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Bio-efficacy of LifeNet, a deltamethrin incorporated long-lasting insecticidal net, as assessed in experimental huts against *Anopheles fluviatilis*, a major malaria vector in east-central India

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ABSTRACT

LifeNet, a deltamethrin incorporated long-lasting insecticidal (polypropylene) net (LLIN), was qualified by the World Health Organization Pesticide Evaluation Scheme (WHOPES) for Phase-II trial in India. The purpose of this trial was to assess the bio-efficacy of unwashed and 20 and 30 times washed LifeNet in comparison to the nets conventionally treated with deltamethrin against the natural population of *Anopheles fluviatilis,* a major malaria vector, in terms of deterring hut entry, inhibiting blood feeding, inducing exophily and causing mortality. The trial was carried out in six experimental huts constructed at Kandhaguda village in Malkangiri district, Odisha State. The efficacy of unwashed and washed (20 or 30 times) LifeNet was compared with untreated polypropylene and conventionally treated (with deltamethrin) polyester net washed to just before exhaustion or washed 20 times. The study showed a significant reduction of entry (treatment: 1.61–4.78; control: 7.61 per hut) and an increase in exit (50.7–64.4% and 39.1%) of *An. fluviatilis* in the treated arms compared to the control arm (untreated net) ($P < 0.05$). Blood feeding rates reduced in treated arms (20.7–68.0%) compared to the control (80.3%) ($P < 0.05$). Total mortality was significantly higher in LifeNet arms (73.8–98.3%) than the control (2.2%) ($P < 0.05$). After 30 washes, the active ingredient (AI) retention in LifeNet was 62%. Performance of the three LifeNet arms against the susceptible population of *An. fluviatilis* met the WHO efficacy criteria of Phase II evaluation for LLINs.

1. Introduction

The launch of insecticide treated mosquito nets (ITNs) has been the turning point in the history of malaria control and many countries up scaled use of ITNs (Lengeler, 2004). However, re-treatment of nets every six to 12 months with insecticides became a major operational challenge in large scale implementation of ITN programme (Teklehaimanot et al., 2007). Earlier studies showed only less than 10% of nets available in Africa and South-east Asia were re-treated (WHO, 2003; Jambulingam et al., 2008). To overcome such low retreatment problem with ITNs in malaria control programme, long-lasting insecticidal nets (LLINs) that retain insecticidal bio-efficacy for three years or more without re-treatment have been developed and promoted. Such wash resistance quality has made LLINs more suitable and acceptable compared to ITNs (Killian et al., 2008). The WHO Pesticide Evaluation Scheme (WHOPES) has already granted full/ interim recommendations to some brands of LLINs and a few more are under initial phases of evaluation.

LifeNet, manufactured by Bayer Environmental Science, is a deltamethrin incorporated long-lasting insecticidal net {a 100 denier polypropylene net with 8.5 g deltamethrin active ingredient (AI) per kg of net corresponding to 340 mg AI/m^2} and it was recommended by the WHOPES for experimental hut evaluation in India. The bio-efficacy of LifeNet, in terms of deterrence, blood-feeding inhibition, induced exophily and mortality was assessed after 20 or 30 washes against the natural population of *Anopheles fluviatilis* in experimental huts in Odisha, east-central India as per the WHO guidelines (WHO, 2005).

