QUALITY ASSESSMENT OF LONG-LASTING DELTAMETHRIN IMPREGNATED INSECTICIDE NETS IN LAGOS, NIGERIA

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ABSTRACT

Current statistics from World Health Organization (WHO) indicated that malaria mortality in Africa has decreased by 54% across all age groups. One of the concerted efforts for this positive control is the use of long-lasting insecticide-treated nets. The objective of this study was therefore to evaluate and compare certain physicochemical characteristics of three long-lasting impregnated Nets (LLINs) for quality control assessment and monitoring.

The physicochemical characteristics of the three commercially available deltamethrin LLINs coded PT, XT and NP such as gram per square meter, mesh count, dimension nets and the deltamethrin content were evaluated in compliant with World Health Organization Pesticide Evaluation Scheme (WHOPES). The extracted deltamethrin active ingredient was assayed using a validated high performance liquid chromatographic method.

Results obtained showed that the dimensional size of net PT and NP were not in conformity with the WHO specifications while net XT complied. All the three nets however satisfied the WHO minimum specifications for mesh count. Only Nets PT and XT conformed to the specified target dose of deltamethrin content in the total nets cut randomly from the nets while Net NP deviated from the specified target dose. In the same sequence Net PT and XT also satisfied the WHO's recommendation for 20 number of washes by not falling below the efficacy concentration of 2g/kg specified by WHOPES.

Hence it is concluded that there is need to ascertain the quality of mosquito nets being imported into the country as many of these available in circulation may not meet the quality specifications.

Key words: Deltamethrin, Malaria, Long-lasting Insecticide Nets (LLINs)

INTRODUCTION

Malaria is one of the most devastating disease conditions in the world affecting both young and old population. It is a life-threatening scourge caused by parasites that are transmitted through bites of infected female anopheles mosquitoes. Malaria is responsible for about 90% of the death recorded in Africa, occurs mostly in young children below the age of five. Malaria, HIV/AIDs and Tuberculosis are major endemic undermining development in Nigeria with momentous morbidity and mortality rate associated with these disease conditions. Some fifteen years ago, African Heads of State met in Abuja Nigeria to further strengthen the roll back malaria goal (RBM) of reducing the African Malaria burden to less than 40% by the year 2010. The Abuja declaration endorsed a concerted strategy to tackle the problem of malaria across Africa [1]. As part of the RBM concerted efforts carried out by Nigeria is the use of environmental interventions which include the use of indoor residual spray, promotion of insecticide treated nets (ITNs), reduction of tariffs and provision of ITNs to make them affordable, improved vector control, prevention and management of malaria in pregnancy [2]. According to the current WHO report, malaria mortality rates, taken into account the population growth, are estimated to have decreased by 60% globally across age while in Africa, the mortality death rates have also gone down by 54% across all age group between 2000 and 2015 [3].

Vector control and prevention remains the safest and most effective measure to stem the surge of malaria transmission, reduction of malaria morbidity and mortality. Insecticide treated nets most especially the long lasting insecticide impregnated nets (LLINs) are being used as a vector control tool in endemic regions. Pesticide has been found to be one of the suitable vector control measures that can be used to curtail the burgeoning tide of malaria and they include the following: Organochlorines [Dichlorodiphenyltrichloroethane (DDT), Heptachlor]; Organophosphate [Malathion, Parathion, Dichlorvos]; Carbamates [Carbofuran]; Organosulphur compounds [Thiocarbamates, Dithiocarbamates]; Dinitrophenols [Dinocap]; Thiocyanate [Thanite]and Pyrethroids [Cypermethrin, Ellethrin, Permethrin, Deltamethrin]. Of all the pesticides in use, deltamethrin has been seen to be the most potent and efficient [4,5]. Deltamethrin belongs to the chemical class of pyrethroids, naturally occurring insecticidal compounds that are synthesized from chrysanthemum flowers [6]. As a lipophilic compound, deltamethrin is not soluble in water and therefore is highly stable in the physical environment. Unlike many pyrethroids, deltamethrin is also stable in air and sunlight: when exposed to either, it does not degrade, even after two years' time at 40 degrees Celsius [7]. Deltamethrin has historically been used for a variety of purposes, including vector-control for insects such as the mosquito and the tsetse fly and the associated public health threats they pose [8]. As a pyrethroid, deltamethrin paralyzes the nervous system of its insect target, leading to eventual death. Insects absorb, quickly metabolize, and are poisoned by the compound, through both oral and dermal means. The susceptibility of various insects to deltamethrin depends on not only their own physiological structure, but also the surrounding environmental conditions. For example, flies are more vulnerable to the compound at dawn. Some of the WHO recommended LLINs of deltamethrin origin are PermaNet® 2.0 (deltamethrin coated on polyester); PermaNet® 2.5 (deltamethrin coated on polyester with strengthened border; PermaNet® 3.0; DawaPlus®2.0 (deltamethrin coated on polyester); Netprotect® (deltamethrin incorporated into polyethylene and Yorkool® (deltamethrin coated on polyester. With the advent of LLINs, proliferation of different brands of mosquito nets in Nigeria has been on the increase. There is therefore the need to constantly monitor and evaluate the physicochemical properties of these nets as a quality control measure in line with WHOPES through the determination of their physicochemical properties and also be able to sustain the improvement achieved by WHO in the war against malaria.

