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Use of a portable field-adapted liquid chromatographic system (C-Vue machine) to estimate the quantity of deltamethrin on insecticide-treated nets paired with WHO cone bioassays to determine ITN bioefficacy as part of three-year durability monitoring in Mali

Moussa B. M. Cisse[1,2*], Ibrahim Traore[1,2], Mamadou Sow[2], Yacouba Dansoko[1,2], Alice Dembele[1,2], Lazeni Konate[2], Jean-Marie Sanou[1,2], Youssouf Diarra[2], Samah Sissoko[3], Abdourhamane Dicko[3], Mahamadou Magassa[3], Lansana Sangare[4], Jules Mihigo[4], Celestin Kouambeng[4], Phillipe Rwatana Mutwa[5], Paula Marcet[6], Michael D. Green[6] and Ousmane Koita[1,2]

Abstract

Background Monitoring insecticide levels and physical integrity over time is essential for assessing the durability of insecticide-treated nets (ITNs), which largely depends on the net handling habits of users. This study determined the insecticide content and effectiveness of ITNs (Yorkool and PermaNet 2.0) at 6, 12, 24, and 36 months after a mass distribution campaign in Mali.

Methods At 6 months (May, 2018), 12 months (December, 2018), 24 months (November–December, 2019), and 36 months (November–December, 2020), 30 nets were randomly collected from households in the districts of Kenieba and Kita in the southern part of Mali, together with information about ITN use and washing practices. The insecticidal effectiveness of the ITNs was assessed with the World Health Organization (WHO) cone test using a laboratory-reared, susceptible colony of *Anopheles coluzzii*. The residual insecticide content was measured by a non-destructive sampling technique with a portable field-adapted high-performance liquid chromatographic (HPLC) system (C-Vue®) validated by running samples in parallel with standardized WHO HPLC methods.

Results At each survey time, nets were washed an average of three times over the previous 6 months, most commonly using Local soaps containing sodium hydroxide, detergent, or bleach. Using HPLC_CVue, the average deltamethrin concentration was 55 mg/m^2 at 6 months and gradually decreased to 14 mg/m^2 at 36 months for Yorkool nets. The values for the PermaNet 2.0 nets were 45 mg/m^2 at 6 months and 6 mg/m^2 at 36 months. Until the 24-month

*Correspondence:
Moussa B. M. Cisse
moussa.cisse@lbma.edu.ml
Full list of author information is available at the end of the article

evaluation, the proportion of nets with minimal effectiveness was greater than 80% for both net types and sites. At 24 and 36 months, less than 80% of nets from both products and sites met the WHO optimal effectiveness criteria.

Conclusion The WHO standardized cone test and C-Vue evaluation demonstrated that net type effectiveness and insecticide content were consistently lower than expected at 3 years, and users washed nets with local soaps containing sodium hydroxide, detergent or bleach. The C-Vue portable chromatographic device was used successfully for the first time in Mali to measure the insecticide concentration of ITNs.

Keywords Insecticide-treated nets, Minimal effectiveness, Optimal effectiveness, Insecticide content, Field-adapted liquid chromatographic system C-Vue®

Background

Insecticide-treated nets (ITNs) play a significant role in the prevention and control of malaria. They provide personal protection and can reduce transmission, protecting an entire community (i.e., a mass effect) in areas with sustained high levels of coverage and anthropophilic vectors (exophily) [1, 2]. For malaria control with ITNs, the World Health Organization (WHO) currently recommends universal coverage, defined as "one ITN for every two people in a household" [3]. In many ITN distribution programmes, it is assumed that ITNs have a relatively uniform lifespan of approximately 3 years per manufacturer claims. Thus, mass distribution campaigns at 3-year intervals supplemented by routine distribution through antenatal, immunization services, and shools are considered sufficient to maintain adequate and sustained net coverage levels [4]. However, recent longitudinal studies and surveys have revealed that the assumption of a uniform 3-year lifespan may be overly optimistic and that the rate at which net coverage declines after a campaign may be substantially more rapid than previously assumed [5–8].

ITNs are a key component of the malaria vector control strategy in Mali, where donors such as the U.S. President's Malaria Initiative (PMI) and the Global Fund to Fight HIV, TB and Malaria (GF) have distributed millions of nets since programme implementation began in 2007 [9]. ITN distribution strategies in Mali are based on providing access through mass distribution campaigns to the entire target population at a rate of 2 people per net, as well as routine distribution to pregnant women, and children under one year of age during prenatal consultations for women and the Expanded Programme on Immunization for Children [4, 10]. These strategies are reinforced by educational communication encouraging net care and use at the community level. Mali began with a ITN mass distribution campaign in 2011 and has conducted additional campaigns in 2014 and 2017. In 2021, 91% of households in Mali had at least one ITN, as substantial improvement compared to 2006, when only 50% of households in Mali had at least one ITN [11]. However, only 44% of households had one ITN for every two household members in 2021.

