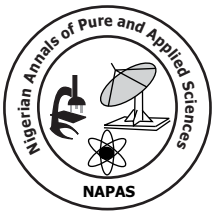

Evaluation of Perma Net® 2.0 in the Control of Culex quinquefasciatus and Aedes aegypti from Awka, Anambra State, Nigeria.

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Abstract

Long Lasting Insecticide Nets (LLINs) reduce human – mosquito contact through provision of physical barrier, repellent effect or mortality effect. This study evaluated the physical integrity and residual effect of insecticides in Perma Net® 2.0 of 6 months, 1 year, 2 years, 3 years and 4 years duration of use. Perma Net® 2.0 used were obtained from systematically selected households within Ifite Awka and new Perma Net® 2.0 serving as control were collected from Society for Family Health. Adult mosquitoes were collected from selected households using Pyrethrum knockdown Collection method before the Perma Net® 2.0 were removed. The top and four sides of the nets were examined for presence of holes. Also, net pieces measuring 25cm × 25cm were obtained from Perma Net® 2.0 of different duration and subjected to WHO cone bioassay using Randomized Block Experimental Design. The *Culex quinquefasciatus* and *Aedes aegypti* used for the bioassay were collected from breeding sites within Ifite Awka, reared and maintained in an insectary. There was at least one hole in 10%, 30% and 40% of LLINs with 2 years, 3years and 4 years duration of use respectively. Significant differences were found in the physical integrity of the nets ($P < 0.05$). *Culex quinquefasciatus* and *Aedes aegypti* constituted 40.9% and 27.0% of all mosquitoes collected indoors respectively. There was no significant difference in the indoor abundance of the two mosquito species ($P > 0.05$). However, their abundance depended on the duration of LLINs usage ($P < 0.05$). The mortality effects of 16%, 8%, 7%, 5%, 5% and 2% on *Culex quinquefasciatus* were recorded for Perma Net® 2.0 of 0 month, 6 months, 1 year, 2 years, 3 years and 4 years respectively. The mortality effects of 23%, 12%, 9%, 6%, 5% and 3% on *Aedes aegypti* were recorded for Perma Net® 2.0 of 0 month, 6 months, 1 year, 2 years, 3 years and 4 years respectively. The new LLINs produced significantly highest mortality effect ($P < 0.05$) on both mosquito species than nets of other duration of usage. The mortality effect of Perma Net® 2.0 on *Culex quinquefasciatus,* and that on *Aedes aegypti* were similar ($P > 0.05$). It shows that Perma Net® 2.0 offer physical barrier more than repellency and mortality effect against *Culex quinquefasciatus,* and *Aedes aegypti*.

Key words: *Culex quinquefasciatus*, *Aedes aegypti*, Perma Net® 2.0, LLINs, Mortality, Repellency.

