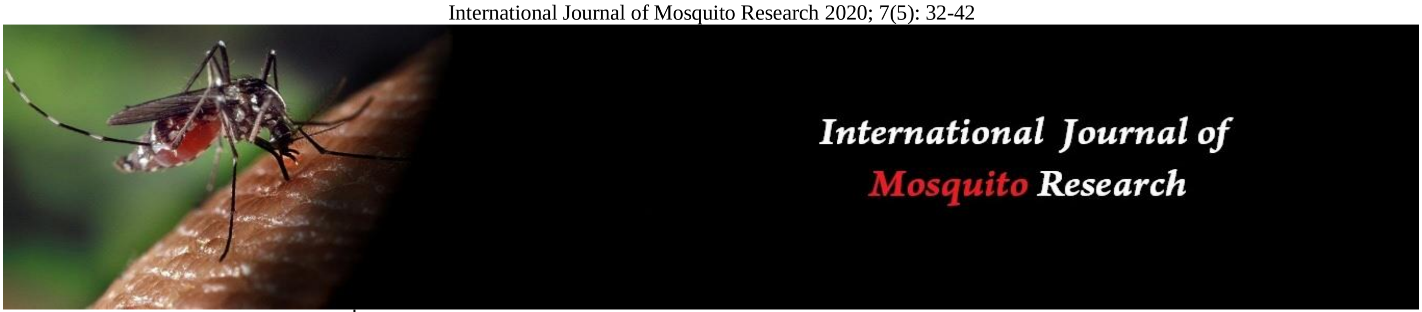

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Chemical barrier and survivorship: Comparative study of two brands of polyester nets and one brand of polyethylene nets in different conditions of used in Benin

Idelphonse B Ahogni, Rock Y Aïkpon, Jean-Fortuné Dagnon, Roseric Azondekon, Bruno Akinro, Germain G Padonou and Martin C Akogbeto

Abstract
Background: The long-lasting insecticide-treated mosquito net (LLIN) has established itself in a few years as a privileged tool in the fight against malaria. To date, several brands have received either a provisional authorization from World Health Organization Pesticide Evaluation Scheme. However, the chemical efficacy and survival of these brands differ depending on the conditions of use from one country to another. What are the factors that impact this efficiency and sustainability?.
Methods: LLIN follow-ups (every 6 months) were carried out in three malaria-endemic district of Benin: Toffo and Tori-Bossito and Ouesse for 2 years. This cohort study involved 900 LINs, 300 per brand (DawaPlus®2.0, DuraNet® and PermaNet®2.0) and aims to assess the bio-effectiveness and survival of LLINs. The colorimetric test allowed us to quantify the insecticide lost over time.
Results: Overall, 283 out of 900 LLINs (enrolled at the start of the study) were found and assessed after 24 months of use. The reasons for the losses were: LLINs destroyed (27.3%), LLINs moved (13.9%) and LLINs used for other purposes (10.1%). A significant decrease in the survivorship of DawaPlus®2.0, PermaNet®2.0 and DuraNet® LLINs was observed after 2 years, 51%, 53.3% and 34% respectively. The efficacy of the three LLINs also didn't meet WHO requirements (80% mortality for WHO cone tests).
Conclusions: The decrease in the survival of LLINs due to the increasing accidental destruction during this study highlights the need to develop and implement new strategies such as behavior change awareness to manage this vital means of fighting malaria.

Keywords: Malaria, efficacy, survivorship, LLIN

Introduction
Benin is located in West Africa in the tropical zone between the equator and the Tropic of Cancer (between the parallels 6 ° 30 'and 12 ° 30' of Latitude North and the meridians 1° and 30° 40 'of East longitude). It has a population of around 10 million. It is limited to the North by the Niger River which separates it from the Republic of Niger; to the northwest by Burkina Faso, to the west by Togo, to the east by Nigeria and to the south by the Atlantic Ocean [1]. The geography of Benin translates to the south by an equatorial climate with high humidity. Alternating dry seasons (November to March and mid-July to mid-September) and rainy seasons (April to mid-July and mid-September to October). In the center and north, a tropical climate. A dry season from November to April and a rainy season from June to September. This results in an additional level of complexity, when certain areas are difficult to access, and the epidemiology of malaria and the vector varies considerably. In this context, the prevention of malaria, and therefore of the vector, relies on several means including vector control [2]. Vector control is an essential element in the prevention of malaria. It targets mosquitoes capable of transmitting the parasites responsible for malaria. Vector control has been shown to reduce or interrupt the transmission of malaria when coverage is wide enough. The two basic widely applicable vector control measures are long-lasting insecticide-treated mosquito nets (LLINs) and indoor residual insecticide spraying [2].
LLIN is a very effective and cost-effective intervention, which has grown considerably in sub-Saharan Africa in recent years [3].

Given that many countries have now reached high LLIN coverage and are approaching the WHO goal of universal coverage of one mosquito net for two people at risk, the question of how these successes can be sustained becomes a subject of debate [4]. Benin, compared to this objective is not late because 80% of households have a LLIN for two people [5]. However, the importance of the durability of nets and the "average useful life" of a net is increasingly recognized as critical factors in the design of purchased malaria control programs, as these determine what type of nets should be purchased and how often should the nets be replaced [6, 7, 8]. This is reflected in the WHO guidelines for monitoring LLINs in the field, which recommends that a regularly monitoring of the nets [9]. Several reports, including an assessment of the net sustainability of insecticide-treated nets three years later in 2011 [8, 10] and 2017 [11], funded by the PMI, indicate a rapid decline in net survival in Benin. This highlights the need to establish LLIN monitoring activities similar to those carried out following the massive distribution of previous campaigns, to justify, quantify and prioritize future replacement needs. This includes analysis of the bioassay, as WHO guidelines state that LLINs must have adequate insecticidal activity after 20 standard washes and a minimum of 3 years of regular field use [12]. Monitoring the sustainability of the nets focuses on three indicators: the survival of the nets, which is the percentage of nets still present and used in the household to which they were distributed; physical integrity, a quantification of the size and number of holes in the MILDs; and bio-efficacy, a measure of the insecticidal effect of MILDs. Following the 2014 mass campaign, which took place in October, a net sustainability study was carried out in three endemic regions that received the LDA DawaPlus®2.0, DuraNet® and PermaNet®2.0. The objective of this manuscript is to highlight after a two-year evaluation (follow-up at 6, 12, 18 and 24 months) the main factors responsible for the loss of survival and the bio-efficacy of the MILDs under study.

