Overview of Some Factors Affecting the Durability and Efficacy of LLINs Used by the Communities in South Region of Cameroon, Central Africa

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Research

Keywords: LLINs, brands, maintenance methods, bio-efficacy, physical integrity, Kribi, South Cameroon

Posted Date: November 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-876554/v2

Abstract

Background: Long-lasting insecticidal nets (LLINs) are widely used in sub-Saharan Africa to control the transmission of malaria parasites by Anopheles mosquitoes. However, their operational lifespan depends on numerous factors that are often not considered. This paper investigates on some of the factors likely associated with the rapid breakdown of LLINs efficacy in urban and rural settings of the seaport city of Kribi, South Region of Cameroon.

Methods: A cross-sectional survey was conducted in June 2019 including: (i) a household survey using standard questionnaire for brands of LLINs used, their maintenance, and coverage rates in study communities, (ii) assessment of the physical integrity of nets, and (iii) WHO cone bioassays to determine the residual efficacy of the nets against wild strains and susceptible reference laboratory strains of *Anopheles gambiae* s. l. species.

Results: A total of 540 households were surveyed, 235 in Kribi- urban and 305 in Kribi-rural. The overall net coverage rate per sleeping space was 68.82%, irrespectively with a similar trend between Kribi-rural (68.98%) and Kribi-urban (68.62%) ($p = 0.929$). Of the 1,211 LLINs recorded, 64.73% were Interceptor®, 25.44% were PermaNet®, 4.68% were Olyset Net ; the remainder (05.13%) belonged to Yorkool®, Royal Sentry®, Netto®, Super Net® and Panda Net®. The hole indices were significantly correlated to the frequency of washes in kribi-urban as well as kribi-rural settings ($p \leq 10^{-3}$). Nets washed with ordinary soap had lower hole indices compared to nets washed with corrosive detergents in the two groups of settings in the study area ($p \leq 10^{-4}$). Likewise, nets dried under the sun significantly recorded a higher hole indices compared to nets dried in a shaded area ($p \leq 0.04$) in the two groups of settings. Polyethylene fibers were significantly less degraded than polyester fibers. The bio efficacy results revealed 21.6% - 99.6% mortality rates of the Kisumu strain exposed to LLINs washed more than 20 times and from 0.8% to 76.50% for the wild strain. LLINs washed with ordinary soap significantly retained their lethal properties more than LLINs washed with corrosive detergents ($p=10^{-4}$). Similarly, LLINs dried in the shade retained their lethal properties more than those spread in the sun.

Conclusion: Excessive washes, Corrosive detergents and drying regimes are factors that degrade the toxic effect of insecticide impregnated on LLINs. Awareness campaigns are needed to encourage people to adopt good LLIN maintainance practices.

Background

Malaria is the most deadly parasitic diseases in the world. In 2018, about 228 million people contracted malaria among whom about 409.000 deaths was recorded **[1].** Sub-Saharan Africa is the most vulnerable area. In Cameroon, malaria is the main cause of morbidity and mortality, and a major public health problem **[2]**. In 2019, it was responsible for nearly 11. 233 deaths and negatively impacted the economy at the family unit and hence the economy of the entire nation **[2]**. Pregnant women and children under five years of age remain the vulnerable targets in various parts of the country **[3]**.

In view of the magnitude of the problem, the Cameroonian government, through the National Malaria Control Program (NMCP), has defined a number of strategic orientations, of which prevention via the use of insecticide-treated mosquito nets is the corner stone.

The use of mosquito nets, against culicidal nuisance and then to reduce the transmission of vector-borne diseases, including malaria, is a very old practice **[4]**. In the context of malaria control, early trials in different countries demonstrated the impact of insecticide-treated nets on the incidence of the disease **[5]**. However, these nets had the disadvantage that they had to be re-treated regularly, at least once a year **[6].**

Long Lasting Nets (LLINs) were produced as an alternative to re-treating conventional nets. These are nets that are industrially pre-treated with specific processes that allow them to be effective after at least 20 washes and to retain their insecticidal properties for three to five years in standard household use **[7]**. An insecticide of the pyrethroid family, incorporated or coated into the polyester or polyethylene threads during manufacture, is slowly released and migrates from the fabric to the surface of the net, causing a deterrent, repellent or excito-repellent effect. At high coverage levels ($\geq 80\%$), the community-wide benefits of using LLINs have been demonstrated: whereby, a mass effect on the vector population is observed.

Today, LLINs are one of the spearheads of Vector Control Policy (VCP). In sub-Saharan Africa, the proportion of populations with access to a LLINs has increased from less than 2% in 2000 to 67% in 2015, and universal coverage is targeted and achieved in some countries **[7]**. Although they have contributed significantly to the decline in malaria incidence over the past 15 years, the key issue of the actual lifespan (physical integrity and bioefficacy) of LLINs remains questionable. Indeed, there is a lack of investigation into the functional life and performance variation between different LLINs under different conditions of use. Studies in Uganda on the physical integrity of LLINs found that 45-78% of nets were damaged after one year of use under operational conditions **[8]**. In Kenya and Benin, a faster than expected deterioration in bio-efficacy was noted, raising concerns about the effective lifespan of LLINs **[9-10]**. In another study in Laos, about 40% of nets were physically damaged after two to three years of use **[11]**.

Kribi in southern Cameroon is one of the communities that benefited from mass distribution of LLINs in 2016. Since then, little or no study has been carried out to assess the level of operationality of these LLINs. Yet such data are crucial to the implementation of an effective and sustainable prevention strategy.

The present study assesses the physical integrity and bio-efficiency of LLINs used by households in Kribi.