Introduction

The use of Long Lasting Insecticide Nets is one of the most effective ways to control mosquito-borne diseases. LLINs is known to kill mosquitoes, and have proven repellent properties that reduce the number of mosquitoes that enter the house to bite (Curtis *et al*., 2003) as well as reduce the prevalence of mosquito borne diseases (Egbuche *et al*., 2013). LLINs also act as a baited trap that lures mosquitoes to the insecticides by using body gases emitted by humans (Lindblade *et al*., 2015). LLINs still work by providing physical barrier thereby reducing the human – vector contact. It was developed to reduce the need for retreatment and offer protection that last for 3 years (WHO, 2007). Three LLINs fully recommended by the WHO and are: Olyset®, PermaNet® 2.0 and Yorkool LLIN (WHO 2010). There is high level of LLINs ownership and usage in Nigeria with Perma Net® 2.0 as the most commonly used in Awka, Anambra State; Based on pilot survey in Awka prior to this study. Studies on LLINs have largely focused on *Anopheles species* (Koudou et al., 2011; Gouissi et al., 2012; Okia et al., 2013; Abílio *et al*, 2015). Little or no attention is given to other peridomestic mosquito species such as *Culex quinquefaciatus* and *Aedes aegypti. Culex quinquefasciatus* transmit the pathogen that causes urban filariasis. *Aedes aegypti* is the main vector of pathogens that cause dengue fever, yellow fever and other arboviral diseases. These two species are known to breed around human dwellings (Egbuche *et al*., 2016) with high abundance of the adults recorded indoor (Amaechi *et al.*, 2013; Ekesiobi *et al*., 2014; Onyido *et al*., 2016). They are found indoor irrespective of the housing condition (Egbuche *et al*., 2017). For instance, *Culex quinquefasciatus* have been collected indoor from LLINs of less than 3 years duration of use (Ochomo *et al*., 2013). Also, populations of *Aedes aegypti* collected between untreated and the deltamethrin-treated houses were found to be the same (Vazquez-Prokopec *et al.,* 2017). These species of mosquitoes have serious health implications. Lymphatic filariasis is still endemic in some parts of Nigeria (Okorie *et al*., 2015). Recently, 341 suspected cases and 32 confirmed cases of yellow fever were reported from 16 states in Nigeria. This included Abia, Anambra, Borno, Edo, Enugu, Kano, Katsina, Kogi, Kwara, Kebbi, Lagos, Nasarawa, Niger, Oyo, Plateau, and Zamfara states (WHO, 2017). There is also high rate of unrecognized dengue virus infection in parts of the rainforest region of Nigeria (Onoja *et al.,* 2016). These two species of mosquito are greatly increasing in their indoor abundance and as such require research attention as much as *Anopheles species*. Thus the aim of the study was to evaluate the efficacy of LLINs in the control of *Culex quinquefaciatus* and *Aedes aegypti*.

Methodology

Study Area

The study was carried in Awka, the capital city of Anambra State. Its geographical coordinates are 6°12′25″N and 7°04′04″E. Awka has a total of 33 villages, divided into two sections: Ifite and Ezinato sections. Specifically, the study was carried out within Ifite Awka where the largest population of inhabitants comprised students.

Ethical consideration

A letter of intent for the study was obtained from the Head of Parasitology and Entomology Department, Nnamdi Azikiwe University Awka and submitted to the hostel officials for approval. Students whose rooms were used for the study were properly informed and their verbal consent duly obtained.

Experimental design

The study involved both field and laboratory work. First, cross sectional survey of Long Lasting Insecticide Nets was carried out in students' rooms (designated as households) within Ifite Awka, Anambra State. This was to identify the rooms that had people using Perma Net® 2.0, for enrolment in the study. A total of 60 households that used Perma Net® 2.0 of different durations of use were then systematically selected for adult mosquitoes survey; as a metric for deterrent or repellency effect of Perma Net® 2.0. From the selected households, the Perma Net® 2.0 of different durations of usage were retrieved and assessed for physical integrity and also for cone bioassay to determine mortality effect of Perma Net® 2.0.

Indoor survey of adult mosquitoes

Indoor survey of adult mosquitoes was carried out in the selected households before the LLINs were removed. Pyrethrum Knockdown Collection method was used to collect indoor resting/ biting mosquitoes from the selected households. This was done according to World Health Organization (2003).

Assessment of physical integrity of Perma Net® 2.0

The physical integrity of the Perma Net® 2.0 was assessed in order to ascertain its effectiveness in providing physical barrier. Ten

Perma Net® 2.0 of each duration of usage: 0 months, 6 months, 1 year, 2 years, 3 years and 4 years, were held up and the sides, including the top were thoroughly checked for presence of holes / torn parts.

Survey of immature stages of *Culex quinquefaciatus* and *Aedes aegypti*

Potential breeding sites of *Culex quinquefaciatus* and *Aedes aegypti* were surveyed within Ifite Awka Anambra State according to WHO (2003). Larvae collected from different breeding habitats were reared to adult for proper identification to species level and use for the cone bioassay.

