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Authors: Gebremariam, Brhane, Birke, Wondwosen, Zeine, Wuhib, Ambelu, Argaw, and Yewhalaw, Delenasaw

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Brhane Gebremariam[1], Wondwosen Birke[2], Wuhib Zeine[2], Argaw Ambelu[2] and Delenasaw Yewhalaw[3]

[1]Department of Public Health, College of Health Sciences, Aksum University, Axum, Tigrai, Ethiopia. [2]Department of Environmental Health, College of Health Sciences, Jimma University, Jimma, Ethiopia. [3]Tropical and Infectious Diseases Research Institute, Jimma University, Jimma, Ethiopia.

ABSTRACT

BACKGROUND: Long-Lasting Insecticidal Nets (LLINs) efficacy could be compromised due to a lot of influences together with user compliance and vector population insecticide resistance status. Thus, this study was to assess the biological efficacy of DuraNet® with the help of the World Health Organization cone bioassay and field experimental hut.

METHODS: A laboratory and a semi-field conditions experimental huts against *Anopheles* Mosquitoes were conducted in southwestern Ethiopia from September 2015 to January 2016. The bio efficacy of DuraNet® was evaluated using the WHO cone bioassay test and then its field efficacy was evaluated using experimental huts against the malaria vector population.

RESULTS: World Health Organization cone bioassay tests against pyrethroid-resistant *An. arabiensis* led to mean percent mortality and knockdown of 78% and 93%, respectively. Washing of DuraNet® successively reduced its efficacy from 93% knockdown (0 wash) to 45% knockdown (20 washes). Similarly, mean mortality decreased from 84% (0 wash) to 47% (20 washes). A total of 1575 female mosquitoes were collected over 40 nights out of which 1373(87.8%) were *An. gambiae s.l.*, 116 (7.4%) were *Anopheles coustani* and 107 (6.8%) were *An. pharoensis*. The mean blood-feeding rate was significantly lower ($P < .001$) in hut containing unwashed DuraNet® when compared to hut containing untreated DuraNet®. The mean mortality rate was significantly higher ($P < .001$) in hut containing DuraNet® when compared to hut containing untreated DuraNet®. Unwashed DuraNet® showed the highest personal protection 88.7% and 100% against *An. Arabiensis* and *An. pharoensis*, respectively.

CONCLUSION: Both DuraNet® and PermaNet 2.0 moderate efficacy against a pyrethroid-resistant population of *An. arabiensis* from Ethiopia. The bio efficacy of DuraNet® was found below the WHO recommendation. Therefore, the real impact of the observed insecticide resistance against DuraNet® to be further studied under phase-III trials, the need for new alternative vector control tools remains critical.

KEYWORDS: Knockdown, DuraNet®, *Anopheles gambiae*, *Anopheles arabiensis*, experimental hut, cone bioassay, Ethiopia

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CORRESPONDING AUTHOR: Brhane Gebremariam, Department of Public Health, College of Health Sciences, Aksum University, P.O.Box 1010, Axum, Tigrai, Ethiopia. Email: gebremariambrhane@gmail.com

Introduction

Malaria is still one of highest health risks in developing countries with high morbidity and mortality in under 5 children. Assessment of overall economic impact of the disease shows that it accounts for 40% of health cost spending, 30% to 50% of inpatient charges, and up to 50% of outpatient official visits in areas with great malaria.[1] According to the world health organization, there were 216 million new incidents of malaria leading to 445 000 death, of which 90% occurred in African countries.[2]

Long-Lasting Insecticidal Nets (LLINs) are uppermost community health apparatuses and, when used by children and pregnant women, subsidize to improving motherly, neonatal, and infant health, with long-lasting reimbursements to the emerging child.[3] These nets provide personal barrier from bite by mosquitoes in addition those nets lessen also the transmission of malaria and have excito-repellency effect.[4] A total of 505 million insecticidal nets (ITNs) were distributed in developing countries between 2014 and 2016 with household ownership of at least 1 insecticidal net, improved from 50% in 2010 to 80% in 2016. Nevertheless, the ratio of houses with adequate bed nets (ie, 1 net for every 2 people) is very low (43%).[2] Following the high demand of bed nets as key vector control intervention, companies are ramping up production and new brand nets are being introduced for public health utilization.

