Short Communication

Entomological evaluation of PermaNet 2.0® and K-O Tab 1-2-3® treated nets in comparison to nets conventionally treated with deltamethrin, after repeated washing

Mohammad Hassan Kayedi[1], Kiumars Khamisabadi[2,3], Nader Dehghani[4,5], Ali Akbar Haghdoost[6]

[1]Razi Herbal Medicines Research Center and Department of Parasitology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran, [2]Kazeroun field station, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, [3]I.R. Iran National Institute of Health Research, Tehran, Iran, [4]Kazeroun field station, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, [5]Kazeroun Health Center, Shiraz University of Medical Sciences, Shiraz, Iran, [6]Research Center for Modeling in Health, Kerman University of Medical Sciences, Kerman, Iran

The residual insecticidal power of two types of ITNs (PermaNet 2.0® (PN2) and K-O Tab 1-2-3® (KO 123)), compared to K-O Tab® (KO) treated nets, was assessed. The nets were tested unwashed, and after being washed, by hand 5, 15 and 21 times, respectively. After each wash, the nets were dried vertically on a line, in the shade. Two types of bioassays (mean median knock down times (MMKDT) and mortality 24 hours after a 3-minute exposure (%mortality)) were used, along with reared female *Anopheles stephensi*. The number of washes had a great impact on MMKDT and %mortality of all types of nets. This impact was greater for conventionally treated nets, indicating that PN2 and KO 123 nets are significantly more wash resistant than KO nets after 21 washes. There was no significant difference between PN2 and KO 123 with respect to %mortality 24 hours after a 3-minute exposure at 0, 15 and 21 washes. Similarly, the same results were obtained for MMKDT, and the differences between PN2 and KO 123 were not statistically significant. This study demonstrates that the efficacy of KO 123 nets is as beneficial as the efficacy of PN2 nets up to 21 washes.

Keywords: Insecticides, *Anopheles*, Malaria, Permanet, K-O tab

Introduction

One of the greatest problems when using insecticide-treated nets (ITNs) against malaria vectors is that after a few washes of the nets with soaps or detergents (approximately after five washes), the majority of insecticide on the nets is removed and the efficacy of ITNs reduced.[1–3] One method of increasing the insecticide efficacy of ITNs after repeated washing is re-treatment of nets with insecticides, but the treatment and re-treatment rates of nets, especially in most projects in Africa, have been very low.[4–7] In recent years, some manufacturers that produce bednets or insecticides have tried to develop techniques to produce long-lasting insecticide-treated nets (LLITNs), resistant to repeated washing and without need for re-treatment. According to WHO definition, long-lasting nets are ready-to-use pre-treated mosquito nets that have been given a special insecticide treatment which is more durable and resistant to washing than the conventional dipping method. Ideally, and in order to fit the definition of LLITNs, the insecticidal activity should last as long as the nets expected life span (3–5 years).[8]

One of the techniques that has been developed by Vestergaard Frandsen A/S (Lausanne, Switzerland) is the incorporation of 55 mg/m^2 deltamethrin into a resin, which coats the fibre, and their product is PermaNet 2.0® (PN2). This product is a new generation of PermaNet™. WHO has given interim recommendations to PN2 since 2004 and PN2 has been widely distributed and used for the control of malaria in Africa and in other countries and continents.[9] Full recommendation has been granted by WHO for PN2 since 2008.[10]

Correspondence to: Dr Mohammad Hassan Kayedi, Associate Professor of Medical Entomology and Vector Control, Faculty of Medicine, Department of Parasitology, Lorestan University of Medical Sciences, Kamalvand, Khorramabad-Lorestan, Iran. Email: kayedi78@yahoo.co.uk

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Bayer Environmental Sciences (BES) has produced dip-it-yourself tablets (K-O Tab 1-2-3®) (KO 123) with a formulation different to K-O Tab® (conventional method, KO, BES, France) for treating nets and claims that nets treated with the new formulation are lasting insecticide-treated nets (LLITNs). KO 123 has received a time-limited interim recommendation by WHO for the impregnation of polyester nets up to 15 washes.[11] WHO requested that research be carried out with large scale field studies (WHOPES phase III) to test the efficiency and wash resistance of KO 123 as a necessary tool for changing its status for use from interim to full recommendation.[11]

In some of the countries with a high risk of malaria transmission, many people use home-made nets that are not treated with insecticides, and some organizations distribute conventionally treated nets among people that require re-treatment, and therefore, a re-treatment kit with long-lasting efficacy, which is resistant to detergents for more than five washes, could help reduce cases of malaria.

In recent years, there has been a shift towards LLITNs and the present studies' main purpose is to add to the knowledge of net treatment technology.

