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Short and long term evaluation of the efficiency of PermaNet® 2.0 bed net against environmental factors and washing using bioassay tests

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ABSTRACT

The aim of the present study was to examine the resistance of PermaNet® 2.0 bed nets against repeated washing and environmental factors by using bioassay tests. After 5, 15 and 21 washings with detergents and by using bioassay tests, the resistance of 40 PermaNet® 2.0 bed nets was compared with that of 40 bed nets conventionally treated with one K-O tablet. To examine the long-term resistance, 31 PermaNet® 2.0 bed nets were also distributed among villagers, and were re-collected to perform bioassay tests after 1, 2 and 5 years. In the first phase of this study, the insecticidal effect of the conventionally-treated nets significantly decreased due to repeated washings ($P < 0.001$); however, it was not significant regarding PermaNet® 2.0 bed nets ($P = 0.92$ in continuous exposure and $P = 0.12$ in mortality tests). In the long-term phase of this study, the time required for knockdown of PermaNet® 2.0 increased over the first 2 years and then decreased. In addition, the mortality rate decreased over the first 2 years and then increased. In conclusion, it seems that the technique used by the manufacturer for impregnation of PermaNet® 2.0 bed nets has an acceptable efficiency in comparison with conventional techniques.

KEYWORDS: *Anopheles.* Bioassay tests. Insecticide-treated bed nets. Malaria.

INTRODUCTION

Bed nets impregnated with insecticide or insecticide-treated nets (ITNs) are used as an effective tool to control vectors and to prevent the *Anopheles* bites[1-6]. However, the most important problem associated with ITNs is that they lose their properties after several washings[7]. To deal with this problem, in the context of modern industrial methods involved in producing impregnated bed nets, the insecticide is mixed with special types of resins, and then the fiber surface of bed nets is stained. The mentioned technique is used to impregnate the fiber of PermaNet® 1.0 and 2.0 bed nets with minor technical differences[8-11].

In recent years, one of the research priorities of the World Health Organization (WHO) was to evaluate the resistance of bed nets against frequent washings[12,13]. In response to this priority, a number of studies have been devoted to examine the resistance of PermaNet® 1.0 bed nets against the environmental factors and several washings[3-11,14-19]. However, the resistance of PermaNet® 2.0 bed nets against several washings and long-term exposure to environmental factors has not been extensively examined. Hence, the present study was designed to shed more light on this point and examine whether the proposed new type of PermaNet® 2.0 bed net reveals a better function.

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MATERIAL AND METHODS

Assessment of the resistance of PermaNet® 2.0 bed nets against repeated washings

Forty rectangular single polyester fiber PermaNet® 2.0 bed nets, as well as forty rectangular single polyester fiber bed nets, which were non-impregnated with insecticide, were purchased from Vestergaard Frandsen Lausanne, Switzerland in 2004. PermaNet® 2.0 bed nets were introduced as long-lasting mosquito nets, which were industrially impregnated by an innovative technique with deltamethrin at 55 mg/m^2, in the factory. In the conventionally-treated nets, another 40 bed nets were impregnated using tablets with the brand name of K-O Tab®; one tablet was used for each net with deltamethrin at 23 mg/m^2. The insecticide tablets used in this study were provided by Aventis (Aventis Company of England, Guildford, Surrey, UK). The weight of each tablet was 1.6 g, and 25% of its total weight was composed of an effective substance equaling 0.4 g of deltamethrin. A polyester bed net with a total surface area of 11.6 square meters can absorb 0.5 liters of water. To impregnate the bed nets with deltamethrin, a K-O tablet was dissolved in 0.5 liter of water in a plastic dish, and then a net was thoroughly soaked in it. In doing so, the amount of effective substance (deltamethrin) absorbed by the fiber of each net was 25 mg /m^2 [19].

Bed nets washing and drying methods

The treatment groups involved 5, 15, and 21 washes, while the positive control had zero washes and the negative control involved nets without insecticide. Each treatment included 8 PermaNet® 2.0 bed nets and 8 nets impregnated with K-O tab®, with the exception of the negative control group involving four bed nets. The interval of three days was considered between the washing sessions; hence, before performing the bioassay test, the treatments started on 63, 45, and 15 days, and in each of these days the beds received 21, 15, and 5 washes, respectively. Bed nets were impregnated with K-O tablets a week before the first washing.