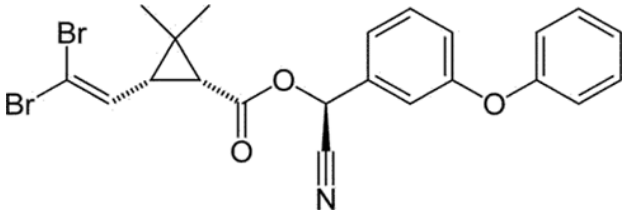


Figure 1: Chemical structure of Deltamethrin

MATERIALS

Three commercially available deltamethrin LLINs coded PT, XT and NP were procured from a licensed supermarkets and pharmacies in Lagos.

METHODS

The physical properties such as gram per square meter, dimensional nets, mesh count and the deltamethrin content were carried out in compliant with World Health Organisation Pesticide Evaluation Scheme (WHOPES). The deltamethrin was extracted from the nets using mixture of acetonitrile and water [1:1]. The extracted deltamethrin active ingredient was assayed using a modified high performance liquid chromatographic method according to CIPAC [9].

PHYSICAL ANALYSIS

Dimensional Test

The individual net was hung on nails attached to the wall and the different sides which include the breadth, length and height were measured using a tape rule.

Table 1: Dimensional Size of Three Different Long Lasting Insecticide Treated Nets of Family Size

Net	Average Length (cm) ± SEM	Average Breadth (cm) ± SEM	Average Height (cm) ± SEM
PT	201.0 ± 7.5	205.8 ± 4.1	204.5 ± 4.7
NP	188.0 ± 2.7	167.0 ± 6.4	152.3 ± 2.3
XT	180.0 ± 0.3	190.0 ± 0.5	150.0 ± 0.4
WHOPES	**180 ± 5%**	**160 ± 5%**	**150 ± 5%**

WHOPES: World Health Organisation Pesticide Evaluation Scheme
SEM: Standard Error of the Mean

Mesh Count

The net, 5cm x 5cm was cut randomly from the different sides of the net and scanned on a plain sheet of paper. Then the number of holes per square inch was manually counted.

Table 2: Mesh count (Holes per square inch) of Three Different Long Lasting Insecticide Treated Nets of Family Size

Net	Mesh count ± SEM
PT	181.0 ± 0.7
NP	167.3 ± 1.8
XT	164.0 ± 0.2
WHOPES	**156 holes/square inch (minimum mesh count)**

WHOPES: World Health Organisation Pesticide Evaluation Scheme
SEM: Standard Error of the Mean

QUANTITATIVE ANALYSIS

Preparation of Solvent for Extraction

A mixture of acetonitrile and water was prepared in the ratio 1:1 volume-volume.

Preparation of deltamethrin standard solution

Deltamethrin reference standard (0.03g) was weighed, transferred into a 50ml volumetric flask, dissolved completely with the extraction solvent and then filled up to mark with the extraction solvent. The mixture was subjected to an ultrasonic bath exposure for one hour at room temperature.

Preparation of Calibration solutions

Gradient concentrations of deltamethrin (52, 60, 68, 76 and 84) µg/mL were prepared from the deltamethrin stock solution and 25µL of sample injected into HPLC. Calibration data were generated and used to plot the graph.

Extraction of deltamethrin from the commercial Nets

A net sample of 25cm by 25cm was cut off from each side of bed net randomly and weighed. The pooled sample was transferred into 500mL volumetric flask. Four Hundred (400)mL of the extraction solvent was added. The mixture was covered and subjected to an ultrasonic bath exposure for one hour at room temperature. The final mixture was properly shaken, filtered with 0.45µm syringe filter and little quantity was transferred into a sample bottle. 25µL of the sample was injected into HPLC machine for analysis.

