.67%)	11/12 (91.67%)
Yorkool (China)	2 (100%)	0	2 (100%)	0	0	0	4 (100%)	4 (100%)
BASF (Thailand)	0	3 (100%)	2 (100%)	3 (100%)	0	0	8 (100%)	8 (100%)
Net protect (India)		1 (100%)	1 (100%)	3 (100%)	0	0	5 (100%)	5 (100%)
Yahe (China)		0	**0**	0	9 (100%)	10 (100%)	19 (100%)	19 (100%)
Total tested nets % efficacy	9/10 (90%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	59/60 (98.33%)	59/60 (98.33%)

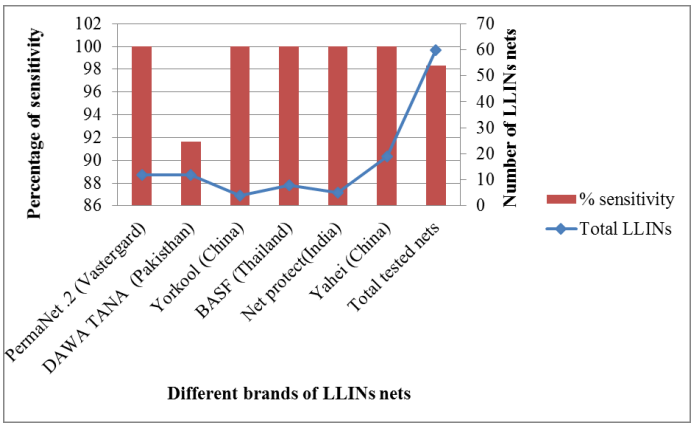

Fig. 2. Bio-efficacy of different brands of one to two years used LLINs nets (0 to >5 washed)

All total of 60 LLINs nets consist of 6 brands of mosquito nets were collected from six different areas of malaria endemic areas. Of this the highest number (19) of Yahe made in China LLINs were collected from Kayin State. Followed by (12) PermaNet 2.0 (Vestergard) Belgium and (12) Dawa Tana (Pakistan) the lowest number (4) of Yakool (China) nets were collected. PermaNet 2.0, Yarkool, BASF, Net protect and Yahei LLINs Nets were found 100% efficacy although DAWA TANA nets was found 91.67% efficacy.

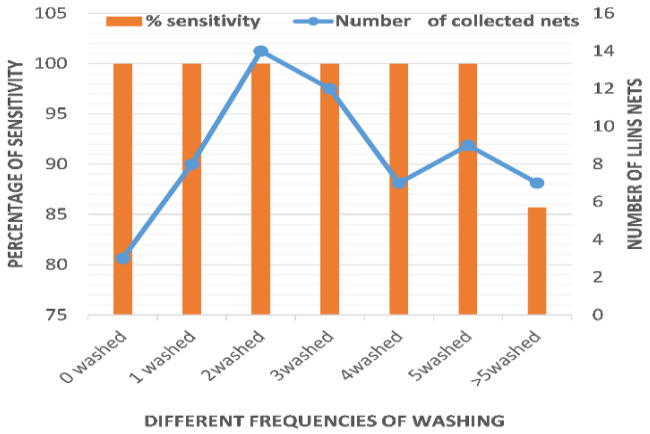

Fig 3. Bio- efficacy of LLINs nets against different washing frequencies

Fig. 3. Shows that all tested different brands of LLINs nets were found highly sensitive to killed 100% of *Anopheles* mosquitoes within 24 hours. Hundred Percent mortality were observed 0-5 time washed of all 1-2 years old LLINs nets within 24hours. Although over 5 times washed nets was found 85.75% sensitivity. Only one DAWA TANA nets which was washed 12 times was found 30% efficacy(mortality). WHO recommended efficacy is 80% mortality of *Anopheles* mosquitoes against cone bio-efficacy test on insecticide treated mosquito nets.