Materials and Methods

Study sites

This study was implemented in three randomly selected districts which received mosquito nets during the massive distribution campaign carried out in October 2014 (DawaPlus®2.0 was distributed in Tori-Bossito, PermaNet®2.0 in Toffo and Yorkool® in Ouesse:) (Figure 1). In each district, an urban area and a rural area were chosen at random. A representative sample of 900 nets (300 per district) was identified and selected from households. The marking and labeling of these mosquito nets was then carried out using a unique identifier (code). Their presence and physical condition in the household were assessed in all the series of surveys, as well as the characteristics of the households, their use, maintenance and behavior. At each follow-up stage (6, 12, 18 and 24 months), sub-samples of field nets were selected to test the effectiveness of the insecticides.

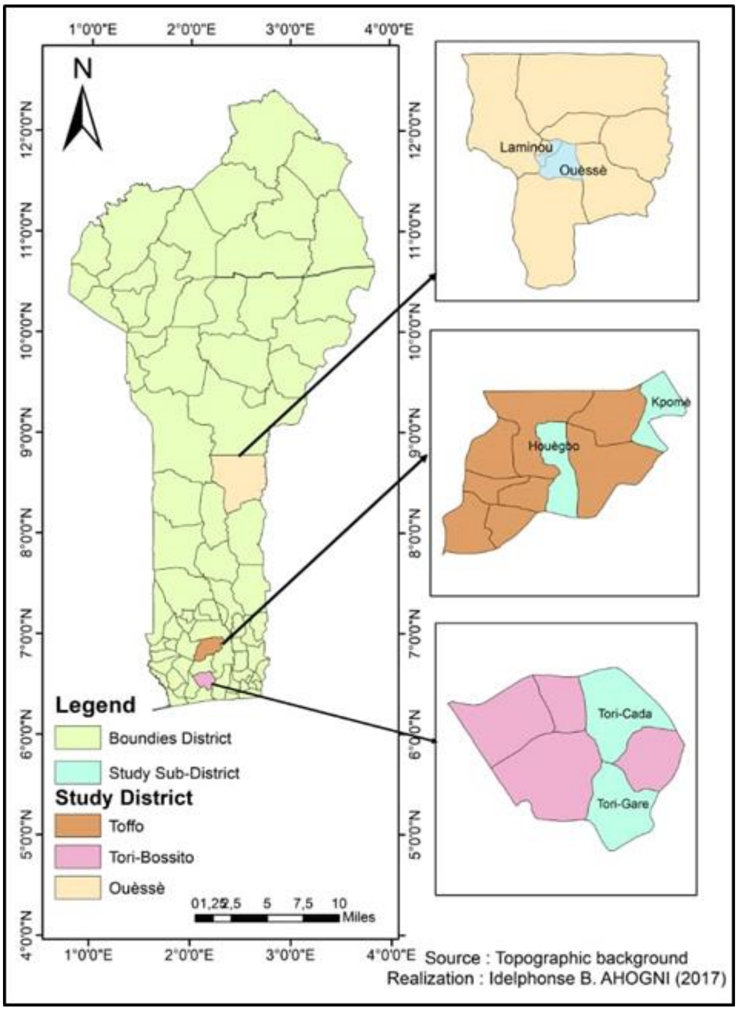


Fig 1: Map of Benin showing the sites

The selection of the three communes of the study, although being made at random, took into account their socio-ecological and epidemiological profiles of malaria. These municipalities represent areas with different malaria transmission potentials with very different climatic or socio-demographic characteristics. The household was the sampling unit and in each household, only one mosquito net was selected from those found by ensuring that it was one of the brands distributed during the mass campaign.

Household Selection
Three teams of three technicians and one village health worker visited and selected the households where the DawaPlus®2.0, PermaNet®2.0 et DuraNet® nets were used in each zone. Three hundred (300) LLINs were selected, marked and enrolled in the study, i.e. 150 LLINs from the central (urban) zone and 150 LLINs from the peripheral (rural) zone. A subsample of 30 MIILD (varying at each pass) representing 10% of the total sample was randomly selected to measure insecticidal activity at each monitoring period (every 6 months) in both areas.

Reference Survey
A questionnaire recommended by the WHO [13] and adapted to the needs of our study, was used to identify the basic characteristics of the households, the sleeping patterns, the level of education, the presence or the absence of the LLINs, the reasons for absence, the number and size of holes. The tools used for the data collection were the Samsung Galaxy Tab 10.1 tablets. An electronic survey form was created on these tablets using the Open Data Kit (ODK) software [7]. This technique allowed the recording and the instantaneous measurement of data. The interviews were conducted at 6, 12 and 18 months.

Monitoring the LLINs
The net monitoring was conducted by visiting door-to-door households to record the physical presence/absence and integrity of the net tissue in order to estimate the periodic attrition rate in addition to information on the perceptions and practices of mosquitos nets. Through these home visits, presence or absence of mosquito nets were confirmed and recorded. When a net was no longer present in a household, homeowners were interviewed to determine the reason for its loss or absence.

LLIN fabric integrity
At each net examination, we looked for holes in the nets and, where applicable, were classified them into four distinct groups according to WHO guidelines [14]. The different size categories used were described to the interviewers as follows:
Size 1: Hole smaller than one inch (0.5-2.0cm)
Size 2: Hole larger than the thumb but smaller than the fist (2-10 cm)
Size 3: Hole larger than the fist but smaller than the head (10-25 cm)
Size 4: Hole larger than the head (> 25cm).

Following WHO recommendations, the survival rate of LLINs, the determination of their physical integrity, the proportional Hole Index (pHI) were calculated using formula 1, allowing holes to be weighted according to their size.
The proportional hole index (pHI) for each LLIN [15] was determined as follows:

(1) pHI = 1 × # S1 + 23 × # S2 + 196 × # S3 + 576 × # S4 (# S = number of holes in the size).

