Long-lasting insecticidal nets remain efficacious after five years of use in Papua New Guinea

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Long-lasting insecticidal nets remain efficacious after five years of use in Papua New Guinea

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SUMMARY

Long-lasting insecticide-treated nets (LLINs) have been distributed throughout Papua New Guinea since 2004 as part of the country's malaria control program. This study aimed to evaluate the efficacy of these used bednets over time and with respect to the various household factors related to their use in order to enable the National Department of Health to maximize on the benefits of LLINs. In 2008 and early 2009, used LLINs (0-9 years old) were collected in various villages in Papua New Guinea as part of the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM)-supported National Malaria Control Program and data were collected on net usage. A subset of the nets were tested for residual insecticide content. Net efficacy was measured by the rate of knockdown of *Anopheles farauti* s.s. following exposure to LLINs using the World Health Organization cone bioassay. Optimal effectiveness (>95% knockdown 1 hour post exposure) was observed in 92% of the LLINs. A slight reduction in efficacy was observed after two years of household use and there was a significant relationship between the number of years in use and percent knockdown (p <0.001) as well as deltamethrin concentration (p <0.001). Washing of nets was not associated with a reduction in deltamethrin concentration, but drying them in the sun was (p = 0.008). The physical conditions of these nets also degraded over time with a significant increase in the number of large holes after 5 years (p = 0.02). These findings are in support of the current recommendation to replace LLINs after five years of use, and demonstrate that proper net care can extend the length of efficacy.

Introduction

Malaria is one of the leading diseases in the world that accounts for the majority of morbidity and mortality seen in over 90 countries (1,2). It is transmitted by *Anopheles* mosquitoes (3) and many malaria control campaigns have included the use of vector control to curb transmission of the disease (4).

Vector control programs have primarily focused on insecticide-based interventions: indoor residual spraying (IRS), insecticide-treated nets (ITNs) and, more recently, long-lasting insecticide-treated bednets (LLINs). ITNs offer personal and community protection against mosquito bites (5,6) and are widely accepted as an important malaria control tool (7). ITNs must be manually treated every 6 months with pyrethroid insecticides, the only insecticide class recommended by the World Health Organization (WHO) Pesticide Evaluation Scheme (WHOPES) for bednet impregnation due to their low mammalian

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toxicity and fast knockdown effect against mosquitoes (8,9). With LLINs, technological advances have enabled insecticides to be incorporated into the net fibres (3), allowing for slow and continuous movement of insecticides (10) from the fibre to the surface of the nets. Therefore, the introduction of LLINs has eliminated the need for retreatment, creating a practical and sustainable vector control tool.

Although LLINs remain effective for a longer period of time, they will require replacement when the mosquito knockdown effect is compromised. The current recommendation is to replace after 5 years of use, and many studies have been carried out to examine insecticide reduction and loss of LLIN efficacy over time (4,11-14). Many studies have found LLINs to be effective in the range of 2-5 years. Lindblade and others (11) found LLIN PermaNet 1.0 (Vestergaard Frandsen) to be effective for 2 years (82.2% mortality). PermaNet 2.0 in Uganda performed effectively after 3 years with 90% of the nets under observation resulting in a mortality rate of >80% (12). In Tanzania, LLINs (PermaNet 2.0) used for 5 years induced less mortality than did new nets sealed for the same duration (4). Efficacy was found to be significantly reduced after 5 years (4,9,13).

Under laboratory conditions, PermaNet 2.0 LLINs retained efficacy after 20 or more washes (6). In Colombia, high efficacy was also observed for PermaNet LLINs after 23 washes, having a lower limit for residual deltamethrin at 3 mg/m^2 and achieving at least 80% mortality in *Anopheles* mosquitoes (14). Insecticide loss and reduced LLIN efficacy have been attributed to differences in the use and wear of the nets in households, primarily the washing practices of users (3). The loss of insecticide could be attributed to the strong detergents used for washing and direct sunlight for drying (4,5,9,15-17) or other factors such as firewood smoke and dust particles (4,9,15) that interfere with exposure.