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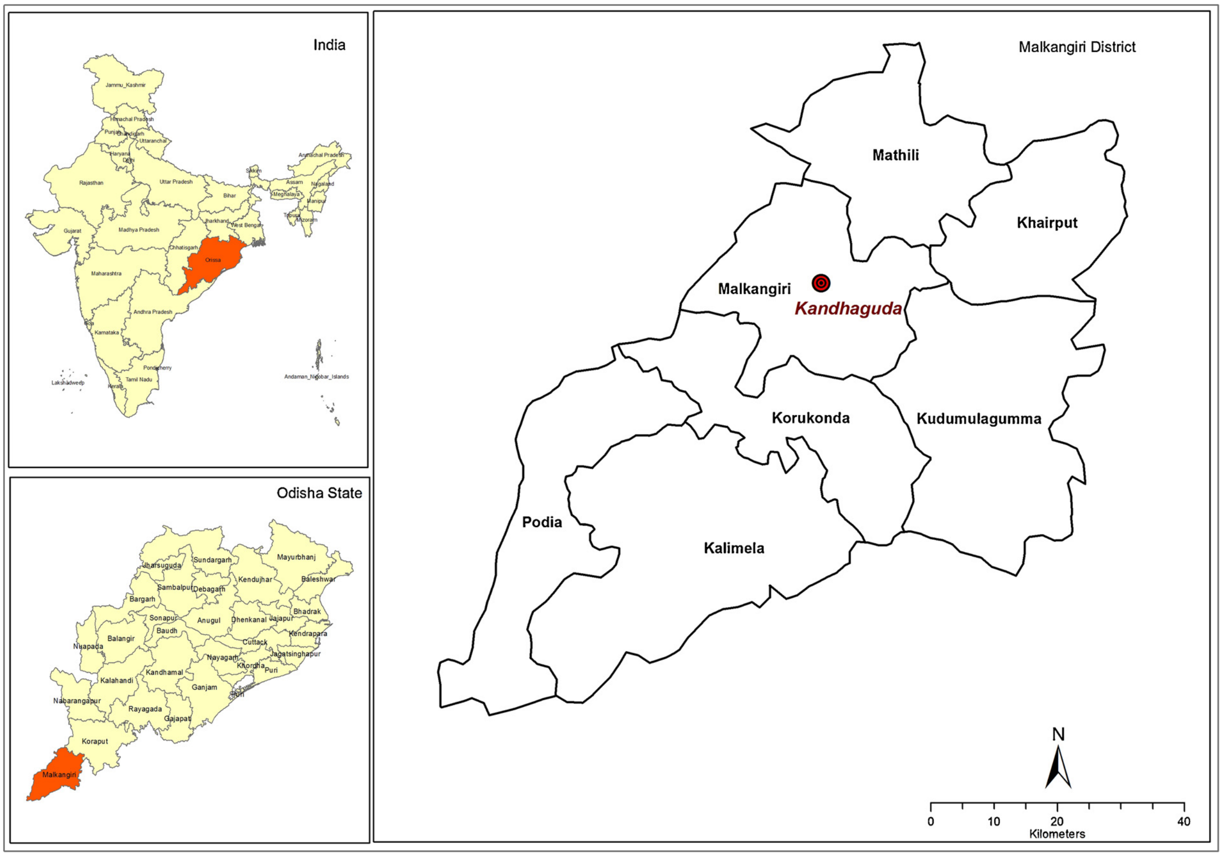

Fig. 1. Map showing the study village, Kandhaguda in Malkangiri district of Odisha State, India.

2. Materials and methodology

2.1. Study area

The trial was carried out in six experimental huts constructed in Kandhaguda village (latitude 18° 25′ N and longitude 81° 58′ E) under Pandripani Community Health Centre in Malkangiri District of Odisha State (Fig. 1). The design of the experimental huts has been described elsewhere (WHO, 2005). The village is located on a hilly terrain surrounded by forests and streams. Three seasons are prevailing there, summer (March to June), rainy (July to October) and winter (November to February). Malaria, mostly with *Plasmodium falciparum* infections, has been endemic in the village with an annual parasite incidence (API) of 9.0–50.9 per 1000 population during 2011–2016. *An. fluviatilis*, the major malaria vector in the area, was endophilic, exophilic, endophagic and anthropophagic (Gunasekaran et al., 1994; Sahu et al., 2008, 2017). The vector species was found breeding mainly in streams and terraced paddy fields and was abundant during September to February (Sahu et al., 2008). It was susceptible to DDT, malathion and synthetic pyrethroids, the commonly used insecticides in malaria vector control programme (Sahu et al., 2015). DDT indoor residual spraying (yearly two rounds) has been carried out in the area since 1953 as a key vector control intervention (Sahu et al., 2015). During 2012–2013, LLINs (PermaNet 2.0) were distributed for the first time.

2.2. Experimental arms

The Phase II study included six comparison arms viz., LifeNet unwashed, 20 times washed LifeNet, 30 times washed LifeNet, conventionally treated (with deltamethrin 10% SC at 25 mg/m^2) polyester net (CTN) and washed to just before exhaustion, conventionally treated (with deltamethrin 10% SC at 25 mg/m^2) polyester net (CTN) washed 20 times and untreated polypropylene net. The test nets (220 × 170 x 150 cm), received from the WHOPES, were coded denoting the six study arms and six replicates and two additional nets of each arm. Each replicate net per arm was tested one night per week. Sixteen (6 replicate + two additional nets per arm) polyester nets for the two CTN arms were treated with deltamethrin 10% SC @ 25 mg/m^2. Except the two arms, unwashed LifeNet and untreated polypropylene net, nets of the other arms were washed 20 or 30 times or until just before exhaustion, as assigned (WHO, 2005).