Materials And Methods

Study site

The current study was conducted in the town of Kribi (02°54'N and 09°55'E), an area of 11,280 km² located in the Gulf of Guinea at the Atlantic Ocean, south Region of Cameroon (Figure 1). Kribi is found in the equatorial domain and the climate includes two rainy seasons and two dry seasons **[12]**. The average annual temperature is between 27°C and 37°C and rainfall is around 2,970 mm per year. Malaria is endemic with a continuous parasite transmission by the major malaria vector species *Anopheles gambiae* s.l. The vegetation is a continuation of the Congolese forest. Several rivers and streams such as Nyong, Ntem, Lokoundje, Lobe, Kienke compose the the local hydrographic network. Riverbanks and surrounding water bodies are suitable habitats for *Anopheles gambiae* larval stages. The main local activity is fishing; however livestock breeding, trade and intensive agriculture are also practiced by inhabitants and corporations. The study took place in two ecologically different sites: Kribi rural and Kribi urban, where a recent KAP studies have indicated that LLINs were the main tool of protection against mosquitoes **[13-14]**.

Kribi-rural (Kribi 1) is a district covering 334 km2 with a population of 22,681 inhabitants **[15]**. This district is undergoing anthropisation due to its proximity to the deep-water port. Several buildings are being constructed across the site, resulting in the destruction of the vegetation cover and the creation of temporary mosquito larval breeding habitats, especially during the rainy season. Kribi-urban (Kribi 2) is a district that covers an area of 125 km2 with an estimated population of 40,000 inhabitants. In this urbanized district, most of the houses are constructed of permanent materials. LLINs are the main tool for the control of mosquitoes.

Characteristics of used LLINs and sampling procedures

Eligible households were those composed of people established in the study areas for at least two years. The effective number of households were n=540. A standard questionnaire was administered to heads of selected households to record the brands of LLINs used, the way they washed and maintained their nets, the effective use of nets by the most vulnerable groups (children under 5 years old and pregnant women). A sample of used LLINs was randomly collected from household users and replaced with new ones in both Kribi rural and Kribi urban. A standard form submitted to households permitted the recording of net characteristics (insecticide coated, type of fiber, brand name) and the washing practices (frequency and type of soap used). Sampled LLINs individually kept in aluminum sheets were stored in a plastic bag at 4°C until further evaluation of their physical integrity and bio efficacy in laboratory.

Assessment of LLINs physical integrity

The LLIN samples collected from the field were later inspected to check for the number and the size of holes, and classify them into four categories as described by Kilian et al. (2008): i) size 1: 0.5-2.5 cm, ii) size: 2.5-10 cm, iii) size 3: 10-25 cm and size 4: > 25 cm. The proportionate Hole Index (pHI) was then calculated as indicator of physical integrity for each net by weighting each hole by size and summing them for each net using the following formula by WHO **[15]**: pHI = (1 × number of size-1 holes) + (23 × number of size-2 holes) + (196 × number of size-3 holes) + (576 × number of size-4 holes).

Cone bioassays

A total of X LLINs were tested. For each net, 5 pieces of 25 cm × 25 cm were sampled from the sides and the roof of each LLIN sample, for the assessment of the residual efficacy after 3 years of household use in rural and urban Kribi. The WHO testing procedure using plastic cones was performed with three strains of *Anopheles gambiae* mosquitoes: a “wild” strain obtained from larval collections in temporary breeding sites from the two study locations (Kribi 1 and Kribi 2) and two laboratory strains (Kisumu and Ngousso) as control references regarding susceptibility to all insecticides. The Ngousso strain identified from the city of Yaounde (Cameroon) as *Anopheles coluzzi* species was established at OCEAC since January 2006. The Kisumu strain (*Anopheles gambiae* species) from a location in Kenya (East Africa) was adapted at OCEAC insectary conditions since 2009. Forty individuals of 2-5 days old female mosquitoes were transferred in 4 plastic cones (10 per cone) previously fixed to each piece of the net sample for 3 minutes of direct contact. After this exposure time, they were transferred back in plastic cups for observation and the recording of mosquito knock down rates within 60 minutes after exposure and mortality 24 hours post exposure. A cotton swab soaked in glucose solution (10%) served to feed mosquitoes during the observation at 27 ± 3°C ambiant temperature and 75 ± 10% relative humidity **[16]**.

Data analysis

The rate of net use was assessed by calculating the ratio of the number of people who slept under the net for three consecutive nights prior to the day of survey. The LLINs coverage rate was determined by estimating the number of nets available in households versus the supposed number of nets required for universal coverage. The rate of protection of sleeping units were estimated by dividing the total number of nets available versus the total number of sleeping units identified.

The proportionate hole index (pHI) used as indicator of physical integrity for each net sample was classified according to the criteria defined by WHO **[15]** as follows: i) “good condition” (no reduction of efficacy) if pHI is between 0 and 64; ii) “acceptable condition” if pHI is between 65 and 642 and “degraded or too torn condition” if pHI > 643. For the bioassay results, the three WHO criteria of residual LLINs bioefficacy to meet were as follows **[16]**: 1: optimal effectiveness: ≥80% mortality or ≥95% knockdown; 2: “minimal effectiveness: ≥50% mortality or ≥75% knockdown, and 3: Not effective: <0.05. Data were entered into an Excel spreadsheet and then analyzed using IBM-SPSS (Statistical package for social sciences) Statistics 25.0 for windows (IBM-SPSS Corp., Chicago USA) software version 22.0. The Kruskall-Wallis H-test was used to compare the differences in sizes between the holes, the Pearson's R-test was used to assess the correlations between the washing frequency of LLINs and the pHIs, and the Chi-square test to compare the mortality rates between wild strains and reference strains. The maximum significance threshold of p-values was 5%

Results

Net characteristics, coverage and washing status

A total of 540 households were surveyed including 235 in Kribi-urban and 305 in Kribi-rural. The overall number of nets counted was 1,211 nets, including 546 in urban Kribi and 714 in rural Kribi (Table 1). Eight (8) brands of mosquito nets were recorded in the study sites, of which Interceptor® (290/448) and PermaNet® 2.0 (114/448) were the most common (Table 2).