Planning for long-term coverage with ITNs requires information on the durability of different ITN products in local settings to choose which products to procure and to determine whether particular products are likely to perform best over time in a given setting. The pattern of net users' age, storage place, type of sleeping place, type of washing, regions and sociocultural are among the critical factors that contribute to the physical deterioration of bed nets [7, 8, 12]. This also informs the necessary rate of replacement in continuous distribution systems, the appropriate interval between campaigns and, when necessary, plans for disposal or recycling of old nets. Monitoring the durability of nets may lead to a better understanding of the factors that determine ITN durability and the laboratory indicators that correlate with these net qualities. It can also provide an opportunity to improve behaviour and communication messages so that users can take better care of their ITNs [3].

The WHO and Vector Control Technical Expert Group (VCTEG) guidelines describe methods for assessing ITN durability based on net attrition, insecticide content, insecticidal activity, and physical integrity [13, 14]. Insecticide levels on ITNs are typically analysed using a straight-phase HPLC technique recommended by the Collaborative International Pesticides Analytical Council (CIPAC). The expense of the instrument and the reagents required for the analysis precludes many malaria-endemic resource-limited countries from carrying out proper monitoring of insecticide levels. Therefore, a method was developed to measure surface levels of deltamethrin on polyester nets. This novel technique uses a portable and inexpensive liquid chromatographic device (C-Vue Chromatography, Simpsonville NC, USA www.cvuechromatography.com) along with a nondestructive net sampling technique. The chromatographic device consists of a spring-driven pump and a battery-powered detector lamp [15]. This study assessed the insecticidal (bioefficacy and insecticide content) durability of ITNs (Yorkool and PermaNet 2.0) in two villages at

6, 12, 24, and 36 months after a net mass distribution was carried out in December 2017.

The objectives of this study were to (1) determine the net use and washing practices within the communities, (2) assess the insecticidal effectiveness using the WHO cone bioassays test method, and (3) estimate the insecticidal content using the C-Vue HPLC method.

Methods

Study sites and period

The ITN mass campaign in the Kayes Region was conducted in December 2017, and data collection was conducted beginning 6 months after distribution (between March and May 2018) and at 12 months (December 2018), 24 months (November–December 2019) and 36 months (November–December 2020). The study was carried out in the districts of Kenieba and Kita, located in the Kayes Region, at sites with similar malaria epidemiological, climatic and socioecological profiles. For the selection of clusters, the campaign ITN distribution registries were used at both sites. A cluster was defined as a community, and the selection was performed with probability proportionate to size, with the number of ITNs distributed per community as the measure of size. In each of the districts, 15 clusters (village) were selected, and a group of 5 households was selected in each (Fig. 1).

ITN brands

Yorkool nets were monitored in Kenieba, and PermaNet 2.0 nets were monitored in Kita, as those were the brands procured and distributed in each region. The technical specifications of the ITNs (material, textile specific, insecticide concentration) are the same for the two brands, except that Yorkool nets are 75-denier and PermaNet 2.0 are 100-denier (a measure of fabric thickness or density). Both nets are made of knitted polyfilament polyester fibers and are treated with deltamethrin to a target concentration of 55 mg/m^2 [16].

Study design

Sampling was carried out in 15 clusters (from villages) per study site (Heath District), and each cluster contained 5 households. At 6 months after distribution (from March to May 2018), a household survey was carried out