Cone bioassay

The cone bioassay was done using the standard of WHO (2006), with slight modification. Net piece measuring 25cm × 25cm was cut from each of the 10 Perma Net® 2.0 of a particular duration of usage. One cone was fixed on each net piece, to give a total of 10 cones for each category of net that constitute a sample test. Batches of only 5 non-blood fed susceptible female mosquitoes aged 2-5 days were introduced into each plastic cone for 3 minutes. This was carried out in a randomized block design with two replicates of each sample test. Perma Net® 2.0 of different duration of usage and reared mosquito species (*Culex quinquefaciatus* and *Aedes aegypti*) were used as the treatments. The response variable was mortality records of mosquito species after 24 hours post exposure (WHO, 2006).

Statistical analysis

Statistical analysis was done at 5% level of significance by using SPSS version 25.0. Chi square was used to test for the physical integrity of Perma Net® 2.0 of different duration of use. ANOVA was used to compare the indoor abundance of *Culex quinquefaciatus* and *Aedes aegypti*. ANOVA was also used to test the mortality effect of Perma Net® 2.0 on the two species of mosquito studied. Paired t-test was used to analyze the mortality effect of Perma Net® 2.0 on the two species of mosquito studied.

Results

The indoor abundance of mosquitoes was used as a metrics for assessing the repellency effect of Perma Net® 2.0. *Culex quinquefasciatus* and *Aedes aegypti* constituted 40.9% (56/137) and 27.0% (37/137) of all mosquitoes collected indoors. Other mosquito species collected included *Anopheles species* 22.6% (31/137) and *Aedes albopictus* 9.5% (13/137). The proportion of *Culex quinquefasciatus* collected from households with Perma Net® 2.0 of 0 months, 6 months, 1 years, 2 years, 3 years and 4 years duration of use were 33.3% (1/3), 45.5% (5/11), 44.4% (8/18), 41.4% (12/29), 29.3% (12/41) and 51.4% (18/35) respectively. The proportion of *Aedes aegypti* collected from households with Perma Net® 2.0 of 0 months, 6 months, 1 years, 2 years, 3 years and 4 years duration of use were 0.00% (0/3), 27.3% (3/11), 22.2% (4/18), 20.7% (6/29), 24.4% (10/41) and 40.0% (14/35) respectively. There was no significant difference in the indoor abundance of *Culex quinquefasciatus* and *Aedes aegypti* (P = 0.124). The abundance of *Culex quinquefasciatus* and *Aedes aegypti* collected from households with Perma Net® 2.0 of different duration of usage is shown in Figure 1. Highest number of *Culex quinquefasciatus*, 32.1% (18/56) was collected in households with Perma Net® 2.0 of 4 years duration of use while the least number, 1.79% (1/56) was collected in households with new Perma Net® 2.0. Perma Net® 2.0 of 6 months, 1 year, 2 years and 3 years duration of use recorded 8.9% (5/56), 14.3% (8/56), 21.4% (12/56) and 21.4% (12/56) abundance of *Culex quinquefasciatus* respectively. There was significant difference in the indoor abundance of *Culex quinquefasciatus* (P = 0.017) from the different households. Highest number of *Aedes aegypti*, 37.8% (14/37) was collected in households with Perma Net® 2.0 of 4 years duration of use while the least number, 0.0% (0/37) was collected in households with new Perma Net® 2.0. Perma Net® 2.0 of 6 months, 1 year, 2 years and 3 years duration of use recorded 8.1% (3/37), 10.8% (4/37), 16.2% (6/37) and 27.0% (10/37) abundance of *Aedes aegypti* respectively. There was also significant difference in the indoor abundance of *Aedes aegypti* (P = 0.035) from the different households.