Table 1
General parameters relating to the coverage and use of LLINs in Kribi

Modalities	Kribi urban	Kribi rural	Total
Number of households surveyed	235	305	540
Number of people surveyed	1343	1750	3093
Number of children under 5 years	275 (20.47%)	460 (26.28%)	735 (23.76%)
Number of children (under 5) using the LLINs	242 (88%)	409 (88.91%)	651 (88.57%)
Number of pregnant women using the LLINs	4/6 (66.67%)	1/3 (33.33%)	5/9 (55.55%)
Brands received in antenatal consultation	Permanet	Permanet	Permanet
Total sleeping spaces	631	761	1392
Prevention method used by households (LLINs)	210 (89.40%)	248 (81.31%)	458 (84.81%)
LLINs available	546	714	1211
LLINs in regular use	433	525	958
Number of washes	3597	3646	7243
Detergent use	124	52	176
Average LLIN/household	2.34±0.127	2.31±0.128	2.33±0.091
Households with at least one LLIN	211 (89.78%)	245 (80.32%)	456 (84.44%)
Households with at least one LLIN for 2 persons	42 (19.90%)	50 (20.41%)	92 (20.17%)
Coverage rate (%)	86.53	93.82	90.17
Utilization rate (%)	79.30	73.53	76.42
Sleeping space protection (%)	68.62	68.98	68.82

Table 2
Characteristics of nets in use collected from Kribi -urban and Kribi- rural in 2019

Brand Name	Coated insecticide (mg/m^2)	Type of fiber /resistance	Kribi-Rural	Kribi - Urban	Total (%)	Size/ Inch^2	WHO pre-qualification
Interceptor®	Alpha-cypermethrine (200)	Polyester /75, 100	161	128	333 (67.8%)	156-177	Complete
PermaNet® 2.0	Deltamethrine (55)	Polyester /75, 100	54	60	114(23.2%)	156-177	Complete
Olyset Net®	Permethrine (1,000)	Polyethylene /150	9	12	21(4.3%)	75	Complete
Yorkool®	Deltamethrine (55)	Polyester /75, 100	13	4	17(3.5%)	156-177	Complete
Royal Sentry®	Alpha-cypermethrine (260)	Polyethylene /145	-	1	1(0.2%)	132	Complete
Netto®	Deltamethrine (80)	Polyester /75, 100, 150	2	-	2 (0.4%)	156-177	In process
DuraNet®	Alpha-cypermethrine (260)	Polyethylene /145	1	1	2 (0.4%)	132	Complete
Panda Net®	Deltamethrine (63)	Polyethylene 100, 115/	1	-	1 (0.2%)	136 and 200	In process

In addition, 19.9% of households (n= 42) had at least one net for every two people in urban Kribi compared to 20.41% (n= 50) in rural Kribi (Table 1).

The rate of net use was comparable in rural Kribi (79.3%) and urban Kribi (73.53%). The number of children under five years of age using the net was 651 (88.57%) while the number of pregnant women sleeping under a net was 5 out of 9 (55.55%) who agreed to take part in the study (Table 1).

The coverage rates for LLINs were 86.53% and 93.82% in rural and urban Kribi respectively (Table 1). In addition, 68.98% and 68.62% of sleeping spaces were covered in the two sites respectively (Table 1).

Status of physical integrity of used LLINs

The physical inspection showed that the nets had holes. Polyethylene fibers were not significantly more degraded than polyester fibers (Table 3). However Table 5 show that the Duranet LLIN brand resisted the most to the negative effects of the corrosive detergents in both study locations, meanwhile Royal sentry and Interceptor resisted the most to the adverse effects of the sun respectively in Kribi-urban and kribi-rural.

Table 3
LLINs maintenance methods (frequency of washing, type of soap, drying method and life span)

		Rural Kribi		Urban Kribi	
		Workforce	**pHI/ Physical condition**	**Workforce**	**pHI/ Physical condition**
Maintenance method	**Frequency of washing**	**241**		**206**	
	[0-5 [	1	420 (acceptable)	2	2232±2224 (degraded)
	[5-10 [	26	591±126.244 (degraded)	30	1006±97.013 (degraded)
	[10-15 [	106	610±68.999 (acceptable)	55	713±61.119 (degraded)
	[15-20 [	45	1193±123.509 (degraded)	16	1316±153.683 (degraded)
	20 ≤	63	1519±96 (degraded)	103	1636±76.604 (degraded)
		p=1,000	$p \leq 10^{-3}$	$p \leq 10^{-3}$	$p \leq 10^{-3}$
	Type of soap	**241**		**206**	
	Ordinary soap	190	624±38.775	83	557±34.618
	Corrosive detergent	51	1830±84.007	124	1754±57.058
		$p \leq 10^{-3}$	$p \leq 10^{-4}$	$p \leq 10^{-3}$	$p \leq 10^{-4}$
	Drying mode	**114**		**100**	
	Sun	52	2258±60.669	68	1871±83.631
	Shade	62	93±11.236	32	225±32.239
		P=0.299	p=0.001	$p \leq 10^{-3}$	p=0.042
Validity period (months)		**235**		**205**	
	[0-10 [	6	1134±433.342	12	689±99.318
	[10-20 [	34	826±121.571	58	967±76.287
	[20-30 [	82	493±61.174	26	844±105.664
	[30-40 [	94	1246±87.573	93	1528±87.163
	40≤	17	1528±233.473	16	2037±178.759
	Test R		**0.612**		**0.600**
Types of fibers	Polyesther	231	957,90±54,91	191	1264,15±57,80
	polyethylen	10	967,18±220,96	15	1468,47±234,29
		P=0,835	P=0,259	P=0,779	P=0,421
Mean number (± standard error, SE)					

Table 5
Holes indices per brand of mosquito net according to the maintenance mode.

