Asian Pacific Journal of Tropical Disease

journal homepage: http://www.apjtcm.com

Original article https://doi.org/10.12980/apjtd.7.2017D6-368

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

Akbar Bagheri[1], Hassan Vatandoost[1,2*], Mansoreh Shayeghi[1], Mohammad Reza Abai[1], Ahmad Raeisi[3], Nutifafa Gidiglo Godwin[1], Morteza Akbari[1], Soraya Sheikhi[1]

[1]*Department of Medical Entomology & Vector Control, School of Public Health & National Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran*

[2]*Department of Chemical Pollutants and Pesticides, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran, Iran*

[3]*Department of Malaria, CDC, Ministry of Health & Medical Education, Iran*

ARTICLE INFO

Article history:
Received 12 Oct 2016
Received in revised form 23 Oct 2017
Accepted 10 Nov 2017
Available online 29 Nov 2017

Keywords:
PermaNet® 2.0
Wash resistance
Deltamethrin

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 dthali*, *Anopheles fluviatilis*, *Anopheles superpictus*, and *Anopheles pulcherrimus*, *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/m^2 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/m^2) by the manufacturer. One piece (25 cm × 25 cm) of net was cut and used

*Corresponding author: Hassan Vatandoost, Department of Medical Entomology & Vector Control, School of Public Health & National Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran.
Tel: +982188954441
E-mails: hvatandoost1@yahoo.com, vatando@tums.ac.ir
Foundation Project: Funded by Tehran University of Medical Sciences under code number of 09211263003.
The journal implements double-blind peer review practiced by specially invited international editorial board members.

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

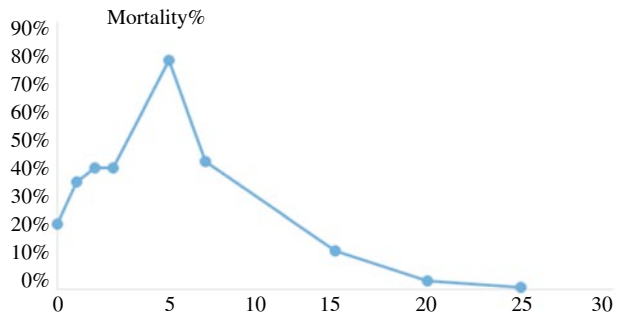

Figure 1. Morality of *An. stephensi* exposed to PermaNet® 2.0 nets.

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	05
20	100	4	0.2
25	100	2	0.3

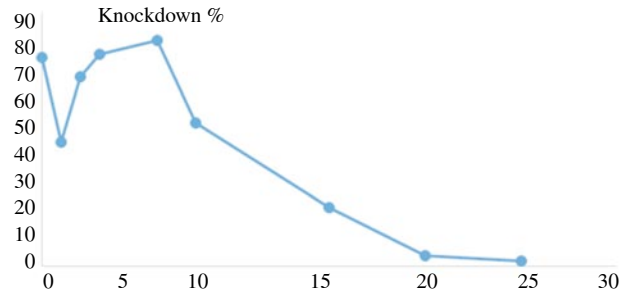

Figure 2. Knockdown of *An. stephensi* exposed to different washes of PermaNet® 2.0.

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/m^2) 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 resistance and bio-efficacy of Olyset® Plus, against malaria vector, *An. stephensi* was evaluated. They found a positive correlation was seen between residues of permethrin on nets, knockdown rate and mortality rate of female *An. stephensi* exposed to different regimes of washed net[28].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This research is funded by Tehran University of Medical Sciences under code number of 09211263003.

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. Malaria management: past, present, and future. *Annu Rev Entomol* 2010; **55**: 569-91.

[4] Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends Parasitol* 2011; **27**: 91-8.

[5] Ceesay SJ, Casals-Pascual C, Erskine J, Anya SE, Duah NO, Anthony JC, et al. Changes in malaria indices between 1999 and 2007 in the Gambia: a retrospective analysis. *Lancet* 2008; **372**: 1545-54.

[6] Fegan GW, Noor AM, Akhwale WS, Cousens S, Snow RW. Effect of expanded insecticide-treated bednet coverage on child survival in rural Kenya: a longitudinal study. *Lancet* 2007; **370**: 1035-9.

[7] Lengeler C. Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database Syst Rev* 2004; CD000363.

[8] Magesa SM, Wilkes TJ, Mnzava AE, Njunwa KJ, Myamba J, Lines JD, et al. Trial pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. Part 2. Effects on the malaria vector population. *Acta Trop* 1991; **49**: 97-108.

[9] Trape JF, Sauvage C, Ndiaye O, Douillot L, Marra A, Diallo A, et al. New malaria-control policies and child mortality in Senegal: reaching millennium development goal 4. *J Infect Dis* 2012; **205**: 672-9.

[10] Coluzzi M, Sabatini A, Petrarca V, Di Deco MA. Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. *Trans R Soc Trop Med Hyg* 1979; **73**: 483-97.

[11] Czeher C, Labbo R, Arzika I, Duchemin JB. Evidence of increasing LeuPhe knockdown resistance mutation in *Anopheles gambiae* from Niger following a nationwide long-lasting insecticide-treated nets implementation. *Malar J* 2008; **7**: 189.

