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Bio-efficacy of DuraNet® long-lasting insecticidal nets against wild populations of *Anopheles arabiensis* in experimental huts

Aneth M. Mahande¹, Shandala Msangi², Lucile J. Lyaruu² and Eliningaya J. Kweka^{2,3*} 

Abstract

Background: Mosquitoes have developed resistance to multiple classes of insecticides for malaria vector control. A new generation of long-lasting insecticidal bed nets (LLINs) has been developed with increased efficacy against these resistant mosquitoes. The present study therefore evaluated the efficacy of the pyrethroid-based LLINs, DuraNet versus PermaNet 3.0, in an Eastern Africa hut design in Magugu in northern Tanzania where mosquitoes' population higher proportion (69.3%) has been identified as *Anopheles gambiae* s.l.

Methods: Standard World Health Organization bioefficacy evaluations were conducted in both laboratory and experimental huts. Experimental hut evaluations were conducted in an area with high populations of *Anopheles arabiensis*. All nets used were subjected to laboratory cone bioassays and then to experimental hut trials. Mosquito mortality, blood-feeding inhibition, and personal protection rate were compared between untreated nets, unwashed LN, and LN that were washed 20 times.

Results: Standard WHO laboratory bioefficacy evaluations of DuraNet and PermaNet® 3.0 which were untreated, washed, or 20 times washed showed optimal knockdown and mortality for both net types against a susceptible strain of *An. arabiensis*. In standard experimental hut evaluations, the blood feeding inhibition for PermaNet® 3.0 unwashed and washed was 82.4% (76.3–88.6%) to 91.5% (84.1–98.8%) while for DuraNet was 98.3% (97.0–99.5%) to 96.0% (94.1–88.2%) respectively. The DuraNet LLINs showed a significantly higher killing effect than the other treatment of 90.0% (86.1–94.2%) and 94.0% (90.2–97.9%) for unwashed and washed nets respectively. No significant difference in deterrence or induced exophily was detected between the treatment arms. There were no adverse effects reported among sleepers in the experimental huts.

Conclusion: The findings of this study indicate that the pyrethroid-based net DuraNet LLINs attained required efficacy when evaluated against wild population of *An. arabiensis* from Northern Tanzania. This adds value to the existing vector control tool box which gives community wider choice for vector control.

Keywords: Behavior, Long-lasting insecticidal nets, Mosquito, Northern Tanzania

Introduction

Malaria control efforts with long-lasting insecticidal nets (LLINs) and conventionally treated nets took a new phase of implementation since 2000 with a great impact in decline of disease incidences and mortality [1, 2]. Mortality

cases have declined to 438,000 cases per year in 2015 from formally 2.7 million in 2000 worldwide [1, 2]. Also, this decline in malaria has been attributed by other several malaria control measures used such as artemisinin combined therapy (ACT), Intermittent Preventive Treatment (IPTP) for pregnant women and vector control including long-lasting insecticides nets (LLINs), and indoor residual spraying of insecticide (IRS) [1, 2].

A long-lasting insecticidal net (LLIN) is “a factory-treated mosquito net that is expected to retain its biological activity after a minimum number of standard

* Correspondence: elingaya.kweka@tpri.go.tz

²Division of Livestock and Human Diseases Vector Control, Mosquito Section, Tropical Pesticides Research Institute, P.O. Box 3024, Arusha, Tanzania

³Department of Medical Parasitology and Entomology, School of Medicine, Catholic University of Health and Allied Sciences, P.O. Box 1464, Mwanza, Tanzania

Full list of author information is available at the end of the article



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washes and a minimum period of use under field conditions" [3]. LLIN remains the key for malaria vector control in rural and urban settings in Tanzania and is more effective in malaria prevention when there is a full coverage of all people at risk [4–8]. In addition, LLINs have been reported to be more cost-effective than IRS programs with the average IRS cost/person/year protection of \$ 2.62 as compared to \$ 1.39 for LN in 3 years' time [9, 10]. LLINs have been found to reduce malaria morbidity and mortality at the community level in malaria-endemic countries worldwide [11–14]. But despite the wide use of LLINs in Tanzania, where over 18 million LLINs have been distributed by the Ministry of Health and partners since 2004, malaria remains the major health problem in Tanzania [15, 16]. The wide coverage of LLINs is thought to have contributed to the development of insecticide resistance in the local malaria vectors population due to the insecticide selection pressure encountered by vectors [9, 17]. Many vector species have developed resistance to one or more classes of insecticides commonly used in Tanzania [18–20]. Development of vector resistance may be caused by many factors including overuse of insecticides by ITNs, IRS, and household pest control or by agricultural pesticides use [19, 21–24]. The WHO global insecticide resistance database has reported pyrethroid resistance detection in 78% of the countries that have reported monitoring data, and 80% of these countries have reported resistance in two or more classes of insecticides [25].