In order for any new long-lasting insecticidal nets to be used in public health, it must gain WHOPES approval.[5] The approval process passes through 3 stages of efficacy testing (laboratory, small-scale semi-field, and large-scale field trial). To be classified as a long-lasting insecticidal net, it must

maintain their effective biological activity for at least 20 World Health Organization recommended washes typical washes under laboratory conditions and 3 years of suggested use under semi-field and field condition.

DuraNet® is an LLIN developed by the Shobikaa Impex pvt Ltd (Karur, Tamil Nadu 639006, and India). It contains 0.55% w/w ± 15% alpha-cypermethrin. The net is a polyethylene fiber coated with a proprietary polymer containing target dose of alpha-cypermethrin at 250 mg/m^2. The polymer binds to the fiber and can withstand multiple washings, the active ingredient diffusing in a controlled manner to the surface of the polymer coat to maintain insecticidal efficacy.

Thus, this study was conducted to verify the intended efficacy of the product in the presence of pyrethriod resistance vector population of *An. gambiae* s.l. using cone bioassay and semi-field experimental huts in southwestern Ethiopia. The cone bioassays were conducted in Tropical and Infectious diseases research institute, Jimma University. The experimental hut study trials were undertaken near the Gilgel-Gibe hydroelectric power dam-I.

Methods

Study area and period

This study was conducted from September 2015 to January 2016 near the Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia. The hydroelectric dam is one of the major hydroelectric dams in Ethiopia with artificial reservoir occupying an estimated area of 62 kmsq.[6] It produces around 184 MWh and is located 260 km southwest of the capital Addis Ababa. It has been functional since 2004. The study area is located between latitudes 7°42′50″N and 07°53′50″N and longitudes 37°11′22″E and 37°20′36″E with an altitude ranging from 1672-1864 m above sea level. The area has a sub-humid, warm to hot climate, obtains between 1300 and 1800 mm of rainfall annually and has a mean annual temperature of 19°C. Mostly, the 2 peak seasonal transmissions of malaria occur during the months of September to December and March to May in the study area.

Bio-efficacy testing of LLIN (DuraNet®) under laboratory setting

LLIN sample preparation and washing process. DuraNet® LLINs (Karur, Tamil Nadu 639006, and India) is treated with alpha-cypermethrin at a target dose of 250 mg/m^2 of netting material. It contains 0.55% W/W ± 15% alpha-cypermethrin. The alpha-cypermethrin chemical is coated onto filaments at bursting strength of 450 kPa of netting material and of 145 ± 5% for seam sub-section per denier yarn. Prior to testing the production date and batch number of all nets were recorded. Seven sub-samples per net (1 from the roof, the rest from side of the net) were taken from each net and prepared for standard LLINs cone tests by cutting 25 cm × 25 cm pieces following WHO protocol.[7] In this study, 9 candidate nets were used for bio-efficacy testing. Thus, a total of 63 sub-sample net pieces (7 sub-sample pieces from each net × 9 DuraNet®s) individually rolled up in aluminum foil, labeled (by net type, net number and sample area) and kept in a refrigerator prior to the assay. Concurrently 9 (1 sub-sample piece from each untreated DuraNet® × 9 untreated DuraNet®s) sub-samples (30 cm × 30 cm) were individually rolled up in aluminum foil, labeled and kept in a refrigerator prior to the assay.

Mosquito rearing and cone bioassay testing. Anopheles mosquito larvae were collected from field sites near Gilgel-Gibe hydroelectric power production reservoir and reared to adults under standard conditions (25 ± 2°C temperature, 80 ± 4% relative humidity) in tropical and infectious diseases research institute, Sekoru campus, Jimma University, Ethiopia. For WHO cone bioassay test, five 2-5 days age unfed female *An. gambiae s.l.* (presumably *An. arabiensis* according to Yewhalaw et al., (2009))[6] mosquitoes were used per cone. Twenty mosquitoes (5 mosquito's per-cone × 4 cone-per sub-sample net piece) were introduced into the cone facing net sample for 3 minutes and then moved to holding paper cups. Then mosquitoes were supplied with a 10% sucrose solution. The number of mosquitoes knocked down and the number of dead mosquitoes were recorded every 10 minute within 60 minutes and 24 hours, respectively. Mosquitoes exposed to untreated DuraNet® pieces were used as controls and experiment conditions were set to be 27 ± 2°C temperature and 75 ± 10% relative humidity (RH) throughout the study. Thus, a total of 1260 mosquitoes (63 net sub-samples × 20 mosquitoes per sub-sample net) were used for complete bioassay testing and another 180 mosquitoes (9 untreated net sub-samples × 20 mosquitoes per sub-sample net) were used as control.