Washing and drying of the bed nets were carried out in a village called "Pele-Hawa", Khorramabad, west of Iran (33°37'47" N 48°17'3" E). Two women washed all the nets with cold water provided through a pipeline network from a spring. It must be mentioned that the average temperature during the washings was 28 °C. Barf™ washing powder was used in this study; 6.5 g powder and 4 liters of water (pH 8.9, 17 °C) were used for each bed net. The hand washing procedure lasted for three minutes and was performed in a steel dish, and then the bed nets were rinsed off twice with the same water in the same steel dish. The hard spring water was also chlorinated before entering the water supply network. After each washing, half of the PermaNet® 2.0 bed nets and half of the bed nets impregnated with K-O tabs were shade-dried for three hours, and the other half of nets were sun-dried for three hours[19]. The same procedure was followed for the control bed nets. All of the bed nets were placed in individualized plastic bags after each washing and drying cycle until the next washing turn.

Distribution of PermaNet® 2.0 bed nets among the villagers to perform the long-term evaluation

Thirty-one rectangular single polyester fiber (120 × 150 × 180 cm) PermaNet® 2.0 bed nets were purchased from Danish Vestergaard Frandsen Company. The bed nets were distributed among the villagers of Sarzanguleh, Visian district, Khorramabad city, Iran (33°27'55" N 48°16'58" E) during the spring season of 2005. The villagers were asked to use the bed nets and wash them as usual, if necessary. As a positive control group, 4 nets were kept in their factory packages. After one, two, and five years, the nets were randomly re-collected from the villagers (nine bed nets each time) to check the insecticide activity of nets over time.

Bioassay tests

The bioassay tests were carried out in the Kazeroon Public Health Research Center, affiliated to the School of Public Health, Tehran University of Medical Sciences. Two bioassay methods, namely continuous exposure and three minutes exposure, were performed on all the bed nets by the expert personnel of the center, under direct supervision of the research team.

Upper level, large lateral, and small lateral surfaces of each cube-shaped bed net were marked to be used in the bioassay tests. Bioassay tests on the marked surfaces were in general, performed using wild-caught female *Anopheles stephensi* from Dadin Sofla (29°22'43" N 51°48'49" E) and Dadin Olia (29°18'42" N 51°52'3" E), Kazeroon, South of Iran. The marked parts of the bed nets were wrapped around a cube metal grid with the dimension of 10 × 10 × 10 cm. To perform each bioassay test, a group of eleven mosquitoes were inserted into the metal grid.

The time for knockdown of the sixth mosquito was recorded in the continuous exposure tests. In the three minutes tests, a group of eleven mosquitoes had contact with the net for three minutes, and then the mosquitoes were removed using an aspirator. They were then put in a paper container

and kept in the insectaries at a temperature of 27 °C and at more than 70% humidity for 24 hours. Then, their mortality was recorded. The mosquitoes were fed with cotton soaked in glucose solution during their maintenance in the insectaries. The negative control test was done on an untreated net for each of the nine tests, and the results were recorded using special forms; however, the results of the negative control tests were almost always zero. In any case, if the mortality rate of the negative control was more than 5% in the three minutes contact test, the results of all the bioassay tests were excluded, and all the tests were repeated the next day.

Statistical analysis

The mean values of the median knockdown time (MMKDT) in the continuous exposure test and the percentage of the mortality rate within 24 hours after the bioassay tests in the three minutes exposure test were calculated for each group of nets. Having presented the results of the mentioned tests in the bar charts, the MMKDT of groups were compared using the one-way ANOVA, and the percentage of the mortality rate were calculated using the chi-squared test. The analysis was carried out using the SPSS software, version 13. Moreover, *p*-values < 0.05 were considered as significant.

RESULTS

The effect of repeated washings

There was no significant difference between the results of the bioassay tests when sun- dried and shade-dried bed nets were compared (p = 0.144).

Regarding the results of the continuous exposure test, a significant increase in the MMKDT as the result of more washings in the K-O tab® treated nets (p < 0.001) was observed. This increment was not significant in the PermaNet® 2.0 bed nets (p = 0.92). As a result of the mentioned disagreement in the behavior of the two types of nets, their MMKDT presented a statistically significant difference after 15 and 21 washings (p < 0.001) (Figure 1).