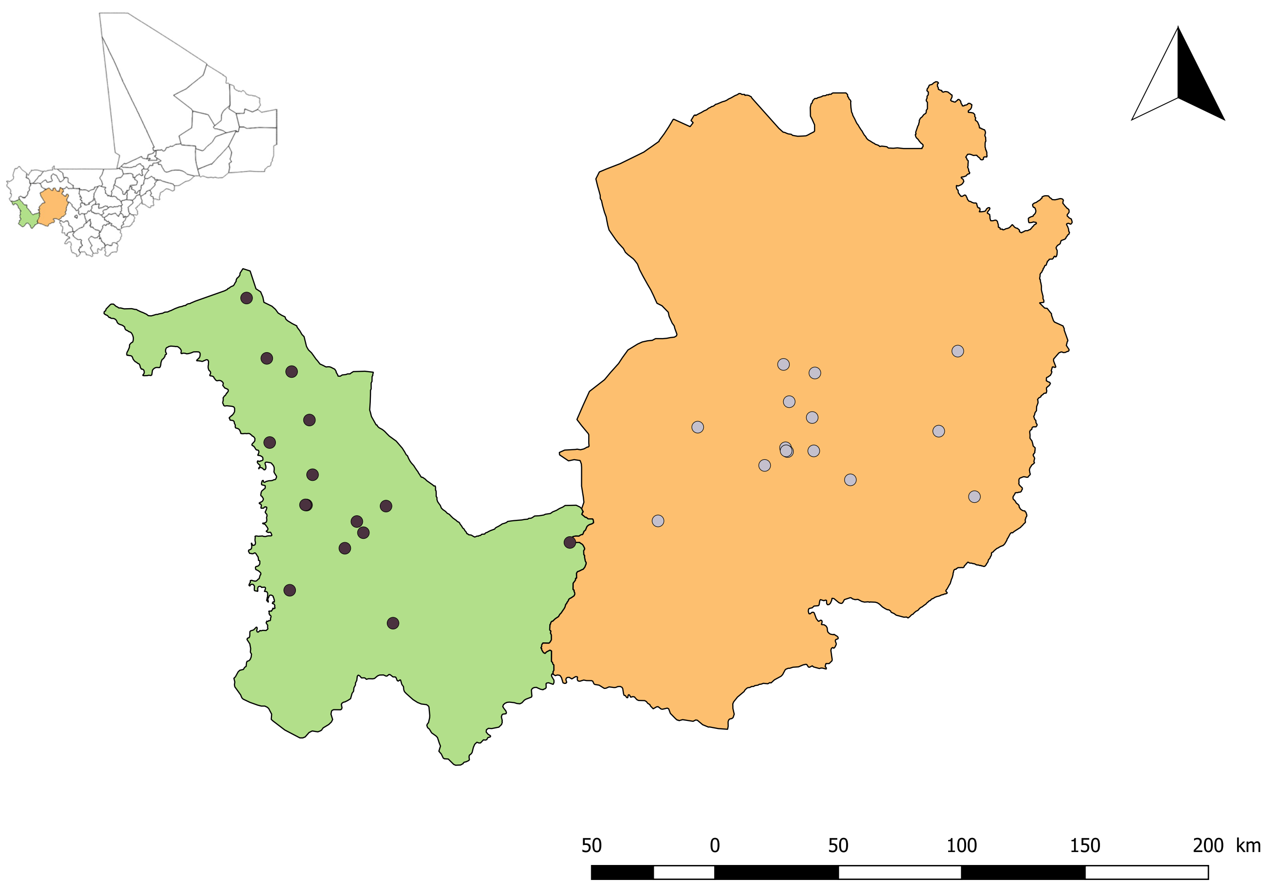

Fig. 1 Net collection sites in Mali, 2018–2020. Grey filled circle: Cluster–Kita. Black filled circle: Cluster Kenieba. Green shade: District Kenieba Yorkool Net. Orange shade: District Kita PermaNet 2.0

at each study site to identify representative households that received campaign nets and request that owners consent to participate in the study. Campaign nets from consenting households were selected to constitute the cohorts of nets for the three-year study period. These nets were labelled with a unique code within 6 months postdistribution. At each survey time (6, 12, 24, and 36 months) (Fig. 2), 30 campaign nets per site (Health District) were selected for insecticide effectiveness testing (bioassays and chemical residue).

Nets sampling

At 6 months, a cohort of ten nets per cluster was tagged for eventual follow-up and removal at the 12-, 24- and 36-month assessments. Using a separate group of two-digit tags (A1-A6, B1-B6), households were selected randomly at 6 months. Following the WHO recommendation for phase III testing of ITNs [14], random samples of 30 campaign nets per site (a total of 60) were selected at each annual assessment. Households were given a new ITN as a replacement. The collected nets were labelled and stored in separate plastic bags for transportation.

Determination of net use and washing practices within communities

Following the WHO [14] and U.S. President's Malaria Initiative [17] guidelines, a one-page questionnaire confirming that the net was received during the 2017 campaign and obtaining basic information on net use, storage, and washing patterns was administered to each participating household at the 6-month, 12-month, 24-month and 36-month assessments. Electronic data capture using Samsung Galaxy Tab A devices with ODK questionnaires allowed for detailed programming which included skip patterns and internal controls to ensure that the data were consistent and complete.

Determination of insecticidal effectiveness using the WHO Cone test at 12, 24, and 36 months post-ITN distribution

Following WHO guidelines [14], the insecticidal activity (biological efficacy) of the ITNs was determined with WHO cone tests using the susceptible mosquito *Anopheles coluzzii* Ngousso reared in a local insectary at the Laboratory of Applied Molecular Biology (LBMA). For each follow-up period, bioassays were conducted with 30 nets per site. For each net, one piece (25 cm × 25 cm) was cut from each side and the top according to WHO guidelines. Each piece was exposed to bioassays and tested with 10 mosquitoes in WHO cone tests [14]. Mortality rate at 24 h and knockdown (KD) rates at 60 min were used to evaluate the bioefficacy of the nets.

Determination of insecticidal content using HPLC at 6, 12, 24, and 36 months post-ITN distribution

The same pieces of nets used for the WHO cone test from the 6-, 12-, 24- and 36-month were used for the determination of insecticide content (surface levels of insecticide) with the portable HPLC C-Vue (C-Vue® Portable Liquid Chromatograph Model CH2B) following the method described [15]. Briefly, values generated by C-Vue chromatography are surface levels (SL) of deltamethrin obtained by rubbing the net with filter paper, which is nondestructive to the nets, and analyzing the residue through liquid chromatography. This method allows us to estimate the mean and median levels of active ingredients (insecticides) in mg/m^2 across the net samples. These values are later compared with manufacturer specifications for the insecticide content of each net brand. A conversion equation was used to estimate the total level (TL) of insecticide from the mean surface level (SL): $T.L. = (Log(SL) + 1.37)/0.0232)$. This equation was obtained through laboratory replication and 2 independent C-Vue devices and validated with gold standard HPLC (Unpublish data) [15].

