THE IMPACT OF REPEATED WASHING ON RESIDUAL EFFICACY OF PYRETHROID LONG LASTING INSECTICIDAL NETS (LLINs) AGAINST DENGUE AND MALARIA MOSQUITO VECTORS

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ABSTRACT

The influence of washing practices on the efficacy of pyrethroid Long Lasting Insecticidal Nets (LLINs) have been evaluated against mosquito vectors, e. i. *Anopheles aconitus* (malaria) and *Aedes aegypti* (dengue). The different active ingredient of pyrethroid insecticides studied, were alfa-cypermethrin 0,2 g/m², deltamethrin 0,055 g/m² and permethrin 1,0 g/m². The evaluation was conducted at of the Institute of Vector and Reservoir Control Research and Development laboratory, Salatiga and washing practices was done using WHO standard method. Field evaluation against *An. aconitus* was done at Tambangan village, Semarang municipality, were washing practices were conducted by the health village cader. The result showed that the influence of washing practices on the LLINs efficacy’s against *Ae. aegypti* and *An. aconitus* (Laboratory study) and *An. aconitus* (field study) were similar. Both study showed that all evaluated pyrethroid LLINs were effective to killed the mosquito vectors for less than 10 washes.

Key words : Long Lasting Insecticidal Net (LLIN), washing resistance, mosquito vectors
INTRODUCTION

The most important malaria vector species tend to bite late at night, mosquito nets would be expected to protect against them effectively. Bed nets have been used to protect people from mosquito bites. The traditional bed net is not effective sufficiently when it is torn and thus mosquitoes can enter through any holes. One way of overcoming this disadvantage is to treat bednets with an insecticide, preferably a pyrethroid, which is having rapid action against mosquitoes. Pyrethroids quickly knocked down and later kill mosquitoes upon contact.

Nets are generally treated by dipping them into an appropriate mixture of a pyrethroid formulation in water and drying them flat, preferably in the shade. A long-lasting insecticidal net (LLIN) is a factory-treated mosquito net that is expected to retain its biological activity for a minimum number of standard WHO washes and a minimum time under field conditions. Currently, a long-lasting insecticidal net would be expected to retain biological activity for at least 20 standard WHO washes under laboratory conditions and 3 years of recommended use under field conditions, as defined in the WHO guidelines (WHO, 2005).

Among the available control measures, vector control using mosquito nets has recently received some attention because it is a simple and effective means of personal protection.

Impregnation of nets offered several advantages including extending the useful life of the net because treated nets are effective even when they are torn: providing a lethal resting site for mosquitoes even during the daytime when they are not used.

Insecticide impregnated bed nets (IBN) have been shown reduce malaria transmission (Binka et al, 1996; Lengeler, 1998). These investigations were based on nets that were dipped in a water emulsion of a pyrethroid insecticide. Such nets lose their efficacy by repeated washes or after about 6-12 months of use, depending on the pyrethroid insecticide and product (Curtis et al, 1996; Binka et al, 1996; Vythilingam, 1996).

One major problem with the current combination of netting materials, insecticides and methods of treating nets is that regular washing quickly diminish the insecticidal effect of the nets. Malaria control programs often ask people using the nets, not to wash them between treatments. The frequency of washing nets varies. However, it is unreasonable to expect families to sleep inside nets that
have not been washed for a long time. It is therefore important that a combination of netting material, insecticide and method of treatment be developed that will allow normal washing without loss of effectiveness (WHO, 2006).

The objective of this study was to determine the impact of repeated washing on residual efficacy of pyrethroid Long Lasting Insecticidal Nets (LLINs) against malaria and dengue vectors, *Anopheles aconitus* and *Aedes aegypti*.

**MATERIALS AND METHODS**

**Washing resistance**

The resistance of an LLINs to washing was determined through standard bioassay carried out on nets washed at intervals required for regeneration, using the standard WHO method, dried and held at 30°C. Bioassay was done after 0, 5, 10, 15 and 20 washes. Each bioassay was conducted just before the next wash (WHO, 2005). The efficacy of pyrethroid mosquito nets LLINs were evaluated under laboratory and field condition.

**Laboratory evaluation**

Determination of time period required for full regeneration of the Long Lasting Insecticidal Nets (LLINs) upon after washing practices. The evaluation was conducted in the laboratory against malaria vector *An. aconitus* and DHF vector *Ae. aegypti*.