Once the pHI value was obtained, the nets were classified as follows [16]:

$0 \leq pHI \leq 64$: Good LLIN
$65 \leq pHI \leq 642$: Damaged LLIN
$pHI \geq 643$: Torn LLIN

LLIN lost
LLIN attrition represents the proportion of LLINs initially enrolled in the study and which have been lost due to different reasons. Attrition can be associated with different reasons. These reasons include: destroyed, so torn and worn; stolen, given, moved; and used for other purposes. The LLINs that were good or damaged are all usable while those that are torn or not. The equations used to calculate the loss rates associated with the different reasons are as follows:

$$\text{-Attrition-1: (physical damage)} = \frac{\text{Total number of LLINs no longer in use because of physical damage}}{\text{(Total number of distributed LLINs)}} \times 100$$

$$\text{-Attrition-2: (displacement)} = \frac{\text{Total number of stolen,given,or sold LLINs}}{\text{(Totl number of distributed of LLINs)}} \times 100$$

$$\text{Attrition-3: (other attrition reasons)} = \frac{\text{Total number of LLINs no longer in use due to other reasons}}{\text{(Total number of distributed of LLINs)}} \times 100$$

Survivorship
Households unavailable during an assessment visit were censored using the non-parametric Kaplan-Meier survival method [13, 15]. The survival of the nets over time was compared to NetCalc's 2 and 3-year life expectancy models (http://www.networksmalaria.org) recommended by Roll Back Malaria. Overall, the survival rate of LLINs was evaluated by the following formula:

$$\% \text{ of Survival} = \frac{\text{Number of LLINs in serviceable condition}}{\textit{Total number of distributed LLINs} - \textit{total number of displaced or absent LLINs}} \; x\, 100$$

In order to compare the physical survival measured at different times, the result of the median net survival was estimated as the time in years until 50% of the LLINs originally distributed were no longer usable. Two approaches were used to estimate the median survival. At each point in time, the proportion in working condition was plotted against hypothetical survival curves with defined median survival [17]. The median survival was taken as the relative position of the data point on a line horizontal between the two adjacent median survival curves. After the final survey, the median net survival was calculated from the last two time points provided that both are less than 85% (when the hypothetical curves are linear), using the following formula:

$$\text{tm} = t1 + \frac{(t2 - t1) * (p1 - 50)}{(p1 - p2)}$$

where tm is the median survival time, t1 and t2 the first and second time points in years and p1 and p2 the proportion surviving the first and second time points respectively as a percentage. The confidence intervals for these estimates were calculated by projecting the 95% CI from the survival estimates in the same manner as described above.

Bioefficacy assessment

Cone test

The cone tests were performed in accordance with the WHO protocol [18]. These tests were carried out on all the five sides of each of the nets. Two standard cones were attached with a plastic plate to each of the five screen fragments. Five females of *Anopheles gambiae Kisumu* unthroated and aged 2-5 days were introduced for 3 minutes in each cone and observed for 24 hours in cups supplied with honey juice. After the 3 minutes of exposure, the Knock-down effect (mosquitoes that felt on their backs) is first measured every 5 minutes for 60 minutes before the mortality reading after 24 hours. A negative control (untreated net) was included in each serie of cone tests. Mortality in the negative control was less than 1%. The application of the mortality corrected with Abbott's formula was no longer necessary [19]. The average bioassay results for the five faces of each sampled LLIN were calculated to determine whether the LLIN meets WHO efficacy criteria, i.e. ≥ 80% mortality on average and/or ≥ 95% Knock-down [16]. The bioassays were performed at a temperature of 25 +/- 2°C and a humidity of 80 +/- 10%. Three brands of threads were tested: DawaPlus®2.0, PermaNet®2.0 et DuraNet®. All nets were tested initially (new nets from packaging), and every at 6 months of use until the current stage. A total of 30 nets per area and per tower were tested.

Chemical decasy: Colorimetric test

WHO recommends the use of the bioassay method (cone test) for monitoring bioeffectiveness. Another method, the colorimetric test, was developed by Green *et al*. (2009) for LLINs treated with deltamethrin [20]. The colorimetric assessment of the bio-efficacy of MIILD involves a two-step process. In the first step, a magnetic sampling device (DEM) is used to sample the amount of insecticide on the surface of the LLIN (without moving it to the laboratory or cutting fragments). The amount of insecticide on the filter paper disc worn by the DEM is proportional to the amount of insecticide on the surface of the net. As the sampling is standardized, the results of the different nets sampled are comparable. The second step in using colorimetric test is to estimate the amount of deltamethrin in the filter paper sample. The colorimetric test results are then validated by comparing them to the results of the WHO bio-tests (cone test) for a series of mosquito nets with different amounts of deltamethrin on their surfaces. The use of a standard threshold (0.7 µg / disc) made it possible to interpret the colorimetric results in terms of "whether or not a LLIN meets the WHO minimum requirements" for bio-efficacy (mosquito net causing mortality greater than 80% in a cone test).

Procedure

The bio-efficacy by colorimetric test was only carried out on LLIN treated with deltamethrin (PermaNet®2.0 and DawaPlus®2.0) distributed in 2014. DuraNet LLINs are treated with alphacypermethrin and are not taken into account for this analysis. 30 LLIN PermaNet 2.0 and DawaPlus 2.0 were then selected at each evaluation and tested.

Sampling

For each mosquito net, the collection of samples for the colorimetric test relates to sides B and C because these two sides better represent the mosquito net. This choice is justified by the fact that several owners can put blankets on the roof of the mosquito net (position D) or fold the lower edges under the mattress or mat (position A), thus causing a loss of the insecticide molecules (Figure 2).

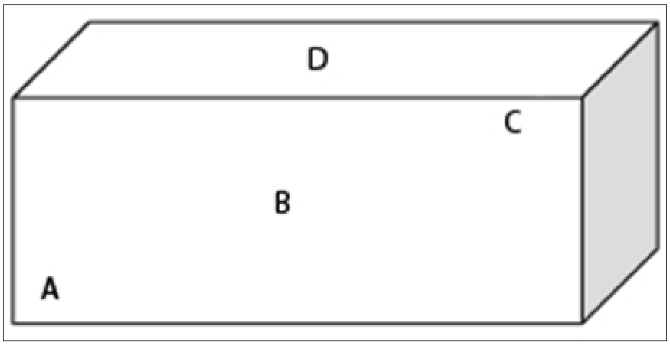


Fig 2: Identification of the different sampling locations

The mosquito net to be tested must be hung at the attachment points. Sides B and C (Figure 2) were immediately identified and attached to a Magnetic Sampling Device (DEM) containing washers of Whatman # 1 filter paper. The ends of the device were fixed to the mosquito net inside the plate so that the washers of Whatman # 1 filter paper absorb part of the insecticide contained in the fibers of the mosquito nets after a back and forth movement - comes ".

According to Green *et al*., 2009 30 “back and forth movements” ensure good impregnation of the Whatman # 1 filter papers for the insecticide. Whatman # 1 filter papers (two per side) soaked in insecticide were wrapped and stored at 4 °C.

