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# The effects of washing and duration of use of long-lasting insecticidal nets (PermaNets) on insecticidal effectiveness

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A B S T R A C T

The use of insecticide-treated nets (ITNs) is one of the most feasible weapons available for malaria control in Africa today. One of the important requirements for ITN use is regular re-treatment at an appropriate time. As a response to the low re-treatment rate when the ITN users are expected to purchase insecticide and re-treat their nets, manufacturers have developed long-lasting insecticidal nets (LLIN) that are pre-treated in the factory, and are claimed to require no further treatment during their lifespan. A study was conducted to assess the possible effects of number of washings, frequency of washing and the duration of use on the effectiveness of PermaNets, a LLIN, against mosquitoes. The study was done for 9 months at Chekereni village, Northeastern Tanzania. The LLINs and untreated control nets were distributed to villagers in three groups. Group 1 nets were used without being washed for the whole study period. Group 2 nets received one wash per month, while group 3 received two washes per month. The effectiveness was assessed by contact bioassays using World Health Organization (WHO) bioassay cones. Mosquitoes were exposed to the netting for 3 min. The knock down and mortality was scored after 3 min and 24 h, respectively. Results showed that the number and frequency of washes had no significant effect on the efficacy for up to 18 washes. Similarly, we could not detect a significant effect of duration of use and conditions of use on the efficacy for up to 9 months. PermaNets caused short-lived irritation and sneezing but appear to be well accepted by the community in which the study was conducted.


## 1. Introduction

Malaria continues to be a major impediment to health and development in Africa, South of Sahara where it takes its greatest toll on very young children and pregnant women (WHO/UNICEF, 2003). The use of insecticide-treated nets (ITNs) is one of the most powerful interventions available to decrease the malaria burden in Africa today. It has been shown, for instance, that proper and regular use of ITNs can reduce malaria mortality in children aged under 5 years by 20% and by 50% when combined with early diagnosis and treatment of malaria cases (UNICEF, 2003). The World Health Organization (WHO) recommended the increased use of ITNs as one of the strategies to reduce the burden of malaria in Africa during the initiation of the global campaign against malaria, the roll back malaria (RBM) (WHO, 1998). Similarly, the Abuja declaration on rolling back malaria, set a target of 60% ITN coverage for children less than 5 years of age and pregnant women in Africa by 2005 (WHO, 2000). In Tanzania, the national bednet voucher scheme, aims at providing as many pregnant women and their children as possible with vouchers to enable purchase of ITNs at subsidized and affordable prices.

Unfortunately, a number of reports revealed very low overall coverage of ITNs in Africa (<20%) and of great concern <1% coverage of young children (UNICEF, 2003; WHO/UNICEF, 2003). Apart from low coverage of ITN across Africa, poor and low re-treatment rates of ITNs at appropriate times has been identified as another major set back in the ITN programs, when ITN users are expected to go to shops to purchase insecticides to re-treat their nets. This has been attributed to the wrong perception by many users that physical barrier of the net is more important in providing protection than the insecticide and possibly the extra costs and efforts involved in obtaining insecticide and retreating the nets (Amstrong-Schellenberg et al., 1999). In response to the low re-treatment rates of ITNs, especially in Africa, WHO prompted manufactures to develop long-lasting insecticidal nets (LLIN). These are ready to use factory pre-treated nets, which are claimed to require no further treatment during their physical lifespan of about 4–5 years, before the net becomes badly torn. The LLINs are considered to be a potential solution to the problem of low net re-treatment rate and a major breakthrough in malaria

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prevention (WHO, 2003). Considering the importance of LLINs in malaria control, a study was conducted to assess the effect of frequency of washing, number of washes and duration of bednet use on insecticidal effectiveness of LLINs in a Tanzanian village. This report provides results of 9 months assessment of the brand of LLIN named PermaNet at Chekereni village, Northeastern Tanzania.

## 2. Materials and methods

### 2.1. Study area

The study was conducted for nine consecutive months between July 2004 and April 2005 at Chekereni village. The village is within the Lower Moshi irrigation scheme area, 10 km South of Moshi town at the foot of Mount Kilimanjaro, Northeastern Tanzania (37°20′E, 3°21′S, 700 m above sea level). Paddy growing in irrigated land is the main activity and conducted throughout the year and therefore mosquito population density is high all year round.

### 2.2. Net distribution

Two types of bednets were distributed to individuals and efficacy of the bednets against mosquitoes tested by bioassays.