The effect of insecticide resistance has been reported to reduce the LLIN effectiveness and increase malaria risks in areas with high insecticide resistance for mixed classes of insecticide [26]. In Northwest of Tanzania, insecticide resistance of Bendiocarb (Carbamates) against *Anopheles gambiae* s.l. was reported and found a sharp reduction in mosquito mortality from 84% in December 2011 to 31% in December 2012 [18, 27]. Similarly, pyrethroids resistance against *An. arabiensis* and *An. gambiae* s.s. has been reported in different parts of Tanzania, such as Muleba, Mabogini, Arumeru, and Muheza [18, 26, 27]. Efforts are underway to develop new vector control products with different modes of action against mosquito that will effectively control resistant strains to currently used classes of insecticide.

In this study, the pyrethroid (alphacypermethrin, 5.8 g of active ingredients/kg \pm 25%)-based LLINs DuraNet[®] was evaluated in experimental huts in comparison to pyrethroid-synergist PermaNet 3.0 and an untreated net. The work was done following the standard WHO procedures [28] to determine comparative efficacy against a free-flying wild resistant population of *An. arabiensis* as per standard measured outcomes from experimental hut trials. The LLIN efficacy was measured in terms of

blood-feeding inhibition, deterrence, induced exophily, and mortality.

Materials and methods

Study sites

This study was conducted during July–December 2015, in malaria epidemic-prone site, Magugu ward, Babati district in Manyara region [29]. The area is located in the Great Rift Valley of northern Tanzania (3° 53' S, 35° 42' E) which is 150 km from Arusha Town. The main economic activities include livestock keeping and crop cultivation mainly maize and rice farms. This area has seasonal malaria prevalence and sometimes epidemics caused mainly by *Plasmodium falciparum* transmitted by *Anopheles gambiae* s.l. mosquitoes during the rainy season and transmitted by *Anopheles funestus* mosquitoes during the dry season [30]. Malaria vector susceptibility status to pyrethroid insecticides was found to have mortality ranging from 98 to 99%, during susceptibility tests conducted in 2011 [31].

Hut design

The experimental huts used were of East Africa design, which were constructed with burnt bricks and roved with corrugated iron sheets. These included a double verandah trap resembling that described by Smith and others [32] with brick walls plastered with mud on the inside, a wooden ceiling lined with hessian sackcloth, open eaves, and window traps and verandah traps on each side. The huts were built on concrete plinths and surrounded by a water-filled moat to deter entry of scavenging ants. Mosquitoes fly upward to enter through the eaves and then fly downwards to exit; this precludes or greatly limits exodus through the aperture enabling the majority of entering mosquitoes to be accounted for. Two opposite sides of the huts had closed verandas, screened to capture mosquitoes that left via the eaves. The other two verandas were left open so mosquitoes could enter through the eaves.

Each night's collection inside the two screened verandah traps was multiplied by two and added to the room and window trap collections. The multiplication is to adjust for the unrecorded escapes through the two verandas which were left unscreened to allow routes for entry of wild mosquitoes via the 4-cm gaps under the eaves [32].

Treatment arms

Washed and unwashed LLINs were evaluated using experimental huts for their effects on free-flying wild mosquitoes and for their ability to deter entry, repel or drive mosquitoes out of houses, induce mortality, and inhibit blood-feeding. PermaNet 3.0 was used as a positive control, and untreated polyester net was used as a negative

control. The following treatment arms were tested using six nets per arm for the study:

1. Untreated polystyrene net
2. PermaNet® 3.0 unwashed
3. PermaNet® 3.0 washed 20 times
4. DuraNet® unwashed
5. DuraNet® washed 20 times

Before testing in the experimental huts, the nets (including the untreated control) were deliberately holed. Six holes were made in each net, two holes in each of the long side, and one hole at each end. Each hole measured 4 cm × 4 cm. Each net was individually coded with two numbers: treatment number and net number.