Fig 3: Different stages of the insecticide collection technic

Insecticide dosage

Whatman # 1 filter papers soaked in insecticide representing the samples previously collected and stored at 4° C were placed in the wells of the plate provided for this purpose. Previously, four standards of 0; 0.5; 1 and 2 µg / disc of deltamethrin were also placed in the wells of the upper row of the plate (Figure 4).

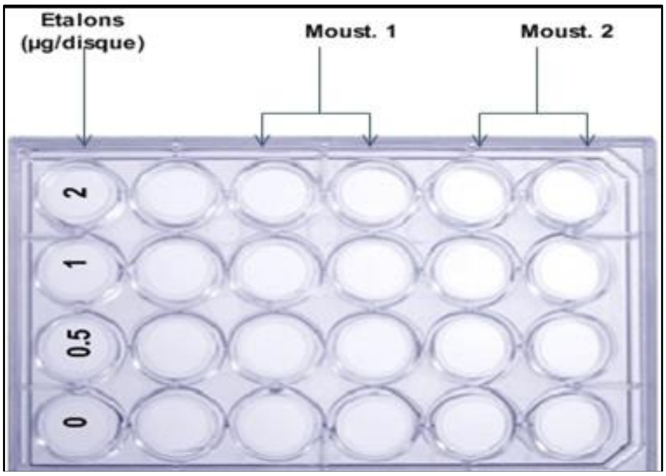


Fig 4: Test plate

Two reagents with the standard names A and B were required for the reaction. Reagent A is was composed of o-dinitrobenzene (30.2 mg / ml) and p-nitrobenzaldehyde (27.2 mg / ml) dissolved in 80% of a solvent, 2-methoxyethanol. Reagent B is was composed of a solution of sodium hydroxide at 16 mg / ml in 80% 2-methoxyethanol. The colorimetric test consists of carrying out two reactions of 5 minutes each. First, 200 µl of reagent A were dispensed into each of the wells of the previously prepared plate. After 5 minutes of waiting when the filter papers have been well impregnated and the insecticide supposed to be in suspension, 50 µl of reagent B were added to each of the wells to activate the reaction while slightly agitating the plate. To obtain an optimal result, another 5 minutes of reaction were necessary. After this reaction time, an intense or light purple color is obtained depending on the amount of insecticide contained in each filter paper (Figure 4).

To measure the intensity of the colors, the plate is was placed on a light source, all protected from interference from outside light by a polaroid (Figure 5 & 6). Using an appropriately adjusted digital camera (without flash and macro activated) placed on the polaroid hole, the reaction photo is was scanned. This digital image of the reaction taken is was transferred to a computer and then analyzed with the MVHimagePCv8 digital image analysis software for measuring the intensity of the color of the images.

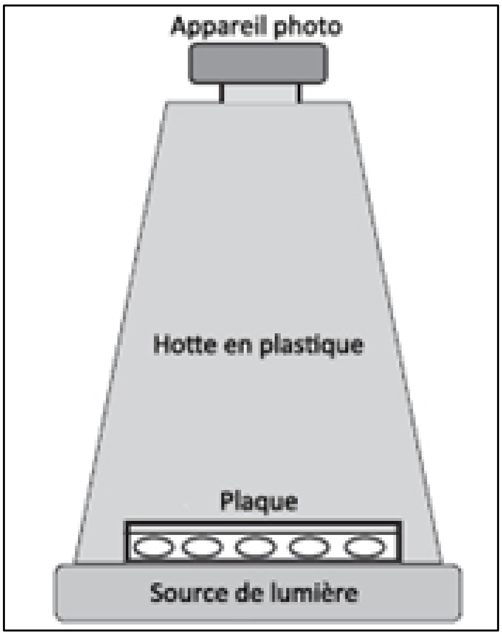


Fig 5: Photo taking

Fig 6: Plate after reaction

Statistical analysis

The binomial test was used to calculate the survival and loss rates and the confidence intervals. The proportional hole index (pHI) for factors that can affect physical integrity was determined by using the poisson regression. Data analysis was performed using R Core Team software version 3.5.1 (2018).

Results

Lost LLINs

Two years after using the LLINs, an average loss of 51.3% was observed on the LLINs selected at the start of the study. The attrition of LLINs was similar across the three brands of LLINs, 49.7% for DawaPlus®2.0, 46.7% for PermaNet®2.0 and 57.7% for DuraNet® (Figure 7). The most frequently cited cause for the loss of LLINs was physical deterioration, 29.7% versus 15% for displacement and 11.2% for other reasons (Figure 8).

