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Research Article

Physical Integrity and Bioefficacy of Long-Lasting Insecticidal Nets Against Resistant *Anopheles Gambiae s.l.* at Ebolowa (Cameroon)

Intégrité Physique et Efficacité Biologique des Moustiquaires Imprégnées d'Insecticide de Longue Durée Contre la Population Résistante d'Anopheles Gambiae s.l. à Ebolowa (Cameroun)

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ABSTRACT

Introduction. Cameroon massively distributed long-lasting insecticidal nets (LLINs) between 2004 and 2015. In the Southern Region, 75% of households had access to at least one LLIN, compared with 54% who used them. Despite these efforts, the malaria prevalence rate among children under the age of 05 remained one of the highest (33%), just behind the East Region (35%). To understand the paradox between the high prevalence rate despite the high use of LLINs, the aim of this study was to assess the physical integrity and biological efficacy of LLINs used in households in Ebolowa.
Methods. Physical integrity was assessed by calculating the proportional hole index (pHI) of each net inspected according to WHO recommendations, using the formula: pHI = (n tr1) + (n tr2 × 23) + (n tr3 × 196) + (n tr4 × 576). The biological efficacy of LLINs was assessed by comparing the mortality rate of field mosquitoes with that of sensitive laboratory mosquitoes of the "kisumu" type exposed to used field LLINs on the one hand, and to new LLINs of the same brand on the other. **Results.** Assessment of physical integrity showed that: 72% was degraded; 20% acceptable and only 8% in good condition. In terms of biological efficacy, all the LLINs tested were effective only against the sensitive laboratory mosquito "kisumu". PBO-based LLINs are more suitable for vector control in Ebolowa. **Conclusion.** The majority of LLINs used in Ebolowa households are degraded.They are effective only against laboratory-susceptible mosquitoes. Resistance mechanisms would explain the loss of effectiveness of LLINs against field mosquitoes.

RESUME

Introduction. Le Cameroun a massivement distribué des moustiquaires imprégnées d'insecticide à longue durée d'action (MIILDA) entre 2004 et 2015. Dans la Région du Sud, 75% des ménages ont pu disposer d'au moins une MILDA contre 54% qui en ont fait usage. Malgré ces efforts, le taux de prévalence du paludisme chez les enfants de moins de 05 ans y est resté l'un des plus élevé (33%) juste derrière la Région de l'Est (35%). Pour comprendre le paradoxe entre le taux élevé de prévalence malgré la forte utilisation des MILDA, l'objectif de cette étude était d'évaluer l'intégrité physique et l'efficacité biologique des MILDA utilisées dans les ménages à Ebolowa. **Méthodes.** L'intégrité physique a été évaluée en calculant l'indice proportionné de trou (pHI) de chaque moustiquaire inspectée suivant les recommandations de l'OMS, selon la formule : pHI = (n tr1) + (n tr2 × 23) + (n tr3 × 196) + (n tr4 × 576). L'efficacité biologique des MILDA était évaluée en comparant le taux de mortalité des moustiques de terrain à celui des moustiques de laboratoire sensibles de type "kisumu" exposés aux MILDA usagées de terrain d'une part, et aux MILDA neuves de même marque, d'autres part. **Résultats.** L'évaluation de l'intégrité physique a montré que : 72% était dégradé ; 20% acceptable et seulement 8% en bon état. Pour l'efficacité biologique, toutes les MILDA testées étaient efficaces seulement contre les moustiques sensibles de laboratoire "kisumu". Les MILDA à base de PBO sont plus indiquées pour la lutte anti-vectorielle à Ebolowa. **Conclusion.** La majorité des MILDA utilisées dans les ménages à Ebolowa sont dégradées. Elles sont efficaces seulement contre les moustiques sensibles de laboratoire. Les mécanismes de résistance expliqueraient la perte d'efficacité des MILDA contre par les moustiques de terrain.

HIGHLIGHTS

What is known of the subject

After massive distributions of long-lasting insecticidal nets (LLINs) in Cameroon between 2004 and 2015, studies show that 75% of households have at least one LLIN compared to 54% who use them in the Southern Region. However, malaria prevalence remains high among children under the age of 05 in the region, despite high household use of LLINs

The aim of our study

Physical integrity and biological effectiveness of LLINs used in households in Ebolowa.

Key Results

1. The evaluation of physical integrity showed that 72% of LLINs were degraded; 20% acceptable and only 8% were in good condition.
2. As for biological effectiveness, all of the tested new and used LLINs were effective only against laboratory "kisumu" mosquitoes (knock-down (KD60) > 95% and/or average mortality rate (TM24) > 98%), but ineffective against field mosquitoes (knock-down (KD60) < 50% and/or average mortality rate (TM24) < 50%).
3. However, the "Olyset plus®" brand LLINs appeared to be the most effective because of the presence of piperonyl butoxide.

Implications for future practices and policies

Strengthen household capacities in the proper use and maintenance of LLINs, study resistance mechanisms in vector species in Ebolowa and adapt LLINs to the resistance context.