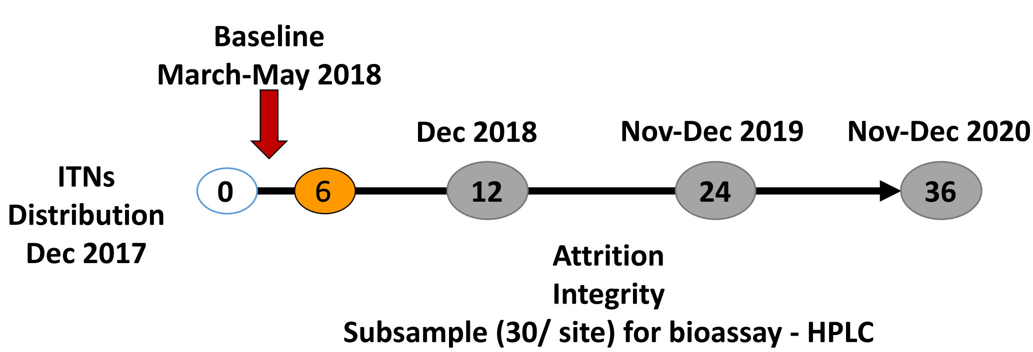

Fig. 2 Study design from 2017 to 2020

Data analysis

Once data collection for 6-, 12-, 24-, and 36-month rounds were completed and the data were verified, the data were transferred to a statistical software package (STATA for Windows, Version 14.0) for further consistency checks and preparation for analysis. The data analysis was conducted by using STATA do files (macros). The *chi-square* test was used for the comparison of proportions and mean values between the two sites. Bioassays were performed using the WHO cone bioassay method. Net effectiveness was determined based on bioassay results, following the World Health Organization Pesticide Evaluation Scheme (WHOPES) Phase III evaluation criteria. According to the WHOPES, at least 80% of recommended ITN brands should maintain optimal effectiveness 36 months post-distribution, where optimal effectiveness is defined as KD 60 mn ≥ 95% or mortality ≥ 80%) [14]. The tunnel tests was not in this study. The minimal effectiveness criteria, as defined by Kilian, were KD60 mn ≥ 75% or functional mortality ≥ 50% [6]. The surface-level and the total insecticide levels from each assessment period were compared by using a *t*-test.

Results

Net use practices

The locations of nets (hanging loose, folded/tied or not hanging) were the same at both sites at 12 months ($p = 0.066$) and 36 months ($p = 0.214$) but differed ($p = 0.001$) at 24 months (Table 1). The proportion of nets hanging loose decreased from 12 to 36 months in Kenieba (30.0–10.3%) and Kita (60.0–6.6%) (Table 1). The main type of sleeping place was a bed at both sites at all sampling times (Table 1). The nets were mainly used by older children (> 13 years old) and adults in Kenieba at all times in both locations, except for the first year postdistribution, when most nets were used by young children or young children plus adults in Kita (Table 1).

The variables related to net use for the nets sampled over time are shown in Table 2. The proportion of nets used the previous night was lower in Kenieba (56.6–53.3%) than in Kita (93.3–90.0%) at 12 ($p = 0.007$) and at 24 months ($p = 0.001$), but no difference in net use was observed ($p = 0.096$) at both sites at 36 months (Table 2).

The proportion of nets used every night in the last week was also lower in Kenieba (56.6–27.5%) than in Kita (83.3–50.0%) at 12 months ($p = 0.008$) and 24 months ($p = 0.003$) at 36 months ($p = 0.055$) (Table 2). Overall, a greater proportion of net use every night last week was observed in Kita throughout the study. Although the proportion of nets not used increased for both sites over time, the proportion not used remained at 30% in Kita at the 36-month point but increased to 69% in Kenieba. Nets were used equally in the rainy and dry seasons at both sites at 12 months ($p = 0.283$), but there was a difference between the sites at 24 months ($p < 0.001$) and 36 months ($p = 0.008$) (Table 2).

Table 1 Variables related to the use and handling of postdistribution campaign bed nets in Mali, 2018–2020

Variable	12 months	24 months	36 months
Kenieba/Yorkool	*N = 30*	*N = 30*	*N = 29*
Location found			
Hanging loose	30.0%	16.6%	10.3%
Hanging folded/tied	36.6%	46.6%	34.4%
Not hanging	33.4%	36.6%	55.1%
Type of sleeping place			
Bed	63.3%	63.3%	72.4%
Mattress	20.0%	0.0%	3.4%
Mat/ground	3.3%	6.6%	10.3%
Net users			
Young child only	26.6%	7.1%	0.0%
Young child + adult	0.0%	28.5%	0.0%
Older child, adult only	73.3%	64.2%	100%
Kita/PermaNet 2.0	*N = 30*	*N = 30*	*N = 30*
Location found			
Hanging loose	60.0%	70.0%	6.6%
Hanging folded/tied	30.0%	23.3%	56.6%
Not hanging	10.0%	6.6%	36.6%
Type of sleeping place			
Bed	86.6%	83.3%	73.3%
Mattress	0.0%	10.0%	0.0%
Mat/ground	0.0%	6.6%	26.6%
Net users			
Young child only	50.0%	5.0%	8.3%
Young child + adult	50.0%	20.0%	16.6%
Older child, adult only	0.0%	75.5%	75.0%

The variables related to the washing of the sampled nets were also assessed. The proportion of nets washed was high (≥ 70%) at both sites at 12, 24, and 36 months (Table 3). The mean number of washes among all nets in the last six months ranged between 2.0 to 3.2 across both sites at 12, 24, and 36 months. The main soaps used at both sites at 12, 24, and 36 months included local soap bar (containing sodium hydroxide), detergent, or bleach (containing sodium hypochlorite, sodium chloride, sodium carbonate, and sodium hydroxide).

