Evaluation on the bioefficacy of PermaNet® 2.0, a long lasting net against Anopheles stephensi

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ABSTRACT

Objective: To evaluate the bioefficacy of PermaNet® 2.0 as long lasting net against main malaria vector, Anopheles stephensi (An. stephensi).

Methods: In order to determine the persistence of PermaNet® 2.0, the cone bioassay tests were conducted on nine pieces of the nets which underwent nine different washing regimes. The washing procedure and bioassay tests were carried out according to the World Health Organization recommended methods.

Results: The mortality rate of An. stephensi on net underwent nine washing regimes (1–25 times) resulted 22%, to 1%, as well as the knockdown rate were 74% to 2%, respectively.

Conclusions: The mortality rate of An. stephensi exposed to long-lasting PermaNet® 2.0 showed an increasing trend following exposure to washed nets from 1–8. This phenomenon indicated a slow release of deltamethrin from inside of the net fibers to the surface which led to increased efficacy of the long-lasting PermaNet® 2.0. In the control group, no mortality occurred in cone bioassay tests.

1. Introduction

Malaria is the most important vector-borne disease in the world. In 2015 alone, the global tally of malaria reached 212 million cases and 429,000 deaths. There are several measures for vector control[1]. Insecticide treated materials, reducing density, survival, contact with humans and feeding frequency[2-4], resulting decrease in malaria transmission, prevalence, morbidity and mortality[5-9]. Anopheles vectors are able adopt themselves with environment[10]. The use leads to the selection of resistant strains[11-14]. There are several mechanisms of resistance to insecticides, including target site insensitivity (kdr) and increased metabolic detoxification[15]. Pyrethroids are today the only group of insecticides advocated for the impregnation of mosquito nets. Recently, World Health Organization (WHO) recommended several long lasting insecticidal mosquito nets for malaria control[16]. The long term efficacy of long-lasting insecticide-treated bed nets (LLINs) in reducing malaria morbidity has recently been questioned in Western Africa[17]. Malaria is still a health problem in the country especially south and southeastern part with border of Pakistan and Afghanistan. Several Anopheles are considered as vector including Anopheles culicifacies, Anopheles stephensi (An. stephensi), Anopheles dhalii, Anopheles flaviatilis, Anopheles superpictus, and Anopheles pulchermissus, Anopheles sacharovi and Anopheles maculipennis[18-20]. According to the national strategic plan the LLINs are recommended for malaria control purposes. Strategic plan of each country should be carefully designed in order to preserve the effectiveness of this method of control. PermaNet® 2.0 net is deltamethrin LLINs, which is manufactured by Vestergaard Frandsen, with the target dose of 55 mg/m² of the polyester fabric. Applied objectives of the current research were to examine the bioefficacy of this product against the main malaria vector, An. stephensi for future use.

2. Materials and methods

2.1. Net purchasing

The net, PermaNet® 2.0, was provided by Vestergaard Frandsen. This polyester mosquito net was 100 denier. Mosquito nets were industrially treated with Deltamethrin EW (55 mg/m²) by the manufacturer. One piece (25 cm × 25 cm) of net was cut and used
for cone bioassay. A net was left untreated (negative control). One piece from the same net was used for cone bioassay before and after every washing (1–25), ensuring WHO standards of washing procedure. The pieces net were washed and dried once a week.

2.2. Washing procedure

This procedure is recommended by WHO[21].

2.3. Detergent “Le chat” supply

The detergent was supplied by the Institut de Recherche pour le Development, Montpellier Cedex 1, France.

2.4. Mosquito rearing

The non-blood fed, 2–3 days old susceptible female *An. stephensi*, susceptible to all pyrethroids were reared in the insectary and then used for all experiments.

2.5. Conical bioassay

The netting samples were subjected to standard WHO bioassays[21].

3. Results

The mortality of mosquitos exposed to PermaNet® 2.0 nets by using conical test which underwent nine washing regimes (0–25 times) resulted 22%, 36%, 41%, 41%, 77%, 43%, 13%, 3%, 1% (Table 1 and Figure 1). Six washes resulted the highest mortality and 25 washes the least. The knockdown rate was 74%, 44%, 67%, 75%, 80%, 51%, 21%, 4%, 2% (Table 2 and Figure 2). Six washes had the highest knockdown, however the least knockdown was observed in 25 washes.