HPLC Chromatographic Conditions

The chromatographic elution was performed under gradient conditions at ambient temperature. An Agilent® HP-1200 with auto-sampler, diode array detector, binary pump and work station (HPLC 3D Chem station). The column used was Lichrospher®100 RP-18, 5 µm particle size with column temperature of 40°C. The Mobile phase is Acetonitrile:Water (70:30 $^{v}/_{v}$). The injection volume was 25 µL and the measurement was taken at a detector wavelength of 254 nm while the run time was 6 min.

WHO Washing Procedure

Net samples (25 cm x 25 cm) were cut from the net PT, XT and NP. They were introduced separately into 3-one Litre beaker containing 0.5 Litre deionized water with 2 g/Litre soap (Royamed® P29). The beakers containing the nets were immediately introduced into a water bath at 30°C and shaken for 10min at 155 movements per minute. The samples were then removed and rinsed twice for 10 min in clean, deionised water in the same shaking conditions. The nets were then dried at room temperature and stored at 30°C in the dark between washes. This washing was done 20 times in accordance with WHO's specifications. Net samples were analysed at 5, 10, 15 and 20 washes only using the procedure for net analysis and deltamethrin contents were evaluated.

RESULTS

The results of physicochemical properties of the three long lasting insecticides nets are presented in table 13. Figure 2 represents the calibration curve deltamethrin standard while figures 2, 3, 5 represent the picture of the cut nets for mesh

Figure 2: Calibration plot of deltamethrin standard

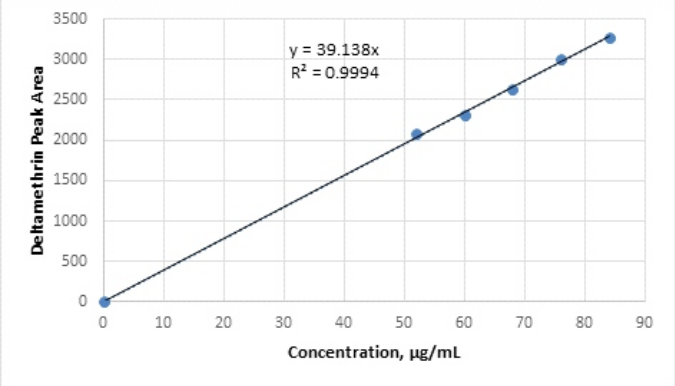


Figure 3: Cut net for Mesh Count Determination of net NP

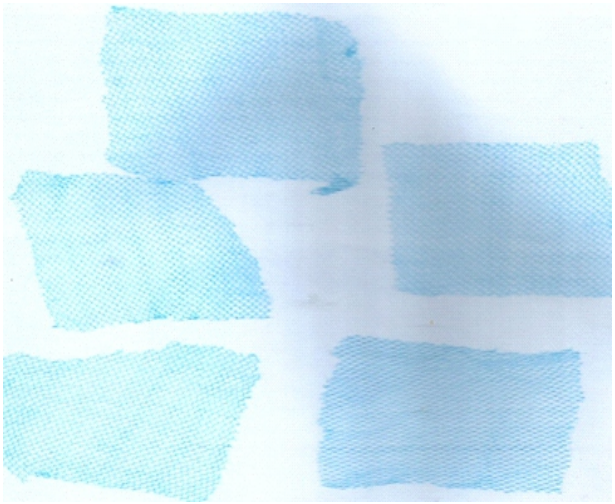


Figure 4: Cut net for Mesh Count Determination of net PT

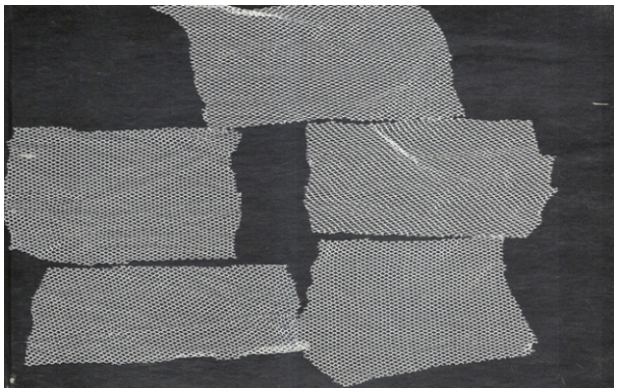
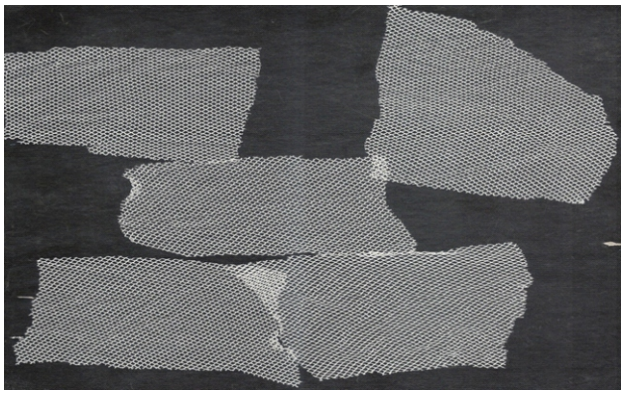


Figure 5: Cut net for Mesh Count Determination of net XT



count determination of net PT, XT and NP respectively and figure 6 shows the chromatograms of deltamethrin standard and sample.