Drying mode		**Shade**		**Sun**	
	Mosquito net brands	**Workforce**	**Phi**	**Workforce**	**Phi**
Rural Kribi	Interceptor®	55	101,42±11,81	29	2368,45±83,41
	Olyset Net ®	1	5	2	1906
	Permanet® 2.0	3	6,67±2,75	17	1355,07±127,41
	Yorkool®	3	55,33±44,73	3	1912,33±5,25
	Royal®	/	/	1	1103
	Total 1	**62**	**/**	**52**	
Urban Kribi	Interceptor®	22	247,27±37,479	30	1644,20±116,181
	Olyset Net®	3	153,67±123,323	9	2078,22±236,648
	Permanet® 2.0	7	189,43±78,044	26	1916,04±138,980
	Yorkool®	0	0	3	1936±453,763
	Total 2	**32**	**/**	**68**	
Type of soap		**Ordinary soap**		**Detergent corrosive**	
	Mosquito net brands	**Workforce**	**Phi**	**Workforce**	**Phi**
Rural Kribi	Interceptor®	133	481,21±37,881	24	1875,86±121,064
	Olyset Net ®	8	1141,13±244,834	4	1418±273,126
	Permanet® 2.0	34	890,71±93,463	17	2049±119,241
	Yorkool®	11	1125,91±208,027	4	1780,75±125,170
	Duranet®	4	714,75±259,968	2	1015,50±224,500
	Total 1	**190**	**/**	**51**	**/**
Urban Kribi	Interceptor®	62	594,65±40,547	66	1630,50±63,808
	Olyset Net ®	5	427,40±182,666	8	1917,13±155,091
	Permanet ®2. 0	16	464,31±74,945	43	1916,77±104,766
	Yorkool®	1	438	4	2004,75±328,833
	Duranet®	/	/	1	1103
	Total 2	**84**	**/**	**122**	**/**

Maintenance of LLINs

The frequency of net washing varied from site to site (Table 3). The number of LLINs that had been washed between ten and fifteen times after 36 months of use was higher in rural Kribi, whereas the number of LLINs that had been washed more than twenty times was higher in urban Kribi (Table 3). A positive and significant correlation between washing frequency and proportionate hole indices in both rural Kribi ($R=0.612$, $p \leq 10\text{-}4$) and urban Kribi ($R=0.600$, $p \leq 10\text{-}4$) was observed.

Furthermore, the use of ordinary soap was more frequent in rural Kribi (190/241 LLINs) than in urban Kribi (83/206 LLINs). In contrast, the use of corrosive detergents was more marked in urban Kribi (124/206 LLINs) than in rural Kribi (51/241 LLINs). Table 4 shows that washing the LLINs with ordinary soap induced significantly fewer proportionate indices of holes than washing the LLINs with corrosive detergents in both rural Kribi (p≤ 10-4) and urban Kribi ($p \leq 10^{-3}$) (Table 3).

Table 4
Mortality rate (%) of *Anopheles gambiae* (Field) 24H after exposure to 10 LLINs.

Insecticides	**Rural Kribi**			**Urban Kribi**		
	Numbers	**Bioefficacy (mortality %)**	**Brand**	**Numbers**	**Bioefficacy (mortality %)**	**Brand**
Permethrin	1		**Olyset Net**	2		**Olyset Net**
[0-10 washes [	1	1.8%		1	50%	
[10-20 washes [	0			1	41.3%	
Deltamethrin	2		**PermaNet rectangular**	1		**PermaNet rectangular**
[0-10 washes [	1	60%		0		
[10-20 washes s [	1	45.5%		1	4.3%	
Alpha-cypermethrin	2		**Interceptor**	2		**Interceptor**
[0-10 washes [	1	1.4%		1	1.5%	
[10-20 washes s [	1	0.8%		1	2.9%	
Total	**5**			**5**		

Sun drying of LLINs after washing was a widespread practice in urban Kribi (68 households /100). While shade drying was a common practice in rural areas (62 households//114) (Table 3). Hole proportionality indices were significantly higher for sun-dried than shade-dried LLINs in both rural (p=0.001) and urban Kribi (p=0.042) (Table 5). On the other hand, there was no correlation between the life span of the LLINs and the hole proportionality indices in the two study sites (Table 3).

Mosquito Nets made of polyester fiber were proportionally more represented than those made of polyethylene in both rural and urban areas (Table 3). Analysis of this table indicated that the nets that were available or made available to householders were more likely to be made of polyester fiber.

Residual bioefficacy of used LLINs

The mortality rates of field strains according to brands and LLINs washing frequencies are recorded in Table 4. It can be seen from this table that mortality rates decrease with increasing washing frequencies of Olyset Net, PermaNet and Interceptor LLINs in both rural and urban Kribi (Table 4).

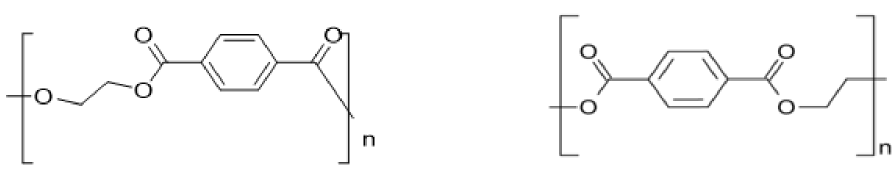
Discussion

LLINs technology was developed to address the low re-treatment rate of locally treated nets, which should be compulsorily re-treated after six months of use **[17, 33]**. Given the benefits of LLINs, their distribution and use have become a priority for national malaria control programs **[18-21]** because, according to Carnevale **[19,22]**, net promotion will only be effective if it is part of a package of measures to improve the quality of life of the population.