For net pieces washing each net sub-samples (25 cm × 25 cm) were introduced individually into 1-l beakers containing 0.5 l deionized water, with 2 g/l WHO recommended soap (Savon de Marseille; pH 10-11) added and fully dissolved just before washing. The beakers were then introduced into a water-bath at 30°C and shaken for 10 min at 155 movements per minute. The samples were then removed, rinsed twice for 10 min in clean, deionized water under the same shaking conditions as above, dried at room temperature and stored at 30°C in the dark between washes.

Bio-efficacy testing of DuraNet® LLIN using experimental huts

Experimental hut establishment. Five experimental huts of West African style, each with 1 room and screened veranda trap were established approximately 500 m from Gilgel-Gibe reservoir shore, southwestern Ethiopia following world health protocol developed for field evaluation of long-lasting insecticidal nets.[5] The huts were made from concrete bricks with a corrugated iron roof, a ceiling of white cotton sheeting and a concrete base surrounded by a water filled moat to prevent the entry of ants. Details of the dimensions of the hut, the Verenda trap and

Figure 1. Experimental hut design of the study.

materials from which the tukuls made were described elsewhere.[8] The slits were constructed from pieces of metal shutters, fixed at an angle of 45° to create a funnel of 1 cm between slits. The design of window slit allows the inward flight of mosquitoes coming from filed but it will be hardly possible for them to escape once they entered the hut. A veranda trap made of iron mesh (22 mm diameter) was set at the back of each hut for trapping exophilic mosquitoes (Figure 1). It is presumed that some mosquitoes (inherently exophilic or mosquitoes repelled due to the exito-repellency effect of the chemicals impregnated into LLINs) will exit the hut once they enter and then after feeding on their host of choice. Thus, the verenda trap was designed to trap and quantify the proportion of mosquitoes exit the experimental hut and by virtue the efficacy of LLINs under test for its exito-repellent effect. Each night mosquitoes were allowed to enter into the hut via the window slits from the environment and freely move between the room and the verenda trap.

Treatment arms and sleepers rotation

In this experiment, 5 different treatment arms were used. These include (1) Untreated unwashed DuraNet® (Negative control) (2) Unwashed treated DuraNet® (3) treated DuraNet® 20 times washed (4) PermaNet® 2.0 20 times washed, and (5) unwashed PermaNet® 2.0 (positive control). All nets were 75 denier polyethylene and polyester nets respectively. To simulate wear and tear condition of nets under usage 6 (4 cm × 4 cm size) holes were cut in each net (2 holes on each of the sides and 1 hole at each end). The DuraNet® LLIN and PermaNet® 2.0 were washed according to World Health Organization Stage II washing procedure.[5] For washing, the nets were put in to aluminum bowl with 10 l of tap water and 2 g/l of savon de Marseille soap. The nets were agitated for 3 minutes, then soaked for 4 minutes, and agitated again for 3 minutes. The nets were agitated manually by stirring them with a pole at 20 rotations per minute. Thus, each net was washed for a total of 10 minute and then rinsed with clean water by a similar procedure, dried horizontally in the shade and stored at ambient temperature between washes. WHO recognized PermaNet® 2.0 LLIN washed 20 times, was used as a positive control to assess DuraNet® LLIN performance.

In each data collection night, 2 non-smoker male volunteers aged 20 to 25 were allowed to sleep in each experimental hut with its door remain closed between 19:00 and 07:00 hours. Each team was rotated between treatments on successive nights within a week to avoid possible bias which could arise due to individual attractiveness to mosquitoes. There were 5 successive collection nights (Monday to Friday) and 2 successive break nights for hut ventilation per week. Thus, there were 25 collection nights in order to complete the whole study. Informed written consent was obtained from each sleeper.