INTRODUCTION

Malaria remains a major public health issue in the world in general and in sub-Saharan Africa in particular (WHO, 2021). In Cameroon, malaria killed 4121 people in 2020, 64% of whom were children aged 0-5 years (NMCP, 2020). During the same period, 2,646,139 confirmed cases were reported in 2020 (compared to 2,628,191 cases in 2019), with 32% of children aged 0-5 years affected. Malaria accounts for 29% of consultations in health facilities and 40% of hospitalizations (NMCP, 2020). To reduce the burden of this disease, Cameroon has adopted the vector control strategy based the use of long-lasting insecticide-treated nets (LLINs) like most African states since the 2000s (Bhatt et al., 2015). The latter aims at eliminating or limiting human-vector contact to prevent infection by parasites (Pagès et al., 2007). In fact, insecticide-treated nets saved about 7 million people in Africa between 2000 and 2015, an average of 1,200 per day (Bhatt et al. 2015a). In addition, they reduce morbidity by 50% and overall mortality by 20-30% in children aged 0-4 years (Zaim et al. 2000). Given these beneficial effects associated with the use of insecticide-treated nets (ITNs), the Government of Cameroon, organized several free distribution campaigns to populations, of ITNs of different brands (PermaNet, Olyset, Interceptor...) between 2004 and 2015 (Antonio-Nkondjio et al., 2019): (i) the first, in 2004-2005, distributed up to 2 million ITNs to pregnant women and children under 5 years old;(ii) the second campaign, conducted in 2011, shifted from ITNs to long-lasting insecticidal nets (LLINs) by distributing up to 8 million LLINs to the general population; (iii) while the third, in 2015, distributed more than 12 million long-lasting insecticidal nets (LLINs) to the general population.

It should be noted that LLINs were produced as an alternative to re-treating conventional nets. These nets are industrially pre-treated by specific processes that allow them to be effective after at least 20 washes and to retain their insecticidal properties for three (03) to five (05) years in normal use (WHO, 2005). They are impregnated with insecticides of the pyrethroid family, which are incorporated into the polyester or polyethylene fibers during manufacturing. These slow-release insecticides migrate from the fabric to the surface of the net, causing a deterrent, repellent or excito-repellent effect. In Cameroon, studies showed that 77% of the population owned at least one treated net and 58% of the population used them regularly (Antonio-Nkondjio et al., 2019). However, given the conditions and duration of use of these LLINs by households, critical questions regarding their functional life and variation in performance in operational situations arise. According to the results of studies conducted on the physical integrity of LLINs, Nopowo et al., (2020), in a study carried out at AYOS in Cameroon, showed that a significant number of LLINs (17%) were no longer able to play their physical barrier role effectively because of holes observed after three years of use. In Senegal and Uganda, 45-78% of nets were damaged after one year of use in operational conditions (Kilian et al., 2008; Diouf M et al., 2018). Furthermore, results from studies conducted in Kenya and Benin on the bioefficacy of LLINs showed a faster than expected deterioration in bioefficacy, highlighting, the effective lifespan of LLINs (Githinji et al., 2010; Azondekon et al., 2014). In another study conducted in Laos, it was shown that approximately 40% of nets were physically damaged after two to three years of use (Shirayama et al., 2007). As a result of the mass distribution campaigns that were initiated, people in the southern region in Cameroon, as well as in other parts of the country, had access to millions of LLINs. Household ownership of LLINs reached 75% with 54% usage (DHS, 2018). However, malaria remained endemic and continues to claim families in Southern Cameroon. The South region is second among the 10 regions of the country in terms of malaria prevalence among children under 05 years of age (33%), just behind the EAST region (35%) (DHS, 2018). The objective of this study was to assess the physical integrity and biological efficacy of seven brands of household-used LLINs sampled in four (04) neighborhoods of the city of Ebolowa in the Southern Region after the 2015 mass distribution campaign.

SUBJECTS AND METHODS

Study Site

Students This study was conducted between September and October 2022 (rain season) in Ebolowa, (2° 54′ North, 11° 09′ East) (Fig 1). This town is located at the heart of the equatorial forest in the Southern Region and is linked to Yaoundé, the political capital of Cameroon, by a 168km tarmac road. This locality of 56 km^2 had an estimated population of around 250,000 in 2019, with an approximate density of 4,464 inhabitants/Km2 (BUCREP, 2020).