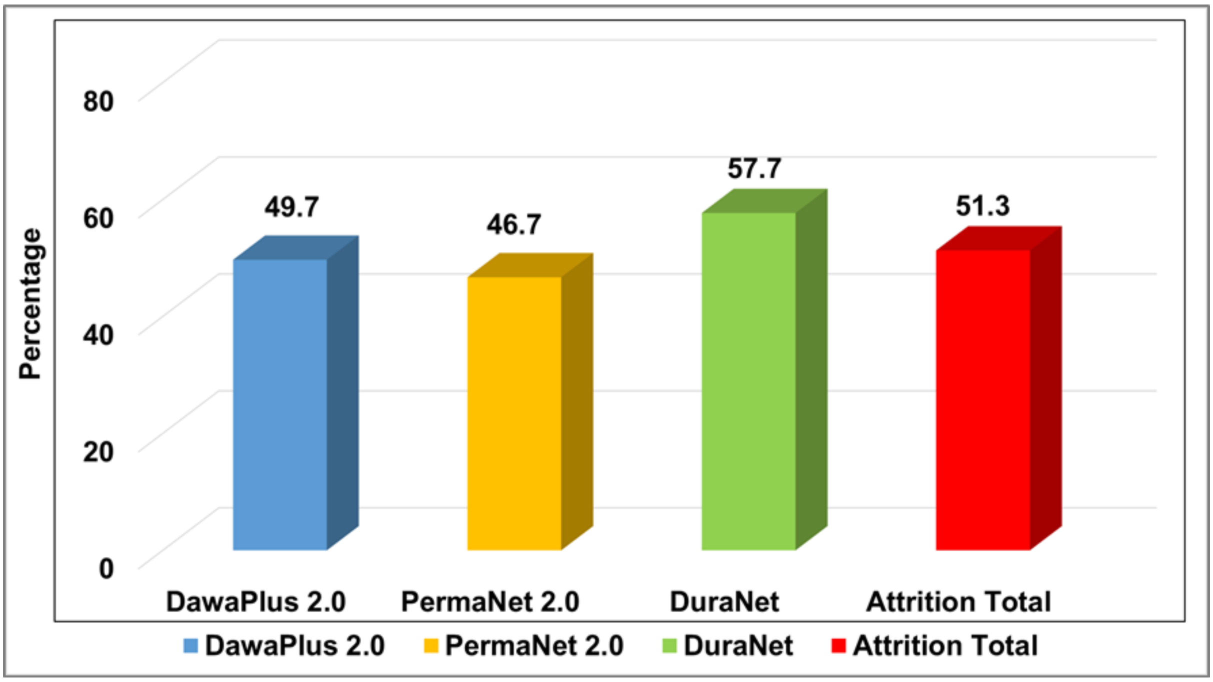
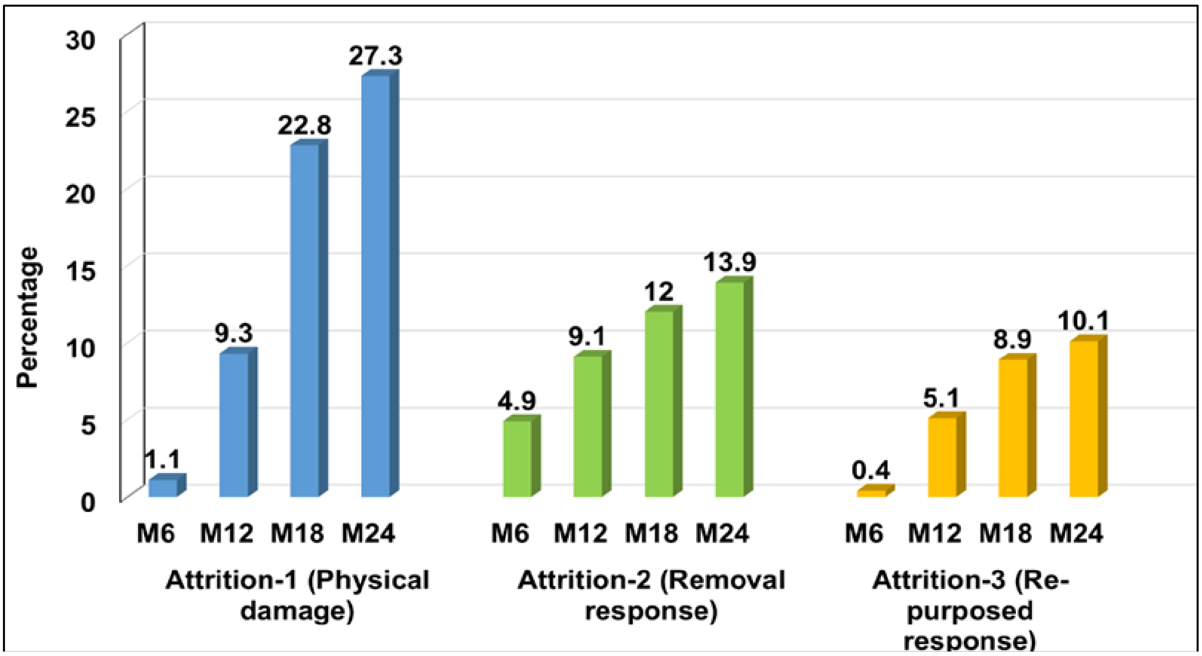


Fig 7: Proportion of LLINs lost after 30 months of use

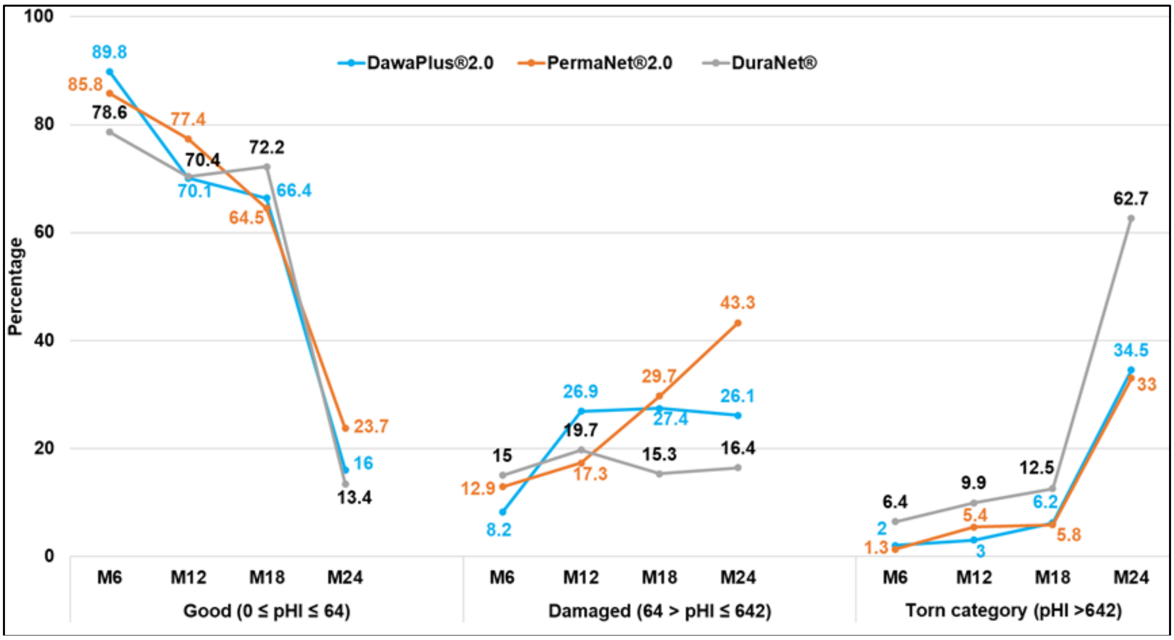


Legend: **M6:** 6 Months, **M12:** 12 Months, **M18:** 18 Months, **M24:** 24 Months

Fig 8: Reasons for loss of LLINs

LLINs fabric integrity

The characterization of the holes according to the WHO guidelines in terms of durability allowed us to classify the LLINs according to several categories in our study. Thus, rapid deterioration has been observed after two years of use. The proportion of DawaPlus®2.0 LLINs in good condition was 13.4% compared to 16% for DuraNet® and 23.7% for PermaNet®2.0. This resulted in an increase in damaged LLINs 43.3%, 16.4% and 26.1% respectively for PermaNet®2.0, DawaPlus®2.0 and DuraNet®. The proportion of LLINs replaced after two years was 33% for PermaNet®2.0, 62.7% for DawaPlus®2.0 and 34.5% for DuraNet® (Figure 9).