2.3. Bioassays

Bio-assays were conducted using WHO-prescribed cones on the nets prior to any wash and after 20 or 30 washes or washed up to just before exhaustion. *An. fluviatilis* could not be used for bioassays, as laboratory reared colony of this species was not available, where net washing was carried out. Therefore, blood-fed *An. stephensi* females reared at Indian Council of Medical Research-Vector Control Research Centre (ICMR-VCRC) laboratory, Puducherry were used for the bioassays. *An. stephensi* was susceptible to DDT, malathion and deltamethrin (Anuse et al., 2015). On each net, five sites were selected and on each site, two cone tests were performed. Five female mosquitoes, released in to each cone, were exposed to the net at the selected sites for three minutes. Thus, a total of 50 mosquitoes were exposed to one net. After exposure, the mosquitoes were kept for 24 h and during this holding period they were given access to sugar solution. Knock down after 60 min of exposure and mortality after 24 h of holding were recorded. At the end of the hut evaluation, fully fed wild caught *An. fluviatilis* females were used for bio-assay on a net selected randomly from each experiment arm tested in the experimental huts.

Table 1
Comparison of performance of the six experimental arms against wild *An. fluviatilis* in experimental huts.

Arms	Untreated polypropylene net	Unwashed LifeNet	LifeNet washed 20 times	LifeNet washed 30 times	Polyester treated net washed until just before exhaustion	Polyester treated net washed 20 times
Number of collections	36	36	36	36	36	36
Number of females entered/ caught	274	58	69	80	146	172
Number of females caught per night	7.61[a]	1.61[b]	1.92[b]	2.22[bc]	4.06[cd]	4.78[d]
Deterrence in % (95% CI)	–	78.8[a] (74.0–83.7)	74.8[a] (69.7–80.0)	70.8[a] (65.4–76.2)	46.7[b] (40.8–52.6)	37.2[b] (31.5–43.0)
Exit rate in % (95% CI)	39.1[a] (33.3–44.8)	53.5[b] (40.6–66.3)	50.7[ab] (38.9–62.5)	55.0[b] (44.1–65.9)	64.4[b] (56.6–72.2)	51.7[b] (44.3–59.2)
Blood feeding in % (95% CI)	80.3[a] (75.6–85.0)	20.7[b] (10.3–31.1)	29.0[b] (18.3–39.7)	27.5[b] (17.7–37.3)	52.1[c] (44.0–60.2)	68.0[c] (61.1–75.0)
Blood feeding inhibition in % (95% CI)	–	74.2[a] (63.4–86.4)	63.9[a] (53.3–75.8)	65.8[a] (56.1–76.6)	35.1[b] (29.2–41.8)	15.3[c] (11.8–19.2)
Total mortality in % (95% CI)	2.2[a] (0.5–3.9)	98.3[b] (95.0–101.6)	91.3[b] (84.6–98.0)	73.8[bc] (64.2–83.4)	39.7[cd] (31.8–47.6)	12.2[d] (7.3–17.1)

Numbers in the same row sharing a letter superscript do not differ significantly ($P > 0.05$).
Total mortality = Immediate mortality + Delayed mortality.
Confidential intervals (CIs) for percentages are based on normal approximation to binomial distribution.

2.4. Analysis for insecticide content

The cut pieces of the test nets (prior to washing, after washing 20 times or 30 times or until just before exhaustion and used-net from each arm at the end of the hut evaluation) were analysed for deltamethrin content at the Walloon Agricultural Research Centre, CRA-W, Gembloux, Belgium. The analytical method used for determination of deltamethrin in samples of LifeNet and conventionally treated nets has been described elsewhere (WHO, 2005).

2.5. Recruitment of volunteers

The process of selection of human volunteers participated in the trial has been mentioned elsewhere (Gunasekaran et al., 2016). Informed consent was obtained from all the selected volunteers and each volunteer was given remuneration. For involving 12 human volunteers in the study, approval from the Institute's Human Ethics Committee's was obtained.