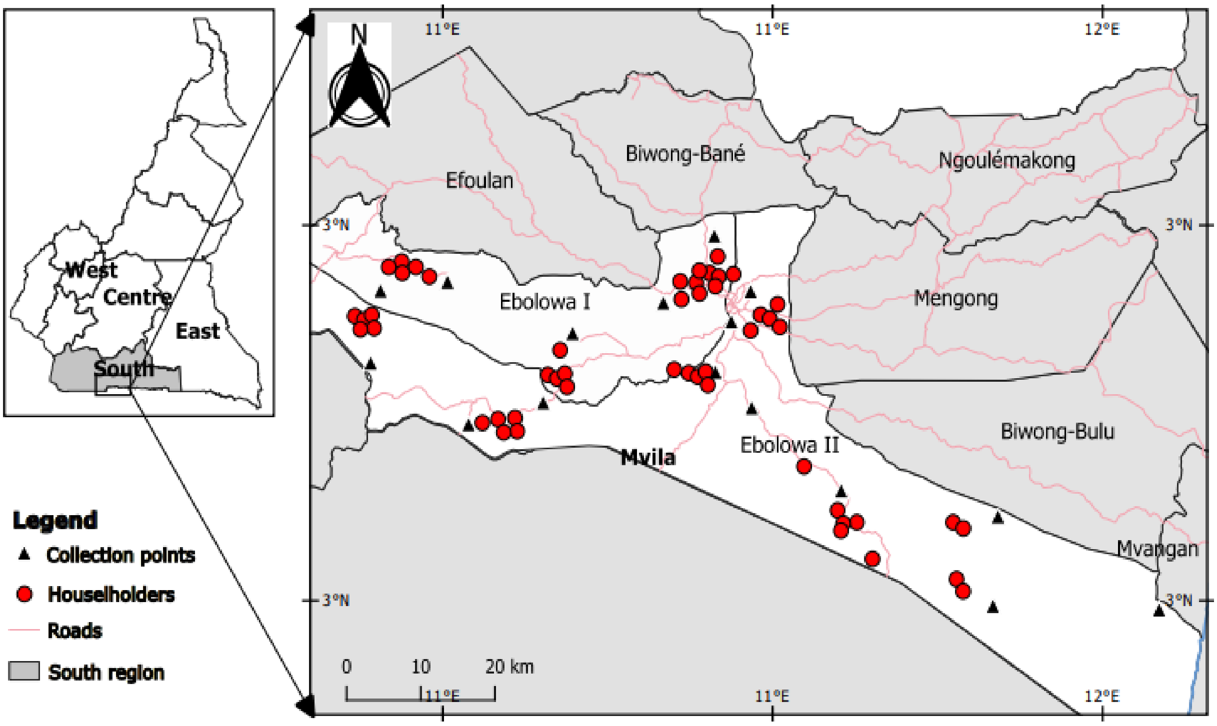

Figure 1. Map showing location of Ebolowa, larval and LLINs collection points

The vegetation around the town consists of an equatorial forest that has been degraded by farming activities. The climate is of equatorial Guinean type, with two rainy seasons (August-October and April-June) and two dry seasons (November-April and June-July). The average annual rainfall is 2,000 mm, while the average annual temperature is 25°C and the average annual humidity 95%. The town is also characterized by numerous marshy areas and above all the presence of an artificial lake, surrounded by vegetation that can favors development of aquatic stages of several mosquito species. People live in mud, plank and/or breeze-block houses with tin roofs, with several opens between the roof and the walls through which mosquitoes can enter or leave the houses. Moreover, the area is known to be hyperendemic for malaria whom transmission is supported by highly insecticide resistant *An. gambiae* s.l. mosquitoes (Efa et al.,2022). The study was carried out in four (04) neighborhoods of the town, namely: Nko'ovos, Mekalat, Ebolowa Si 1 and Ebolowa Si 2, chosen from two health areas because of their high population density.

Sample collection

LLINs survey and sampling

Nets of different brands in use in different households at the time of the study were collected during a cross-sectional survey. In each of the four-study neighborhood, 12 households were randomly selected and their heads or his/her representative were submitted to a questionnaire to assess origin, coverage, use and maintenance of LLINs. Subsequently, one used LLINs, preferably that of the respondent (head of household) was collected and replaced by a new LLINs of the same type. The level of use was defined and classified in three groups:

1. “regular” if the respondent declared the household use their LLINs every night of the week preceding the survey;
2. “irregular” if the respondent declared the household only used LLINs for a few nights;
3. “not used” if the respondent declared the LLINs were not used during the whole week.

All the nets sampled were labelled by a code formed using initial letters of the brand and the neighborhood, and they were stored in plastic bags for later evaluation of their bioefficacy.

Mosquito colonies

Two mosquito colonies were used for the experiments. The An. gambiae Kisumu strain bred at the CRID (Centre for Research in Infectious Desesases) laboratory. This is a reference strain that is susceptible to all insecticides. It has been colonized from mosquitoes collected in the locality of Kisumu (Kenya) and had been maintained for several generations at the insectary of the Kenya Medical Research Institute and has since been extensively used in bioassays experiments across Africa.

The wild F1 An. gambiae s.l. colony from Ebolowa that has been established using larvae collected from typical sunny water pools throughout the four (04) selected neighborhoods. Larvae of all stages were collected using the "dipping" method (Silver, 2008), and were transferred to the insectary. They were fed on TetraMin® fish food (Tetra Holding, Inc., Blacksburg, VA, U.S.A.). Pupae were sorted daily and placed into cages covered with mosquito netting until the emergence of the imagos. The latter were then fed with a 10% glucose solution.

LLINs physical integrity assessment

The sides and the roof of each LLINs sampled were carefully visually inspected. Then the number, size and location of tears were assessed following WHO recommendations (WHO, 2005). The tears were grouped into four categories as per size (Kilian et al.,2008):

- size 1: smaller than one inch (0.5 cm ≤ size 1 ≤ 2 cm);
- size 2: larger than an inch but smaller than a fist (2 cm < size 2 ≤ 10 cm);
- size 3: larger than a fist but smaller than a head (10 cm < size 3 < 25 cm);
- size 4: larger than a head (size 4 > 25 cm).

The proportionate hole index (pHI) was calculated for each net inspected by weighting each hole by its size and summing for each LLINs following WHO recommendations (WHO, 2013) according to the following formula: pHI = (n tr1) + (n tr2 × 23) + (n tr3 × 196) + (n tr4 × 576)

The results from the calculation of the proportionate hole index (pHI) allowed us to categorize the LLINs into three types according to the damage observed:

- good physical condition: if the pHI is between 0 and 64;
- acceptable physical condition: if the pHI is between 65 and 642;
- Degraded physical condition: if the pHI is 643 or higher.

Assessment of bio-efficacy of LLINs

The biological efficacy of the LLINs was assessed using cone tests following the WHO protocol (WHO, 2016). Tests were carried out at 27°C±2ºC and 70% ± 10% relative humidity Five net pieces, each 25 cm × 25 cm in size, were cut from 5 locations of each tested net (used, new or untreated) including the four sides and the roof. Four cones at a time were attached to a piece of netting, and ten 2-5 day-old An. gambiae females (Kisumu or wild type) at one time were introduced in each cone using a mouth aspirator. Ten Mosquitoes were exposed to each piece of netting for 3 min after which they were held in a paper cups with access to 10% sugar solution. . Mosquitoes exposed to untreated net pieces were used as negative controls and those exposed to new net pieces were used as positive controls. When the mortality rate in the controls was > 20%, the tests were discarded and new tests were carried out. On the other hand, when it was ≤ 20%, the mortality observed for insecticide exposure was corrected using Abbott's formula. Results were interpreted according to WHO criteria (WHO, 2016). Thus, an LLINs was said to be "effective" against the susceptible laboratory population strain "Kisumu" when it resulted in: a knockdown (KD60) > 95% and/or an average mortality rate (TM24) > 98%.