Mosquito collection, identification and key parameters measured in determination of LLINs efficacy

Mosquitoes were collected from 6:00 to 7:00 each morning inside bed nets, floors, walls, ceilings, and verandas of each experimental hut by the help of mouth aspirators and torches. Then the collected mosquitoes were recorded as dead or alive. Live mosquitoes were held in paper cups and supplied with 10% sucrose solution. The collected mosquitoes were transported to Asendabo Vector Biology Laboratory, Jimma University, where mosquitoes were sorted by genus, sex and morphologically identified using taxonomic keys.[9] Mosquitoes were also scored for their physiological state as unfed, fed, half gravid and gravid. Delayed mortality was recorded after 24 hours. To evaluate the efficacy of DuraNet LLIN against the resistant populations of *An. arabiensis*, different entomological parameters (deterrence, exit, blood feeding inhibition, and mortality rates) were derived from basic measurements.

The primary outcomes were:

1. Deterrence—the reduction in entry in to treatment hut relative to the control hut (ie, huts holding untreated nets);
2. Mortality—the proportion of mosquitoes killed relative to the total catch size;
3. Killing effect—the numbers killed by a treatment relative to the untreated control, as derived from the formula;

$$\text{Killing effect } (\%) = \left(\frac{Kt - Ku}{Tu}\right) * 100$$

Where **Kt** is the number dead mosquitoes in the huts with treated nets, **Ku** is the number dead mosquitoes in the huts with untreated nets, and **Tu** is the total entering the huts with untreated nets.

4. Blood Feeding Inhibition–The proportional reduction in blood feeding in huts with treated nets relative to controls with untreated nets.

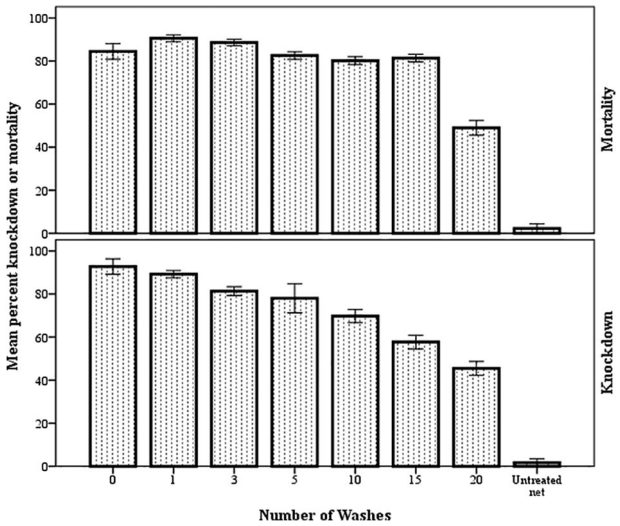

Figure 2. Mean percent knockdown and mortality of *An. arabiensis* exposed in 3 minutes cone bioassay to DuraNet®, Jimma, and southwestern Ethiopia.

5. Personal Protection—The reduction in mosquito biting by treated nets relative to untreated nets, as derived from the formula

$$\%\ \text{personal protection} = \left(\frac{Bu - Bt}{Bu}\right) * 100$$

Where Bu is the total number blood fed mosquitoes in the huts with untreated nets, and Bt is the total number blood fed in the huts with treated nets.

Data analysis

Association between response variables (Percent knockdown and percent mortality) and wash time was analyzed using linear regression. We compared the number of mosquitoes entered to the different treatment arms using negative binomial regression model. Proportion of mosquitoes entering in to the treatments like Mortality, blood feeding, and exiting were estimated by using logistic regression model.