The presence of holes in LLINs also reduces their effectiveness (4,13,18). Large holes can enable a mosquito to easily enter a net, minimizing or eliminating insecticide contact thus compromising the protective efficacy of the bednets (3). There was a rapid increase in the proportion of nets with holes observed in Uganda after only a year of use (12). Kilian and others attributed the physical degradation of nets to the number of washes and socioeconomic status (12).

LLINs are employed throughout many areas of the world as part of malaria control programs. However, most LLIN efficacy studies have been performed in Africa (4,5,9,15,18) or Asia (6,19). In Papua New Guinea (PNG), the National Department of Health has been distributing LLINs throughout the country as part of the malaria control program since 2004 (20). The main malaria vectors are members of the *Anopheles punctulatus* group including *Anopheles farauti* s.s*, Anopheles hinesorum, Anopheles farauti* 4*, Anopheles koliensis* and *Anopheles punctulatus* (21). These vectors have been shown to be highly susceptible to pyrethroids, the insecticides used in LLINs (22); however, there have been no studies on LLIN efficacy in PNG after use in the home. Due to different geographical, environmental and socioeconomic factors present in PNG, previous studies may not be able to provide applicable information that can guide LLIN redistribution schedules. This study aimed to address these gaps in our knowledge of local LLIN efficacy and help inform the National Malaria Control Program.

Methods

Anopheles farauti s.s. colony

A colony of *An. farauti* s.s. was maintained in an insectary at the Papua New Guinea Institute of Medical Research in Madang, PNG. The colony was established from mosquitoes collected in Rabaul, East New Britain Province. Preliminary studies showed the colony to be equally susceptible to insecticides as local wild *An. farauti* s.s. The insectary was maintained at ambient temperature (25-28°C) and adult cages were covered with damp towels to attain 80% relative humidity (RH) throughout the day.

LLIN collection

Between October 2008 and August 2009, used PermaNet 2.0 LLINs (n = 83) were collected from 21 villages in 9 provinces – Western (Fly), Simbu, Eastern Highlands, Western Highlands, Morobe, Madang, East Sepik, West Sepik (Sandaun) and Central Province – in Papua New Guinea, in the frame of a country-wide household survey (20). Collected nets were replaced with new nets of the same brand. At the time of the collection, owners were asked to recall the age of the nets, number of washings, whether they were washed in soap or water alone and whether they were dried in sun or shade. The nets were then sealed individually in resealable storage bags and stored in the dark until the bioassays were conducted.

Residual deltamethrin testing

The used bednets (n = 83) were cut in half and sent to PermaNet® manufacturer Vestergaard Frandsen Laboratories in Hanoi, Vietnam for residual insecticide analysis. Chemical analysis was done using X-ray fluorescence spectroscopy (XRF) to quantify deltamethrin concentration. Those nets with deltamethrin content under the quantification limit of XRF were tested again with high-performance liquid chromatography (HPLC).

WHO cone bioassay

Adult female mosquitoes aged 2 to 5 days were exposed to a subset of used LLINs (n = 51) that were quantified for deltamethrin concentration, using standard WHO cone bioassays (23). They were exposed at 25^{o}C (± 2^{o}C) and 70-80% relative humidity for three minutes and held in the insectary for sixty minutes with access to 10% sucrose-soaked cotton pads. 5 female mosquitoes were placed in each cone, with each net subjected to 10 cones distributed across the side and top of the net (50 mosquitoes per net). During each bioassay, 1 to 5 cones (5 mosquitoes each) were placed on an untreated net as a control. Knockdown was recorded at three minutes, ten minutes, thirty minutes and sixty minutes post exposure and functional mortality was recorded 24 hours post exposure. Mortality observed on untreated nets under 20% was then adjusted for using the Abbott's formula (23). If greater than 20% mortality was observed in the control mosquitoes, the exposure was repeated.

Physical condition observation

Physical condition of the nets was evaluated by estimating the average surface area lost to wear and tear. The number of holes on each net half was counted, and each hole placed in a size category determined by diameter: very small (<1 cm), small (1-5 cm), medium (5-15 cm), large (15-30 cm) and very large holes (>30 cm). The hole index was calculated by multiplying the number of holes in each category by the minimum surface area in cm^2 per hole for each category. The presence of smoke odour was qualitatively recorded as either no odour, faint, moderate or strong smoke odour. Two researchers independently scored each net and a third observer was included if the two observations were discordant.