Insecticidal activity

The results for the determination of insecticide bioefficacy for Yorkool nets collected in Kenieba and PermaNet 2.0 nets collected in Kita at 6, 12, 24 and 36 months post distribution are shown in Table 4. At each point in time, the proportion of nets with optimal effectiveness (at least 95% K.D. or 80% mortality) was < 80% for both

Table 2 Variables related to the use of bioassay test nets, Mali, 2018–2020

Variable	12 months	24 months	36 months
Kenieba/Yorkool	*N = 30*	*N = 30*	*N = 29*
Used last night	56.6%	53.3%	31.0%
Use last week			
Every night	56.6%	50.0%	27.5%
Most (5–6)	0.0%	10.0%	3.4%
Some (1–4)	6.6%	10.0%	0.0%
Not used	36.6%	30.0%	68.9%
Do not know	0.0%	0.0%	0.0%
Seasonal use			
Equally rain and dry	53.3%	56.6%	44.8%
Mainly rain	36.6%	10.0%	37.9%
Rain only	10.0%	33.3%	17.2%
Kita/PermaNet 2.0	*N = 30*	*N = 30*	*N = 30*
Used last night	90.0%	93.3%	53.3%
Use last week			
Every night	83.3%	93.3%	50.0%
Most (5–6)	6.6%	0.0%	3.3%
Some (1–4)	6.6%	3.3%	6.6%
Not used	3.3%	3.3%	30.0%
Do not know	0.0%	0.0%	10.0%
Seasonal use			
Equally rain and dry	73.3%	100.0%	86.6%
Mainly rain	23.3%	0.0%	6.6%
Rain only	3.3%	0.0%	6.6%

Table 3 Variables of related to the washing Bioassay Test Nets, Mali, 2018–2020

Variable	12 months	24 months	36 months*
Kenieba/Yorkool	*N = 30*	*N = 30*	*N = 29*
Ever washed	70.0%	93.3%	79.3%
Washes last 6 months (all)			
Mean	3.2	2.8	2.0
Median	1	2	2
Washes last 6 months (if washed)			
Mean	4.5	3	2.6
Median	2	2	2
Soaps used			
country soaps bar	38.1%	32.1%	21.7%
detergent or bleach	52.3%	57.1%	43.4%
mix	9.5%	10.7%	26.0%
Kita/PermaNet 2.0	*N = 30*	*N = 30*	*N = 30*
Ever washed	83.3%	96.6%	93.3%
Washes last 6 months (all)			
Mean	2.4	3.1	3.0
Median	2	2	3
Washes last 6 months (if washed)			
Mean	2.9	3.2	3.2
Median	2	2	3
Soaps used			
Country soaps bar	36.0%	37.9%	25.0%
Detergent or bleach	64.0%	27.5%	50.0%
Mix	0.0%	34.4%	3.5%

types of nets and at both sites (Table 4). The proportion of nets reaching minimal effectiveness (75% K.D. or 50% 24-h mortality) was > 80% at 6, 12, and 24 months. At 36 months, the proportion of nets with minimal insecticidal effectiveness decreased for both types of nets and at both sites (Table 4). The proportions of nets with optimal and minimal effectiveness were not statistically different ($p > 0.05$) between the two net types during the 36-month survey (Table 4). The proportion of net minimal effectiveness was > 80% at both sites until the 24-month assessment but decreased dramatically at 36 months to 51.7 and 60.0%, respectively, with Yorkool nets in Kenieba and PermaNet 2.0 in Kita (Table 4).

Insecticide surface level

The mean surface and total concentrations of insecticide residue on ITNs collected at 6 (baseline), 12, 24, and 36 months and measured by C-Vue are presented in Fig. 3 and Table 5. The surface concentration of insecticide residue was greater on Yorkool nets (0.69 mg/m^2) than on PermaNet 2.0 nets (0.46 mg/m^2) ($p = 0.021$) at 6 months, but it was not statistically different at 12, 24 or 36 months (Fig. 3). By 36 months after distribution, the mean insecticide concentrations decreased by 74.5% (Yorkool nets) and 86.6% (PermaNet 2.0 nets) of the baseline insecticide concentration. For both types of nets, the surface concentration of insecticide decreased significantly from 6 to 36 months ($p < 0.001$) (Fig. 3).