Net washing procedure

The nets were washed according to a protocol adapted from the standard WHO washing procedure used in phase II, over a 30-day period interval (i.e., by applying the regeneration time value that was established under phase I of 24 h) [33]. Nets were washed in aluminum bowls containing 10 l of well water having a maximum hardness of 5 dh and containing 2 g/l of soap ("Savon de Marseille") using manual agitation. For each wash, nets were agitated for 3 min, left to soak for 4 min, and re-agitated for 3 min for a total of 6-min agitation during a 10-min washing/soaking period. Agitation was done by stirring the net with a pole at 20 rotations per minute. Rinsing was done twice using clean water (10 l per rinsing, i.e., 20 per net). Nets were dried horizontally in the shade then stored at ambient temperature between washes.

Cone bioassays

Six nets (one net per treatment arm, including the untreated control) of each treatment were bio-assayed the day before the first wash. Bioassays were done again for a second time when all washings were completed and for a third time at the end of the field experiment with nets used in huts. Bioassays were conducted according to the WHO procedures for cone tests [28]. For each net, five cones were placed on the five sections of the net (roof and four sides). Five (5) non-blood fed females of *An. gambiae* susceptible Kisumu strain from TPRI insectaries were introduced per cone and exposed for 3 min to the net surfaces. Bioassays were replicated five times for a total of 25 mosquitoes tested per position on each net. Knockdown was recorded 60 min after exposure, and mortality was scored 24 h after exposure. During the observation period, mosquitoes were provided with 10% sugar solution.

Experimental hut study design

One net was hung in each hut, with one net per treatment type deployed concurrently in the five test huts. Each week, the treatment arms were rotated among the huts according to a Latin square scheme. Six nets were used per treatment arm, and each of the six nets was tested one night during the week. At the end of the week, the huts were carefully cleaned and aired for 1 day to remove potential contamination. The treatment was then rotated to a different hut. The trial continued for 6 weeks to ensure complete rotation through the huts. In this study, 6 weeks, i.e., one complete Latin square, was long enough to obtain sufficient numbers of mosquitoes for adequate statistical analysis.

In each of the hut, one sleeper occupied a bed and slept inside the tested net during the night as from 18:00 to 06:00 in the morning. Each morning, between 06:00 and 08:00 am, two experienced mosquito technicians collected all dead mosquitoes on floors, those resting inside, verandah traps, and window traps of each hut using hand aspirators for estimating deterrence and exophily. The collected mosquitoes were put separately in paper cups, sorted into their live status and abdominal conditions and then identified to species level using identification key [34, 35]. The killing effect was observed for all mosquitoes collected from each treatment arms.

Perceived side effects

The sleepers in the huts were interviewed at the end of the experiment about perceived adverse or beneficial side effects of the LLINs and of the untreated nets when implemented in accordance with standard procedures [28]. The hut sleepers were recruited from the study area and were requested to notice any noticeable adverse side effects in the course of the whole study. A short questionnaire was prepared and used to seek information from the hut sleepers regarding possible itching, dizziness, or running nose. Hut sleepers were given questionnaire to respond every morning slept in each net brand. Each hut had one adult sleeper as recommended in huts trial for bed nets in WHO protocol, and they were rotated within huts to avoid the biasness effect on mosquito attraction [33].

Statistical analysis

The proportion of mosquitoes that exited early, the proportion that were killed within the hut, and the proportion that successfully blood fed were compared by species and then analyzed by logistic regression or generalized linear mixed models (using Statistical Package for Social Scientists (SPSS version 25 (SPSS Inc., Chicago, IL)), which provide a framework for regression modeling of non-normal outcome data (such as mosquito mortality) while naturally adjusting for clustering effects. The

clustering of observations made in one hut night and any variation between huts and sleepers was controlled for by adjusting the models.

Outcome measured was calculated as per standard procedures [28]. The primary analysis was a test of the non-inferiority of the DuraNet washed 20 times relative to PermaNet® 3.0 unwashed and that washed 20 times. According to WHOPES, a candidate LLINs is considered to meet the phase II efficacy criteria if, after 20 washes, it performs as well as or better than the reference LLINs when washed 20 times in terms of blood feeding inhibition and mortality.