#### 2.2.1. Type 1—treated nets

These were long-lasting insecticidal nets with 55 mg/m$^2$ residual deltamethrin, factory pre-treated nets. The nets were 100% polyester, white in colour and approximately conical in shape with the circumference of the top and bottom rings 180 and 1200 cm, respectively and the distance along the sloping surface of the net 220 cm, with the commercial name of PermaNet® 2.0, made by the Vestergaard Frandsen Company. Sixty nets were divided into three groups of 20 nets and distributed to individuals in families who were assessed as unable to purchase nets and having no bednets at home. The nets were labelled according to the intended number of washings per month. The first group of nets was not washed at all (W0), the second group was washed once a month (W1), the third group was washed twice a month (W2). This division aimed at assessing the effect of number and frequency of washing. Half of the nets in each of these groups (10 nets) were given to families with mud houses, grass-thatched roofs with no cemented floor. The other half was given to families with brick built houses with iron sheet roofs and cemented floors. This division separated households with different socioeconomic status, which in turn reflected different net use conditions. In this regard, the variation in cleanliness, dust and smoke from cooking firewood and charcoal between the two was evident. Therefore this division aimed at assessing the effect of different use conditions; dirty and dusty on one hand or relatively clean environment on the other.

#### 2.2.2. Type 2—untreated nets

These were similar to type 1 except that they were not treated. This group was also divided into three groups of 20 each and distributed to individuals in the same area in a similar way. The group was used as negative controls for type 1 nets.

Before net distribution, the prospective net users were informed of the aims of the work, the methods to be used in collecting the data in their houses, benefits of the work and in particular, the long-lasting insecticidal type of bednets and the possible side effects. During the tests, individual informed consent was sought before entering each house for bioassay tests. The study participants were shown the results of the bioassays regularly, especially results of exposing mosquitoes to their nets. At the end of the study, the individuals with untreated nets were allowed and assisted to treat their nets with deltamethrin.

### 2.3. Net washing

Hand washing was done once or twice per month or not at all. Washing was done using clean water and normal alkaline detergent soaps available in local shops, for a duration of about 10 min and rinsed twice. They were then spread on flat surfaces in shade to dry. The users of the nets were requested not to do any washing or treatment on their own. This request was made to all members of each family receiving a net during the handing over of the net to avoid washing not intended as part of the trial. Effort was also made to get information on net washing by regularly asking children when they were alone if they had seen their elders washing the nets in our absence. Washing was done under the investigators' supervision on specific days every month.

### 2.4. Bioassay tests

#### 2.4.1. Test mosquitoes

Two strains of *Anopheles gambiae* Giles were used for the tests. A strain of An gambiae sensu lato (R-70) maintained in the laboratory at the Tropical Pesticides Research Institute (TPRI) insectaries for several decades and known to be susceptible to most insecticides, was used for the test. Two to three days old, female mosquitoes were blood fed and then left overnight and tested when they were semi-gravid. In order to simulate field conditions, wild adult female An gambiae sensu lato were collected from local houses and cattle sheds in the study area and also used for tests. These resting mosquitoes were almost all blood fed. Collection was done early in the morning and mosquitoes put in paper cups in cool boxes, transported to a field laboratory and the tests were done the following morning when the mosquitoes were semi-gravid, alongside the laboratory strain.

#### 2.4.2. Test procedures

The first bioassay was done just before the net started to be used and subsequent tests were conducted monthly. The tests were done early in the morning before 10:00 h to avoid high temperatures during exposure. Contact bioassay tests, using WHO plastic bioassay exposure cones were employed. Four exposure cones were attached at four different positions on each net, with rubber bands. A net flap was made inside the cone to reduce the chance of mosquitoes resting on the cone instead of on the treated net. About five to seven mosquitoes were then gently introduced into the cone using a sucking tube. The cone-hole was then covered with cotton wool. The exposure was for 3 min, after which mosquitoes were gently removed using the sucking tube and put in paper cups. Knock down was scored immediately by counting all individuals unable to fly. The testing of laboratory mosquitoes was followed immediately by testing wild caught mosquitoes using the same way. While in the paper cups, mosquitoes were supplied with cotton wool with glucose solution, placed on the net covering the paper cups. The paper cups with the mosquitoes were then put in cool boxes and transported to the field laboratory. They were left overnight in a holding room, maintained at $27 \pm 2$ °C and $76 \pm 5$% RH. Mortality was scored after 24 h. Five nets were used for the test for each of the three groups, that is those unwashed (W0), washed once a month (W1) and washed twice a month (W2). Each of the five bednets was tested with a minimum of about 20 mosquitoes (four cones with a minimum of five mosquitoes each). Therefore, a minimum of 100 mosquitoes was tested in each level of washing each month. This was done for both the LLIN group and control (untreated), using both laboratory-reared and field-collected mosquitoes separately. The tests were done at homes where the nets were in use. To make sure that all nets were included in the tests, the nets to be tested were changed each month in rotation, until all the 20 nets in each