Results

Cone bioassay and washing resistance

Exposure of wild population of *An. arabiensis* mosquitoes to net sections of DuraNet® LLIN in WHO bioassay tests gives mean mortality rate of 78% as well as mean knockdown effect of 93%. Mean percent knockdown and percent mortality of DuraNet® LLIN showed significant relationship ($R^2 = 0.797$, n = 260, $P < .001$). The wash resistance activity of DuraNet® LLINs, measured in terms of percent knockdown and mortality is presented in Figure 2. The mean percent knockdown of wild population of *An. arabiensis* decreased from 93% to 45% up on exposure to the DuraNet® washed 0 and 20 times respectively. The mean percent mortality decreased from 84% to 47% on exposure to the DuraNet® washed 0 and 20 times, respectively. Wash resistance has negative correlation with percent knockdown and percent mortality which means as number of wash increase, percent knockdown and percent mortality declined ($r^2 = 0.333$, %KD = 89.16 ± 2.15, $P < .001$ and $r^2 = 0.236$, %Mt = 81.94 ± 1.68, $P < .001$), respectively.

Experimental hut trial

Mosquito entry into the huts. A total of 1575 Anopheles mosquitoes were entered and collected in the 40 nights during the trial. These consisted of 1373 (87.8%) *An. gambiae* s.l. presumably *An. arabiensis* (Yewhalaw et al., 2009), 116 (7.4%) *Anopheles coustani* (Laveran), and 107 (6.8%) *An. pharoensis* (Theobald). The mean number of mosquitoes caught per night was 8.75 for *An. arabiensis*, 5.52 for *An. Coustani*, and 6.33 for *An. pharoensis*. The mean mosquito entry is not significantly different among the treatment arms ($F = 1.277$, $P > .05$). There was no clear evidence of deterrence associated with any of the treatments however; there were fewer *An. Arabiensis* in huts with the Unwashed DuraNet® compared to treated DuraNet® and PermaNet® 2.0 (Table 2).

Mosquito mortality, blood feeding and exit rates. Mosquito blood feeding rates, mortality rates and exit rates of the 5 treatments are presented in Table 1. The mean blood feeding rate was significantly lower ($P < .001$) in hut containing unwashed DuraNet® when compared to hut containing untreated DuraNet®. However, there was no significance difference ($P > .05$) in blood feeding rate among huts containing untreated DuraNet®, 20 times washed DuraNet®, unwashed PermaNet® 2.0 and 20 times washed PermaNet®2.0. The mean mortality rate was significantly higher ($P < .001$) among huts containing treated DuraNet®, 20 times washed DuraNet®, unwashed PermaNet® 2.0 and 20 times washed PermaNet® 2.0 when compared to hut containing untreated DuraNet®. Higher mean number of mosquitoes were caught while exiting the huts containing treated DuraNet®, 20 times washed DuraNet®, unwashed PermaNet® 2.0 and 20 times washed PermaNet® 2.0 when compared to hut containing untreated DuraNet®, however, there was no significant difference ($P > .05$) among the treatments.

Blood feeding inhibition and personal protection. Unwashed DuraNet® showed strong and better bio-efficacy in terms of blood feeding inhibition when compared to untreated DuraNet® and the rest of the treatment arms. The mean blood feeding rate of mosquitoes collected from a hut with unwashed DuraNet®, Unwashed PermaNet 2.0, twenty times washed DuraNet®, 20 times washed PermaNet 2.0 and Untreated DuraNet®, was 0.8/person-night, 4.1/person-night, 5.7/person-night, 6.0/person-night, and 7.0/person-night respectively. There was significant reduction ($P < .001$) in blood feeding inhibition in all the treatment arms (hut with unwashed DuraNet®, hut with 20 times washed DuraNet®, hut with

Table 1. Mean blood feeding, mortality and exit rates of populations of *An. arabiensis* exposed to different bed net types in field experimental huts in southwestern Ethiopia.

TREATMENTS	BLOOD-FEEDING RATE MEAN ± SE	MORTALITY RATE MEAN ± SE	EXIT RATE MEAN ± SE
Untreated DuraNet®	77.70 ± 7.30	6.61 ± 3.80**	26.77 ± 5.51
Unwashed DuraNet®	5.80 ± 2.32**	52.38 ± 5.62	40.42 ± 5.22
20× washed DuraNet®	55.94 ± 6.78	28.17 ± 6.57*	36.24 ± 5.50
Unwashed PermaNet® 2.0	53.39 ± 5.89	61.74 ± 6.09	48.16 ± 5.53
20× washed PermaNet® 2.0	53.35 ± 7.01	39.87 ± 6.34	35.32 ± 5.78

Means within the same column and with different letters are significantly different using Tukey means separation test.
**Significant at $P < .001$. *Significant at $P < .05$.