In contrast, when exposing "resistant" field mosquito populations, LLINs were:

- of "optimal efficacy" when the average mortality rate was > 80%, after 24 hours of observation.
- minimally effective" when the average mortality rate was between 50% and 80% after 24 hours of observation.
- "Ineffective" when the average mortality rate was < 50%, after 24 hours of observation.

Data analysis

Data were entered into Excel 2019 and analysed using SPSS 2020 software (IBM statistics version 20). Student's t-test was used to compare the mean mortality rate between the laboratory KISUMU susceptible strain and the field resistant strain for each new net and to assess the Knock-down (KD) effect per net for both strains of mosquito populations.The ANOVA test was used to compare the performance of the new nets. For the LLIN bioefficacy test, statistical analyses were performed using MedCalc's Comparison of proportions calculator, VassarStats and MS. Excel, 2013. Graphpad prism version 7.00 (GraphPad Software Inc., La San Diego, CA, USA) was used to construct the graphs. The statistical test was significant at the $\alpha = 5\%$ threshold, for a p value < 0.05.

RESULTS

A total of 54 nets were used for testing in this study, namely: 50 used LLINs sampled from households in the field and 04 new pyrethroid-only LLINs purchased from the National Malaria Control Programme. Seven brands of LLINs were inventoried in the field: DURANET®; INTERCEPTOR®; OLYSET®; OLYSET PLUS®; PERMANET 2.0®; SIAMDUTCH® and YORKOL®.

Origin, use and maintenance of LLINs

Overall, the results of the LLINs survey showed that 92% (46/50) of LLINs used by households in Ebolowa came from free mass distribution campaigns by Ministry of Public Health. The majority (84%) of nets sampled in households has been used for more than 2 years. The frequency of use was regular in 79% (n = 37) of cases, irregular in 18% (n = 9) and null in 8% (n = 4) of cases. As for the maintenance of the LLINs, 80% (n = 40) have been washed between 2 and 3 times, compared with 20% (n = 10) which have never been washed. The washing materials used were mainly standard recommended soap (36%, n = 18) and detergent powders (44%, n = 22). Upon washing, 62% (n = 31) of the nets were dried in the sun compared with 18% (n = 9) in the shade.

Physical integrity of LLINs used in households

The assessment of physical integrity showed that very few LLINs were in good condition overall, irrespective of brand. 72% (36/50) were degraded; 20% (10/50) acceptable and only 8% (4/50) in good condition. Taking into account the brand of LLIN, the study showed that Duranet® brand nets were the most deteriorated: 75% (3/4), followed by Olyset® brand LLINs: 73.52% (25/34); Olyset plus® brand LLINs: 60% (3/5) and finally Permanet 2.0® brand LLINs: 50% (2/4). However, there was no statistically significant relationship (P = 0.475) between physical condition and brand of LLIN, as shown in Table I below.

Biological efficacy of LLINs

Biological efficacy of LLINs collected from households

All the LLINs collected in households and tested showed were effective against the reference susceptible An. gambiae "Kisumu" strain, with regard to knock-down rates (KD60) > 95% and/or the average mortality rate (MR24) > 98%. No knockdown and very low mortality (2.5%) were observed in the test ran with untreated net, as shown in Table II below. A very contrasting pattern was observed when testing field F1 *An. gambiae* s.l. local mosquitoes since all the LLINs appeared ineffective. Overall, knockdown rates (KD60) after 60 minutes of exposure and 24-hour post-exposure mortality rates (MR24h) were extremely low (< 50%) for all brands of LLINs (table III). . Olyset plus® brand LLINs had the highest average mortality rate at 4.60%. For the other brands of LLINs, the average mortality rate varied between 0.25% and 4%. as shown in **Table III below.**

Biological efficacy of new LLINs against An. gambiae s.l. from Ebolowa

Exposure of the susceptible "kisumu" population to new LLINs

Exposure of a subsample of 250 'kisumu' laboratory mosquitoes to four (04) new Olyset®, Olyset plus®, Duranet® and Permanet 2.0® LLINs demonstrated that all were effective against 'kisumu' individuals. A KDR 60 rate of 100% and a mortality rate after 24 hours post-exposure (MR 24h) of 100% were obtained for Olyset® and Olyset plus® brand LLINs. For Duranet® and Permanet 2.0®, the KDR 60 rates were 100% and the 24-hour post-exposure mortality rate was 97.5% respectively.