**Field evaluation**

Determination of time period required for full regeneration of the Long Lasting Insecticidal Nets (LLINs) after washing practices which was done monthly by the village caders. Evaluation residual efficacy of LLINs was carried out against rice fields malaria vector *An. aconitus*.

Both studies were on January – August 2008 conducted, in the Institute for Vector-Reservoir Control Research and Development (IVRCRD), Salatiga Municipality and in the rice fields’ areas of Tambangan Village, Mijen Subdistrict, Semarang Municipality, Central Java Province, using bioassay test method (WHO, 2005).

**LABORATORY EVALUATION**

1. Materials:

   Bioassay test kits, sucking tube, plastic cups, cotton wool, rubber band, glucose solution, powder soap, alcohol, Three types of pyrethroids Long Lasting Insecticidal Nets (LLINs) evaluated. The nets were impregnate with three different active ingredient (a.i), of pyrethroid insecticides : Alphacypermethrin (0,2
Damar T.B. et. al, The Impact of Repeated washing

Deltametrin 0,055 g/m² and Permetrin (1,0 g/m²).

Each LLIN type was selected and cut in pieces for 25 cm X 25 cm (three pieces for each mosquito net’s side). Bioassay tests of LLINs were done for: none (before washing), 5, 10, 15 and 20 times washes. Female mosquitoes An. aconitus or Ae. aegypti, 3-4 days old, glucose fed condition (colonies maintained in the laboratory of the IVRCRD) were used for evaluation.

2. Methods
a. Net washing (WHO, 2005)

The net samples (25 cm X 25 cm) of each LLINs type was individually introduced into blue cap bottle (1000 ml volume), containing 500 ml of deionized water and 2 g/l soap (“Savon de Marseille” recommended the standard soap), pH 10-11, added just before and fully dissolved. Bottles were shaken immediately for 20 minutes at 155 movements per minute. The samples were removed and rinsed twice for 20 minutes in clean deionized water in the same shaking conditions as stated above. Nets were dried at room temperature, wrapped in aluminium foil and stored at 30°C in the dark between washes.

b. Bioassay test

Five (susceptible strain) sugar fed, 2-4 day old tested mosquitoes, An. aconitus and Ae. aegypti, were exposed to netting materials (25 cm X 25 cm) for 3 minutes, under standard WHO cones. After sixty minutes of contact the knocked down (KT) mosquito were counted and kept under observation for 24 hours in laboratory, to calculate mortality. At least 180 mosquitoes were tested on each sample (4 sides, three replicates, 15 mosquitoes/cone). Tested mosquitoes were also exposed to untreated nets, used as control/UTC. They were then exposed for 3 minutes in the exposure cone, gently taken out after exposure and kept into the holding plastic cups to determine the mortality after 24 hours holding in the laboratory, with access to sugar solution. All bioassay tests were conducted in the laboratory maintained at room temperature 25-29°C and the humidity of 68-92%.

FIELD EVALUATION

1. Materials:

Bioassay test kits, sucking tube, plastic cups, cotton wool, rubber band, glucose solution, powder soap, alcohol, three type of pyrethroid
LLINs sample, as stated on the laboratory evaluation.

Nine village houses (similar in condition) were chosen for the study. Three other selected houses were used for untreated insecticide mosquito nets which were used as untreated control/UTC. Each LLIN type was provided to be used by the people, in each selected house (three replicates for each LLIN type).

2. Methods
   a. Net washing
      Net samples measure (180x160x150 cm) were distributed to nine selected families in the study village (two units for each family). A total of 18 unit LLINs (6 unit for each type), were evaluated for the study. The nets were individually washed by the village cader, using well water and ordinary soap which is normally used by the villagers for clothes washing. Ten grams of washing soap powder added into the bucket which was provided with 10 liters of well water. Washing method applied as commonly done by the villagers and rinsed twice using well water. The nets were dried at room temperature and heavy shade place (not direct to the sun rise), before fixed at the sleeping bed. Washing practices of the LLINs were conducted biweekly, before the bioassay test performed. Evaluations were done for 10 times (during the study), before washed and (1-9) post washed practices. Evaluation was terminated when tested mosquito’s mortality was less than 80%.
   b. Bioassay test
      Five female susceptible strain mosquitoes (glucose fed, 3-4 days old) An. aconitus were exposed to the mosquito nets (which have been regularly used by the villagers). Exposure was done for 3 minutes, under WHO standard cones. Same bioassay test method of laboratory evaluation was applied for this evaluation.