Based on the above explanation, in our study the authors compared the residual insecticidal power of two types of insecticide-treated nets (ITNs) (PN2 and KO 123), which are claimed by their manufacturers to be LLITNs with conventionally treated nets (KO), using hand washing, to assess if there exist any differences between the nets.

Materials and Methods

Net treatment

Twelve manufactured single size ($180 \times 150 \times 130$ cm) rectangular treated nets (PN2, white colour, 100 denier) and 33 single size nets, similar to the PN2 (white colour, 100 denier) but untreated, were bought in 2005 from Vestergaard Frandsen (Denmark). The fibres of these nets were made from 100% polyester. The long-lasting nets (PN2) had been treated with 55 mg/m^2 deltamethrin in the factory. Sixteen untreated nets were treated with deltamethrin using one KO for each net, $\sim$28.6 mg active ingredient (a.i.) per square metre (supplied by the Aventis company, Sanofi UK, Guildford, UK) and the other 16 untreated nets were treated with deltamethrin using one KO 123 for each net (supplied by the Bayer company, Tehran branch, Tehran, Iran). One remainder untreated net was used for negative controls. Each net was rubbed for 3 minutes and then dried horizontally in the shade.[12]

Washing and drying

All nets were vigorously washed and then dried, in a village in Western Iran, by two women, with 3-day intervals between washings. For washing, each group of nets was divided equally between the two women. The method of hand washing commonly used in the area for washing the clothes and the authors make sure that later comparisons were valid. Detergent (local brand: 'Barf') of 6.5 g that is commonly used in the area was used. Each net was washed in a flat wash-tub made of metal and was hand rubbed for 3 minutes using four litres of cold, hard and chlorinated tap water sourced from a mountain spring (17(°C, pH 8.9). There were two rinses for each net in the same water and conditions with 3-minute hand rubbing for each rinse. The average temperature was 28°C (mean temperature taken from data loggers that ran continuously through the day).

Washing of the '21 wash' and '15 wash' group of nets started at 63 and 45 days before the bioassay tests, respectively, and for the 'five wash' group it started 15 days before testing. Therefore, all groups of nets were ready for bioassay tests at the same time. The authors did not received sufficient numbers of PN2 for washing and testing, and therefore made the decision to delete the five wash PN2 group from the project.

After each wash, the nets were hung up in the shade under a thatched shelter and left for 8 hours and then were put indoors in plastic bags, till they were washed again. The '0 wash' group of nets (positive controls), (i.e. four PN2, four KO 123 and four KO) were not washed. The '0 wash' nets were put in their drying positions on the same day that the washing of the other nets commenced.

Bioassay tests

Bioassay tests were carried out with the collaboration of field staff at the Kazeroun field station (Fars province, southern Iran) on nets which had received 0, 5, 15 or 21 washes and negative controls (untreated, unwashed net). Females of *Anopheles stephensi* (3–5 days old) that had been reared and blood fed in the laboratory were used for bioassay tests. For each bioassay test, a group of 11 mosquitoes was used.

Continuous exposure test

Small areas of five sides of each net were repeatedly wrapped around a cubic wire frame ($10 \times 10 \times 10$ cm). A batch of 11 mosquitoes was subsequently inserted inside the frame by a mouth aspirator and at the same time, the timer was started. Therefore, five replicates were carried out per net. In total, 240 continuous exposure tests were carried out using 2640 mosquitoes. Each knock down (KD) mosquito was removed from the netting and the time of sixth KD mosquito was noted. One test was carried out on each side of the nets.[3,12–18]

Three-minute exposure test

The same procedures carried out for the continuous exposure test was repeated for this section of tests. There was limited exposure time for these tests. After a 3-min exposure of mosquitoes to the netting, all of them were removed to a paper cup by a mouth aspirator. The paper cups were kept for 24 hours in an insectary and supplied with cotton glucose solution. The %mortality of the mosquitoes was noted after 24 hours. In total, 240 three-minute exposure tests were carried out using 2640 mosquitoes.[19]

As negative controls, a small area on one side of an untreated net was wrapped around a frame and a batch of 11 mosquitoes inserted inside the frame. In this case, the exposure time was 15 min. One control exposure was carried out between each batch of nine tests. The overall mortality was calculated at the end of each day's testing. This was generally zero, but if it exceeded 5%, the autthors re-tested the nets the following day.

Statistical methods

SPSS version 15 was used for the analysis of data. Two main outcomes of the tests were %mortality and mean median knock down times (MMKDT). One-way ANOVA was used to compare the values in different nets. Mann–Whitney tests were used when the assumptions of ANOVA were not met. The outcomes are statistically independent from one another.