The mean numbers of unfed and fed mosquitoes from indoor, verandah, and window traps from treated and untreated huts were compared, and the induced exophily was calculated using the Abbott formula [36] $[(N_c - N_t)/$

$N_c] \times 100\%$, where N_t is the number of mosquitoes from verandah and window traps of treated hut while N_c is the number of mosquitoes from verandah and window traps of untreated hut. Similarly, feeding inhibition was estimated using the formula $[(F_c - F_t)/F_c] \times 100\%$, where F_c is the number of mosquitoes found fed in untreated control hut while F_t is a number of mosquitoes found fed in treated hut.

Results

Species composition

A total of 1020 mosquitoes were collected in five experimental huts. Of all the mosquitoes collected, *An. arabiensis* mosquitoes were the most predominant (69.3%) species. Other mosquito species collected were *Culex*

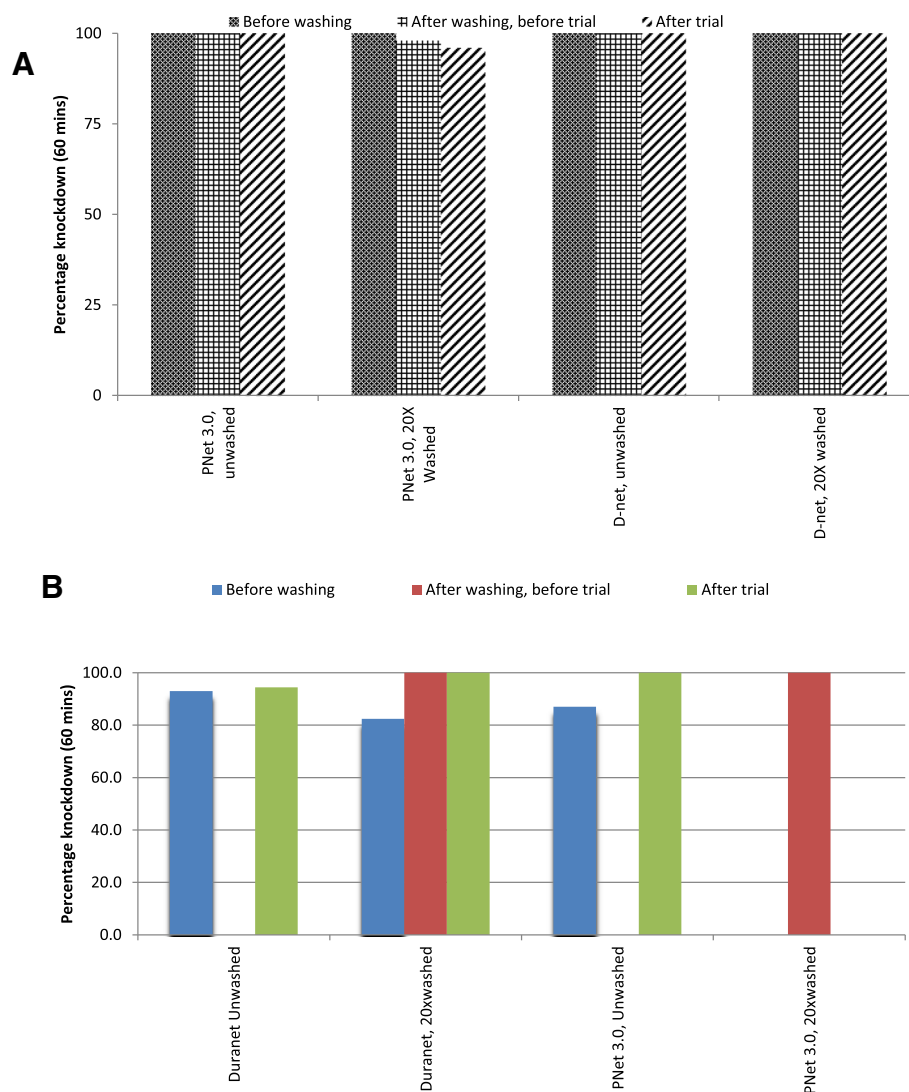


Fig. 1 Bioefficacy (percentage knockdown) in WHO cone bioassays of sub-samples from LN against **a** a susceptible laboratory strain and **b** resistant field strain of *An. arabiensis*