**Table 1**
Mortality (corrected for control mortality) of mosquitoes exposed to PermNets with various numbers of washings

| Washes per month | Max total washes | Wild-collected mosquitoes | | | Laboratory-reared mosquitoes | | |
|---|---|---|---|---|---|---|---|
| | | Total exposed mosquitoes | % mean mortality | % mortality range | Total exposed mosquitoes | % mean mortality | % mortality range |
| 0 | 0 | 880 | 99.1 | 96.0–100 | 993 | 98.4 | 95.4–100 |
| 1 | 9 | 842 | 98.6 | 95.8–100 | 970 | 99.3 | 96.0–100 |
| 2 | 18 | 869 | 97.8 | 95.0–100 | 1010 | 98.6 | 95.2–100 |
| *P*-value | | | 0.66 | | | 0.54 | |

group were equally involved in the tests. Bioassay tests were done once a month for 9 months to assess the effect of duration and conditions of use on the efficacy. The duration of net use was grouped as short (0–3 months), medium (4–6 months) and long (7–9 months).

### 2.5. Questionnaire

A simple questionnaire was designed to seek information on the acceptability of the nets. Fifty-six participants in the LLIN group responded while 52 responded from the untreated control.

### 2.6. Data analysis

The proportion of dead mosquitoes (mortality) from each tested net for each level of washing and duration of net use for the whole study period were listed and tested for normality, which gave a fairly good normal distribution. The mean percentage knock down (KD) at 3 min and mortality at 24 h were estimated for each level of washing per month and duration. A simple analysis of variance was used to compare mean percent mortalities and KDs obtained from the three levels of washings (W0, W1 and W2), duration of use (short, medium and long) and the two conditions of use. Paired *t*-tests used to compare the overall mean percentage mortality of laboratory and field strains. Where control mortality was between 5% and 20%, the treatment observed mortality figures were corrected by the formula of Abbott (1925).

## 3. Results

The study participants appeared to have complied well with our request not to wash the nets as we never saw any sign of the net being washed in our absence. The first bioassay test done before net use gave 100% mortality. The monthly bioassay test results are presented in Tables 1–4. There was a mean 24 h corrected mortality of above 97% with no significant difference ($P > 0.05$) among the different levels of washing for both strains of mosquito (Table 1). Similarly, our results show that there were corrected mortalities of above 97% among the different durations of net use, with no significant difference between them (Table 2). On the other hand the KD effects were variable, ranging from 0% to 87% with laboratory-bred mosquitoes and 8.7–80% with wild-collected mosquitoes. There was no significant difference ($P > 0.05$) in mean percentage KD at 3 min from different levels of washing with each strain (Table 3). However the duration of use showed some effect on the KD of laboratory-bred mosquitoes, with significantly higher ($P < 0.05$) KD in short duration of use. This effect was not observed with the wild-collected mosquitoes (Table 4). The overall percentage mortalities of wild and laboratory mosquitoes, showed no significant difference ($t = 0.184$, $P = 0.855$). The control mortalities of laboratory mosquitoes exposed to untreated bednets varied from 0% to 18.2% (mean = 6, S.E. = 1.05) while in wild mosquitoes it varied from 0% to 14.3% (mean = 5.7, S.E. = 1.04).

Our questionnaire indicated that 85% of respondents among LLIN users complained that they had sneezing and slight irritation for the first week or two. There were no such complaints from the control group. However, almost all respondents appreciated that the nets have been of much help to them in reducing night biting of mosquitoes. Moreover, the mortality of mosquitoes exposed to bednets up to 9 months without treating the nets impressed the participants to the extent of asking the price and availability of this type of nets.

## 4. Discussion

It is known that washing and conditions of use are important factors determining the amount of insecticide in nets, its effectiveness and therefore the requirement for re-treatment. This is because the use of alkaline soaps and repeated vigorous washing can remove some of the pyrethroid deposit from nets (Najera and Zaim, 2002). This is the reason for the need for annual re-treatment of conventional nets (Maxwell et al., 2002). Our result shows that after a maximum of 18 washings with alkaline detergent soaps, the biological efficacy of PermaNets against laboratory-bred and field-collected mosquitoes were similar to unused and unwashed nets. Such high performance and wash resistance of PermaNet was also reported by Graham et al. (2005) and Yates et al. (2005). Similarly, we could not detect a major effect of duration and conditions of bednet use on the efficacy of the LLINs on mosquitoes after up to 9 months of continuous use in two different field use conditions. Lindblade et al. (2005) also found that the number of months that LLINs were in use was not associated with net failure. In our study, the group of bednets that were not washed at all (W0) for 9 months became very dirty to the extent of changing the original colour due to smoke from cooking fires, charcoal and dust, especially in mud houses with no cemented floors, but the nets maintained their efficacy against mosquitoes, as also found by