DATA ANALYSIS

The data was analyzed descriptively and also by using varian analysis of tested mosquito mortality An. aconitus and Ae. aegypti after bioassay evaluations.
RESULTS

Laboratory evaluation

The results of the bioassay test on *Ae. aegypti* carried out in the laboratory for all evaluated LLINs (Alphacypermethrin 0,2 g/m$^2$, Deltamethrin 0,055 g/m$^2$ and Permethrin 1,0 g/m$^2$), for unwashed and after repeated washing, presented on Table 1 and Figure 1.

The mortality of *Ae. aegypti* on unwashing of all evaluated nets were 100%. The mortalities on LLINs of Alphacypermethrin 0,2 g/m$^2$, Deltamethrin 0,055 g/m$^2$ and Permethrin 1,0 g/m$^2$, were respectively dropped to 68,44%, 60,22% and 55,56% at the 10th washes.

Table 1. Mortality (%) of tested mosquito *Ae. aegypti* after exposure on the LLIN (Laboratory evaluation).

<table>
<thead>
<tr>
<th>LLINs</th>
<th>Number of washing practices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Alphacypermethrin</td>
<td>100</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>100</td>
</tr>
<tr>
<td>Permethrin</td>
<td>100</td>
</tr>
</tbody>
</table>

The influence of washing practices of (LLINs) Alphacypermethrin (0,2 g/m$^2$), Deltamethrin (0,055 g/m$^2$) and Permetrin (1,0 g/m$^2$), against malaria vector *An. aconitus* in the laboratory, presented on Table 2 and Figure 2. The efficacy of evaluated LLINs Alpha-cypermethrin (0,2 g/m$^2$) and Deltamethrin (0,055 g/m$^2$), were killing *An. aconitus* effectively for 10 washes. The average mortalities for 10 washes for Alphacypermethrin and Deltamethrin were 87,78% and 86,89% (> 80%). In addition, Permethrin LLIN was only effective for less than 10 washes.
Table 2. Mortality (%) of tested mosquito *An. aconitus* after exposed on the LLIN (Laboratory evaluation).

<table>
<thead>
<tr>
<th>LLINs</th>
<th>Number of washing practices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Alphacypermethrin</td>
<td>100</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>100</td>
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<tr>
<td>Permethrin</td>
<td>100</td>
</tr>
</tbody>
</table>

**Field evaluation**

The influence of washing practices of (LLINs) Alphacypermethrin (0,2 g/m$^2$), Deltamethrin (0,055 g/m$^2$) and Permethrin (1,0 g/m$^2$), against malaria vector *An. aconitus* field evaluation, presented on Table 3 and Figure 3.

Table 3. Mortality (%) of tested mosquito *An. aconitus* after exposed on the LLIN (Field evaluation).

<table>
<thead>
<tr>
<th>Number of washing practices</th>
<th>Percent (%) Mortality of tested mosquito</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alphacypermethrin</td>
</tr>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>99,26</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
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<tr>
<td>4</td>
<td>99.63</td>
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<td>5</td>
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<td>6</td>
<td>94.69</td>
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<tr>
<td>7</td>
<td>87.04</td>
</tr>
<tr>
<td>8</td>
<td>81.79</td>
</tr>
<tr>
<td>9</td>
<td>78.15</td>
</tr>
</tbody>
</table>

Mortality of *An. aconitus* post exposed on pyrethroids LLINs Alphacypermethrin 0,2 g/m$^2$, Deltamethrin 0,055 g/m$^2$ and Permethrin 1,0 g/m$^2$, were respectively effective for 8, 9 and 7 washes, and the average mortalities were 81.79%, 82.47% and 81.91% respectively (> 80% mortality).
DISCUSSION

LABORATORY EVALUATION

The evaluated mosquito nets of long lasting insecticidal nets (LLINs) made of polyester multi-filament 75 denier, which were impregnated with a.i Alphacypermethrin 0.2 mg/m$^2$, and Deltamethrin 0.055 g/m$^2$, effective to kill 
Ae. aegypti for less than 10 washes.