By using the relationship between surface and total levels, surface levels were converted to total levels of deltamethrin. The insecticide concentration decreased from 55 mg/m^2 at 6 months (Baseline), to 29 mg/m^2 at 12 months, to 28 mg/m^2 at 24 months and to 14 mg/m^2 at 36 months for the Yorkool nets (Table 5). It decreased from 45 mg/m^2 at 6 months to 32 mg/m^2 at 12 months, to 26 mg/m^2 at 24 months, and to 6 mg/m^2 at 36 months for the PermaNet 2.0 nets (Table 5).

Discussion

This ITN durability study compared the use practices, insecticidal effectiveness and residual insecticide content of nets from two communities following their distribution through a mass campaign. The nets distributed

Table 4 Cone bioassay results using a susceptible colony of *An. coluzzii* on Yorkool and PermaNet 2.0 ITNs, Mali, 2018–2020

Site/Net	6 months (Baseline)	12 months	24 months	36 months
Kenieba/Yorkool	*N = 30*	*N = 30*	*N = 30*	*N = 29*
KD60				
Mean (95% CI)	94.6% (90.5–98.6)	85.6% (78.2–93.5)	90.5% (83.5–97.5)	67.3% (55.2–79.5)
Median (IQR)	98% (94.0–99.7)	94% (82–99.7)	96% (96.0–100.0)	78.0% (38.0–96.0)
Mortality 24 h				
Mean (95% CI)	54.4% (47.8–60.9)	74.1% (63.5–84.7)	54.2% (44.3–64.2)	42.4% (29.7–55.1)
Median (IQR)	54.0% (42.0–66.0)	79% (72–89.7)	57.0% (44.2–72.4)	36.0% (22.0–60.0)
Optimal effectiveness				
Estimate (95% CI)	66.6% (45.8–82.5)	70.0% (47.9–85.5)	70.0% (47.9–85.5)	31.0% (15.1–53.1)
Minimal effectiveness				
Estimate (95% CI)	96.6% (77.7–99.5)	100%	93.3% (75.8–98.4)	51.7% (32.7–70.1)
Kita/PermaNet 2.0	*N = 30*	*N = 30*	*N = 30*	*N = 30*
KD60				
Mean (95% CI)	94.9% (92.8–96.9)	93.7% (89.4–98.1)	84.2% (76.5–91.8)	64.5% (53.7–75.3)
Median (IQR)	97.0% (94.0–100.0)	98% (94.2–100)	95% (78.0–99.7)	66.0% (42.0–94.0)
Mortality 24 h				
Mean (95% CI)	62.0% (53.6–70.5)	71.2% (63.8–78.6)	65.2% (57.2–73.3)	39.8% (33.8–45.8)
Median (IQR)	59.0% (48.0–72.0)	69% (64.2–77.7)	62.0% (58.2–74.0)	40.0% (28.0–54.0)
Optimal effectiveness				
Estimate (95% CI)	66.6% (53.1–77.8)	76.6% (48.9–91.8)	56.6% (37.0–74.3)	20.0% (8.0–41.5)
Minimal effectiveness				
Estimate (95% CI)	100.0%	100%	96.6% (77.7–99.5)	60.0% (38.9–77.8)

were multifilament polyester-based ITNs treated with 75-denier deltamethrin (Yorkool) or 100-denier deltamethrin (PermaNet 2.0). To assess the influence of the net use environment and behavioural factors, this study was conducted in two health districts, Kenieba and Kita, located in the Kayes Region, which have similar malaria epidemiological, climatic, and socioecological profiles.

Net use practices

The pattern of net users' age, storage place, type of sleeping place, and type of washing are among the critical factors that contribute to the physical deterioration of bed nets [7, 8, 12]. The locations where nets were found (hanging loose, hanging folded/tied, and not hanging) were similar at 12 and 36 months. However, the location of the net was different at 24 months, with a greater proportion of nets hanging loose in Kita than in Kenieba. The risk of net physical damage increases when hanging loose or not hanging after use[17]. This risk was high at each survey time at both sites. The main type of sleeping place was a bed frame (bamboo sticks) at both sites at the 12-, 24- and 36-month surveys. A similar result was found in a study of Magnet and PermaNet 2.0 nets in Ethiopia [18] and with DawaPlus 2.0 and DuraNets in a durability study in the Democratic Republic of Congo [12]. This type of sleeping place increases the risk of net physical damage because some holes that form in the ITN are mechanical, caused by being snagged on sharp materials and bed edges [4]. In most instances, the primary users of nets were older children (> 13 years old) and adults. This is likely due to fact that this age group represents a large proportion of the population. Similar results were observed in a study on Olyset and PermaNet 2.0 in Tanzania [19].

The proportion of nets used the previous night was lower in Kenieba (where the nets were Yoorkol) than in Kita (where the nets were PermaNet 2.0) at 12 and 24 months. However, net use decreased between 12 and 36 months at both sites, and net use was not statistically different at 36 months. The same results were observed for the proportion of nets used in the last week. There was no evidence of seasonal variation in net use, with nets used equally in both sites during the rainy and dry seasons. The ITN use, mainly depends on their physical and chemical conditions, but it is also influenced by a plethora of factors including product quality, geographically specific use practices, awareness of benefits, and seasonal factors such as mosquito nuisance levels, temperature, and humidity, all of which affect comfort within the ITN [20–22].