Likewise, the results of the three minutes exposure test showed that only in the K-O Tab® nets, the mortality rate has declined as a result of more washings (p < 0.001). However, the difference between the results of two types of nets was only significant after 21 washings (p < 0.001) (Figure 2).

Results of long-term exposure to environmental factors

The findings presented in Figure 3 and Table 1 revealed that the MMKDT in the positive control group was 366.05 seconds, which was significantly longer only after two years of exposure (502.92 seconds, p = 0.028) (Table 1).

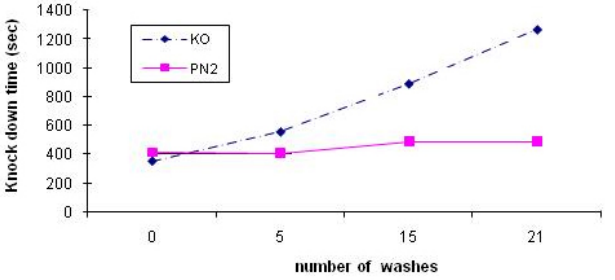

Figure 1 - Mean median knock down time of *Anopheles stephensi* in continuous exposure test to KO treated nets (KO) in comparison to PermaNet® 2.0 (PN2).

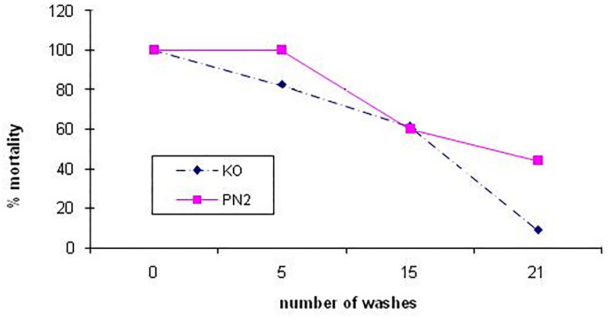

Figure 2 - 24 hours mortality of *Anopheles stephensi* after 3 minute exposure to KO treated nets (KO) in comparison to PermaNet® 2.0 (PN2).

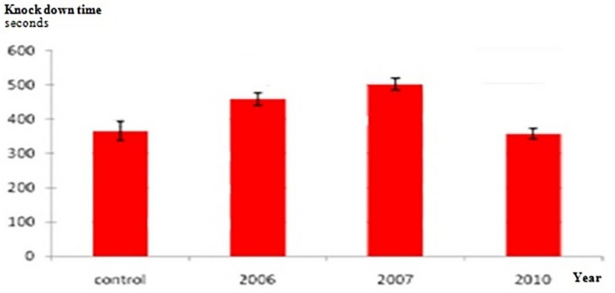

Figure 3 - Mean median knock down time of *Anopheles stephensi* on the Bioassay test's findings based on the continuous exposure in PermaNet® 2.0 nets during 2006, 2007 and 2010 in comparison with control group.

Table 1 - Results of the bioassay tests of PermaNet® 2.0, mean values of the median knock down times (mmkdt) and the mortality rate of *Anopheles stephensi* in the long term studies

Year of test	P-values for mmkdt	P-values for mortality rate
2006 & Control +	0.256	0.310
2007 & Control +	0.028	0.333
2010 & Control +	0.998	0.995
2006 & 2007	0.306	0.996
2006 & 2010	0.004	0.056
2007 & 2010	<0.001	0.037

According to Figure 4, the highest mortality rate was observed in the positive control group (98.9%), and the lowest one was observed after one year of exposure (89.4%). In addition, based on the results shown in Table 1, the mortality rate was more or less significant only after one year of exposure to the environmental factors.

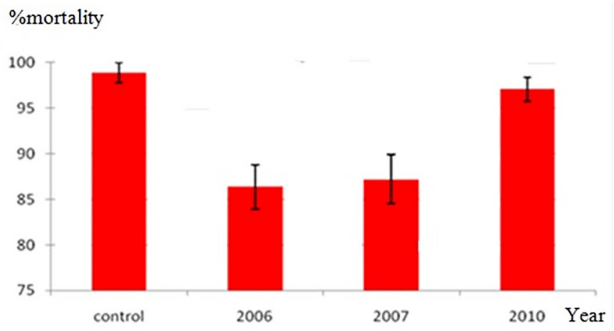

Figure 4 - Mortality percent 24 hours after 3 minutes exposure with PermaNet® 2.0 in bioassay test results during 2006, 2007 and 2010 in comparison with control group.