The rate of use of LLINs was 73.53% in rural Kribi and 79.30% in urban Kribi. This relatively high rate of LLINs use could be correlated with the high culicid density found in some studies in the study area, which is considered malaria endemic **[20, 30, 33]**. Our results are consistent with those reported in Benin by Moiroux *et al* **[31]**. The latter showed a positive correlation between culicidal nuisance and effective use of LLINs by vulnerable groups. The results show that the coverage rates reported in our work are well above the minimum coverage rate recommended by WHO for universal and effective coverage. These high coverage rates are to the advantage of the study areas because, according to WHO, a large coverage of a population (>80%) with impregnated mosquito nets would provide protection even for those who do not sleep under them **[33]**. It would also reduce transmission, but especially the incidence rate of malaria fevers, as shown by the mass introduction of lambda-cyhalothrin-impregnated nets in the northern savannah zone of Côte d'Ivoire **[25-26]**. Furthermore, the coverage rate in rural Kribi was significantly higher than in urban Kribi. This result can be explained by the availability of shops in urban areas, which favours the acquisition of other means of protection such as sprays and fans by local residents. Similar results were obtained during a survey conducted in Manoka and Youpwé, rural and urban localities in the city of Douala and Mvoua, in south-Cameroon **[18, 27]**. In addition, the absence of some heads of households at the time of distribution could also explain this difference in coverage in the two areas.

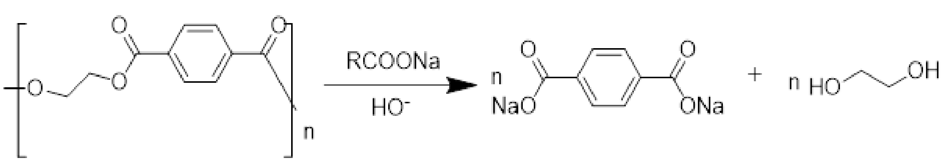
To be effective in its protective role, a mosquito net should constitute an impenetrable physical barrier to mosquitoes. Indeed, given its architectural characteristics (polyester or polyethylene fibers, mesh size 156, i.e. 25 holes/cm^2), a LLIN was designed to allow only organisms the size of an adult mosquito to pass through **[23-24]**. However, wear and tear over time, maintenance (frequency of washing, type of soap used, method of drying) etc. are all factors that can impact on the physical integrity of LLINs. Our study showed a positive correlation between the proportionality indices of holes (pHI) of LLINs and the frequency of washing in rural Kribi and urban Kribi. These results are consistent with those recorded in a study conducted in Ayos in the central region of Cameroon **[28-29]**. Indeed, the stresses placed on the net fibers by the high frequency of washing would lead to the appearance of type I holes which, and with further usage and washing, these holes will enlarge to size II, III or even IV. Similar observations have been made in neighborhoods in the city of Douala (Cameroon) [**28**]. The nature of the detergent used during washing could also be the cause of net deterioration. Our work shows that the hole proportionality indices were lower for LLINs washed with ordinary soap (lumpy) than those washed with corrosive detergents (powdered soap), both in rural and urban Kribi. This result is attributed to the fact that dirty LLINs are soaked in water containing detergent for several minutes or even hours before being washed. This washing process causes chemical interactions between the detergent molecules on the one hand and the polyester or polyethylene of the LLINs on the other hand; the chemical reactions thus established could contribute to the embrittlement of the LLINs fibers. In contrast, with lump soap, such a soaking process does not take place and the LLINs are washed directly. The majority of today's polyester fibers are composed of terepthalic acid and ethylene glycol (PET) **[34]**.

As an illustration, some nets are made from polyethylene terephthalate plastic (A) and polyester (B) with the following structures

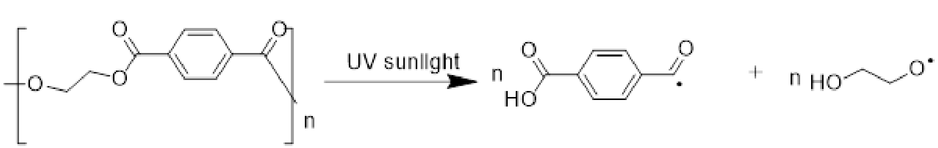


A: Polyethylene terephthalate structure B: Polyester structure

In a soapy solution (basic medium), these esters could undergo basic hydrolysis leading to long-term degradation of the net.

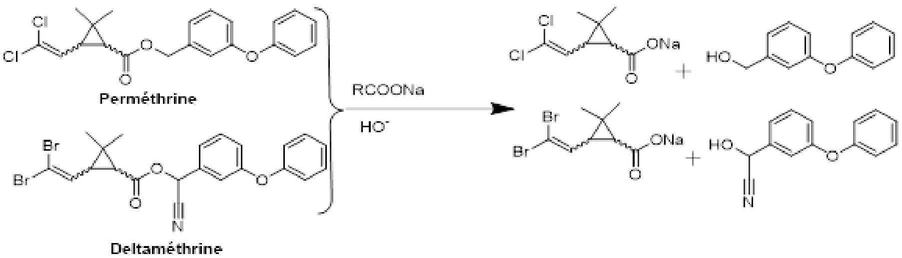


A similar analysis could be undertaken to explain the fragility of LLINs spread out in the sun after washing, compared to those spread out in the shade, as observed in our study.