The proportion of nets washed was high (> 70%) at both sites at 12, 24 and 36 months. The mean number of

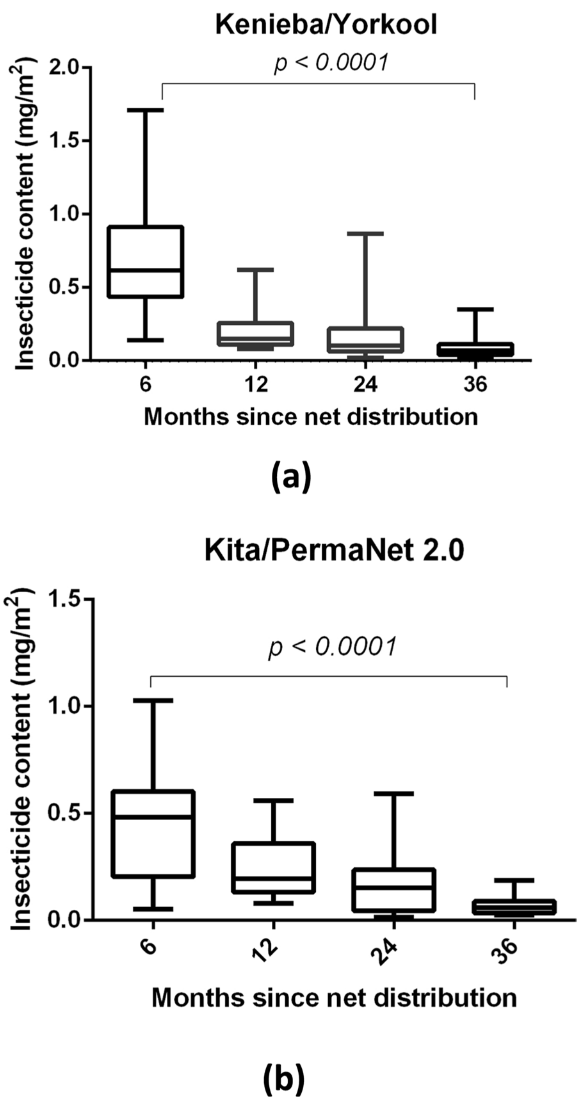

Fig. 3 Surface insecticide concentrations of the ITNs in Mali, 2018–2020: **a** Kenieba/Yorkool and **b** Kita/PermaNet 2.0

washes in the last six months (all/if washed) was moderate (≤3 wash), at approximately 3.0 at both sites at 12, 24, and 36 months. Similar results were observed in the Democratic Republic of the Congo with DawaPlus 2.0 in the DuraNet study [12]. The main soaps used were country soaps, detergent, or bleach at both sites at 12, 24, and 36 months. One factor that may have contributed to the rapid decrease in insecticide use is the use of country soaps, detergent or bleach during washing [23, 24].

Insecticidal effectiveness using the WHO cone test

Based on the WHO [14] criteria at each survey, the proportion of Yorkool and PermaNet 2.0 nets with optimal effectiveness was < 80%. The proportion of both types of nets reaching minimal effectiveness was > 80% at 6, 12, and 24 months but at 36 months, decreased to 51.7% for the Yorkool net and 60.0% for the PermaNet 2.0. The proportions of nets with optimal and minimal effectiveness were similar for both types of nets during the entire study period, despite the differences in handling and use patterns observed in the sampled sites.

These findings differ from studies conducted in Ethiopia and Senegal, where PermaNet 2.0 retained its optimal effectiveness for up to 24 and 36 months, respectively [18, 19, 25]. Similarly, a study in Tanzania demonstrated an excellent insecticidal durability of PermaNet 2.0, with mean 60-min knockdown rates greater than 95% in the WHO cone bioassay tests at all time points, alongside a moderate decline in mean 24-h mortality rates from 93% at 12 months to 72% at 33 months[19]. A similar pattern was observed, in a study conducted in Benin on Yorkool nets, where, an average, 58% of nets still met WHO optimal effectiveness criteria after 18 months [26]. Conversely, in Madagascar, the insecticidal effectiveness of Yorkool net dropped below the optimal effectiveness level at the 12-month assessment, with an average mortality rate of 23% [27]. These findings suggest greater variation in ITN durability between countries than between net brands [20].