Statistics

Since very high rates of knockdown were observed 1 hour post exposure, we also calculated time to 50% knockdown and used this to measure the impact of usage variables on net efficacy. This was done by fitting the knockdown data to the equation [$y = a/ (1.0 + \exp(-(x-b)/c))$]. The relationships between net efficacy and usage variables were analysed by 2-sample t-test or single factor analysis of variance when there were 3 or more groups. GraphPad Prism 6.0b was used for statistical analyses and construction of graphs.

Results

Number of years in use

The ages of LLINs included in this study were 9 years (n = 1), 7 years (n = 3), 4 years (n = 7), 3 years (n = 9), 2 years (n = 5), 1 year (n = 23) and less than 1 year (n = 3). Of the 51 LLINs tested, 47 (92%) exhibited optimal effectiveness (>95% knockdown 1 hour post exposure) and all nets resulted in over 85% knockdown of exposed mosquitoes. However, the nets started showing reduced efficacy after 2 years of home use (Figure 1) and percent knockdown was significantly associated with the number of years in use ($p < 0.001$). Residual deltamethrin (DM) concentration ranged between 70 mg/m^2 and undetectable levels. There was a significant relationship between DM concentration and number of years in use ($p < 0.001$) (Figure 2).

Washing practices

The number of washes were very low (range: 0-6 washes), with a median of 2, 0, 1, 2, 1, 1, 0 washes for initial years of net use in 2009, 2008, 2007, 2006, 2005, 2002 and 2000 respectively, with over 90% of these nets washed three or fewer times. Median DM concentration between the washes was 49.2 mg/m^2, 18.6 mg/m^2, 11.55 mg/m^2, 19.55 mg/m^2, 20.6 mg/m^2 and 31.00 mg/m^2 for 0, 1, 2, 3, 4, 6 washes respectively. There was not a significant relationship between number of

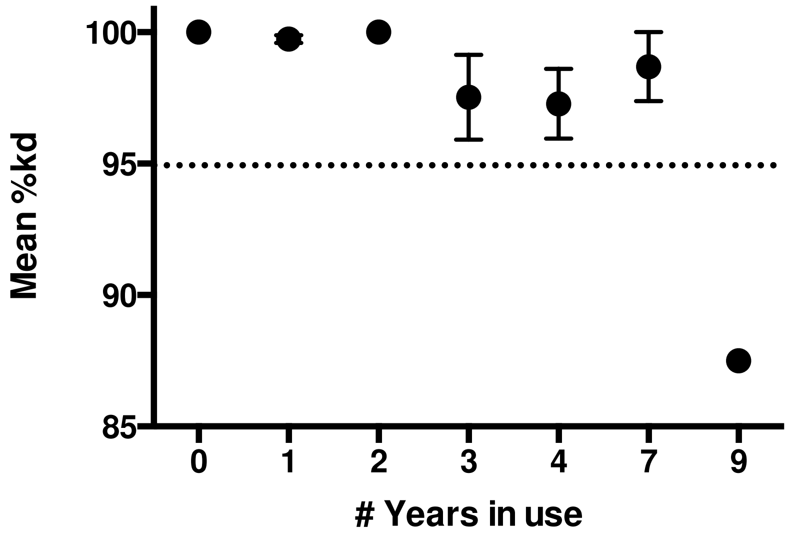

Figure 1. Mean percent knockdown in mosquitoes 60 minutes post exposure to LLINs that had been removed from household use after the given number of years. The dotted line represents the World Health Organization criterion for net efficacy. Error bars represent standard error of the mean.

