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Deltamethrin and permethrin residue on long-lasting insecticidal nets after 18 months of use in a visceral leishmaniasis-endemic area in Nepal

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ABSTRACT

The insecticide residue on two types of long-lasting insecticidal nets (LLIN), Olyset Net and PermaNet 2.0, used in a visceral leishmaniasis-endemic village in eastern Nepal was quantified using HPLC. After two washes during 18 months of use the mean insecticide residues on PermaNet 2.0 and Olyset Net were 53.5 mg/m^2 (97.3% of the target dose) of deltamethrin and 911.8 mg/m^2 (91.2% of the target dose) of permethrin, respectively. These residues were close to the insecticide loads specified by the manufacturers of the two LLINs. The use of LLINs has been postulated as an alternative or complementary method to indoor residual spraying. Our results suggest that LLINs should be washed 4–5 times each year throughout their lifespan by specifically requesting villagers to wash nets on certain dates. The insecticide residue on the nets and their bioefficacy against sand fly vectors should be monitored after each wash, in various cultural settings, to assess their durability and long-term retention of the insectide.

1. Introduction

Visceral leishmaniasis (VL), also known as kala-azar, is a fatal disease if left untreated. It has been reported from 52 districts in India, 45 districts in Bangladesh and 12 districts in Nepal, putting approximately 200 million people at risk of the disease.[1] In the Indian subcontinent (Bangladesh, India and Nepal), VL incidence is as high as 21 cases/10 000.[2] Indoor residual spraying (IRS) is the only strategy being adopted in India and Nepal for VL vector control but there have been difficulties in its implementation. Previous studies have shown the efficacy of insecticide-treated nets (ITN) as a protective tool against the sand fly vectors of anthroponotic cutaneous leishmaniasis,[3] zoonotic cutaneous leishmaniasis[4] and zoonotic visceral leishmaniasis.[5] Similarly, long-lasting insecticidal nets (LLIN) reduced the indoor density of the VL vector (*Phlebotomus argentipes*) in endemic regions of India and Nepal.[2,6]

LLINs are considered to be the most technologically advanced form of ITNs and are intended to remain effective after many washes, with the manufacturing companies claiming that they retain their efficacy for 4–5 years.[7] By 2004, the World Health Organization Pesticide Evaluation Scheme had approved two types of LLINs, PermaNet and Olyset Net, for community interventions.[8,9] Later five more

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nets were given interim clearance.[10] LLINs were found to be superior to ITNs and were effective after 20 standard washes.[11] Despite the large body of experience documenting the efficacy of LLINs after washing we could not find any published documents about the insecticide residue on LLINs after being used to control VL in communities in the WHO South-East Asian Region. This study analyzed the insecticide residue on two brands of LLINs after 18 months of use under field conditions in a VL-endemic village of eastern Nepal. Such data is fundamental for health planners and researchers for VL vector control.

2. Materials and methods

2.1. Long-lasting insecticidal nets analysed

The insecticidal residue on two brands of LLIN, PermaNet 2.0 and Olyset Net, was analysed. The specifications for PermaNet 2.0 were 100 denier fibre thickness; mesh size 1.5×1.5 mm; target 55 mg/m^2 deltamethrin concentration coated on polyester fiber (manufactured by Vestergaard-Frandsen Company, Lausanne, Switzerland), and for Olyset Net were 150 denier thickness; mesh size 4×4 mm; target 1000 mg/m^2 permethrin concentration incorporated in polyethylene fiber (manufactured by Sumitomo Chemical Co., Osaka, Japan).

2.2. New long-lasting insecticidal nets

Five new PermaNets were randomly collected from different stacks of the same production batch by date before being distributed in different villages in VL-endemic districts in the eastern part of Nepal. Composite samples of 16 swatches (4 from the roof panel and 3 from each of the 4 side panels) of size 30 (5×6) cm^2 for each LLIN were cut off using sharp scissors. Samples from each net were kept separately in re-sealable bags to obtain the average target concentration of insecticide on each net.

The PermaNet 2.0 and Olyset Net were distributed during a pilot test to compare the two types of net[12] and the latter was selected for the KALANET field trial during this study. Thus we were unable to test the permethrin loaded on new Olyset Nets.

2.3. Used long-lasting insecticidal nets

Two brands of LLINs (PermaNet and Olyset Net) were distributed in a VL-endemic village of Morang district in eastern Nepal in September 2005 and the community was educated on the proper use and washing of the nets.[12] After 18 months of use, five LLINs of each brand were withdrawn from the villagers and replaced by new LLINs. Each collected LLIN was cut vertically into two halves and one-half of each net was sent for residue analysis.

2.4. Insecticide residue analysis

Residue analysis was performed in the Vestergaard Frandsen quality control laboratories in Hanoi, Vietnam. Insecticide determination was performed by normal phase HPLC with UV Diode Array Detection (HPLC-DAD). The principle of this method was proposed in 2006 for adoption by the Collaborative International Pesticides Analytical Council (CIPAC).