Results

MMKDT

The results of the bioassays with continuous exposure are summarised in Fig. 1. At 0 and 5 washes, all nets had similar MMKDT and these were not significant (Table 1). At 15 washes, the conventionally treated and PN2 nets gave the longest (indicating lower insecticidal activity) and shortest (indicating higher insecticidal activity) MMKDT, respectively, and the differences between them (including KO123) were significant (Table 1). Similarly, the authors recorded the same results for 21 washes of nets (Table 1). Results of one-way ANOVA and multiple comparisons of KO and KO 123 nets showed that hand washing up to 21 washes significantly reduced the insecticidal efficacy of nets ($P<0.001$ for both of nets), although PN2 showed better wash resistance, and its MMKDT value did not increase significantly even after 21 washes (Fig. 1).

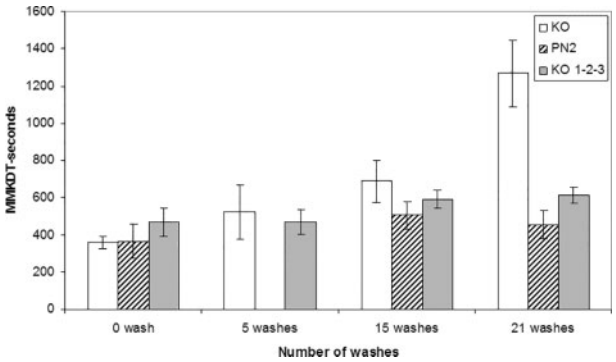

Figure 1 Mean median knock down time with 95% CI of *A. stephensi* with continuous exposure to KO, PN2 and KO 123.

Mortality 24 hours after 3-minute exposure

The results of the bioassays with %mortality 24 hours after a 3-minute exposure are summarised in Fig. 2. As expected, 0 wash mortalities measured 24 hours after a 3-minute exposure to any ITN were close to 100% with no significant difference between nets (Table 1). With an increase in the number of washes mortality of mosquitoes decreased. However, this reduction of mortalities for KO nets is much higher than the two other ITNs at 21 washes (Fig. 2). At 5 and 15 washes the confidence intervals of nets overlapped and the authors found no statistical differences (Fig. 2). As indicated in Fig. 2, after 21 washes, PN2 and KO 123 nets gave greater mortalities than the KO nets with a significant difference (Table 1). The differences between PN2 and KO 123 were not, however, statistically significant ($P=0.668$).

Discussion

The results of the one-way ANOVA indicate that the number of washes had a great impact on MMKDT and %mortality of all types of nets. This impact was greater for conventionally treated nets, indicating that PN2 and KO 123 nets that are claimed by manufacturers to be LLITNs are significantly more wash resistant than KO nets after 21 washes. Hand washing removed insecticide from fibres, which caused a general increase in MMKDT and a general decrease in mortality from 0 to 5 washes, from 5 to 15 and from 15 to 21 washes, although the pattern of increase or decrease differed with net type.

Mortality at 15 washes was relatively high for the conventionally treated nets. There is inconsistency for this result between different laboratories and field studies around the world.[17] This could be because of different hand washing methods, varieties of detergents or soaps and chemical or physical factors of water used for washing. Previous studies published by the first author of this study reported almost identical results (40% and 64.8% mortality in comparison to 62% mortality in the present study).[3,17] Other researchers have reported different results.[2,15]

There was no significant difference between PN2 and KO 123 with respect to %mortality 24 hours after a 3-minute exposure at 0, 15 and 21 washes (Table 1). Similarly, the authors noted the same

Table 1 Results of one-way ANOVA of MMKDT and mortality 24 hours after a 3-minute exposure

Number of washes	Type of nets	*P*-values for MMKDT	*P*-values for %mortality
0 wash	KO, KO123	0.052	0.364
	KO, PN2	0.994	0.987
	KO 123, PN2	0.065	0.453
5 washes	KO, KO123	0.042	0.887
	KO, PN2	–	–
	KO 123, PN2	–	–
15washes	KO, KO123	0.220	0.477
	KO, PN2	0.013	0.344
	KO 123, PN2	0.095	0.899
21 washes	KO, KO123	<0.001	<0.001
	KO, PN2	<0.001	<0.001
	KO 123, PN2	0.453	0.668

MMKDT: mean median knock down times.