Legend: **M6:** 6 Months, **M12:** 12 Months, **M18:** 18 Months, **M24:** 24 Months

Fig 9: Physical integrity of LLINs

LLINs Survivorship

Overall, the LLINs found and evaluated were 651, 511, 556 and 283 respectively after 6, 12, 19 and 24 months. By combining the results of the three types of LLINs, the survival rate is was 95% after 6 months of use. After two years, this rate has dropped to 47%. When analyzing each type of LLINs, 53% was observed for the average survival rate of DawaPlus 2.0; 53.33% for PermaNet 2.0 and 34.33% for DuraNet. The observed survival rates were compared to those of the NetCalc model which predicted a 74% survival with LLINs that could be useful for 3 years and 50% for those of 2 years. Our results were significantly lower than that predicted for the three types of LLINs (74%). But it is similar to the rate predicted for a lifetime of 2 years (50%) for DawaPlus®2.0 and PermaNet®2.0. However, for DuraNet®, this rate is was much lower than two years (p<0.001) (Figure 10).

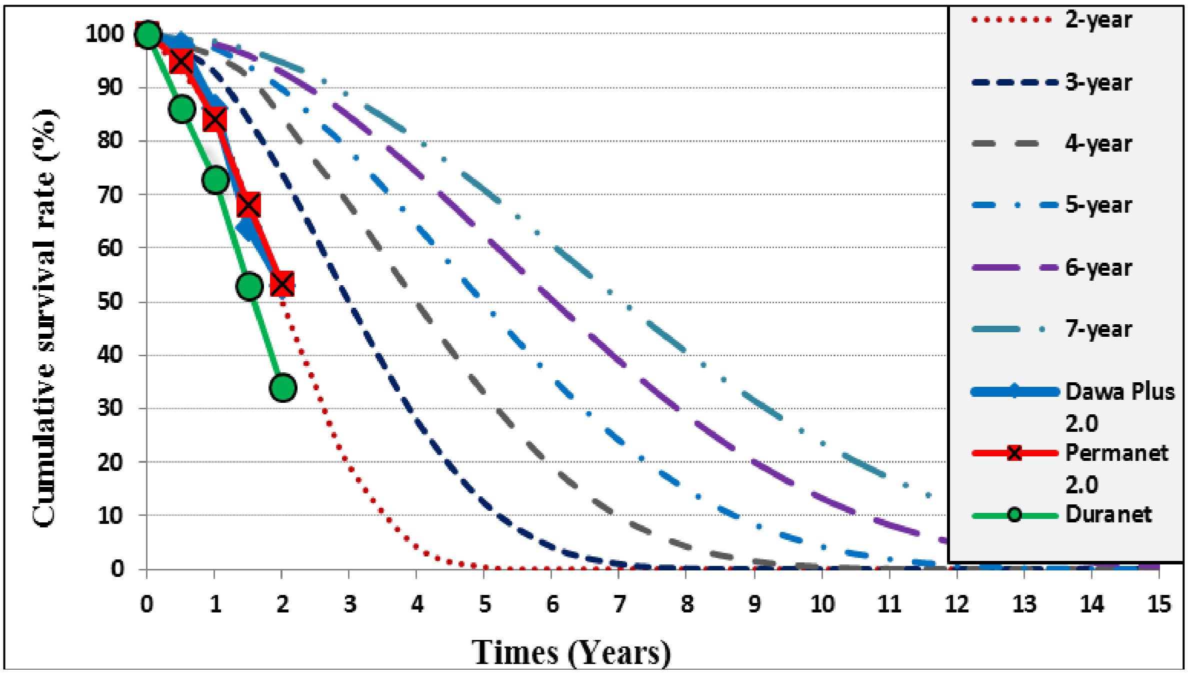


Fig 10: Survival rate of three types of LLINs observed two years after distribution via national campaign

Median survival

In order to facilitate comparisons with other sustainability data, the has been calculated from the last two data points and the results are shown below. The median survival calculated was 2.1 years at Tori-Bossito (DawaPlus®2.0), 2.2 years at Toffo (PermaNet®2.0) and 1.7 years at Ouesse (DuraNet®). The estimates obtained from the graph were very similar to those calculated in the final survey. Given the confidence intervals around median survival, it can be said that at the three sites, the performance of the LLINs tested was clearly below the mark of three years at Tori-Bossito and Toffo, but also below the mark of 2 years with regard to Ouesse.

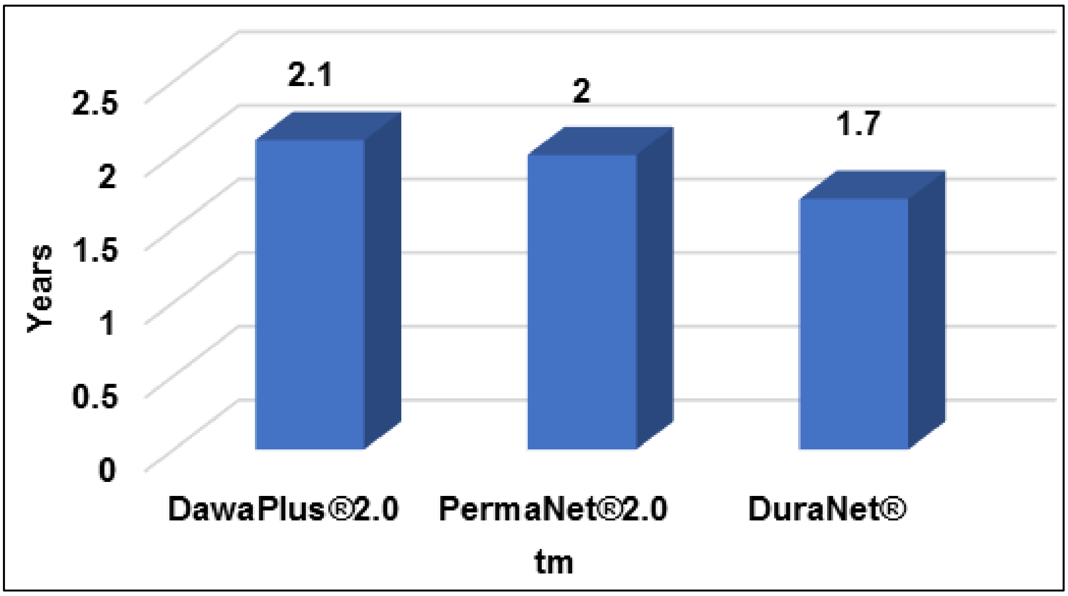


Fig 11: Median survival of the three LLINs after two years of use

Assessment of bio-efficacy

WHO cone test

Table 1 shows the mortalities observed with the WHO cone tests. Six months after distribution, more than 96% of knock-down effects (mosquitoes fell on the back) and 94% of mosquito net mortality were observed. Similar results were recorded one year after distribution, with death rates everywhere greater than 90%. Two years after distribution, the estimate of biological efficacy has dropped over time. The results did not confirm the good performance of the previous monitoring. The rates of mosquitoes falling on their backs after 60 minutes were around 80%. In contrast, the average mortality observed after 24 hours was 46.2% for DuraNet®, 72.2% for PermaNet®2.0 and 54.3% for DawaPlus®2.0.