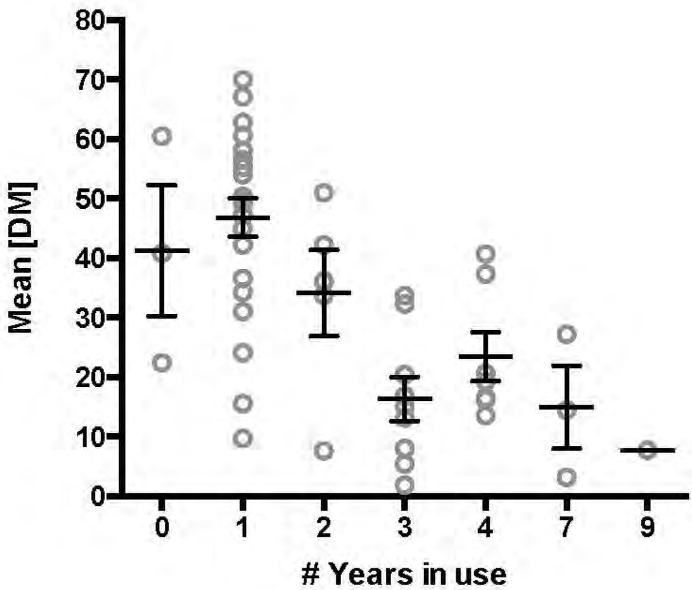

Figure 2. Mean deltamethrin concentration (mg/m[2]) of long-lasting insecticidal nets (LLINs) after the given number of years of household use. Error bars represent standard error of the mean.

washings and DM concentration or percent knockdown. The majority of washed nets (84% of 25 nets) had been washed using soap. There was no significant relationship between whether soap was used in the washing and DM concentration or time to 50% knockdown.

Drying practices

The majority of washed nets (80% of 24 nets) were hung to dry in the sun and this practice was significantly correlated with low DM concentration (Figure 3, p = 0.008). The mean concentration in nets dried in the sun was 20.2 ±3.0 mg/m^2 compared to 36.3 ±7.9 mg/m^2 in the shade. There was not a significant relationship between drying in the sun and time to 50% knockdown.

Physical conditions and odour

The wear and tear of the nets (measured by hole surface area, Figure 4) was significantly correlated with number of years in use (p <0.001). In addition nets over 4 years old were more likely to have holes that were categorized as large or very large than nets under 5 years old (Table 1; p = 0.02), a condition that could greatly reduce net effectiveness. A total of 85% of nets up to 4 years old were classified as being in good condition with less than 50 cm^2 total hole surface area on half of the net (24). The presence of some smoke odour, an indicator of proximity to a cooking fire, was detected in 56% of the nets. However, there was no relationship between smoke odour and time to 50% knockdown or DM concentration.

Discussion

The WHO cone bioassay was used in this study to look at the survival rate of the mosquitoes after exposure to the LLINs. This study presents the proportion knockdown observed 1 hour post exposure, and no recovery was observed in knocked-down mosquitoes 24 hours post exposure. Since the majority of nets remained highly efficacious according to WHO standards, we also measured decreases in deltamethrin concentration as well as increases in time to 50% knockdown to determine if the usage variables had any relationship with efficacy. Results are presented as proportion or time to knockdown rather than mortality; however, we did not observe any recovery of knocked-down mosquitoes after 24 hours. One limitation of the study is that the age of the nets was self-reported and may be subject to recall bias. Additionally the age of nets may not directly correspond with the number of years of in-home use and may also include time that the

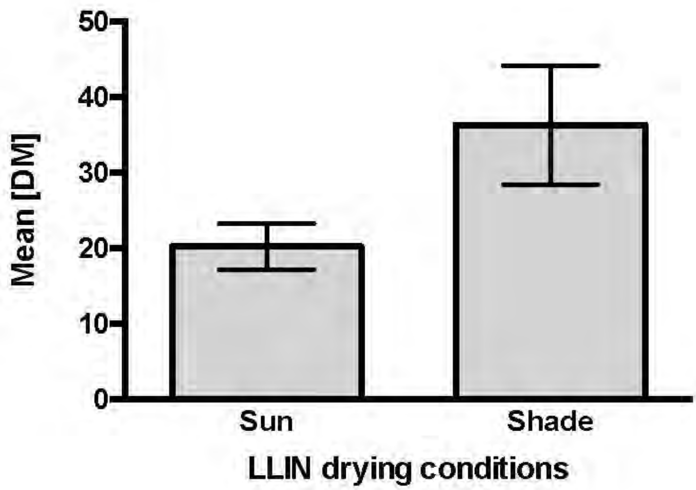

Figure 3. Mean deltamethrin concentration (mg/m^2) of washed long-lasting insecticidal nets (LLINs) that had been dried in the sun or shade (p = 0.008). Error bars represent standard error of the mean.