2.6. Rotation of experiment arms and sleepers

The procedure of acclimatization and assessing suitability of the experimental huts has been described elsewhere (Gunasekaran et al., 2016). There were six comparison arms and each arm had six replicate nets; each one was tested one night per week in the six experimental huts. Six sleeper teams, each with two persons, were formed at the beginning of the trial, and not changed till the end. Following the Latin Square Rotation scheme, test nets and sleepers were rotated between the huts (WHO, 2005). Sleepers were rotated daily and the arms weekly (WHO, 2005). The beddings together with the white cloth, which was used to spread on the floor of the room and the verandah, and the test nets were removed from the huts at the end of each week. Since, the beddings and the white clothes (marked with arm code) came in close contact with the treated nets, they were also rotated along with the arms. After every full rotation (six nights), the experimental huts were cleansed and kept opened for ventilation to remove contamination, if any, from the preceding treatment.

Six holes, each of 4 × 4 cm, were made on four sides of the treated and untreated nets to resemble the state of a torn net (WHO, 2005). This would set to emphasize on testing, whether the synthetic pyrethroid used in the nets effectively prevented mosquito biting on sleepers rather than the nets. The procedure of mosquito collection from bed-net, verandah and walls, roof and floor of the room has been communicated in our earlier publication (Gunasekaran et al., 2016).

2.7. Statistical analysis

The data on mosquito collections were analysed to estimate the effect of the LifeNet arms in terms of deterrence, induced exophily, blood-feeding inhibition and mortality (clubbing immediate and delayed mortality together). The number of *An. fluviatilis* obtained from each hut/arm was recorded day wise and analysed for variance and mean. Since, both variance and mean were equal, negative binomial regression was used to analyze the number of the vector species entered the huts (hut entry) and logistic regression analysis was done to compare the exit, blood feeding and mortality rates between the LifeNet arms and the untreated net (Stata 9).

3. Results

3.1. Species composition

In total, 36 collections (6 collections per week for 6 weeks) were carried out in each of the six experimental huts (i.e. 36 collections for each experiment arm). Of the total mosquitoes collected (n = 2468); *An. fluviatilis* was predominant constituting 32.4%. *An. culicifacies,* the malaria vector of secondary importance, formed 14.5% of the collections; 33.8% was other anophelines and 19.3% was culicines. The number of *An. culicifacies* collected during the trial was very low; hence, further analysis was limited to *An. fluviatilis.*

3.2. Hut entry

The entry of *An. fluviatilis* in to the experimental huts with unwashed LifeNet, LifeNet washed 20 times, LifeNet washed 30 times, CTN washed to just before exhaustion, CTN washed 20 times and untreated polypropylene net ranged from 0 to 7, 0 to 7, 0 to 8, 0 to 9, 0 to 12, and 1 to 20, respectively over the 36 collections during the six weeks period, and the mean density ± SD was 1.61 ± 1.89, 1.92 ± 2.02, 2.22 ± 2.03, 4.06 ± 2.18, 4.78 ± 2.56 and 7.61 ± 4.49 in these arms (Table 1).

To see the effect of LifeNet in preventing entry of *An. fluviatilis* in to the huts, negative binomial regression analysis was done with the number of mosquitoes entered as dependent variable and the six experiment arms as independent variable. The entry of the vector species in to the experimental huts was found to be over-dispersed (non-random) {alpha that measures over-dispersion = 0.23 (95% CI = 0.142–0.375), $\chi^2 = 43.95$, df = 5, $p < 0.05$} justifying the analysis using negative binomial regression as well.

Table 2
Results of cone-bioassays.[a]

Arms	Before any wash		After washing		Prior to experimental hut study		After hut study	
	NE	CM (%)	NE	CM (%)	NE	CM (%)	NE	CM (%)
Untreated polypropylene net	50	0	50	0	25	0	25	4
Unwashed LifeNet	50	100	50	100	25	100	25	100
LifeNet washed 20 times	50	100	50	100	25	100	25	100
LifeNet washed 30 times	50	100	50	100	25	100	25	100
Polyester treated net washed until just before exhaustion	50	100	50	78[b]	25	76	25	72
Polyester treated net washed 20 times	50	100	50	60	25	60	25	56

NE: Number of mosquitoes exposed; CM: Corrected mortality.
[a] *An. stephensi* was used for cone-bioassays before any wash and after washes, whereas prior to hut evaluation and after hut evaluation, *An. fluviatilis* was used for the cone-bioassays.
[b] After 9 washes, mortality was 82%; After 10 washes, mortality was 78%.