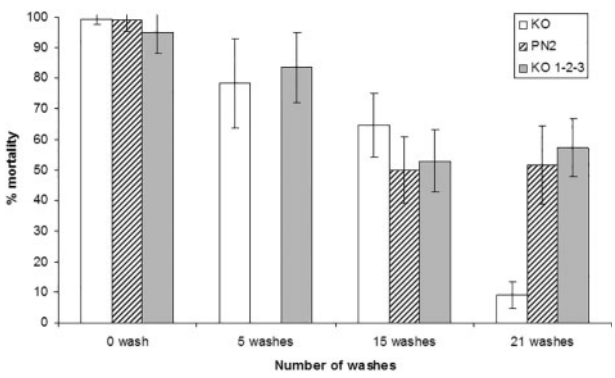

Figure 2 Mortality of *A. stephensi* 24 hours after 3-minute exposure to KO, PN2 and KO 123 with 95% CI.

results for MMKDT and the differences between PN2 and KO 123 were not statistically significant (Table 1). These results show that the technique used for the impregnation of nets in KO 123 to increase the wash resistance of treated nets works as well similar to the technique used for the impregnation of PN2 nets.

Sreehari *et al.* compared wash resistance of PN2 and KO 123 treated nets up to 20 hand and machine washes.[15] Their method of hand washing differs from the authors' method in the present study. There was no significant difference between PN2 and KO 123 treated nets with respect to %mortality and KD time after 20 washes which is confirmed by the authors' study. They reported 80% and 82.5% mortality for PN2 and KO 123, respectively, with hand washing, after 20 washes. Our results showed >50% mortality for both nets after 21 washes; lower mortality than the Sreehari *et al.* results.[15] This inconsistency may be because of differences in hand washing methods, differences in detergent or soap used for washing and various chemical or physical factors of the water, or because of tolerance of *Anopheles* used for bioassay tests.

In previous studies, the wash resistance of PermaNet™ was found to be similar to the results of wash resistance of PN2 (new generation of PermaNet™) up to 15 washes.[2,17,18,20,21] In the present study, the results showed better wash resistance of PN2 in comparison to PermaNet™ up to 21 washes.[21] However, there is still scope for the manufacturer of PN2 to improve wash resistance.

Kroeger *et al.*[22] and Gunasekaran and Vaidyanathan[23] evaluated the wash resistance of PermaNet® in the field and laboratory respectively. The hand washing method used in the Kroeger *et al.*'s research was one with an intensive rubbing of the nets, although Gunasekaran and Vaidyanathan used a rotary shaker for their washing.[22,23] Both studies showed >80% mortality of *Anopheles* up to 23 and 26 washes, respectively, >30% mortality compared to the results of our study with PN2 after 21 washes.

Gimnig *et al.*[24] used more or less the same laboratory washing method as Gunasekaran and Vaidyanathan[23]; their results showed >50% mosquito mortality after 20 washes, which is more consistent with the authors' results. Although the method of washing of Rafinejad *et al.* was similar to the methods of Gimnig *et al.* and Gunasekaran and Vaidyanathan, they reported 90% mosquito mortality exposed to PermaNet® after 20 washes, which is more consistent with the results of Kroeger *et al.* and Gunasekaran and Vaidyanathan.[22–25]

Msangi *et al.* reported >95% mortality of *Anopheles* after 18 washes and 9 months use of PermaNet®. His results are more consistent with the results of Kroeger *et al.* and Gunasekaran and Vaidyanathan.[22,23,26]

In two studies that were carried out by Yates *et al.* and Oxborough *et al.* on wash resistance of KO 123, although their studies were laboratory based and they used WHO laboratory washing methods, they reported similar results to our study and confirmed that KO 123 is LLITNs.[13,27,28] The results of Oxborough *et al.* are more consistent with our results.[27] They showed ~39% mosquito mortality of cone bioassays exposed to 20 washed KO 123 treated kits in comparison to near 55% mosquito mortality after 21 washes in our study. Yates *et al.* reported 100% or near 100% mortality after 30 wash cycles.[13]

Conclusion

Here, the insecticidal activity of all nets decreased up to 21 washes. Although the pattern of insecticide loss was different between net types, PN2 and KO 123 showed best insecticidal efficacy at 21 washes. Thus, it can be recommended that PN2 and KO 123 are LLITNs and KO 123 probably should receive full recommendation from WHO.

According to WHO definition of LLITNs,[8] if we consider that expected life span of a net will be 3 years, nets usually will be washed not more than 20, but for 5 years maybe people wash their nets up to 30 washes.[8] Meanwhile some researchers in their trials have washed nets up to 30 or 40 washes. [23,29] Therefore, more research is necessary to evaluate wash resistance of both nets up to 30 washes and manufacturers should improve their technique of impregnation.

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Disclaimer Statements

Contributors MHK, AAH, KK and ND contributed to the proposal and planning of the study; MHK, KK and ND collected the mosquitoes; MHK, KK and ND did the bioassay tests; MHK and AAH analysed data; MHK, AAH, KK and ND drafted and revised the paper. All authors read and approved the final manuscript. MHK is guarantor of the paper.

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Conflicts of interest The authors declare that no conflicts of interest.

Ethics approval None.

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