Table 2. Blood-feeding inhibition and personal protection due to DuraNet® LLIN in the experimental huts, southwestern Ethiopia.

TREATMENT	PERSONAL PROTECTION		
	AN. ARABIENSIS (WILD)	*AN. PHAROENSIS (WILD)*	*AN. COUSTANI (WILD)*
	N (%)	N (%)	N (%)
Untreated DuraNet® (NC)	232 (0**)	43 (0**)	30 (0*)
Unwashed DuraNet®	24 (88.7**)	00 (100**)	2 (93.3*)
20× washed DuraNet®	176 (42.4)	27 (62.8)	5 (83.3*)
Unwashed PermaNet® 2.0 (PC)	131 (45.6)	11 (83**)	16 (46.6)
20× washed PermaNet® 2.0	192 (38.6)	6 (75)	33 (0*)

*Significant at $P < .05$. **Significant at $P < .01$.

unwashed PermaNet 2.0, hut with 20 times washed permanent 2.0) when compared to hut with untreated DuraNet®·(control). Blood feeding inhibition can be also expressed as personal protection and unwashed DuraNet® showed the highest personal protection 88.7%, 100%, and 93.3% against *An. arabiensis, An. pharoensis*, and *An. coustani*, respectively. Both PermaNet 2.0 twenty times washed and DuraNet® washed twenty times showed low to moderate personal protection (45.6% against *An. arabiensis* to 83% against *An. pharoensis*) when compared to untreated DuraNet® (Table 2).

The killing effect DuraNet®. DuraNet® (both unwashed and 20 times washed) showed low to moderate killing efficacy (65.93% to 54.03%) against vector population of *An. arabiensis*. Whereas, against *An. pharoensis*, both unwashed and washed DuraNet® showed no evidence of killing effect (0.00 to 9.0%). Similarly, PermaNet 2.0 (unwashed and washed) showed low to moderate killing effect against population of *An. arabiensis* (Table 3).

Discussion

Insecticide resistance remains major threat against the efficacy of long-lasting insecticidal nets, which are key intervention tools in controlling malaria vector. In this study laboratory bioassay was initially conducted using field collected larvae raised to adult population of *An. arabiensis* to assess the bio-efficacy of unwashed DuraNet® (candidate bed net) and untreated DuraNet® (negative control). Furthermore, field experimental study was conducted on bio-efficacy of unwashed DuraNet® and PermaNet 2.0 following WHOPES standard guidelines in order to corroborate the laboratory findings.[5]

The cone bioassay tests indicated that exposure of population of *An. arabiensis* mosquitoes from Gilgel-Gibe area, southwestern Ethiopia, to sections of DuraNet® LLIN resulted in mean knockdown and mean mortality of 93% and 78%, respectively. The mortality result is slightly lower than the range of WHO recommendation (>80%) for public health application. The knockdown effect also is slightly below the required WHOPES recommended levels of 95% and above. In contrast to the above results earlier bio efficacy tests conducted using unwashed DuraNet® in India (Sood et al[10]; Gunasekaran et al[11]) showed 100% efficacy when tested against populations of different species of *anopheles*. The slight decline in bio-efficacy of DuraNet® in this study may be due to resistance development of vector populations of *An. arabiensis* in the study area.[8,12] In Ethiopia, Previous studies indicate that populations of *An. Arabiensis* have developed resistance against insecticides used for net impregnation.[13-17]

Table 3. The overall killing effect of DuraNet® LLIN in experimental hut.