Net samples were cut into small pieces (less than 2×2 cm in size) and deltamethrin was extracted into solution using a mixture of the solvents iso-octane (HPLC grade) and 1,4 dioxane (HPLC grade) with 0.15% (v/v) water (80/20). Dibutyl phthalate was added as the internal standard. The extraction bottle was sonicated in a water bath set at 80 °C and then shaken vigorously for at least 15 min. A volume 10 μl of solution was filtered through a 0.45 μm syringe filter into a vial. A volume of 5 μl of filtered solution was injected into a normal phase isocratic HPLC equipped with PDA/UV detector and deltamethrin was quantified using an internal standard calibration curve.[13]

2.5. Data analysis

The one sample *t* test was applied to see the difference between the calculated mean (see Table 1) and the standard mean (target concentration claimed by the manufacturers) of deltamethrin and permethrin residue.

3. Results

Deltamethrin residue on the new PermaNets was (mean ± SD) 59.0 ± 5.4 mg/m^2 and on the used PermaNets after two washes over 18 months was (mean ± SD) 53.5 ± 5.4 mg/m^2 which was 97.3% of the target dose (Table 1). The residue of permethrin on the used Olyset Nets under the same conditions was (mean ± SD) 911.8 ± 56.8 mg/m^2 which was 91.2% of the target dose (Table 1). After washing, the calculated mean value of deltamethrin residue on the PermaNets was not

Table 1
Deltamethrin and permethrin residue on new and used long-lasting insecticidal nets distributed in a visceral leishmaniasis-endemic village in eastern Nepal

Residue on new nets (n = 5)		Residue on nets after two washes and 18 months of use	
PermaNet		PermaNet (n = 5)	Olyset Net (n = 5)
Place in stack	Deltamethrin (mg/m^2)	Deltamethrin (mg/m^2)	Permethrin (mg/m^2)
C1 (top)	52.4	51.3	881.8
C2	55.4	54.2	1012.8
C3	66.5	53.8	882.7
C4	59.5	55.1	896.3
B (bottom)	61.3	53.0	885.3
Mean	59.0	53.5	911.8

significantly different from the standard mean value ($p = 0.077$). However, the calculated mean value of permethrin residue on the Olyset Nets after washing was significantly less than the standard mean value ($p = 0.025$). Over the 18 months some villagers had washed the nets twice as there were two festivals in this time period. Several of the villagers had not washed the nets at all and none of the villagers had washed the net more than twice, hence we were unable to collect LLINs that had been washed more than twice.

4. Discussion

For the five new PermaNets, the average deltamethrin loaded (59 mg/m^2) was higher than the target concentration (55 mg/m^2). The lowest concentration of insecticide was found in the net from the upper layer of the stack and the highest from the one in the middle. Though the number of samples tested was small ($n = 5$), the differences observed may be due to the fact that the insecticide coating was not homogeneous or the upper layer of nets was more exposed to high temperatures. A high concentration of deltamethrin on unwashed PermaNets 2.0 was also reported in earlier studies.[14,15]

In our study, after two washes within 18 months of use, both LLINs retained more than 90% of insecticide residue. PermaNet 1.0 had a significantly lower risk of failure among six net types tested after 2 years of use in a study in western Kenya.[16] The second-generation PermaNet retained insecticide well and had 41.5% of baseline dose after 36 months in another study.[13] The residues of deltamethrin persisted well on impregnated bed nets for at least 15 weeks after impregnation in a study in Iran.[17] Chemical residue loss on Interceptor LLINs and conventionally treated nets, after a 1-year follow-up period, was 22% and 93% respectively in another study.[18] Decreases of deltamethrin on PermaNet by 60–80% after 23 washes,[19] to about 50% over the first 10 washes,[14] to 15% after 20 washes have been reported.[15]

In a previous study, the average permethrin content after 7 years of use was 33–41% of the initial insecticide dose of 20 000 mg/kg^2.[20] Two other studies have reported that the efficacy of Olyset Net remained at about 90% even after 20 washes at an interval of 24 h[21] and that there was no significant difference in efficacy between LLINs washed 20 times and unwashed ones.[22] The bioavailability of insecticide on Olyset netting fibre was consistent, killing 100% *Anopheles minimus* in India during up to 10 months of monitoring, and was observed to be wash resistant after the twentieth wash at fortnightly intervals.[23] The wash resistance and bioefficacy of Olyset nets showed 100% mortality in *A. culicifacies* after up to 11 washings, whereas 100% mortality was observed in *A. fluviatilis* after 20 washings.[11] The efficacy of Olyset nets increased by heat-assisted regeneration (replenishment of permethrin to the surface of the net after washing).[24]