The percentage mortality of 
Ae. aegypti exposed to Alphacypermethrin 0.2 g/m$^2$, Deltamethrin 0.055 g/m$^2$ and permethrin 1.0 g/m$^2$ after 5 washes were respectively 93.78%, 95.33% and 90.0%, while for 
An. aconitus with mortality as 89.33%, 99.56% and 81.56%. After the nets had been washed 10 times, the percentage mortality of 
Ae. aegypti exposed to Alphacypermethrin 0.2 g/m$^2$, Deltamethrin 0.055 g/m$^2$ and Permethrin 1.0 g/m$^2$ was 68.44%, 60.22% and 55.56%, while for 
An. aconitus the mortality was 87.78%, 86.89% and 58.0% (Table 1 and 2, Figure 1 and 2).

Mortality of 
Ae. aegypti exposed to all evaluated LLINs was dropped below the cut off value of 80.0% after 10

washes. While mortality of 
Ae. aegypti exposed to Permethrin 1.0 g/m$^2$ LLIN was dropped below the cut off value, after 10 time washes, it was 55,56%.

After 10 washes, mortality of 
An. aconitus exposed to all evaluated LLINs was dropped below the cut off value of 80.0% (Table 2).

Laboratory study of Interceptor® LLIN (Alphacypermethrin, with the target dose of 0.2 g/m$^2$ of polyester fabric) was reported by WHO (2007) as good performance against An. gambiae in term of efficacy and was resistance. This net met the WHOPES phase criteria of KD affect above the 95% after 20 washes. Mortality dropped below the cut-off value of 80% after 20 washes.
The efficacy of Olyset LLIN (Deltamethrin 0.055 g/m²) was studied in Malaysia. The study revealed that the percentage mortality of *An. maculatus* exposed to Olyset, nylon multifilament and polyethylene nets after 15 washes with water was 95%, 83% and 26% respectively, while for *Ae. aegypti* with mortality of 100%, 91.7% and 81.7%, respectively. After the nets had been washed four times with soap and water, the percentage mortality of *An. maculatus* exposed to Olyset, nylon and polyethylene nets was 86.7%, 80.3% and 3.3% respectively. While for *Ae. aegypti* the mortality was 90.3%, 50% and 5% respectively (Vythilingam 1996).

This study revealed that mortality of *Ae. aegypti* exposed to all evaluated LLINs impregnate with pyrethroids insecticide such as: (Alphacypermethrin 0.2 g/m², Deltamethrin 0.055 g/m² and Permethrin 1.0 g/m²), were remained at closed to 80% for less than 10 washes (Table 1, Figure 1).
FIELD EVALUATION

The average mortality of An. aconitus exposed on three type of LLINs Alphacypermethrin 0.2 g/m², Deltamethrin 0.055 g/m² and Permethrin 1.0 g/m² after 9 washes were respectively 78.15%, 82.47% and 73.52%. These results mean that the efficacy of LLINs Alphacypermethrin 0.2 g/m², Deltamethrin 0.055 g/m² and Permethrin 1.0 g/m² were respectively effective for 8, 9 and 7 washes, due to the mortality of tested mosquito of An. aconitus that dropped below the cut off value of 80.0% (Table 3, Figure 3)

Varian analysis (one–way, multiple comparation) showed that the influence of washing practices of the efficacy evaluated LLINs: Alphacypermetrin 0.2 g/m², Deltametrin 0.055 g/m² and Permethrin 1.0 g/m², were found similar.

Mortality of An. aconitus after exposed on all evaluated LLINs before washing was not significantly different (P < 0.05), compared to 1, 2, 3 and 4 times washes.

The efficacy of three LLINs tested for 5 and 6 washes were not significantly different (P < 0.05). Same results were also found for 7, 8 and 9 washes, which were not significant different (P < 0.05).
Both studies, laboratory evaluation (conducted against *Ae. aegypti* and *An. aconitus*) and field evaluation against *An. aconitus*, showed that the net samples were only effective at least for less than 10 washes (mortality more than the cut off value of 80.0%). These results were actually less than WHO recommendation that long-lasting insecticidal nets (LLINs) should be expected to retain biological activity for at least 20 standard WHO washes under laboratory conditions. In addition to that, the LLINs at least suppose to be used for 3 years under field conditions, as defined in the WHO guidelines (WHO, 2005 and 2006).

**SUMMARY**

The effectiveness of pyrethroids long lasting insecticidal nets (LLINs), impregnated of Alpha-cypermethrin 0.2 g/m², Deltamethrin 0.055 g/m² and Permethrin 1.0 g/m² against *Ae. aegypti* and *An. aconitus* both in the laboratory and *An. aconitus* in the field evaluations were only less than 10 washes.

**REFERENCES**