Tableau I.Physical integrity of LLINs according to brand

Brand of MILDA	Numbers of different brands of LLINs sampled from households	Physical Status						P
		Acceptable		Good		Degraded		
	N	N	%	N	%	N	%	
DURANET®	4	1	25	0	-	3	75	
INTERCEPTOR®	1	0	-	0	-	1	100	
OLYSET®	34	7	20.6	2	6	25	74	
OLYSET PLUS®	5	0	-	2	40	3	60	P = 0.475
PERMANET 2.0®	4	2	50	0	-	2	50	
SIAMDUTCH®	1	0	-	0	-	1	100	
YORKOL®	1	0	-	0	-	1	100	
Total	50	10	20	4	8	36	72	

Tableau II. Mortality rate of kisumu strain mosquitoes exposed to field LLINs

LLINs brands	Number of LLINs tested (n)	Number of mosquitoes tested (n)	Kisumu mosquitoes' strain KDR 60 (±SE)	KDR 60 (±SE)	Status of field LLINs following exposure to laboratory "kisumu" mosquitoes' strain
Contrôle	7	350	0.00 (±0.00)	2.50 (±2.50)	
Yorkol	1	50	97.3 (±2.30)	98.52 (±2.67)	Optimum efficiency
Siamduct	1	50	96.7 (±2.30)	97.5 (±2.50)	Optimum efficiency
Interceptor	1	50	98 (±2.67)	100 (±0.00)	Optimum efficiency
Duranet	4	200	96.7 (±2.30)	97.5 (±2.50)	Optimum efficiency
Permanet 2.0	4	200	96 (±2.30)	97.5 (±2.50)	Optimum efficiency
Olyset	34	1700	97.3 (±2.40)	98.52 (±2.67)	Optimum efficiency
Olyset Plus	5	250	98 (±2.67)	100 (±0.00)	Optimum efficiency

Tableau III. Mortality rate of wild strain mosquitoes exposed to field LLINs

LLINs brands	Number of LLINs tested	Number of mosquitoes tested	field mosquito strains (*An. Gambiae s.l.*) KDR 60 (±SE)	MR 24h (±SE)	Status of field LLINs following exposure to field mosquito strains
Contrôle	7	350	0.00 (±0.00)	0.00 (±0.00)	
Yorkol	1	50	2.22 (±1.33)	2.00 (±1.33)	Ineffective
Siamduct	1	50	2.22 (±1.33)	2.00 (±1.33)	Ineffective
Interceptor	1	50	2.22 (±1.33)	4.00 (±4.00)	Ineffective
Duranet	4	200	0.00 (±0.00)	0.25 (±0.25)	Ineffective
Permanet 2.0	4	200	0.00 (±0.00)	0.50 (±0.33)	Ineffective
Olyset	34	1700	0.29 (±0.00)	1.41 (±0.13)	Ineffective
Olyset Plus	5	250	0.88 (±0.33)	4.60 (±1.63)	Ineffective

Tableau IV. Mortality rate of kisumu strain mosquitoes exposed to new LLINs

LLINs brands	Number of LLINs tested	Number of mosquitoes tested	Kisumu mosquitoes' strain		Status of new LLINs following exposure to laboratory "kisumu" mosquitoes' strain
			KDR 60 (±SE)	KDR 60 (±SE)	
Control	1	50	0.00 (±0.00)	2.50 (±2.50)	
OLYSET	1	50	100 (±0.00)	100 (±0.00)	Optimum efficiency
OLYSET Plus	1	50	100 (±0.00)	100 (±0.00)	Optimum efficiency
PERMANET 2.0	1	50	100 (±0.00)	97,5 (±2.50)	Optimum efficiency
DURANET	1	50	100 (±0.00)	97.5 (±2.50)	Optimum efficiency

As the knockdown rate at 60 minutes (KDR 60) and the mortality rate 24 hours post-exposure (MR 24h) for individuals of the susceptible strain "kisumu" were respectively greater than 95% (KDR 60) and 98% (MR 24h) for all the LLINs in the study, we concluded that all these new LLINs were "effective" against individuals of the susceptible laboratory strain "kisumu". as shown in Table IV below.

Table V. Mortality rate of wild strain mosquitoes exposed to new LLINs

LLINs brands	Number of LLINs tested	Number of mosquitoes tested	Field Strain (*An. gambiae* s.l)		Status of new field LLINs following exposure to field mosquito strains
			KDR 60 (±SE)	MR 24h (±SE)	
Control	1	50	0.00 (±0.00)	0.00 (±0.00)	
OLYSET	1	50	15.55(±6.29)	4.00(±2.67)	Ineffective
OLYSET Plus	1	50	97.77 (±2.67)	42.00 (±6.29)	Ineffective
PERMANET 2.0	1	50	13.33 (±4.42)	10.00 (±3.33)	Ineffective
DURANET	1	50	33.33 (±7.92)	36.00 (±9.33)	Ineffective

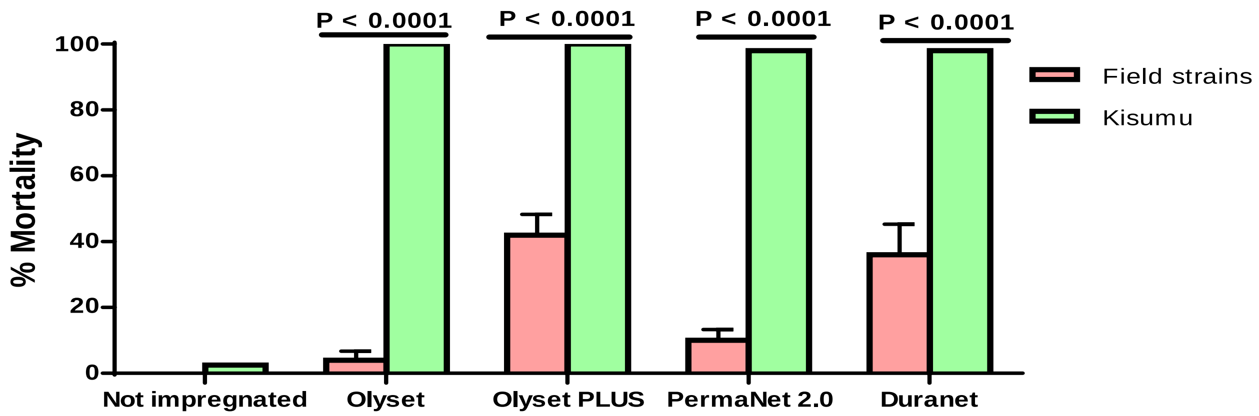

Figure 2. Comparison of mortality between the susceptible Kisumu strain and the field strain An. gambiae s.l. expoqed to new LLINs