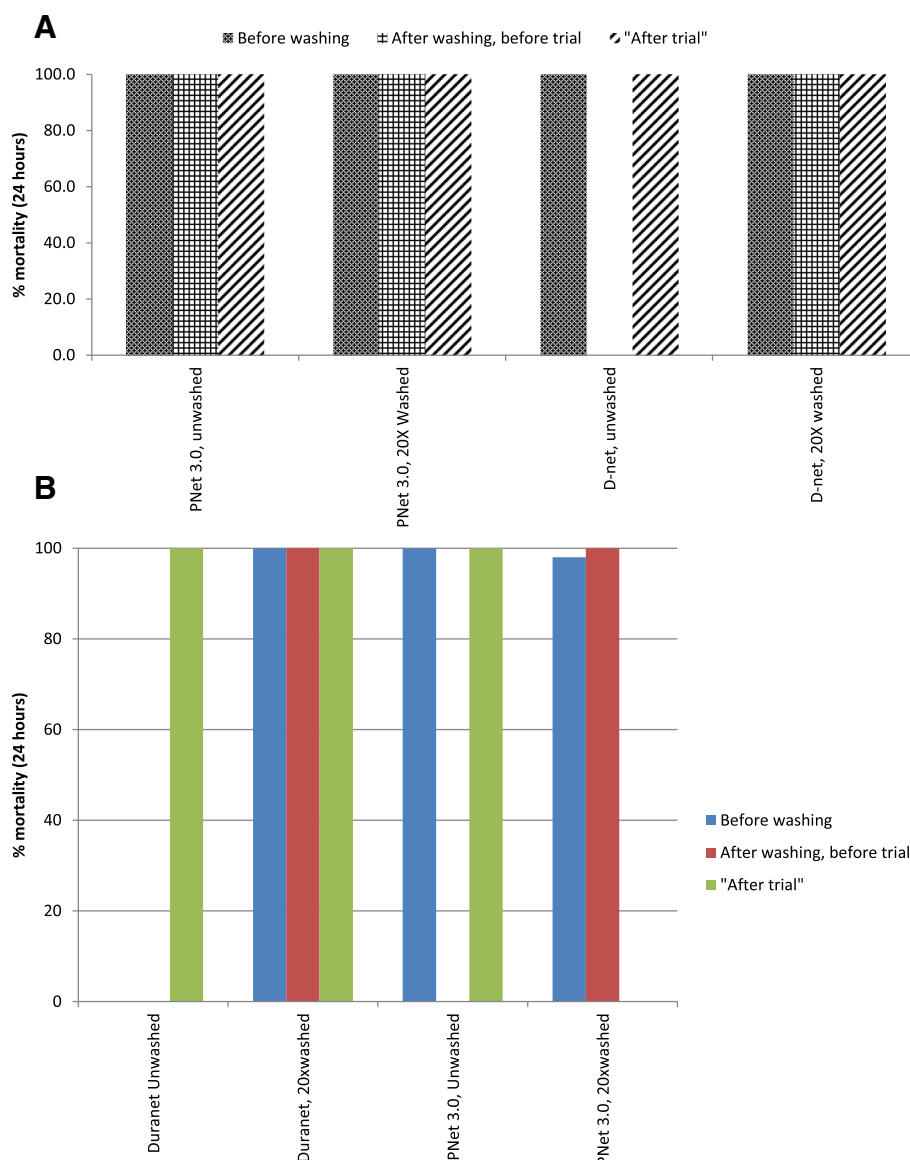


Fig. 2 Bioefficacy (percentage mortality) in WHO cone bioassays of sub-samples from LN against **a** a susceptible laboratory strain and **b** a resistant field strain of *An. arabiensis*

quinquefasciatus and *Mansonia spp.* which made 29.6% of the population.

Bioefficacy of nets in laboratory

Efficacy testing of LLIN sub-samples via cone bioassays with a susceptible laboratory strain of *An. gambiae s.s.* (Kisumu) indicated that DuraNet and PermaNet® 3.0 had optimal bioefficacy at all three time points, including before washing, after washing and before the trial, and after the trial (Fig. 1a, b and 2a, b). All nets therefore surpassed WHO bioefficacy cutoffs of $\geq 80\%$ mortality or $\geq 95\%$ knockdown.