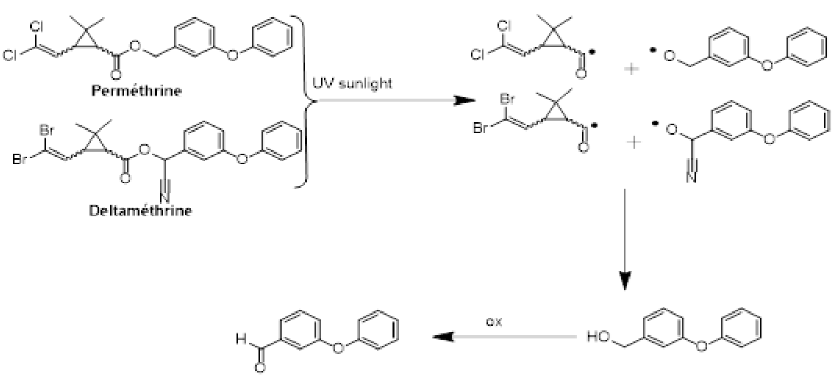
In our view, awareness campaigns should be organized on a regular basis by community health workers to teach people how to maintain a LLIN.

The results of insecticide tests inform us about the impact of poor LLINs maintenance on the effectiveness of the impregnating insecticide. Our results show a gradual loss of insecticide impregnation with the frequency of washing (Table 4). This was also the case for Olyset Net in Kribi-urban, PermaNet in Kribi-rural and Interceptor in rural and Kribi-urban (Table 4). The Olyset Net LLINs is a new type of net developed by Sumitomo Chemical Co Ltd (Japan), in which the insecticide (permethrin used at a concentration of 2% w/w, i.e. about 900mg/m2) is incorporated by fusion into a fiber made of polyethylene resin. Compared to impregnation by dipping, where the product is simply deposited on the fibers, the Olyset Net® manufacturing process integrates the insecticide into the support at the time of its polymerization. According to Darriet *et al* (2007), these nets have an efficacy period of three years and may retain their effectiveness after 30 washes. However, when washed with a corrosive detergent, there will be a loss of insecticide efficacy according to the chemical equation:



Indeed, Permethrin and Deltamethrin are very unstable in basic media so that at pH = 9, their life span is considerably reduced, in fact these molecules undergo basic hydrolysis which denatures them. In contrast, both insecticides are very stable in acidic media. It is therefore advisable to wash the net with water containing vinegar, which contains acetic acid and is less irritating in small doses (Azebaze, comm. pers.).

As for the PermaNet® brand mosquito net, it is a net in which the insecticide, in this case Deltamethrin dosed at 50mg/m2, is mixed with a resin that coats the polyester fibers. The pyrethroid attached to the substrate is gradually released by the resin, so that the net remains effective even after repeated washing. Laboratory tests on *A. gambiae* with WHO cones showed that after 20 washes, a three-minute contact of mosquitoes with the treated netting still induced a 100% knock-down effect (KD), while in terms of mortality, a PermaNet® washed 20 times still killed 50% of the batches of anopheles **[29,32]**. From the above, it can be suggested that the early decline in efficacy obtained in our results after 20 washes of polyester fiber nets is also due to washing with corrosive products and exposure to the sun after washing. Indeed, if the corrosive product hydrolyses the insecticides in question according to the chemical equations stated above, the sun's rays act on these insecticides according to the following photochemical reaction:



This equation shows that the sun has a highly damaging effect on permethrin and Deltamethrin. Beyond the fact that it causes cleavages, oxidations and cis or trans isomerization’s, it generally leads to the formation of free radicals which would be carcinogenic.

Conclusion

From this study, it appears that the frequency of washing, the detergents used and the place of drying (shade or sun) are factors likely to affect the physical integrity and biological effectiveness of a LLINs. While it is not always easy to standardise washing and drying procedures under local conditions, awareness of maintenance and good housekeeping can enable the adoption of good practices that can contribute to increasing the duration of the physical integrity and effectiveness of the LLINs in an operational situation.

Abbreviations

LLINs= Long-Lasting Insecticidal Nets

NMCP= National Malaria Control Program

VCP= Vector Control Policy

PHIs= Proportionate Hole Index

PET= Poly-Ethylene Terephthalate

Declarations

Informed consent and ethical considerations

Ethical approval for this study was obtained from the Faculty of Science of the University of Douala (UD). Administrative authorization was obtained jointly from the sub-prefects of the two districts and the prefect of the Ocean Department (South Cameroon) at N°497/AR/L11/A1. And all study participants gave their informed consent.

Competing interests

The authors declare that they have no competing interests

Consent for publication

Not applicable

Funding

Not applicable

Authors' contributions

LSEN, PAN and RSM designed the study for the study. RSM, OENH, FNNT, RN carried out the field work. RSM, WDT, RN and FNNT performed the laboratory analyses. SRM, PAA, FNNT and WDT performed the data analysis. LSEN, CT and PAN supervised the study. RSM, PAN, PAA and CT wrote the original draft. EWE, PNA, LSEN, CT, PAA and RSM reviewed and edited the final draft. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank the entire population of the Ocean Department for their collaboration.

References

1. Organisation mondiale de la santé (OMS), 2019 Rapport sur le paludisme dans le monde 2018
2. Ministère de la santé du Cameroun (MINSANTE) 2019. Prévalence du paludisme au Cameroun. Severe Malaria Observatory
3. Ministère de la santé du Cameroun (MINSANTE) 2016. Plan stratégique national de lutte contre le paludisme
4. Lindsay SW, Gibson ME (1988) Bednets, revisted-old idea, new angle. Parasitol Today 4:270–2
5. Lengeler C (2004) Insecticide treated bed nets and curtains for preventing malaria (Cochrane review) Cochrane Database Syst Rev 2:CD000363
6. Maxwell CA, Chambo W, Mwaimu M, et al (2003) Variation of malaria transmission and morbidity with altitude in Tanzania and with introduction of alphacypermethrin treated nets. Malaria Journal 2:28