Table 5 Chemical content results on Yorkool and PermaNet 2.0 ITNs, Mali, 2018–2020

Variable	6 months (Baseline)	12 months	24 months	36 months
Kenieba/Yorkool	*N = 30*	*N = 30*	*N = 30*	*N = 29*
Surface levels of insecticide (mg/m^2)	0.69	0.20	0.19	0.08
Converted total levels of insecticide (mg/m^2)	52	29	28	12
% Decrease of total levels of insecticide compared to baseline	–	47.3%	49.0%	74.5%
Kita/PermaNet 2.0	*N = 30*	*N = 30*	*N = 30*	*N = 30*
Surface levels of insecticide (mg/m^2)	0.46	0.24	0.17	0.06
Converted total levels of insecticide (mg/m^2)	45	32	26	6
% Decrease of total levels of insecticide compared to baseline	–	28.8%	42.2%	86.6%

Based on the PMI [17] guidelines, if less than 80% of the nets in a given region meet the optimal effectiveness criteria, it is recommended to evaluate whether at least 80% of the nets meet the minimal effectiveness criteria (75% K.D. or 50% 24-h mortality). Although this is not an official WHO-recommended threshold, it can provide an assessment of net performance. If ≥ 80% of ITNs meet the minimal effectiveness evaluation criteria, then there is a potential problem with the bioefficacy of ITNs, and further investigation of likely causes should be performed. If < 80% of nets meet minimal effectiveness evaluation criteria, then a significant problem with the bioefficacy of the nets is likely, and urgent investigation related to the possible causes should be performed. This study revealed that potential causes could include the use of country soaps, a detergent, or bleach.

Insecticidal effectiveness using HPLC

The HPLC C-VUE method [15] showed that the mean deltamethrin concentration in Yorkool and PermaNet 2.0 nets decreased significantly over the study period. At the end of the survey (36 months after distribution), the mean concentrations of available insecticide residue represented 25% (Yorkool nets) and 13% (PermaNet 2.0 nets) of the baseline insecticide concentration, which was in line with other studies in Senegal [25] and Ethiopia [18] with PermaNet 2.0 using the Collaborative International Pesticide Analytical Council (CIPAC) method 454/LN/M/3.2 [28]. In these studies, after 24 and 36 months, the mean chemical concentrations of alphacypermethrin and deltamethrin were 23.9 and 24.6% of the baseline concentration, respectively [18]. A studyin Burkina Faso, using the same CIPAC method [28], PermaNet 3.0 showed a retention of 16% of its deltamethrin content on side panels and 65% on the roof panel after 36 months of field use, compared to the manufacturer's target dose [29]. In Kenya, Olyset Plus nets demionstrated a more rapide decline in in insecticidal content over time, with permethrin content decreased by 36, 40 and 52% at the end of years 1, 2 and 3, respectively. In contrast, the PBO content saw a more significant decrease, dropping by 66, 81, and 87% across the same period. For Olyset Net, the loss of permethrin content was comparatively slower, with a decrease of 16% in year one, and ony small increase in years 2 and 3 (19 and 24%, respectively) [30].

Conclusion

This 3-year survey showed that the bioefficacy of both Yorkool and Permanent 2.0 nets was below the WHO criteria of optimal effectiveness, evidenced by a rapid loss of deltamethrin. This could have been caused as users did not follow the manufacturer's recommendations, washing the nets with local soaps, detergent or bleach. Better community education is therefore essential to ensure the appropriate use and care of nets in areas with ITN-based interventions to maximize the operational impact on the prevention, control, and eventually achieving the elimination of malaria. Failure to preserve the efficacy of nets could have operational implications, requiring early replenishment with new nets to ensure that such an ITN-based malaria intervention remains effective. The C-Vue portable chromatographic device was used successfully for the first time in Mali to measure the surface-level insecticide concentration of ITNs. This simple technology allows for an affordable and locally available, nondestructive method to monitor the insecticide content of ITN in malaria-endemic countries.

Abbreviations

ITN	Reated net
PMI	President Malaria Initiative
WHO	World Health Organization
CIPAC	Collaborative International Pesticide Analytical Council
HPLC	High-Performance Liquid Chromatography
WHOPES	World Health Organization Pesticide Evaluation Scheme
KD	Knock down
CE	Comite Ethique
INRSP	Institut National de Recherche en Sante Publique
SL	Surface level
TL	Total level

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Disclaimer

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Author contributions

Design of the study and tools: MBMC; I.T.; PM implementation and supervision: MBMC; I.T., MS, Y.D., A.D., JMS, S.S., A.D. and OK; bioassay: MBMC, I.T., MS, Y.D., A.D., JMS; and L.K.; chemical testing: MBMC; IT and Y.D., L.K.; and MG; analysis and interpretation: MBM, TSA; I.T and MG, L.S., manuscript: all.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study protocol was translated into French and presented to the Mali National Institute of Public Health Research ethical committee for review. The clearance data were obtained on January 22, 2018, under reference number 02/2018/CE-INRSP. The staff implementing this study complied with all the policies and procedures of both the PMI and the local ethics board. Informed oral consent was obtained from all participants in this study prior to conducting the interviews.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]USAID PMI ITN Durability Monitoring Project, Bamako, Mali. [2]Laboratoire de Biologie Moléculaire Appliquée, Université Des Sciences, Techniques Et Technologies de Bamako, Bamako, Mali. [3]Programme National de Lutte Contre Le Paludisme, BP E3206, Bamako, Mali. [4]President's Malaria Initiative, USAID, Bamako, Mali. [5]U.S. President's Malaria Initiative, US Centers for Disease Control and Prevention, Bamako, Mali. [6]Entomology Branch, US Centers for Disease Control and Prevention, Atlanta, GA, USA.

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