Table 1: Mean mortality observed with cone tests in the WHO after 6 and 12, 19 and 24 months

Periods	LLIN tested		N mosquitoes tested	% KD after 60 minute	CI 95%	Mortality (%)	CI 95%
6 months	DuraNet®	N=30	1553	100	99.2-100	100	99.2-100
	PermaNet®2.0	N=30	1529	96	93.8-97.5	94	91.3-95.8
	DawaPlus®2.0	N=30	1538	100	99.1-100	100	99.1-100
12 months	DuraNet®	N=30	1559	93.6	90.6-95.7	95.3	92.6-97.0
	PermaNet®2.0	N=30	1510	95.1	92.6-96.8	92.7	89.8-94.8
	DawaPlus®2.0	N=30	1540	96.4	99.1-100	94.1	99.1-100
19 months	DuraNet®	N=30	1491	94.7	92.3-96.5	85.3	81.9-88.4
	PermaNet®2.0	N=30	1559	92.2	89.3-94.4	78.2	74.2-81.9
	DawaPlus®2.0	N=30	1525	62.5	58.2-66.6	69.3	65.2-73.3
24 months	DuraNet®	N=30	1596	89.1	86.0-91.7	46.2	41.7-50.7
	PermaNet®2.0	N=30	1528	80.1	76.0-83.8	72.2	67.7-76.9
	DawaPlus®2.0	N=30	1501	92.2	89.5-94.4	54.3	49.8-58.7

CI: Confidence interval

Assessing insecticidal decasy: Colorimetric test

At the start and immediately after the mass distribution campaign, 30 samples of each deltamethrin-based LLIN were evaluated by a colorimetric method. All new LLINs were above the threshold concentration of 0.35 µg / sample, which represents an efficiency rate of 100% (Figure 6). The average quantities of deltamethrin tested were 2.50 µg / sample for PermaNet®2.0 and 3.58 µg / sample for DawaPlus®2.0. After six months of use, the efficiency of LLINs with a quantity of deltamethrin ≥ 0.35µg decreased to 77% (95% CI: 59.1-88.2) in Toffo (PermaNet®2.0) but remained 100% (CI to 95%: 88.7-100) in Tori-Bossito (DawaPlus®2.0) (table 2). Overall, 12% (n = 7) of our PermaNet®2.0 samples were no longer effective after six months of use. In contrast, DawaPlus®2.0 LLINs the threshold amount of deltamethrin (Table 2). After 12 months of use, the DawaPlus®2.0 LLINs were still effective their effectiveness with an amount of deltamethrin >0.35µg. But of the 30 PermaNet®2.0 LLINs tested, 23 quantity of deltamethrin of had a ≥0.35 µg.

After 19 months of use, 64.7% (6/17) of DawaPlus®2.0 LLINs retained their efficacy with an amount of deltamethrin ≥0.35µg. But with the 17 PermaNet®2.0 LLINs tested, 11 did not reach the threshold amount of deltamethrin.

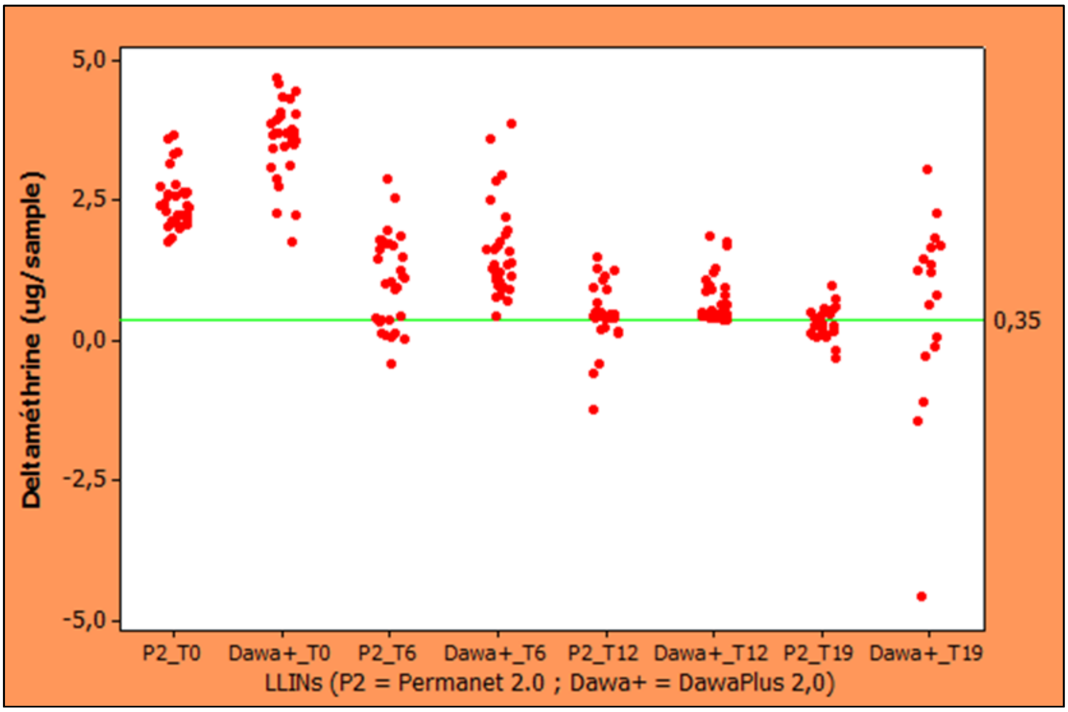


Fig 12: Plot of nets with insecticide levels below and above effective concentration at T0, T6, T12 and T19