7. WHO (2005) Guidelines for laboratory and field testing of longlasting insecticidal mosquito nets, WHO/CDS/WHOPES/GCDPP/2005.11, 24p
8. Kilian A, Byamukama W, Pigeon O, et al (2008) Long-term field performance of a polyester-based long-lasting insecticidal mosquito net in rural Uganda. Malaria Journal 7:49. doi: 10.1186/1475- 2875-7-49
9. Githinji S, Herbst S, Kistemann T, Noor AM (2010) Mosquito nets in a rural area of Western Kenya: ownership, use and quality. Malaria Journal 9:250. https://doi.org/10.1186/1475-2875-9-250
10. Azondekon R, Gnanguenon V, Oke-Agbo F, et al (2014) A tracking tool for long-lasting insecticidal (mosquito) net intervention following a 2011 national distribution in Benin. Parasites and Vectors 7:6. https://doi.org/ 10.1186/1756-3305-7-6
11. Shirayama Y, Phompida J, Kuroiwa C, Miyoshi M, Okumura J, Kobayashi J. comportement d'entretien et moustiquaires imprégnées d'insecticide de longue durée (LLITN) précédemment introduites dans le discrit de Bourapar, province de Khammouane, RDP Lao. Santé publique 121(2), 122-129, 2007.
12. service météorologique de la région du Sud : Ebolawa, 2018
13. Anonyme, 2010 Anonyme, Enquête démographique et de santé et à indicateurs multiples, Côte d'Ivoire. Institut National de la statistique, 42 p, 2011-2012.
14. Mbongue RS, Akono PN, Ngo Hondt OE, Magne Tamdem G, Nopowo NT, Offono LE, et al. Knowledges, Attitudes and Practices of Household Heads on Malaria in Urban and Rural Areas of Kribi, South-Cameroon. Austin Journal of Public Health and Epidemiology. 2020; 7(1): id 1088
15. WHO, 2011 Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions, WHO/HTM/NTD/Whopes/2011.5, 44p, 2011
16. Maxwell CA, Chambo W, Mwaimu M, et al (2003) Variation of malaria transmission and morbidity with altitude in Tanzania and with introduction of alphacypermethrin treated nets. Malaria Journal 2:28
17. Guillet P, Alnwick D, Cham MK, et al (2001) Long-lasting treated mosquito nets: breakthrough in malaria prevention. Bull World Health Organ 79:998
18. OMS. Rapport sur le paludisme dans le monde 2018. Le rapport de cette année en un clin d'œil. Novembre 2018.
19. Carnevale Pierre, Robert Vincent, Manguin Sylvie, Corbel Vincent, Fontenille Didier, Garros Claire, Rogier Christophe, Roux J. (préf.). (2009). *Les anophèles : biologie, transmission du Plasmodium et lutte antivectorielle.* Marseille : IRD, 391 p. (Didactiques). ISBN 978-2-7099-1662-2. ISSN 1142-2580.
20. Ngninpogni D.M, Ndo C, Akono P.N, Nguemo A, Nguepi A, Metitsi D.R, Tombi J, P Awono-Ambene, Bilong Bilong C. F. Insights into factors sustaining persistence of high malaria transmission in forested areas of sub-Saharan Africa: the case of Mvoua, South-Cameroon. (2021) 14:2 https://doi.org/10.1186/s13071-020-04525-0
21. Atkinson J, Bobogare A, Fitzgerald L, et al (2009) A qualitative study on the acceptability and preference of three types of long lasting insecticide-treated bed nets in Solomon Islands: implications for malaria elimination. Malaria Journal 8:119. https://doi.org /10.1186/1475-2875-8-119
22. Richard A, Zoulani A, Lallemant M, Trape JF, Carnevale P, Mouchet J , Malaria in the forest region of Mayombe, People's Republic of the Congo. I. Presentation of the region and entomologic data. Annales de la Societe Belge de Medecine Tropicale, 01 Dec 1988, 68(4):293-303
23. J. Etang, F. Chandre, P. Guillet , L Manga. Reduced bio-efficacy of permethrin EC impregnated bednets against an Anopheles gambiae strain with oxidase-based pyrethroid tolerance. Malaria Journal 2004, 3:46 doi:10.1186/1475-2875-3-46
24. WHO (2013) Guidelines for laboratory and field-testing of long lasting insecticidal nets, WHO/HTM/NTD/WHOPES/2013.3, 89p,
25. MC Henry, SB Assi, C R, J Dossou-Yovo, F Chandre, P Guillet, AND P Carnevale, protective efficacy of lambda-cyhalothrin treated nets in anopheles gambiae pyrethroid resistance areas of côte d'ivoire, The American journal of tropical medicine and hygiene 73(5), 2005, pp. 859–864
26. S.B. Assi, M.C. Henry, C. Rogier, I. Nzeyimana I.et Kone M., Evaluation de la sensibilite de Plasmodium falciparum a la chloroquine dans la region de savane au Nord de la Cote d'Ivoire. Bulletin de Société Pathologique et Exotique ; 97 (3) : 177-179, 2004.
27. Mbida AM, Ntonga PA, Awono-Ambene P, Tchoffo R, Talipouo A, et al. (2018) Insecticidal Nets versus kdr-Resistant Mosquitoes. Current Trends in Entomology and Zoological Studies : CTET-106. DOI: 10.29011/CTEZS-106. 100006
28. F.N. Nopowo • L. Offono Enama • H.G. Tsila • A. Mbida Mbida • C. Tonga • E. Ngo Hondt • R. Mbongue •S. Kekeunou • J. Mekoulou Ndongo • P.N. Akono. Évaluation de l'efficacité des moustiquaires imprégnées 36 mois après leur distribution dans le Sud