Bioefficacy of nets in experimental hut trial

In the experimental huts trial, there was no significant difference in the number of mosquitoes entering each of the treatment arms therefore indicating that there was no difference in deterrence among treatments except for unwashed DuraNet LN. Induced exophily ranged from 86.9% (83.6–90.1%) to 89.1% (87.2–91.9%) for both DuraNet® washed and unwashed. However, there was an apparent significant difference in induced exophily when compared PermaNet 3.0 and DuraNet either washed or unwashed (Table 1).

Blood feeding was 45.7% (41.1–51.1%) higher for the untreated control than for the treatment arms, and the

Table 1 The experimental huts response for *Anopheles arabiensis* collected during the 6 weeks trial for the behavioral analysis of DuraNet and PermaNet3.0 unwashed and washed

Parameter	Summary data	Untreated net (u)	DuraNet, unwashed	DuraNet, washed 20 times	PermaNet 3.0, unwashed	PermaNet 3.0, washed 20 times
Deterrence	Total number of females caught	385	84	257	241	169
	Females caught/night	15.4	3.4	10.3	9.6	6.8
	Deterrence (%)	0	78.18	33.25	37.40	56.10
Exophily	Number females in exit traps and verandah	118	73	229	188	131
	Exophily (%)	30.6	86.9	89.1	78.0	77.5
	95% confidence limits	26.6–35.3 ^a	83.6–90.1 ^b	87.2–91.9 ^b	76.3–80.2 ^c	74.2–81.1 ^c
Blood feeding	Number of blood-fed females (B)	176	3	7	31	15
	Percentage of blood-fed	45.7	3.6	2.7	12.9	8.9
	95% confidence limits	41.1–51.1 ^a	1.9–4.9 ^b	1.3–4.0 ^b	10.3–15.3 ^c	5.9–12.3 ^c
	Blood feeding inhibition (%)	0	98.3	96.0	82.4	91.5
Mortality	Number dead females morning (immediate mortality)	0	4	8	6	5
	Number dead females after 24 h (delayed mortality)	1	6	3	4	5
	Total number dead females (K)	1	10	11	10	10
	Overall mortality (%)	0.3	11.9	4.3	4.1	5.9
	95% confidence limits	0.11–0.43 ^a	8.8–14.8 ^b	2.5–6.0 ^c	2.4–5.1 ^c	2.8–8.7 ^c
	Mortality corrected for control (%)		11.7	4.0	3.9	5.7
Summary	Personal protection (%)		98.3	96.0	82.4	91.5
	Killing effect (%)		90.0	100	90.0	90.0

Those 95% CI values in the same row with different superscript letters differ significantly

calculated personal protective effect was 82.4% (76.3–88.6%) to 91.5% (84.1–98.8%) for both PermaNet® 3.0 unwashed and washed while for DuraNet ranged between 96.0% (94.1–88.2%) and 98.3% (97.0–99.5%) for 20 times washed and unwashed nets respectively (Table 1). Mortality corrected for controls was highest for DuraNet unwashed and least in PermaNet 3.0 unwashed (4.3% (2.4–6.1%) to 11.9% (8.8–15.3%)). The overall killing effect of nets varied between unwashed and washed 20 times DuraNet and PermaNet3.0 and was significantly ($P = 0.032$) highest for DuraNet washed 20 times (Table 1).

Perceived side effects

There were no reported negative side effects such as itching, dizziness, or nose running among the five hut sleepers who participated in the interview for the all experimental days.

Discussion

The current study has found the wild population been composed of *An. arabiensis* by 69.3% and 29.6% was made up by *Cx. quinquefasciatus* and *Mansonia spp.* This species composition is similar to what has been found by previous studies conducted in Magugu formerly Ubugwe land [30, 37, 38].

The findings of this study have shown that the lowest deterrence was observed in DuraNet washed 20 times and PermaNet 3.0 unwashed while the highest was found in DuraNet unwashed (78%). The low deterrence rate observed in this current study was similar to what was observed in other study based on PermaNet 3.0 nets evaluation [19, 39]. The findings of this study have revealed that the deterrence of DuraNet evaluated is comparable to standard nets of PermaNet 3.0 when tested against wild free-flying mosquitoes [19, 39]. In the study conducted in India, DuraNet had deterrence similar to other LLINs such as Olyset, Netprotect, PermaNet, and interceptor [40]. This study results have revealed that the DuraNet can be widely use as physicals and chemical barriers against malaria vectors. This is highly concurring with study conducted in Solomon Island which has shown that community acceptance of DuraNet usage is 68.7% [41]; hence, community protection with deterrence efficacy shown can have impact on malaria decline.