Overall, *An. fluviatilis* entry differed significantly between the six experiment arms ($\chi^2 = 94.85$, df = 5, $p < 0.005$). Compared to the untreated net (reference category), the entry was significantly lower ($p < 0.005$) in all the five treated arms. Among the treated arms, the three LifeNet arms viz., unwashed LifeNet, LifeNet washed 20 times and LifeNet washed 30 times produced significantly higher deterrent effect (reduced entry) than the two conventionally treated polyester net arms (CTN), except that the entry was at the same level with polyester nets washed to just before exhaustion and LifeNet washed 30 times, as indicated from the 95% CI for the incidence rate ratio (IRR). The IRR (95% CI) of entry of *An. fluviatilis* was 0.21 (0.15–0.30), 0.25 (0.18–0.36), 0.29 (0.21–0.41), 0.53 (0.39–0.72) and 0.63 (0.47–0.84) for unwashed LifeNet, LifeNet washed 20 times, LifeNet washed 30 times, CTN washed to just before exhaustion and CTN washed 20 times, respectively.

3.3. Exit rate (induced exophily)

The exit rate of *An. fluviatilis* from the huts with unwashed, 20 times washed and 30 times washed LifeNet ranged from 50.7% to 55%. With CTN washed to just before exhaustion and CTN washed 20 times, the exit rate was 64.4% and 51.7%, respectively. In the huts with untreated nets also, the exit rate was found to be 39.1% (Table 1). The number exited from the hut on each day of collection in each arm over the experiment period was subjected to logistic (binomial) regression analysis by taking exit of mosquitoes as dependent variable and the six experiment arms as categorical covariates, keeping the control arm as reference category. There was a significant difference inthe exit rate of *An. fluviatilis* between the six experiment arms (Wald statistics = 25.955, df = 5, p = 0.000). Compared to the untreated control, the number exited was significantly higher in the five treated arms, except LifeNet washed 20 times. The odds ratio (95% CI) of the number of *An. fluviatilis* exited with unwashed LifeNet, LifeNet washed 20 times, LifeNet washed 30 times, CTN washed to just before exhaustion and CTN washed 20 timeswas 1.79 (1.01–3.71), 1.61 (0.95–2.73), 1.91 (1.15–3.15), 2.85 (1.86–4.28) and 1.67 (1.14–2.46), respectively. The 95% CIs for the odds ratios showed that the exit rate did not differ significantly between the five treatment arms.

3.4. Blood feeding rate

Blood feeding of *An. fluviatilis* was not completely inhibited by any of the five treated arms. The feeding rate was 20.7%, 29.0%, 27.5%, 52.1%, and 68.0%, respectively with the three LifeNet arms and the two CTN arms, whereas the feeding rate was 80.3% with the untreated control arm. Thus, there was a significant difference in blood feeding rates between the six experiment arms (Wald statistics = 134.002, df = 5, p = 0.000). Compared to the control, the feeding rate was significantly lower in the five treated arms, and among the treated arms, the three LifeNet arms greatly reduced the blood feeding than the two CTN arms. The odds ratio (95% CI) of blood feeding (inhibition) of *An. fluviatilis* was 0.06 (0.03–0.13), 0.10 (0.05–0.18), 0.09 (0.05–0.16), 0.27 (0.17–0.41) and 0.52 (0.34–0.81), respectively for the five treated arms. The blood feeding rate between the three LifeNet arms did not vary significantly and similarly between the two CTN arms. Thus, LifeNet, whether unwashed or washed (20 or 30 times) greatly inhibited blood feeding of the vector species.