Exposure of the wild population to new LLINs purchased from the NMCP

The exposure of 250 field mosquitoes from the "wild" strain to the same new LLINs showed that all these LLINs, although new, were "ineffective" against field mosquitoes, with mortality rates of less than 50% for all 04 LLINs. The 24-hour post-exposure mortality rates (TM24) obtained were 4% ± 2.67 for Olyset®, 42% ± 6.29 for Olyset plus®, 36% ± 9.33 for Duranet® and 10% ± 3.33 for Permanet 2.0®, respectively. Assessing the KD60 effect, we found a statistically significant relationship between the rate of field knockdown and mortality for Olyset® (t = - 2.05, ddl = 18, P = 0.027) and Olyset plus® (t = - 7.9, ddl = 18, P < 0.0001) LLINs. On the other hand, no statistically significant relationship was observed between the knock-down rate and the mortality rate for Permanet 2.0® (t = - 0.36, ddl = 18, P = 0.723) and Duranet® (t = 0.16, ddl = 18, P = 0.437) LLINs, as shown in Table V below. These results show that the performance of the 04 nets in the study is reduced overall. However, it was lower for Olyset® and Permanet® 2.0 and higher for Olyset plus® and Duranet®, which performed better than the first two (F = 0.75; ddl = 3; P < 0.0001). Since the mortality rates of individuals from field strains were less than 50% for all 04 new LLINs to which they were exposed, it can be said that these new LLINs are "ineffective" against field strains. Comparing the mortality rates of individuals belonging to the sensitive laboratory strain "kisumu" with those of the field strain, following their exposure to the same LLINs, we obtained a statistically significant difference in mortality ($P < 0.0001$) in favour of the Kisumu strain for all 04 nets, as shown in Figure 2 below. This result shows that the four (04) new Olyset®, Olyset plus® Permanet®2.0 and Duranet® LLINs are indeed "effective" against the laboratory susceptible strain, but "ineffective" or have a "reduced effectiveness" against individuals of the Ebolowa field strain, due to the resistance they would have developed.

DISCUSSION

The objective of this study was to assess the physical integrity and biological efficacy of seven brands of household-used LLINs (Olyset®, Olyset Plus®, PermaNet® 2.0, and Duranet®, Interceptor®, Siamdutch®, and Yorkol®) sampled in four (04) neighborhoods of the city of Ebolowa in the Southern Region after the 2015 mass distribution campaign. The results shows that the majority of LLINs in the field came from free distribution campaigns organized by the NMCP, while a minority came from market purchases. The Olyset® brand nets distributed free during the campaigns were the most frequent in households. It was observed a good use of LLINs in households in the different neighborhoods selected, with an average rate of regular use of LLINs of 79%. This rate of use could be explained by a strong nuisance of mosquitoes.ourresults corroborate those reported in Benin by Moiroux et al., (2012), who showed a positive correlation between nuisance and effective use of LLINs (Moiroux et al., 2012)., It would be important to raise public awareness of the importance of this tool in the fight against malaria.

The assessment of the Physical integrity of these LLINs showed that they were mostly degraded regardless of brand. This result is similar to those obtained in other studies conducted in others areas in Cameroon (Boussougou-sambe et al., 2017; Nopowo et al., 2020; Ngogang-Yipmo et al., 2022) and in others countries in Africa like: Senegal, Chad and Uganda, respectively, which showed that 45-78% of nets in operational use were damaged and only 30% were still in good condition after one year of use (Diouf et al., 2018; Allan et al., 2012; Killian et al., 2008). Unlike studies in Mozambique and Benin, (Morgan et al., 2015; Ossé et al., 2013), our results showed no statistically significant association between loss of physical integrity and LLIN brand. However, it is still worth noting that Duranet® brand LLINs were the most damaged followed by Olyset® brand LLINs. The evaluation of the bioeffectiveness of LLINs (Olyset®, Olyset Plus®, Permanet® 2. 0 and Duranet®) showed that both field and new LLINs were effective against individuals of the laboratory-susceptible strain "Kisumu" in terms of KD60 and mortality rates after 24 hours of exposure (between 90 and 100%). These results show that despite the poor maintenance of the LLINs by the population (in terms of the washing equipment used, the number of washes, the drying conditions, and their physical integrity), the LLINs in the field were able to retain a sufficiently effective dose of insecticide against the sensitive laboratory strain Kisumu. In contrast, exposure of field mosquitoes, known as "wild" strains, to LLINs collected from households in Ebolowa, showed that they were ineffective. Although LLINs are designed to withstand repeated, excessive, or aggressive washing that can rapidly reduce their useful life, insecticide retention and loss of residual bioeffectiveness could be explained by a wide range of variables such as the number of times a net was washed, the manner in which it was washed, exposure to UV light, handling and wear of the net, exposure to dust and rain, and the use of harsh detergents (Etang et al., 2013). One other reason that could explain the loss of efficacy of field mosquitoes towards LLINs collected from households could be the expression of resistance mechanisms in these mosquitoes (Efa et al., 2022). This idea is reinforced in our study by the results obtained when field mosquitoes were exposed to new LLINs. The results showed that new LLINs were just as ineffective against field mosquitoes as household LLINs. These results are comparable to those obtained from studies conducted in other regions of Cameroon where it was shown that pyrethroid-impregnated LLINs lost efficacy against field strains of An.gambiae s.l due to resistance mechanisms (Antonio Nkondjio et al., 2019; PNLP, 2018). In addition, Menze et al, (2018) obtained similar results with the same LLIN brands in An. funestus in Mibelon, Cameroon. The loss of efficacy of LLINs is thought to be due not only to the selection pressure induced by the massive and free distribution of LLINs by the Cameroonian government, but also to the regular use of pesticides in agriculture by the population, which is thought to be at the origin of the development of resistance that affects the efficacy of LLINs (Antonio-Nkondjio et al., 2019; Etang et al., 2016; Etang et al., 2013). Analysis of the performance of four brands of new LLINs showed that the Olyset Plus® brand LLIN impregnated with Permethrin + PBO had the highest mortality rate compared to the other three brands: Olyset®,Permanet® 2.0 and Duranet® impregnated with Permethrin, Deltamethrin and Alphacypermethrin respectively. This result shows that, despite the resistance of field mosquitoes to Pyrethrinoids in general, the combination of insecticide and PBO could mitigate this resistance. The level of performance observed with Olyset plus®, PBO-based nets in this study, is similar to results reported in other locations in Cameroon (Menze et al., 2018); Malawi (Riveron et al., 2015), DR Congo (Riveron et al., 2018). These studies have shown that PBO-treated nets should be considered as an alternative to pyrethroid nets in areas of high resistance, due to metabolic resistance mechanisms, including cytochrome P450s (Riveron et al., 2013).