Table 2: Average deltamethrin surface level tested by location at T6, T12 and T19

	CD (mg/m²)	ADB	AD6M	loss rate(%)	AD12M	loss rate(%)	AD19M	loss rate(%)
PermaNet®2.0 (n=30)	55mg/m²	2,50	1,06	58	0,45	82	0,31	88
DawaPlus®2.0 (n=30)	80mg/m²	3,58	1,59	56	0,76	79	0,57	84

CD: Concentration of deltamethrin (mg/m², ADB: Average deltamethrin at Baseline, AD6M: Average deltamethrin after 6 months

Discussion

This study assessed the performance of DawaPlus®2.0, DuraNet® and PermaNet®2.0, three LLINs recommended by WHOPES with an emphasis on survival and bio-efficiency, two key indicators of sustainability. A mosquito net is considered to meet the WHO LLIN requirements if, after three years of use, at least 73.8% of the LLINs sampled survive, and 80% of them retain their bio-efficacy (this is a knockdown ≥ 95% (rate of mosquitoes fallen on the back) and a mortality after 24 hours ≥ 80%) with a standard WHO cone bioassay [12]. According to these criteria, the three LLINs in the studied have shown results which not meet the expectation for for after 2 years of use. The survival of the three brands monitored (53% for DawaPlus®2.0 and PermaNet®2.0, and 34% for DuraNet®) in this study remained well below the three-year mark, generally considered to be the average useful life of a LLIN. The median survival calculated in our study showed that the DawaPlus®2.0 LLIN at this period had 2.1 years of survival versus 2 years for PermaNet®2.0 (therefore similar to what was predicted by the NetCalc model for a LLIN of 49.9%) and 1.7 years for DuraNet®. This is in line with the results of the unpublished sustainability study undertaken in the DRC in 2015 [19]. Low estimates of the median physical survival of LLINs have also been reported in other studies for other LLINs. In Zambia, Olyset® and PermaNet®2.0 had an estimated median survival of 2.0 years [20], Olyset® in Benin, 1.5 to 2.0 years [10] and PermaNet®2.0 in Ethiopia, only 1.0 year [21]. In contrast, median survival times over three years were also noted. A retrospective durability study of the DawaPlus®2.0 LLIN found a median survival varying between 3.0 and 4.7 years at three sites in Nigeria [15]. Recently, these results were confirmed in a prospective study, still in the same country, with a median survival of DawaPlus®2.0 ranging from 3.2 to 5.3 years (Obi *et al*. Pers commun). In Cambodia, the median survival of PermaNet®2.0 was 3.4 years [25]. The median survival of Olyset® has been measured at 4.0–4.5 years [23] in Kenya. These similar physical durability results for the same brands of LLIN in different locations were is much wider than all of the differences observed between the brands of LLIN examined above. This suggested that the differences in net use and environment were telling than the differences in net material. It also implied that the physical sustainability in Benin currently appears weak, and should be improved with more awareness and behavioral change in the use of LLINs.

In our study, DuraNet © showed relatively low insecticidal efficacy. Only 46.2% of LLINs met WHO requirements after 2 years of monitoring. However, the results of the Colorimetric test showed that 64.7% of DawaPlus®2.0 LLIN retained their efficacy with an amount of deltamethrin ≥0.35µg. In the two tests, were compared what 80% (meaning) and more of the optimal performance in the evaluation of 31 months in Congo on the same brand of LLIN (27). In northern Tanzania, DuraNet © also had 94% mortality in bioassays after 20 washes [25]. Regarding DawaPlus®2.0, barely 54% of LLINs were effective after 2 years in our study. This result is far from the optimal performance of 90% obtained at 24 months in Congo [25]. However, in a recent prospective sustainability study in Nigeria, this brand of LLIN showed an optimal efficacy of 97% in two sites in Nigeria after 36 months (Obi *et al*. Submitted). PermaNet®2.0 was also unable to reach 80%

efficiency after 2 years in our study. From our results, no obvious reason for the poor insecticide durability of three LLINs is apparent. It has been shown that drying LLINs in the sun, for example, reduces the content of insecticides by up to 5% points in a study in Kenya [26]. However, another study has shown that when bioassays are used as results, repeated exposure to the sun only reduces vector mortality if the exposure is 3 days after each wash, but not if the drying period is only 3 hours [26]. It is therefore unlikely that drying alone can explain the observed decline in insecticide efficacy in our case.

Limits

Our results could be influenced by certain behaviors and attitudes of the community people were aware of routine monitoring, as it was a prospective study. This does not exclude a potential Hawthorne effect, where being asked about the care and handling of the net four times in the two years may have contributed to behavioral changes. The fact that certain parameters were not taken into account in this study was is also a handicap in terms of understanding and assessing the survival and bioeffectiveness of LLINs in an objective and concrete manner. However, we reassure the scientific world that another paper submitted on the same study took care to address these parameters widely to help understand this difference between LLINs.

Conclusion

Two years after distribution, monitoring the bioeffectiveness and durability of LLINs in natural conditions, DawaPlus®2.0 and PermaNet®2.0 LLINs, both made of 100-denier polyester, showed significantly better median physical survival compared to MILDs. DuraNet© in 150 denier polyethylene, but the three remained well below the expected median survival of two years. The difference could be attributed to some extent to care behavior and several other factors developed in another paper. It seems preferable to distribute 100-denier polyester LLINs, such as DawaPlus®2.0 and PermaNet®2.0 because the polyethylene brand tested in 2011 and the DuraNet made of the same fiber have shown their limits. Insecticide performance was not optimal for any of the three LLINs at two years of follow-up. However, at that time, most of the cohort LLINs were already lost or no longer usable.

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Competing interests

The authors declare that they have no competing interests.

Ethical Clearance and consent to participate

This study received ethical approval (favorable ethical opinion n°05 of 07 November 2017) from the CREC Institutional Ethics Committee (IECC). All heads of households aged 18 years or older who have received the DawaPlus®2.0, PermaNet®2.0 and DuraNet® nets under evaluation were voluntarily included in the study after signing the consent form and were allowed to withdraw if they wish without fear of retaliation.

Authors' contributions

IBA and MCA designed the study. IBA and MCA carried out the field activities. IBA drafted the manuscript and analyzed the data. RYA, FD, RA, AB an GGP critically revised the manuscript. IBA and MCA conceived and designed the study and MCA revised the manuscript for intellectual content. All authors read and approved the final manuscript.

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