3.5. Mortality from hut collection

The total mortality (immediate + delayed) rate of *An. fluviatilis* in the six experiment arms are provided in Table 1. A significant difference was observed in the total mortality rate between the six experiment arms (Wald statistics = 201.002, df = 5, p = 0.000). All the five treated arms produced significantly a higher mortality than the untreated control. Among the treated arms, the mortality caused by the three LifeNet arms was significantly greater than that caused by the CTN arms. Though not significantly different, unwashed LifeNet caused the highest mortality (98.3%) and the lowest (73.8%) was by the LifeNet washed 30 times. Similarly, between the two CTN arms, 20 times washed one produced a lower mortality (12.2%) than the one washed to just before exhaustion (39.7%). The odds ratio (95% CI) of the total mortality (killing effect) of *An. fluviatilis* was 3.067E3 (351.55–2.67E4), 564.90 (167.09–1.910E3), 151.15 (54.77–417.16), 35.46 (13.79–91.20) and 7.48 (2.77–20.25) for unwashed LifeNet, LifeNet washed 20 times, LifeNet washed 30 times, CTN washed to just before exhaustion and CTN washed 20 times, respectively.

3.6. Mortality from cone-bioassays

Prior to any wash, LifeNet and CTN arms produced 100% mortality of *An. stephensi* while the mortality against the control was zero. After washing (test species: *An. stephensi*) and prior to and after the evaluation in experimental huts (test species: *An. fluviatilis*), the LifeNet arms caused 100% mortality, whereas, the mortality caused by the CTN arms ranged from 56% to 78%; the mortality with the untreated net was < 5% (Table2).

3.7. Chemical analysis

The target dose of deltamethrin in unwashed LifeNet was 8.5 g/kg (± 25%). The chemical analysis showed that three samples of unwashed LifeNet complied with the target dose (6.62, 6.47 and 6.64 g/kg) while in two samples the insecticide content (6.07 g/kg and 6.20 g/kg) was just below the tolerance lower limit (6.375 g/kg). There was a good homogeneity of the distribution of the active substance over all the tested net samples as the relative standard deviation (RSD) of the content was 7.0, 5.2, 2.0, 1.1 and 4.2%. In LifeNet samples washed 20

Table 3
Results of chemical analysis for deltamethrin content (g/kg).

Treatment	Deltamethrin content (g/kg)										DM retention (% of wash 0)
	Before any wash		Washed until just before exhaustion		After 20 washes		After 30 washes		After Expt. hut study		
	Mean	Within net RSD (%)	Mean	Within net RSD (%)	Mean	Within net RSD (%)	Mean	Within net RSD (%)	Mean	Within net RSD (%)	
Unwashed LifeNet	6.62	7.0	–	–	6.07	1.1	6.20	4.2	6.69	2.5	–
LifeNet washed 20 times	6.47	5.2	–	–	4.02	10.7	–	–	4.81	4.0	62
LifeNet washed 30 times	6.64	2.0	–	–			4.04	7.5	4.04	13.6	62
Polyester treated net washed until just before exhaustion	0.07	107.5	< 0.01	–		–	–	–	< 0.01	–	< 14
Polyester treated net washed 20 times	0.05	105.2	–	–	< 0.01	–	–	–	< 0.01	–	–
Untreated polypropylene net	0.01	0.0	< 0.01	–	< 0.01	–	< 0.01	–	< 0.01	–	–

Target dose and tolerance limit for deltamethrin in LifeNet (100 denier) = 8.5 g/kg ± 25% [6.375 g/kg – 10.625 g/kg].
RSD: Relative standard deviation; DM: Deltamethrin.

or 30 times, the RSD was found to be lower (10.7% and 7.5%). The (insecticidally inactive) deltamethrin R-alpha isomer content in unwashed LifeNet was 1.19–1.65 g/kg corresponding to 18.0–25.2% of the deltamethrin content and this amount did not increase after washing and also after testing. It was observed after 20 and 30 washes, the average insecticide content was 4.02 g/kg and 4.04 g/kg, corresponding to an overall retention of 62% and 62%, respectively (Table 3).

The conventionally treated polyester net prior to any wash contained 2.3–3.1 mg/m^2; RSD = 105.2–108.0 (0.05–0.07 g/kg; RSD = 105.2–107.5%) deltamethrin (Table 3), which was only 11% of the target dose. The analysis indicated that < 0.4 mg/m^2 (< 0.01 g/kg) deltamethrin was available in the CTN washed to just before exhaustion, which was 14% lesser than the corresponding retention rate. At the end of the hut trial, there was no significant decrease in the deltamethrin content in all the treated arms.

3.8. Side effects

Interview of the volunteers showed that six volunteers (n = 12), who slept under the nets of LifeNet arms had nose irritation and itching on their face and hands for one week, but subsided afterwards. All the 12 volunteers, who slept under nets of the different arms, reported that they did not get any odour from the nets. Reduced mosquito bites in the huts and an undisturbed night sleep throughout the study period were the perceived benefitsby the volunteers.