Cameroun. Bulletin de Société Pathologique et Exotique. (2020) 113:289-297. DOI 10.3166/bspe-2021-0159

29. Corbel V., Chabi J., Dabiré R., Etang J.D., Nwane P., Pigeon O., Akogbéto M., Hougard J.M., 2010. Field efficacy of a new mosaic long-lasting mosquito net (PermaNet® 3.0) against pyrethrinoid-resistant malaria vectors: a multicentre study in Western and Central Africa. Malaria Journal, 9, 13
30. Lindblade K A., Ellen D., Wiliam A., Hawley A., Nabie B., John W., Dwight M., George O., John V., Laurence S. and John G., 2005. Evaluation of long lasting insecticidal nets after 2 years household use. Tropical Medicine & International Health 10, S1141-1150, 2005
31. Moiroux N, Boussari O, Djènontin A, et al (2012) Dry season determinants of malaria disease and net use in Benin, West Africa. PLoS One 7:30558. doi: 10.1371/journal.pone.0030558
32. Morgan J, Abílio AP, do Rosario-Pondja M, et al (2015) Physical durability of two types of long-lasting insecticidal nets (LLINs) three years after a mass LLIN distribution campaign in Mozambique, 2008–2011. American Journal of Tropical Medicine and Hygiene 92:286–93. doi: 10.4269/ajtmh.14-0023
33. P. Carneval, Moustiquaires impregnees et paludismes, Medecine Tropicale 1998 ; 58 : 243-244, 1998.
34. Eun Kyung Choea, Eun Jong Sonb , Beom Soo Leec , Sung Hoon Jeongc , Hyun Cheol Shind , Jang Seong Choid. (2006) NF process for the recovery of caustic soda and concentration of disodium terephthalate from alkaline wastewater from polyester fabrics. Desalination 186 (2006) 29–37

Figures

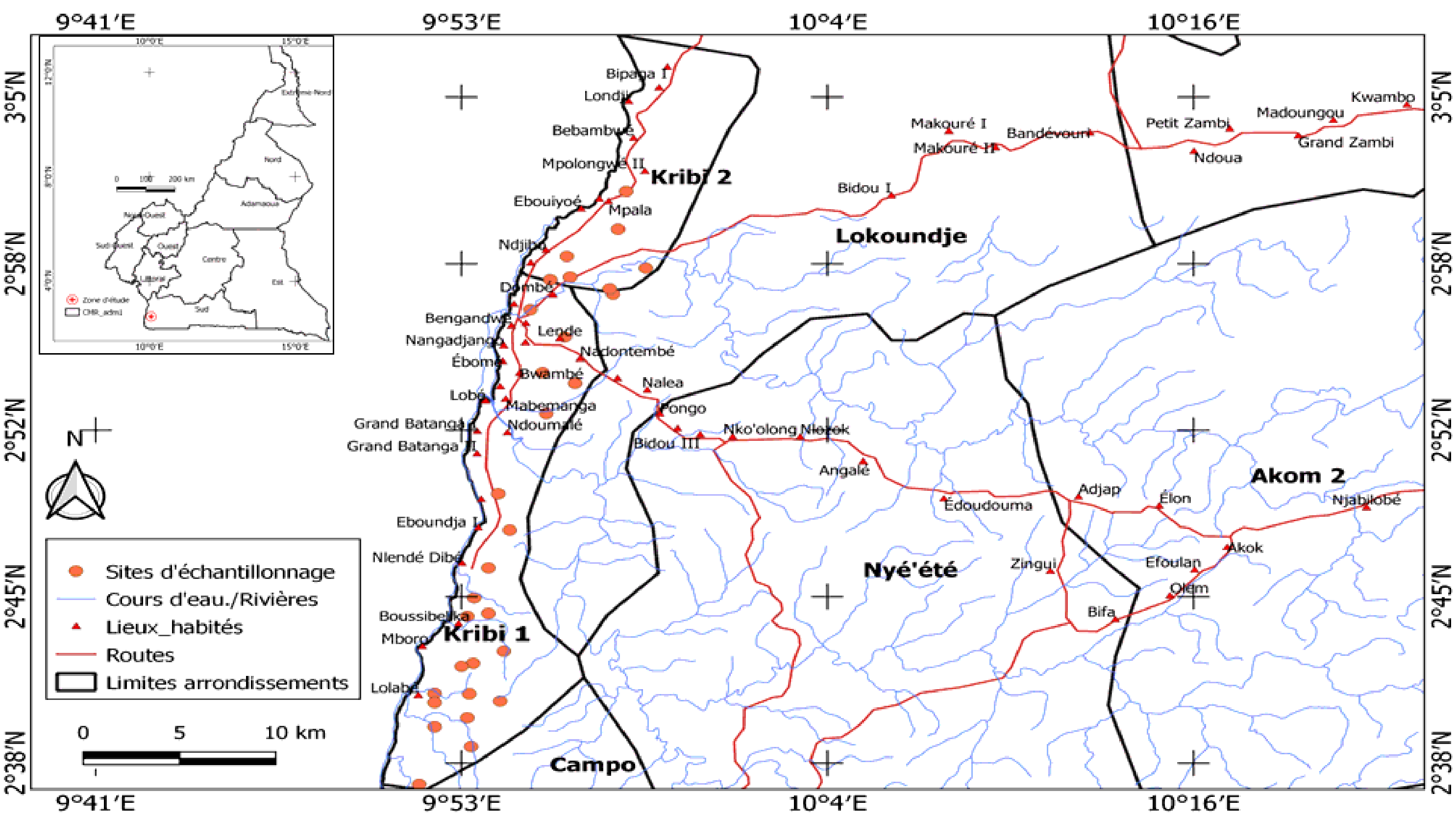


Figure 1

Location of sampling sites in Kribi 1 (rural) and Kribi 2 (urban)

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