**Table 2**
Mortality (corrected for control mortality) of mosquitoes exposed to PermaNets with varying duration of use

| Duration of bednet use | Wild-collected mosquitoes | | | Laboratory-reared mosquitoes | | |
|---|---|---|---|---|---|---|
| | Total exposed mosquitoes | % mean mortality | % mortality range | Total exposed mosquitoes | % mean mortality | % mortality range |
| Short | 860 | 98.8 | 95.8–100 | 984 | 99.0 | 96.0–100 |
| Medium | 879 | 97.8 | 95.2–100 | 973 | 98.6 | 95.2–100 |
| Long | 852 | 98.7 | 96.0–100 | 1016 | 98.6 | 95.4–100 |
| *P*-value | | 0.98 | | | 0.93 | |

**Table 3**
Knock down rates of mosquitoes immediately after 3 min exposure to PermNets with various numbers of washings

| Washes per month | Wild-collected mosquitoes | | | Laboratory-reared mosquitoes | | |
|---|---|---|---|---|---|---|
| | % mean KD | % knock down range | | % mean KD | % knock down range | |
| | | Min. | Max. | | Min. | Max. |
| 0 | 33.6 | 8.7 | 63.6 | 50.5 | 14.3 | 86.9 |
| 1 | 50.0 | 20.8 | 80.0 | 47.7 | 24.0 | 85.7 |
| 2 | 34.0 | 9.1 | 52.6 | 39.1 | 0.0 | 66.7 |
| *P*-value | 0.114 | | | 0.563 | | |

**Table 4**
Knock down rates of mosquitoes immediately after 3 min exposure to PermNets with varying duration of use

| Duration of bednet use | Wild-collected mosquitoes | | | Laboratory-reared mosquitoes | | |
|---|---|---|---|---|---|---|
| | % mean KD | % knock down range | | % mean KD | % knock down range | |
| | | Min. | Max. | | Min. | Max. |
| Short | 43.5 | 8.7 | 74.0 | 63.0 | 21.0 | 87.0 |
| Medium | 39.0 | 9.1 | 80.0 | 36.0 | 0.0 | 57.1 |
| Long | 35.6 | 19.2 | 60.0 | 40.0 | 15.0 | 66.7 |
| *P*-value | 0.74 | | | 0.017 | | |

Njunwa et al. (1991). Our results indicated significantly higher KD effect of the LLINs in nets used for a short duration than those used for long duration in laboratory-reared mosquitoes. However, this effect was not observed with the wild caught mosquitoes and most importantly, the mortality was not affected. It appears that after 9 months of use with 18 washings, the amount of deltamethrin and therefore the activity had started to decline, resulting into low KDs. However, there was still sufficient insecticide to eventually kill the mosquitoes. From the evidence of Maxwell et al. (2006) it appears that the insertion of the mosquitoes inside netting wrapped around wire frames and observation of the time for KD is a more sensitive test for insecticidal power of netting than use of WHO cones and observation of mortality after 3 min plus 24 h holding. Since detergents and washing conditions are extremely variable, wash resistance tests for LLINs may need to be done for each situation.

The KD rates observed showed a wide range and relatively low overall mean. The variability is due to the use of cones for exposing mosquitoes to the treated nets in which mosquitoes could be spending varying times on treated nets and the plastic cones. It is possible that during the exposure, some mosquitoes spent more time on the treated nets than others and therefore picked up more insecticide. The similar performance of the LLINs to both wild and laboratory strains known to be susceptible to deltamethrin, is an indication that the malaria vectors in the study area are still fully susceptible to deltamethrin, which is currently used in bednet treatment in Tanzania.

Although majority of our questionnaire respondents complained of sneezing and slight irritation, this side effect was transient and short lived. This side effect is also observed in conventionally treated nets with deltamethrin at a much lower concentration rate of 25 mg/m$^2$ (WHOPES, 2003), which is the WHO recommended rate for treatment of conventional bednets. Since the insecticide is bonded to the net fibre with resins, PermaNets may reduce both the human exposure and the risk of environmental contamination (WHO/UNICEF, 2003). Despite the short-lived sneezing and irritation to LLINs users, our results show that this type of bednet has been well accepted in the study village. This was also the case with lambdacyhalothrin-treated nets, which led to complaints of sneezing (Njunwa et al., 1991; Maxwell et al., 1999).

Although the use of LLINs avoids the need to organize a system for retreating nets, the manufacturing process and technical requirements could limit the production to few large specialized net manufacturers and consequently higher costs for the nets. To reduce the net costs and make LLINs available to all poor rural communities, a simplified treatment process to increase the effective lifespan of conventionally treated nets would increase the supply of this important weapon against malaria. The K-O tabs 1-2-3® may satisfy this requirement (Yates et al., 2005).

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