TREATMENT	OVERALL KILLING EFFECT (%)		
	AN. ARABIENSIS	*AN. PHAROENSIS*	*AN. COUSTANI*
Unwashed DuraNet®	65.93	0.0*	12.33
20×washed DuraNet®	54.03	9.0	13.4
Unwashed PermaNet® 2.0 (PC)	64.81	2.25	39.7
20× washed PermaNet® 2.0	39.81	9.0	20

Wash resistant long-lasting insecticidal nets treated with pyrethroids are viewed as an important device in the area of vector control that would lessen the difficulties related to re-treating conventional insecticide treated nets.[18] DuraNet®, the alpha-cypermethrin treated net, has similar conditions as that of Interceptor® LLIN and PermaNet® 3.0, which had been given interim but full recommendation since June 2017 by WHOPES.[19]

In current study, DuraNet® showed reduced washing resistance. This is reflected in continues decrease of both mean percent knockdown and mean percent mortality of population of *An. arabiensis* on exposure to the DuraNet® washed 0 and 20 times, respectively. This is in contrast to the study done by Sood et al[10] which showed no significant difference in percent mortality and percent knockdown of population of *An. culicifacies* between unwashed and 20 times washed DuraNet® in India. However, in the same study by Sood et al,[10] DuraNet® showed 100% knockdown but only 45% mortality after 20 washes against *Anopheles gambiae* which is also consistent with current results. Wash resistance technology is added to the LLIN's materials with the assumption of pyrethroid chemicals such as Alpha-cypermethrin should have a strong affinity to the polyester netting fibers so that even after forceful washing a thin layer of pyrethroid, practically undetectable by High Performance Liquid Chromatography yet adequately bio active to encourage knockdown and mortality should still continue bound to the fibers.

Comparison of mosquito density collected from experimental huts with (unwashed DuraNet®, 20 times washed DuraNet®, unwashed PermaNet®2.0, 20 times washed PermaNet®2.0, and untreated DuraNet®) showed no clear evidence of deterrence associated with any of the treatments however; there were fewer *An. Arabiensis* in huts with the unwashed DuraNet® compared to untreated DuraNet®. Likewise, a study carried out by Asale et al[8] using experimental huts in the study area showed the absence of significance variation in deterrence among huts containing unwashed PermaNet®2.0, untreated net and DDT sprayed hut. The possible explanation for the absence of significant variation in mosquito density among treatment arms could be accounted to low number of mosquito catches during the study period but the impact of insecticide resistance on the efficacy of the nets to be further studied in phase-III trials.

In this study significant reduction in blood feeding rate was observed in hut containing unwashed DuraNet® when compared to hut containing untreated DuraNet®. Similarly, higher mean mosquito mortality rate was recorded among huts containing treatment arms when compared to hut containing control net (untreated DuraNet®). The results for DuraNet® are new report from Jimma area thus we could not corroborate but, Vector population of *An. arabiensis* from the study area showed no significant reduction of mortality rate, exit pattern and blood feeding inhibition when tested against PermaNet 2.0.[8]

In this study, DuraNet® and PermaNet 2.0 showed low to moderate killing efficacy against vector population of *An. arabiensis*. Moreover, both DuraNet® and PermaNet 2.0 showed no evidence of killing effect against *An. pharoensis*. According to Kitau et al[20] LLINs and ITNs treated with pyrethroids were more effective at killing *An. gambiae* and *An. funestus* than *An. arabiensis*. Thus, the variations in the outcome variables of Anopheles shown above (DuraNet® and PermaNet 2.0) may be due to different susceptibility status and species differences.

Limitation

In this study, supplementary test like chemical assays were not conducted to measure the total insecticide content of the netting before and after wash resistance studies that support better interpretation of the results.

Conclusion

Cone bioassay study of DuraNet® LLINs against population of *An. arabiensis* in Jimma area showed reduced bio-efficacy when measured in terms of percent knockdown rates which were slightly below the World Health Organization recommendation. The evaluation of the efficacy of DuraNet® and PermaNet 2.0 LLINs using experimental huts showed that both vector control tools showed low to moderate efficacy against pyrethroid resistant population of *An. arabiensis* from Ethiopia. While the real impact of the observed insecticide resistance against key vector control tool (LLINs) to be further studied under phase-III trials, the need for new alternative vector control tools remains a matter of urgency.

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Author's contributions

BG, AA, and DY conceived, designed and participated in conducting the study. WB, WZ, and DY were involved in data management, analysis, interpretation, and drafting the document. All authors read and confirmed the final document.

Ethical considerations

The study proposal was reviewed and approved by the institutional review board (IRB) of the College of Health Sciences, Jimma University (Reference Number RPGC/344/06).

ORCID iD

Brhane Gebremariam https://orcid.org/0000-0002-0824-3221

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