4. Discussion

Development of insecticide resistance is probably the biggest threat to the capacity to control malaria vectors or sustain any drive towards malaria elimination. Currently, the chemical agents that make malaria vector control feasible are the pyrethroids. The best tools for delivering pyrethroids are insecticide treated nets (ITNs). However, the challenges on use of ITNs are unscheduled frequent washing by users which decreases the insecticide content available on the netting and making them ineffective quickly and very low re-treatment compliance (Teklehaimanot et al., 2007). To get rid of these problems, LLINs have been introduced and are being promoted in many malaria endemic areas (Killian et al., 2008). Recent trends confirm that scale up of this tool is making inroads into the malaria burden in many countries (Killian et al., 2008; Enayati and Hemingway, 2010; Gunasekaran et al., 2014). LLINs have the quality of retaining the insecticidal efficacy for three to five years without any re-treatment and are considered to be an important innovation accepted globally for malaria prevention (Tami et al., 2004; WHO, 2007; Banek et al., 2010). The synthetic fibres (polyester or polyethylene or polypropylene) that are used for manufacturing these nets have been mixed with an insecticide. The nets not only kill or repel the mosquitoes, but also work as a physical barrier to them. There are reports that presence of a LLIN also drive out the mosquitoes even from the surroundings (Pennetier et al., 2013). WHOPES has given full recommendation to DuraNet®, Interceptor®, MAGNet, Olyset® Net, PermaNet® 2.0, Royal Sentry® and Yorkool™, while LifeNet has been given interim recommendation based on the results obtained from laboratory studies and field evaluations (WHO, 2008, 2009; WHO, 2014). In India, the experimental hut studies are conducted only by the ICMR-VCRC, Puducherry through its field site at Odisha State. The current evaluation of LifeNet in experimental huts was carried out following the WHO guidelines (WHO, 2005) in an area where malaria was transmitted primarily by *An. fluviatilis*, which was susceptible to synthetic pyrethroids (Gunasekaran et al., 2014).

Compared to the untreated control and the CTN arms, a greater efficacy of LifeNet (both washed and unwashed) in terms of preventing mosquito entry (deterrence) was confirmed. Further, the equal deterrent effect showed by the three LifeNet arms indicated its higher wash resistance (withstanding 30 washes) without losing bio-efficacy of the synthetic pyrethroid treatment. As observed in the current study, a West African study also reported a significant reduction of entry of *An. gambiae* with LifeNet® washed 30 times compared to unwashed Life Net (Agossa et al., 2014). On the other hand, in Benin, when LifeNet was evaluated against *An. gambiae,* which was moderately pyrethroid resistant, there was no significant reduction of entry of the vector species (WHOPES, 2011). The treated nets induced significantly a higher exophily than the untreated nets. The excito-repellent effect of deltamethrin in all the treated arms was sufficient to induce greater exophily compared to the untreated control and continued to exert repulsive action on the *An. fluviatilis* susceptible strain. Though, the exit rate of CTN washed to just before exhaustion (10 washes) was higher than the other treated arms, the difference was not statistically different. LifeNet, both unwashed and washed 20 or 30 times, greatly inhibited blood feeding of *An. fluviatilis* (74% with unwashed and 64 and 66% with washed 20 and 30 times) compared to the untreated control and the CTN arms, as observed in Benin against *An. gambiae* and in Tanzania against *An. arabiensis* during experimental hut studies (WHOPES, 2011; Agossa et al., 2014).

In terms of killing effect, although the five treated arms were not comparable, they caused significantly higher mortality than the control. Both unwashed and 20 times washed LifeNet caused > 90% mortality and after washing 30 times there was a reduction in mortality (73.8%), but not statistically significant. Further, the mortality caused by LifeNet washed 30 times was about two fold higher than the mortality (39.7%) caused by CTN washed to just before exhaustion (10 washes). Similarly, in the Tanzanian trial, the mortality by LifeNet washed 20 or 30 times was nearly twice that caused by CTN washed to just before exhaustion (WHOPES, 2011). However, in the Benin trial, the LifeNet washed 20 times caused significantly a higher mortality than the CTN washed to just before exhaustion, but the mortality caused by the LifeNet washed 30 times was not significantly different from the CTN (WHOPES, 2011).