The blood feeding inhibition ranged between 82.4 to 98.3% for treated nets compared to control. In previous studies, it was observed that unwashed PermaNet 3.0 nets had feeding inhibition efficiency of 91% while after 20 times washes had 63% feeding inhibition [39]. The evaluated nets (DuraNet) had blood feeding inhibition efficiency of 98.3% before being washed and dropped to

96% after 20 times washes but was not as drastic as that of PermaNet 3.0 found in Muheza [39]. The study conducted in India has shown DuraNet to have similar feeding inhibition as other long-lasting nets such as Olyset, Netprotect, PermaNet, and interceptor [40]. The increased blood feeding inhibition has shown to have potential of adding value to the currently existing tools against malaria vectors.

In the assessment of the knockdown effect in laboratory before wash, after 20 times washes, and after experimental hut trial, the knockdown shown was above accepted WHO cutoff point (above 80%) [42]. There was reduced knockdown in all stages of cone bioassays using wild resistant population of *An.arabiensis*. This trend seemed similar to previous study conducted in areas with resistant wild population with reported resistant population in pyrethroids, organophosphates, and carbamates [31]. In this study, the mortality effect of the mosquitoes collected in experimental huts with unwashed net was 11.7% while with 20 times washed net was 3.9%. This was contrary to what was found in trial conducted in India which had mortality effect of 74.5% [40]. The lower mortality effect might have been attributed with increased insecticides resistance in Tanzania vector population for both washed and unwashed DuraNet [18, 26, 27, 31]. This is also similar to numerous experimental hut studies evaluated different net brands in Tanzania [9, 18, 26, 27, 39], and hence, the most important outcomes from such trials were the mortality parameters which are summarized by personal protection and killing effect outcomes [19, 43, 44]. The overall killing effect was found to be significantly higher for DuraNet washed 20 times than unwashed; this might be attributed with the high susceptibility status to variety of the insecticides in this study area [31].

The current findings have shown to have exophily rate of 86.9% when unwashed and 89.1% after 20 times wash. The recorded exit rate in this study was found to be higher than that recorded in previous in India [40]. This might be attributed with the variance in species composition between the study sites involved and variations in insecticides tolerance.

During the trial, there was no any adverse effect reported among experimental hut sleepers and mosquito sampling individuals in the huts where DuraNet was used. Similar response was found with the study conducted in Asia with DuraNet acceptability rate found to be 69.8% [41].

Conclusion

Based on these findings, DuraNet has shown protection efficiency against wild population with mortality high effect in huts. This has added an additional tool in malaria vector control toolbox and selection option.

Abbreviations

ACT: Artemisinin combined therapy; IPT: Intermittent preventive treatment; IRS: Indoor residual spray; LLINs: Long-lasting insecticidal nets

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Availability of data and materials

All data associated with this manuscript conclusion have been presented in this paper.

Authors' contributions

EJK and AM conceived and designed the study. SM, LL, and AM organized the data collection in experimental huts. SM and EJK coordinated the data analysis and interpretations. EJK, SM, LL, and AM revised the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The hut sleepers who slept in each individual hut under net per night were recruited among the inhabitants of the villages close to the site. The sleepers have been working in huts for the past 7 years and were offered chemoprophylaxis during and for 1 month after the experimental hut trial. They were also informed on the objective of this study and signed an informed consent. This study had experimental permit No.1714 issued by Registrar of Pesticides in Tanzania.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

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Author details

¹Division of Livestock and Human Diseases Vector Control, Tropical Pesticides Research Institute, Mabogini Field Station, Moshi, Tanzania. ²Division of Livestock and Human Diseases Vector Control, Mosquito Section, Tropical Pesticides Research Institute, P.O. Box 3024, Arusha, Tanzania. ³Department of Medical Parasitology and Entomology, School of Medicine, Catholic University of Health and Allied Sciences, P.O. Box 1464, Mwanza, Tanzania.

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