DISCUSSION

The findings of the first phase of this study revealed that in comparison with the conventionally-treated bed nets, the technique used to impregnate the PermaNet® 2.0 to deltamethrin in the factory yielded a better efficiency. PermaNet® 2.0 bed nets kept their insecticide power until twenty-one washings. It does not mean that PermaNet® 2.0 bed nets maintained all of their insecticide power until twenty-one washings; however, the amount of insecticide residues was enough to create a notable mortality of mosquitoes exposed to the fibers of the bed net.

In addition, it was found that a three hours exposure to sunlight after each washing did not significantly change the insecticide activity of the treated nets. The mentioned finding is supported by results of other authors who studied these aspects[6,14,15,19] and can be considered as a promising result as, in practice, most people dry their nets under sunlight, and resistance of the treated nets against sun can encourage the use of the recommend ITNs.

Sreehari *et al.* reported high mortality (≥ 80%) of both, *Anopheles culicifacies* and *Anopheles stephensi* after 20 washings of PermaNet® 2.0[20]. Similarly, Sood *et al.* showed that PermaNet® 2.0 is wash-resistant even after 20 washings (≥ 80% mortality of *An. Stephensi* in cone bioassays). However, there was a gradual decline in the insecticide power of the nets[21]. In another study carried out by Prakash *et al.*, PermaNet® 2.0 presented 72.5 % mortality of *An. Minimus* 24 hours after a three minutes contact with PermaNet® 2.0 after 15 washings[22]. All the above-mentioned studies were carried out in India. In addition, in a study carried out in Colombia by Jaramillo *et al.*, PermaNet® 2.0 showed wash resistance up to 15 washings, as well[23].

In the long-term evaluation of PermaNet® 2.0 in the present study, it was shown that even after several years, *Anopheles stephensi* mortality rate was not less than 85%. The mentioned finding indicates that in real situations in which people use them for their protection, wash them by hand, and store them following normal procedures, the bed nets keep their efficiency even up to five years.

In a research carried out using PermaNet® 2.0, Kilian *et al.* studied the insecticide properties of deltamethrin available in the mosquito nets, and found that this compound caused 80% mortality of *Anopheles gambiae* after 36 months of follow up.[24] In another survey, 24 months of household use of PermaNet® 2.0 were evaluated by Picado *et al.*[25]. They reported a decline of deltamethrin concentration in the net fibers from 55 mg/ m^2 to an average of 11.6 mg/m^2 in India after 2 years. Moreover, decline value was 27.9 mg/m^2 in Nepal after 2 years.

The manufacturer claims that PermaNet® 2.0 is a new generation of impregnating bed nets with a better efficacy over time. The results of many studies conducted around the world have shown that in comparison with the conventional treated nets and PermaNet® 1.0, PermaNet® 2.0 has presented stronger resistance against repeated hand washing and exposure to environmental factors. Nevertheless, there are some pieces of evidence revealing that the efficacy of PermaNet® 1.0 and PermaNet® 2.0 is more or less comparable after 30 consecutive hand washings using laundry powder[20-23]. Therefore, it seems that companies still have to spend more time and energy to improve the quality of their next generation products.

It is critical to mention that the Kazeroon station was closed after 2007; therefore, the location of the bioassay tests was changed, which can be regarded as one of the reasons for some of the observed unexpected variations in the mortality rates observed over time (Figure 4).

CONCLUSION

In conclusion, the present study supports the findings of other studies indicating that the PermaNet® 2.0 is resistant against repeated washings and long-term exposure to environmental factors although its resistance is not perfect after many years of use.

AUTHORS' CONTRIBUTIONS

MHK, ZK, and AAH contributed to the proposal and development of the study. MHK, NA, ZK, SF, and KK

collected the mosquitoes and performed the bioassay tests. AAH analyzed the data and interpreted the results. Furthermore, MHK, KK, NA, ZK, SF, and AAH drafted and revised the manuscript. All the authors read and approved the final manuscript. MHK and AAH are guarantors of the paper.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

ETHICS APPROVAL

None.

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