DISCUSSION

Long lasting insecticide treated nets (LLINs) have emerged as a potent tool in lowering morbidity and mortality of mosquito borne diseases. A LLINs nets remains effective over a certain period of time even after several washings. The LLINs nets currently on the market are pre-treated nets that have been industrially manufactured. In Myanmar, WHO recommended PermaNet 2.0 and Olyset net (LLINs) have been widely distributed to reduce malaria transmission in endemic areas. Simultaneously other brands of LLINs nets as DAWA TANA nets, Yorkool, BASF, Net protect and Yahe nets are highly distribution in malaria endemic areas. It has been widely distributed by Government society, NGOs and INGOs to reduce malaria transmission in endemic areas. PermaNet 2.0 with deltamethrin at 55mg/m^2 incorporated into a resin coating the fibers at factory were evaluated for bio–efficacy in Pakistan, Tanzania, India, Uganda, and a few other countries and reported to be performing well even after repeated washing [21-23,34].

Before testing bio-efficacy of LLINs nets, *Anopheles* mosquito were collected in six study areas using animal bait Kanda big mosquitoes catching nets for 5 to 7days in each area. The main vector *Anopheles minimus* was observed in higher density in Beelin, Taunggoo, Pyin Oo Lwin and secondary vectors *An. aconitus*, *An. philippinensis*, *An. annularis, An. Jamesii* were abundantly collected from Beelin, Taungoo, Kamamaung, and Hpa-an. High number of *An. vagus* and *An. barbirostris* were found in all areas. *Anopheles maculatus* was observed in high number in Taungoo, Pyin Oo Lwin and Hpa-an and also *An. culicifacies* was found in Taungoo and Pyin Oo Lwin Townships. Although high numbers of *An. subpictus* and *An. tessellatus* were found in Than Byu Zayat. Khin Maung Kyi [7] revealed that *An. annularis* were abundantly present in Rakhine State and it is a local vector of malaria. *An. dirus* and *An. minimus* were found in coastal areas of Mon and Taninthayi Region and *An. dirus* larvae were abundantly collected in domestic water well in both coastal areas [5, 6, 35]. Similar result has been found in Kamamaung Sub Township, Hpapun District Kayin State, *An dirus* D larvae were abundant observed in domestic water well in raining season and *An. minimus* A and *An. culicifacies* B were co-breeder in sand pools and slowly running water in Yunsalin river in dry season [36]. *Anopheles minimus* and *An. baimaii* have been incriminated by several independent investigators and unequivocally proven to be the major vectors in their state, but a host of other mosquito species, including *An. nivipes* and *An. culicifacies*, are also believed to contribute to the overall disease burden of malaria [37]. In Papua New Guinea, the Solomon Islands and Vanuatu it was found that there was a shift in the behaviour of *An. farauti* vector from feeding indoors late in the night to outdoors early in the night [38-42]. This change in behaviour was most likely due to vector irritability caused by the DDT leading to the vector avoiding insecticide exposure [40-43].

Insecticide susceptibility status of all collected *Anopheles* mosquitoes from different studied areas were found to 100% mortality or susceptibility to WHO insecticides impregnated papers as DDT 4%, Deltamethrin 0.05%, Permethrin 0.75% and Cyfluthrin 0.15%. These WHO recommended insecticides were very sensitive to *Anopheles* mosquitoes from Kamamaung Sub Township Kayin state [36]. However, in the southwest Pacific, there has been no evidence of physiological insecticide resistance despite decades of DDT-IRS followed by pyrethroid-IRS and treated nets [44].Vector control is an integral component of the containment of malaria, and other than indoor residual spraying, long-lasting insecticidal nets are the only

appropriate technologies that are being provided to high-risk states/districts with marginalized population groups [45].