N'Guessan et al. reported a significant loss of activity to a similar level after 20 washes in PermaNet 3.0, PermaNet 2.0 and a conventional deltamethrin-treated net.[15] However, Sreehari et al. reported that mortality of mosquitoes on three different LLINs remained >80% after up to 20 hand washes and up to 15 machine washes.[7] LLINs were found to be superior to ITNs and were still effective after 20 standard washes in a laboratory in Tanzania, Iran, Pakistan and India.[11,14]

In our study, none of the villagers had washed their nets more than twice during the 18 month period and most had washed their nets once. The villagers soaked the LLINs in cold water with detergent for 5–10 min, rubbed them by hand, dipped them in fresh cold water, rinsed them and dried them in a shed. None of the villagers used kerosene lamps to light their house but all of them used firewood to cook food. It has been reported that drying nets for a few hours in the sun was not harmful and exposing the LLINs to dirt, dust and smoke after washing had no significant effect.[25] In a comparative study of four LLINs, between four drying regimens, for PermaNet and TNT there were no significant differences observed but for BASF and Olyset, the differences were significant.[26]

In a comparative study in Nepal using α-cypermethrin for IRS, PermaNet as LLINs and plastering of inner walls of houses with lime as ecological vector management (EVM), the density of *P. argentipes* was significantly low in both the IRS and LLIN arms.[27] The cost of IRS was US$5.9/household/year, of LLIN US$4.5/household/year and of EVM US$8.7/household/year. These results indicate that LLIN and IRS may be cheaper options for the control of sand flies on the Indian subcontinent.[28] It was confirmed in Bihar, India and in a border village of Nepal that in India and Nepal *P. argentipes* sand flies were susceptible to deltamethrin but resistant to DDT.[29] Clusterwide distribution of LLINs in India and Nepal significantly reduced the *P. argentipes* density/house by 24.9% as monitored monthly for 12 months after distribution of LLINs.[6] Insecticide-treated bed nets with slow-release insecticides (KO Tab 123), were effective in terms of a significant reduction in sand fly density and bioassay results.[30] Thus LLINs should be added to VL control programmes as they are cost-effective and efficient in reducing vector density.

The results of bioassays and the chemical analysis of samples from sprayed walls in India and Nepal indicated substandard spraying and suboptimal concentrations of insecticide on sprayed surfaces.[31] In our study both LLINs retained >90% of the insecticide loaded. Hence, the use of LLINs has been postulated as an alternative or complementary method to IRS for vector control. In 1998, 58% and 37% of villagers of two villages had used untreated mosquito nets in Nepal,[32] and in 2000 mosquito nets were used by 38% of people in Nepal.[33] The number of net-users increased to 61% (297/490) during 2007 and among them 30% used the net throughout the year.[34] In another study, 87% of 60 households (80% in India and 93% in Nepal) had an average of 2.6 untreated mosquito nets per house.[12] Hence, behaviour and practices may also favour the use of LLINs, This would likely be more efficient if predistribution education and motivation is done. In 2007 all users liked the textures of both polyester and polyethylene nets but they had a slight preference for those made of polyester (PermaNet) mainly because of their relatively greater softness in comparison to polyethylene LLINs.[12] If Olyset Net is used its mesh size should be reduced so that even after loss of insecticidal effect it may prevent the sand flies from entering inside the net.

In conclusion, in this study PermaNet 2.0 and Olyset Net were found to be equally good at retaining insecticide residue. The manufacturers claim efficacy of LLINs for 5 years even after 25 washes. In future studies, the 25 washes should be spaced out so that the nets are washed 4–5 times each year throughout their lifespan by specifically requesting villagers to wash nets on certain dates. The insecticide residue on the nets and their bioefficacy against sand fly vectors should be monitored after every wash, in various cultural settings, to assess the durability of the nets and long-term retention of the insecticide.

Authors' contributions: MD conceived and designed the study in collaboration with MB and AK; MD, SR and LR implemented the study in the field; MD and LR performed the laboratory work; LR managed the coding, data and records; SR, LR and AP analysed and interpreted the data; MD, SR and LR drafted the manuscript. All authors revised the manuscript critically for intellectual content and read and approved the final version. MD is guarantor of the paper.

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Competing interests: Vestergaard-Frandsen Company is the manufacturer of PermaNet but they tested the residue impartially which is indicated by the fact that both nets retained more than 90% of their residue. Chemicals in the nets have also been tested, in a dengue study, by Vestergaard. Similar results have been found on validation at Liverpool, UK (personal communication with Prof. Axel Kroeger). The authors have no interests to declare.

Ethical clearance: The Ethical Review Committee of the World Health Organization (Geneva, Switzerland) and the Institutional Ethical Review Board of B.P. Koirala Institute of Health Sciences (Dharan, Nepal) approved the study protocol.

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