Figure 6: Chromatograms of Deltamethrin Standard[A] and Extract from Net[B]

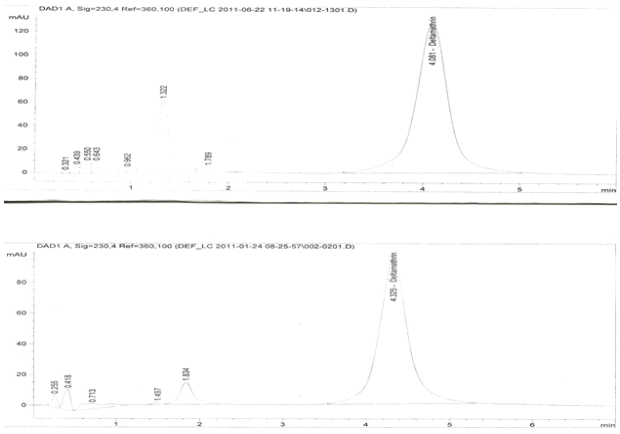


Figure 7: The Histogram showing deltamethrin content before and after 20 washes.

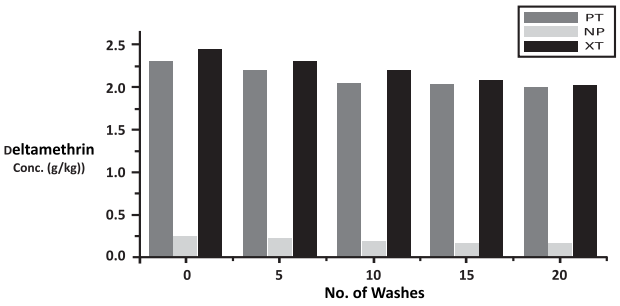


Figure 7 shows the histogram of deltamethrin content before and after 20 washes. The result of the linearity gave the equation of line of regression of y = 39.13x where y is the peak area and x is the concentration of deltamethrin. The coefficient of determination (r^2) was 0.999 with the retention time of the sample not deviated more than 15 sec of that of the standard as recommended by CIPAC.

DISCUSSION

World Health Organisation has emphatically encouraged the use of insecticide treated nets (ITNs) and environmental management to reduce the vector population as key strategies for malaria prevention and control in sub-Saharan Africa [10,11]. Insecticide-treated nets (ITNs) are effective malaria control tool but to remain effective, there is need for treatment with pyrethroid insecticide about once a year [12]. Specifications for and quality control of mosquito nets are important to define acceptable physical properties of mosquito netting and nets for institutional buyers and end users. These specifications help to ensure that the net does what it is intended to do for the user [13]. The net holes determine the size of insects that are kept out and airflow as well as the temperature that the net reaches. The amount of deltamethrin

incorporated into the net determines the efficacy and potency of the net.

WHO specifies a dimension size of 180 cm length × 160 cm width×150 cm height for rectangular nets of family size, it was observed from the results obtained that the dimension size of net PT deviated by 12, 29 and 36%; net NP deviated by 4, 4 and 2% while net XT was found to be in conformity with the specification. WHO specifies a minimum mesh count of 156 holes/square centimetre, it was observed that all the three nets complied with the specification. Although the holes in netting with mesh count are neither rounds nor square and may be irregular in shape and size. To prevent mosquitoes' entry, the size of holes is very important however there is lack of standardized method to assess size of holes hence the consensus that 156 holes/cm^2 (mesh count) remains the quality specification for nets. Deltamethrin, the active ingredient of the insecticide treated net was qualitatively identified by the retention time of deltamethrin in the sample net compared with the chromatograms of the standard. Net PT and XT conformed to the specified target dose of deltamethrin content in the total nets cut randomly from different sides of the nets while Net NP deviated from the specified target dose. The results of the number of washes showed the efficacy of the drug binder and confirmed suitability for use in the next three to five years.

Continuous monitoring and assessment of the efficacy of ITNs/LLINs in use is necessary in order to appropriately modify vector control operations and prevent the development of pyrethroid resistance. Hence it is important to ascertain the quality of the nets available in circulation by assessing their physicochemical properties relating to efficacy.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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