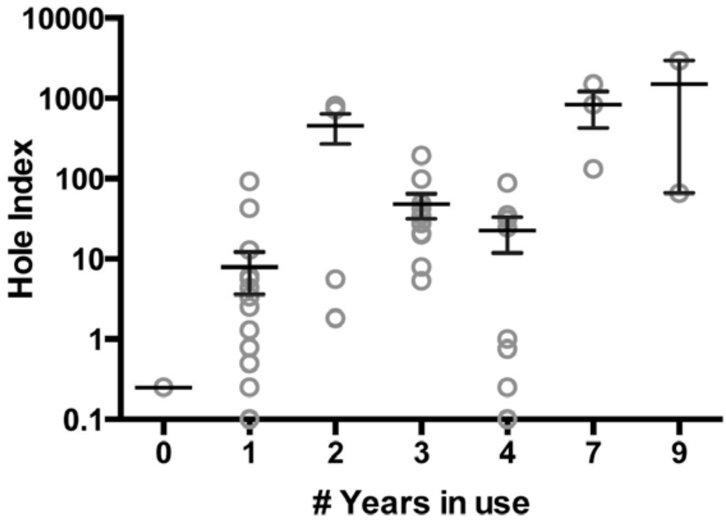

Figure 4. Mean hole index for long-lasting insecticidal nets (LLINs) (estimated cm^2 of compromised net surface on one half of the net) after the given number of years of household use. Error bars represent standard error of the mean.

TABLE 1

MEAN NUMBER AND SIZE OF HOLES IN LLINS COLLECTED AFTER THE GIVEN NUMBER OF YEARS OF HOUSEHOLD USE

Number of years in use	Very small (<1 cm)	Small (1-5 cm)	Medium (5-15 cm)	Large (15-30 cm)	Very large (>30 cm)
0	1.0	0.0	0.0	0.0	0.0
1	7.4	2.2	0.2	0.0	0.0
2	19.6	5.0	0.8	0.2	0.6
3	54.5	9.6	1.2	0.2	0.0
4	29.4	6.8	0.4	0.1	0.0
7	51.3	18.7	3.3	1.3	1.0
9	33.0	10.0	3.5	0.5	2.0

LLINs = long-lasting insecticidal nets

net remained in the package.

Mean percent knockdown was above the WHO criterion for net efficacy (>95%) through 7 years of home use; the 4 individual nets that exhibited slightly lower knockdown (85%<KD<95%) were over 3 years old. In order to preserve maximum deltamethrin residues, users may wash nets as needed but should always dry nets in a shaded area. Although high efficacy was seen in nets over 5 years old, a significant increase in wear and tear was observed in older nets, supporting the recommendation to replace LLINs every five years. This observation may not be a true representation of all nets in use and may have been influenced by owners preferring to donate nets in poor condition. Further studies on the durability of LLINs beyond five years and the relationship between wear and tear and risk of exposure are needed in order to provide comprehensive guidance to the National Malaria Control Program.

These encouraging results are dependent on the susceptibility of local vectors to pyrethroids. In this study the *An. farauti* colony was used in the bioassays in order to remove any individual variations in mosquito populations, allowing us to examine the relative impacts of usage on knockdown effect. Previous studies have shown members of the *An. punctulatus* group to be highly susceptible to pyrethroids (22) and our study observed no difference in knockdown rates between wild and colony *An. farauti*. However, future studies should continue to monitor insecticide resistance, especially as pyrethroid-based control efforts are scaling up, so that it can be mitigated before malaria control is compromised.

The protective effect of an LLIN distribution, and the optimum time for re-intervention, will be governed by entomological factors as well as insecticide degradation (25). LLINs have proven to be a very powerful tool in controlling malaria by reducing mosquito densities and by shortening the lifespan of mosquitoes (7) so that very few live through the extrinsic incubation period for *Plasmodium* spp. The utility of this control measure relies on the vector population entering human dwellings in search of a bloodmeal at the time the user is asleep, two conditions which are not always true in the South-West Pacific region (26,27). Continuous monitoring of mosquito-biting behaviours is also recommended to ensure that LLINs are effectively targeting and reducing the vector population. Otherwise it may be necessary to use an integrated approach to target early outdoor biters, such as larval site reduction, larvicides, odour-baited traps or spatial repellents (28).

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