In the present study among all of the nets [PermaNet 2.0 (Vastergard), DAWA TANA (Pakistan), Yorkool (China), BASF (Thailand), Net protect (India), Yahe (China)] were inspected for physical appearance, 91.67% (55/60) were clean, 8.33% (5/60) were slightly dirty. In villages 90% of households were in possession of two or more LLINs nets. Of the LLINs that were distributed in 2015, all (60/60) of the inspected nets were physically intact and in good condition. The residents were using LLINs during the night time in the villages. High number of LLINs nets were utilized among vulnerable age group of children, woman and higher age group were observed. Other researcher observed similarly LLINs usage was higher among high risk group in the same population [46]. The use of LLINs is more effective than the application of indoor residual spraying with the possibility of high public participation as well as integrating primary health care which shows reduction of overall morbidity and mortality in children aged 1–4 years [20].

In the present study, bio-efficacy tests revealed that LLINs net were found very effective to control *Anopheles* mosquitoes due to high mortality of wild caught *Anopheles* mosquitoes. It is evident that 100% mortality was noted after 24h of exposure time by using WHO cone test against LLINs nets expect one DAWATANA net. Of these, the maximum mosquitoes were knocked down within 30 to 40 min in fields. In case of LLINs nets, maximum mortality was seen after 24h of exposure period and found that 100% mortality was noted on all brands of collected LLINs nets from different areas. Only one DAWA TANA nets was found 30% mortality which was washed 12 times before test. 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% [47]. Delenasaw and his associates found a wider range of maximum mortality i.e. 13.9% - 81.1%, in their study on 6 WHO recommended LLINs in South-Western Ethiopia [48].

LLINs are still the main malaria vector control priority based on WHO recommendation [49] .All total of 60 LLINs nets consist of 6 brands of LLINs mosquito nets were collected from six different areas of malaria endemic areas. PermaNet 2.0, Yarkool, BASF, Net protect, Yahei LLINs Nets and DAWA TANA nets were found 100% and 91.67% efficacy although only one DAWA TANA was found 30% efficacy. It may be due to the fact that high frequency of washing with detergent and also coursed of the leaching of synthetic pyrethroid from impregnated fibers. Other researchers who are working on LLINs nets were observed that the tolerance to some conventional pyrethroid insecticides has been confirmed from field population of *An. stephensi* in endemic foci of southeastern Iran, as a result of operational issues with malaria vectors and agricultural pest control [50]. The values of LT_{50} and LT_{90} for *An. stephensi* tolerant strain was found to be 5.6 and 21.1 min with a mortality rate of 98% after 1-h exposure to permethrin which indicates susceptibility of this strain [51]. Another study by Pennetier and colleagues reported mortality rate and knockdown rates of *Anopheles gambiae s.l.* were exposed to unwashed net samples of Olyset® Plus to be 100% but were decreased to 64.0% after 20 washings whereas the storage of the LLINs surprisingly increased the mortality after washing [52]. Our study showed that the bio-efficacy of long-lasting insecticidal nets PermaNet 2.0, Yarkool, BASF, Net protect and Yahei LLINs Nets were found 100% efficacy as well as DAWA TANA nets was found 91.67% efficacy. These mosquito nets could be affected by susceptibility of *Anopheles* mosquitoes to insecticides especially pyrethroids as Deltamethrin. Present study suggested that WHO recommended LLINs nets and other brands of LLINs net which come from different countries be checked for sensitivity and resistance level with local vectors *Anopheles* mosquitoes before introduction into the community in malaria endemic areas.

CONCLUSIONS

LLINs nets are more effective and hygienic as well as they reduce the mosquito-density and reduce the men vector contact. They could either complement or replace some of the preventive and protective measures against the mosquito compare to other mosquito control techniques. Based on the present study, it is apparent that LLIN-based intervention technology is effective and appropriate for the control of malaria-transmitting *Anopheles* mosquitoes that they are abundantly present in malaria endemic areas in Myanmar and this method is a greater community acceptance, compliance and retention. Although, replacement with new LLINs nets with old LLINs nets in households are necessaries to provide every two years due to the waning residual efficacy (2years or 20washed) and physical integrity of nets. LLINs are needed for prolonged net serviceable lives and needed to develop effective LLINs nets that are more robust and potent against multiple insecticide-resistant insects. The promotion of this study is strongly advocated to ensure greater population coverage and transmission risk reduction for the continued success of malaria control strategies and operations.

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