Before any wash, the treated arms, including the two CTN arms, produced 100% mortality of *An. stephensi* (in cone-bioassays). After washing 30 times, there was no decline in mortality caused by LifeNet, but after washing of CTN to just before exhaustion or after 20 times, the mortality dropped to 78% and 60%, respectively. The insecticide treatment of LifeNet continued to produce 100% mortality of *An. fluviatilis* before and after testing in experimental huts, whereas in the case of CTN arms, treated with the same insecticide, the mortality after hut testing declined to 72% and 56%, respectively. Therefore, it was obvious that up to 30 washes LifeNet could offer adequate protection (with > 80% mortality) and this would be sufficient to cover transmission seasons for at least three years as people of this region are unlikely to wash their nets 30 times during three years (Anuse et al., 2015). Thus, LifeNet, with its higher wash resistance quality (as per the WHO standard, the number of washes any LLIN should withstand is 20), could offer protection for an extended period. In the case of CTN, 20 washes greatly reduced the efficacy of the insecticide treatment and thereby shortening the protection period. The study conducted at Tanzania and Benin also reported 100% knock down (KD) and mortality after washing of LifeNet (20 or 30 washes) and at the end of the hut trial, whereas the CTN washed 20 times caused only 30% KD and 22% mortality (WHOPES, 2011).

The decline in deltamethrin content in LifeNet with 62% retention after 20 or 30 washes was an indication of reduced bio-availability of the active ingredient (AI) on the net surface due to washing. However, this was not reflected from the performance of LifeNet against the susceptible vector in experimental huts, as it produced a comparable effect. There was a relationship between the mortality rates in the bioassays and the available dosages of deltamethrin in different treated nets before and after washing. The relative standard deviation i.e. within-net variation also showed a good homogeneity of the distribution of AI over the net.

5. Conclusions

In summary, the current experimental hut study demonstrated a greater performance of LifeNet washed 20 or 30 times compared to untreated (negative control) and the conventionally treated nets (positive control) in terms of deterrence (preventing hut entry of mosquitoes), induced exophily, blood-feeding inhibition and killing effect against the malaria vector, *An. fluviatilis* and thereby meeting the WHO efficacy criteria of Phase II evaluation of LLINs. All the three LifeNet arms (unwashed, washed 20 times and washed 30 times) caused significant inhibition of blood feeding by *An. fluviatilis* in experimental huts. Further, the LifeNet, even after 30 washes, retained the insecticidal efficacy to cause 100% mortality of *An. fluviatilis* in field condition. The study further confirmed that LifeNets after washing even 30 times can reduce vector densities by killing mosquitoes, reduce mean lifespan of mosquitoes and thereby reducing vectorial capacity, and offer increased personal protection even when the nets are torn with holes. Generally, people wash their nets maximum seven to eight times per year (Anuse et al., 2015). In this context, LifeNet, with its built-in bio-efficacy, could be an efficient vector control mean to interrupt malaria transmission by susceptible vector like *An. fluviatilis* in India.

Ethics statement

The study was approved by the Human Ethics Committee of ICMR-VCRC, Puducherry.

Author summary

The LifeNet LLIN is manufactured by Bayer CropScience as a deltamethrin-treated long-lasting (incorporated into filaments) insecticidal [mosquito] net and was qualified by the WHOPES for Phase-II trial. The current study was a phase II trial of LifeNet LLIN conducted in an adapted version of the west African experimental huts whereby mosquitoes can only escape outside to a single veranda trap; constructed at Kandhaguda village of Malkangiri district in Odisha State. The aim of the study was to assess the bio-efficacy of unwashed and 20 and 30 times washed LifeNet in comparison to untreated nets and nets conventionally treated with deltamethrin against the natural population of *An. fluviatilis,* a major malaria vector (susceptible to synthetic pyrethroids), in terms of deterring hut entry, inhibiting blood feeding, inducing exophily and causing mortality. The study revealed that performance of the three LifeNet arms against the susceptible population of *An. fluviatilis* met the WHO efficacy criteria of Phase II evaluation for LLINs.

Therefore, LifeNet, with its built-in bio-efficacy, could be an efficient vector control mean to interrupt malaria transmission caused by susceptible vectors like *An. fluviatilis* in India.

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Competing interests

The authors declare that they have no competing interests.

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