[12] Diabate A, Baldet T, Chandre F, Akoobeto M, Guiguemde TR, Darriet F, et al. The role of agricultural use of insecticides in resistance to pyrethroids in *Anopheles gambiae* s.l. in Burkina Faso. *Am J Trop Med Hyg* 2002; **67**: 617-22.

[13] Hemingway J, Field L, Vontas J. An overview of insecticide resistance. *Science* 2002; **298**: 96-7.

[14] Vulule JM, Beach RF, Atieli FK, Roberts JM, Mount DL, Mwangi RW. Reduced susceptibility of *Anopheles gambiae* to permethrin associated with the use of permethrin-impregnated bednets and curtains in Kenya. *Med Vet Entomol* 1994; **8**: 71-5.

[15] Donnelly MJ, Corbel V, Weetman D, Wilding CS, Williamson MS, Black WC 4th. Does kdr genotype predict insecticide-resistance phenotype in mosquitoes? *Trends Parasitol* 2009; **25**: 213-9.

[16] World Health Organization. Report of the 12th WHOPES working group meeting 2009b, WHO/HTM/NTD/ WHOPES/2009.1. Geneva: WHO; 2009, p. 120.

[17] Trape JF, Tall A, Diagne N, Ndiath O, Ly AB, Faye J, et al. Malaria morbidity and pyrethroid resistance after the introduction of insecticide-treated bednets and artemisinin-based combination therapies: a longitudinal study. *Lancet Infect Dis* 2011; **11**: 925-32.

[18] Raeisi A, Shahbazi A, Ranjbar M, Shoghli A, Vatandoost H, Faraji L. National strategy plan for malaria control (I. R. Iran, 2004-2008). Tehran: Ministry of Health & Medical Education of Iran Publication; 2004, p. 72.

[19] Vatandoost H, Mashayekhi M, Abaie MR, Aflatoonian MR, Hanafi-Bojd AA, Sharifi I. Monitoring of insecticides resistance in main malaria vectors in a malarious area of Kahnooj district, Kerman province, southeastern Iran. *J Vector Borne Dis* 2005; **42**: 100-8.

[20] Vatandoost H, Zahirnia AH. Responsiveness of *Anopheles maculipennis* to different imagicides during resurgent malaria. *Asian Pac J Trop Med* 2010; **3**(5): 360-3.

[21] World Health Organization. Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets 2005. WHO/CDS/WHOPES/ GCDPP. Geneva: WHO; 2005.

[21] Davari B, Vatandoost H, Oshaghi MA, Ladonni H, Enayati AA, Shaeghi M, et al. Selection of *Anopheles stephensi* with DDT and dieldrin and cross-resistance spectrum to pyrethroids and fipronil. *Pest Biochem Physiol* 2007; **89**: 97-103.

[22] Shaeghi M, Vatandoost H, Mamivanpoor H, Abai MR, Yaghoobi-Ershadi MR, Raeisi A, et al. Evaluation of bioefficacy of alpha-cypermethrin in long lasting impregnated net (Interceptor®) using analytical method. *Asian Pac J Trop Med* 2010; **3**(8): 642-6.

[23] Rozendaal J, Curtis C. Recent research on impregnated mosquito nets. *J Am Mosq Control Assoc* 1989; **5**(4): 500-7.

[24] Alonso P, Lindsay S, Schellenberg JA, Keita K, Gomez P, Shenton F, et al. A malaria control trial using insecticide-treated bed nets and targeted chemoprophylaxis in a rural area of The Gambia, West Africa, the impact of the interventions on mortality and morbidity from malaria. *Trans R Soc Trop Med Hyg* 1993; **87**: 37-44.

[25] Rafinejad J, Vatandoost H, Nikpoor F, Abai M, Shaeghi M, Duchen S, et al. Effect of washing on the bio-efficacy of insecticide-treated nets (ITNs) and long-lasting insecticidal nets (LLINs) against main malaria vector *Anopheles stephensi* by three bioassay methods. *J Vector Borne Dis* 2008; **45**(2): 143.

[26] Dabire RK, Diabate A, Baldet T, Pare-Toe L, Guiguemde RT, Ouedraogo JB, et al. Personal protection of long lasting insecticide-treated nets in areas of *Anopheles gambiae s.s*. resistance to pyrethroids. *Malar J* 2006; **5**: 12.

[27] Anshebo GY, Graves PM, Smith SC, Wills AB, Damte M, Endeshaw T, et al. Estimation of insecticide persistence, biological activity and mosquito resistance to PermaNet® 2 long-lasting insecticidal nets over three to 32 months of use in Ethiopia. *Malar J* 2014; **13**: 80.

[28] Sheikhi S, Vatandoost H, Abai MR, Shayeghi M, Raeisi A, Akbari M, et al. Wash resistance and bio-efficacy of Olyset® Plus, a long-lasting insecticide-treated mosquito net with synergist against malaria vector, *Anopheles stephensi*. *Asian Pacific J Trop Med* 2017; **10**(9): 887-91.