CONCLUSION

The objective of our study was to assess the physical integrity and biological effectiveness of LLINs used by households in the city of Ebolowa. The study found that Olyset®, Olyset plus®, Duranet®, and Permanet 2.0® LLINs are the most commonly used in households in Ebolowa. In operational condition in the field, most of these LLINs have lost their physical integrity, exposing sleepers to mosquito bites. This observation is an alert for the National Malaria Control Program, which must set up a system of regular monitoring of LLINs in households. This will necessarily involve sensitizing households on best practices for the use and maintenance of LLINs, in order to preserve their physical integrity. In addition, the evaluation of the biological efficacy of LLINs showed a loss of efficacy against field mosquitoes for both field and new LLINs, indicating the presence of resistance mechanisms in these "wild" mosquito populations. However, it should be noted that despite their loss of efficacy, Olyset plus® LLINs had the highest mortality rates, indicating the importance of the pyrethroid + PBO combination in controlling resistance. Finally, it is important for the National Malaria Control Program to identify the resistance mechanisms present in Anopheles in the city of Ebolowa for a better control of resistance. In the meantime, insecticide-treated nets with PBOs constitute a better alternative in the framework of vector control in the city of Ebolowa. Indeed, it must be said that despite the ineffectiveness of LLINs due to the development of insecticide resistance observed in field mosquitoes, LLINs primarily protect against infectious mosquito bites, as pyrethroids have a repellent role for mosquitoes. On the other hand, LLINs also provide a physical barrier between sleepers and mosquitoes, as long as their physical integrity remains intact. This means that people must ensure the proper use of LLINs by implementing good practice in their use.