### Table 1

Parameters of mortality of *An. stephensi* exposed to PermaNet® 2.0 nets using conical test under laboratory conditions.

| No. of washes | Number Tested | Mortality % | SE |
|---------------|---------------|-------------|----|
| 0             | 100           | 22          | 0.3|
| 1             | 100           | 36          | 1.0|
| 2             | 100           | 41          | 0.7|
| 3             | 100           | 41          | 1.2|
| 6             | 100           | 77          | 0.7|
| 8             | 100           | 43          | 0.6|
| 15            | 100           | 13          | 0.3|
| 20            | 100           | 3           | 0.3|
| 25            | 100           | 1           | 0.2|

### Table 2

Parameters of knockdown time of *An. stephensi* exposed to PermaNet® 2.0 nets using conical test under laboratory conditions.

| No. of washes | Number tested | knockdown (%) | SE |
|---------------|---------------|---------------|----|
| 0             | 100           | 74            | 2.1|
| 1             | 100           | 44            | 0.2|
| 2             | 100           | 67            | 0.2|
| 3             | 100           | 75            | 0.1|
| 6             | 100           | 80            | 0.2|
| 8             | 100           | 51            | 0.1|
| 15            | 100           | 21            | 0.5|
| 20            | 100           | 4             | 0.2|
| 25            | 100           | 2             | 0.3|

4. Discussion

There are several reports on different aspects of malaria vectors in the country for malaria vector control[21,22]. The present study determined the efficacy and wash-resistance of pyrethroid net named PermaNet® 2.0 nets (LLINs impregnated with deltamethrin with the target dose of 55 mg/m²) using cone bioassay method. The mortality rate of *An. stephensi* exposed to long-lasting PermaNet® 2.0 showed an increasing trend following exposure to washed nets from 0× to 8×. This phenomenon indicated a slow release of deltamethrin from inside of the net fibers to the surface which led to increased efficacy of the long-lasting PermaNet® 2.0. In the control group, no mortality occurred in the cone bioassay tests. Several studies have shown the potential effectiveness of bed nets impregnated with insecticide. What are the industrial and manual methods? (of course industrial technology had decreased due to factors such as washing, direct sunlight, dust, smoke, water and air as well as displacement and folding bed nets during the day. It is effective in reducing the amount of active ingredients in the insecticide to varying degrees[23]. Field studies have shown that pyrethroid insecticide-treated bed nets, if not washed will cause insecticide residual effects from 3 months to one year depending on the type and amount of pesticides remaining, with long-lasting (LLINs) which are even 9 months up to 11 months depending on the number of times the washing was done. Mosquito net in washing cycle, the method and frequency of cleaning and type of detergent is different in different regions. Different studies have shown that residual soap mosquito nets lead to the destruction of molecular structure of pyrethroids[24]. In a study in 2008 on *An. stephensi* measured PermaNet without the effect of washing on mortality was 99.2, and 20 times washing for 70% of deaths were reported[25]. Study in Benin, West Africa, showed unwashed PermaNet 3.0 was associated with a higher mortality than unwashed PermaNet 2.0 or unwashed Olyset[26]. In Vietnam all PermaNet arms were performing slightly better than conventionally...
treated nets. In Burkina Faso, results showed a strong reduction of LLIN efficacy. In this area, a significantly higher mortality and blood feeding inhibition was associated with unwashed and washed PermaNet® 3.0 compared to unwashed and washed PermaNet® 2.0.[26]. By bioassay with susceptible Anopheles in Ethiopia, efficacy dropped significantly to 47.2% at 26–32 months.[27]. Wash of washed net[28]. seen between residues of permethrin on nets, knockdown rate and An. stephensi[2] Curtis CF, Jana-Kara B, Maxwell CA. Insecticide treated nets: impact on World Health Organization. World malaria report. Geneva: World Health References [1] World Health Organization. World malaria report. Geneva: World Health Organization; 2016, p. 186. [2] Curtis CF, Jana-Kara B, Maxwell CA. Insecticide treated nets: impact on vector populations and relevance of initial intensity of transmission and pyrethroid resistance. J Vector Borne Dis 2003; 40: 1-8. [3] Enayati A, Hemingway J. 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