REFERENCES

1. Allan R, O'Reilly L, Gilbos V, Kilian A. An observational study of material durability of three World Health Organization Recommended long-lasting insecticidal nets in Eastern Chad. Am J Trop Med Hyg. 2012; 87:407-11.
2. Antonio-Nkondio, C., Ndo, C., Njiokou, F., Bigoga, J.D., Awono-Ambene, P., Etang, J., Ekobo, A.S., Wondji, C.S. Review of malaria situation in Cameroon: technical viewpoint on challenges and prospects for disease elimination. Parasit Vectors. 2019;12: 501.
3. Asidi A, N'Guessan R, Akogbeto M, Curtis C, Rowland M. Loss of household protection from use of insecticide-treated nets against pyrethroid-resistant mosquitoes, Benin. Emerging infectious diseases. 2012;18(7):1101.
4. Azondekon R, Gnanguenon V, Oke-Agbo F, et al. A tracking tool for long-lasting insecticidal (mosquito) net intervention following a 2011 national distribution in Benin. Parasit Vectors. 2014 ; 7:6. doi: 10.1186/1756-3305-7-6.
5. Bakwo, E., Akono-Ntonga, P., Belong, P., Messi, J. Contribution of mosquito vectors in malaria transmission in an urban district of Southern Cameroon Journal of entomology and nematology. 2010; 2: 13-17.
6. Bhatt S, Weiss D, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The efect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature. 2015;526(7572):207.
7. Boussougou-Sambe ST, Awono-Ambene P, Tasse GC, Etang J, Binyang JA, Nouage LD, et al. Physical integrity and residual bio-efficacy of used LLINs in three cities of the South-West region of Cameroon 4 years after the first national mass-distribution campaign. Malar J. 2017; 17;16(1):31. doi: 10.1186/s12936-017-1690-6. PMID: 28095873; PMCID: PMC5240192.
8. Delbene R. Etude socio-économique. Concession Forestière No 1050, UFA 09-017 et 09018. Département de la Mvila. Arrondissement d'Ebolowa et de Mvangan. MINFOF. Cameroun. 2003; 433.
9. Diouf M, Diouf EH, Niang EAH, Diagne CT, Konaté L, Faye O. Evaluation of Physical Integrity and Biological Efficacy of Two Types of LLINs Aged 5 to 36 Months Sampled in 11 Districts of Senegal. Bull. Soc. Pathol. Exot. 2018 ; 111:126-131.
10. : Efa S, Elanga-Ndille E, Poumachu Y, Tene B, Mikande JZ, Zakariaou N, Wondji CS, Ndo C. Insecticide Resistance Profile and Mechanisms in An. gambiae s.l. from Ebolowa, South Cameroon. Insects. 2022; 13: 1133. https://doi.org/ 10.3390/insects13121133
11. Enquête démographique de santé. Rapport Minsanté. 2018 ; 255-283.
12. Etang J, Nwane P, Piameu M, Manga B, Souop D, et al. Evaluation of New Tools for Malaria Vector Control in Cameroon: Focus on Long Lasting Insecticidal Nets. PLoS ONE. 2013; 8(9): e74929. doi:10.1371/journal.pone.0074929
13. Etang J, Pennentier C, Piameu M, Bouraima A, Chandre F, Awono Ambene P. When intensity of deltamethrin resistance in Anopheles gambiae (s.l.) leads to loss of long lasting insecticidal nets bio-efcacy: a case study in north Cameroon. Parasit Vectors. 2016;9:132.
14. Githinji S, Herbst S, Kistemann T, Noor AM. Mosquito nets in a rural area of Western Kenya: ownership, use and quality. Malar J. 2010 ; 9:250. doi: 10.1186/1475-2875-9-250
15. Kilian A, Byamukama W, Pigeon O, et al. Long-term field performance of a polyester-based long-lasting insecticidal mosquito net in rural Uganda. Malar J. 2008; 7:49.
16. Kilian A, Byamukama W, Pigeon O, et al. Long-term field performance of a polyester-based long-lasting insecticidal mosquito net in rural Uganda. Malar J. 2008 ; 7:49. doi: 10.1186/1475- 2875-7-49
17. Menze, B. D., Wondji, M. J., Tchapga, W., Tchoupo, M., Riveron, J. M. & Wondji, C. S. Bionomics and insecticides resistance profiling of malaria vectors at a selected site for experimental hut trials in central Cameroon. Malaria Journal. 2018; 17: 1-10.
18. Moiroux N, Bio-bangana AS, Djènontin A, Chandre F, Corbel V, Guis H. Modelling the risk of being bitten by malaria vectors in a vector control area in southern Benin, West Africa. Parasites & Vectors. 2013; 6: 71.
19. Morgan J, Abílio AP, Do Rosario-Pondja M, et al. Physical durability of two types of long-lasting insecticidal nets (LLINs) three years after a mass LLIN distribution campaign in Mozambique, 2008–2011. Am J Trop Med Hyg. 2015; 92:286-93.
20. N'Guessan R, Corbel V, Akogbéto M, Rowland M. Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. Emerg Infect Dis. 2007b;13(2):199-206.
21. Ngongang-Yipmo ES, Tchouakui M, Menze BD, Mugenzi LMJ, Njiokou F, Wondji CS. Reduced performance of community bednets against pyrethroid-resistant Anopheles funestus and Anopheles gambiae, major malaria vectors in Cameroon. Parasit Vectors. 2022; 26;15(1):230. doi: 10.1186/s13071-022-05335-2. PMID: 35754045; PMCID: PMC9233849.
22. Nopowo FN, Offono Enama L, Tsila HG, Mbida Mbida A, Tonga C, Ngo Hondt E, et al. Évaluation de l'efficacité des moustiquaires imprégnées 36 mois après leur distribution dans le Sud Cameroun. Bull Soc Pathol Exot.

2020;113(3):289-297. French. doi: 10.3166/bspe-2021-0159. PMID: 33881248.
23. Ossè AR, Aïkpon R, Sovi A, et al. Long lasting insecticidal nets use, efficacy and physical integrity in a vector resistance area after a nationwide campaign in southern Benin, West Africa. J Public Health Epidemiol. 2013; 5:325-35.
24. Pagès F, Orlandi-Pradines E, Corbel V. Vecteurs du paludisme : biologie, diversité, contrôle et protection individuelle. Med Mal Infect. 2007 ; 37:153 – 61.
25. Programme National de Lutte contre le Paludisme (PNLP). Profil entomologique du paludisme au Cameroun. Programme National de Lutte contre le Paludisme. Rapport Minsanté Cameroun. 2018; 1-81
26. Programme National de Lutte contre le Paludisme (PNLP). Rapport d'activités. 2020.
27. Riveron JM, Chiumia M, Menze BD, Barnes KG, Irving H, Ibrahim SS, et al. Rise of multiple insecticide resistance in Anopheles funestus in Malawi: a major concern for malaria vector control. Malaria Journal. 2015;14(1):344.
28. Shirayama Y, Phompida S, Kuroiwa C, et al. Maintenance behaviour and long-lasting insecticide-treated nets (LLITNs) previously introduced into Bourapar district, Khammouane province, Lao PDR. Pub Health . 2007 ;121:122–9
29. Silver JB. Sampling the larval population. Mosquito ecology: field sampling methods. 2008:137-338.
30. World Health Organization. Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets. 2005 ; 24.
31. World Health Organization. Guidelines for laboratory and field-testing of long-lasting insecticidal nets. 2013 ; 89.
32. World Health Organization. World Malaria Report 2021. WHO Global Malaria Programme.https://www.who.int/docs/default-source/malaria/world-malaria-reportsAssessed on December 2021.
33. Zaim M, Aitio A, Nakashima N. Safety of pyrethroid-treated mosquito nets. Med Vet Entomol. 2000;14(1):1-5.