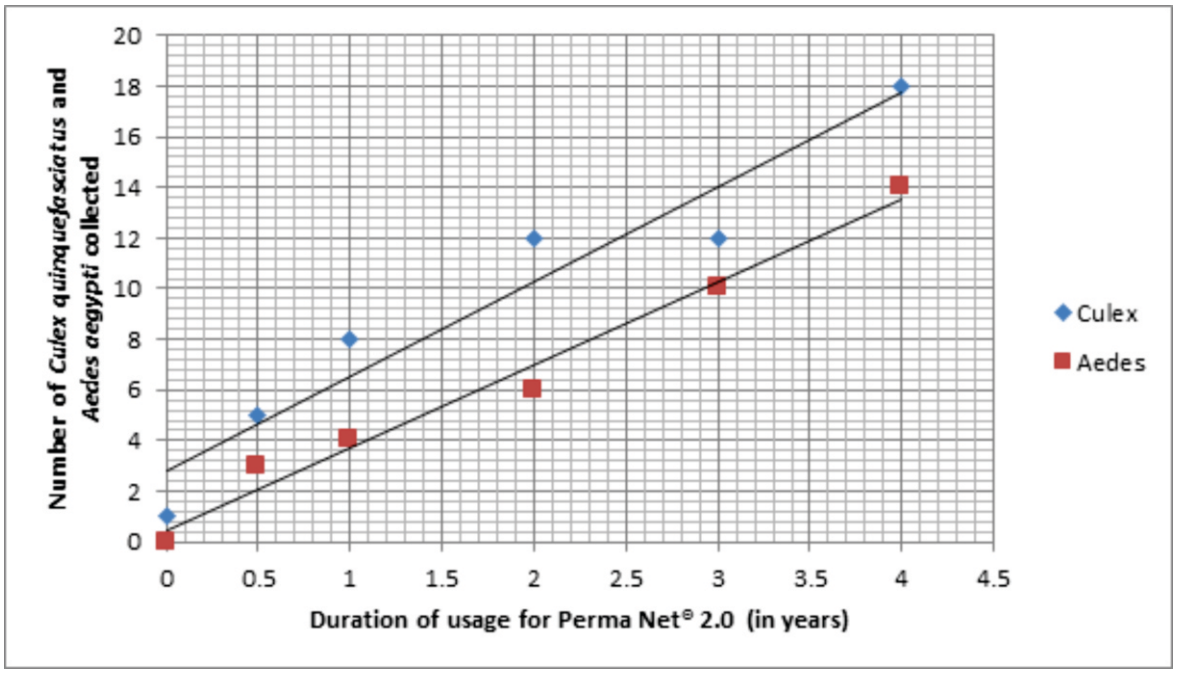

Figure 1: Indoor abundance of *Culex quinquefasciatus* and *Aedes aegypti* in households with Perma Net® 2.0 of different duration of use.

The Mortality effect of Perma Net® 2.0 on *Culex quinquefasciatus* and *Aedes aegypti* is shown in table 1. The new Perma Net® 2.0 produced the highest mortality effect of 16.0% on *Culex quinquefasciatus* and 23.0% on *Aedes aegypti* than nets of other duration of usage. There was significant difference in the mortality effects of Perma Net® 2.0 of different durations of usage on *Culex quinquefasciatus* (P = 0.001). There was also significant difference in the mortality effects of Perma Net® 2.0 of different durations of usage on *Aedes aegypti* (P = 0.00). The mortality effects of Perma Net® 2.0 of different durations of usage on *Culex quinquefasciatus* when compared with the effects on *Aedes aegypti* were not significantly different (P = 0.064).

Table 1: Mortality effect of Perma Net® 2.0 on *Culex quinquefasciatus* and *Aedes aegypti*

Duration	Number of test	Number of mosquitoes exposed per test	*Culex quinquefasciatus*		*Aedes aegypti*	
			Mean ± se	% Mortality	Mean ± se	% Mortality
0 month (control)	2	50	8.0 ± 0.0	16.0	11.5 ± 0.5	23.0
6 months	2	50	4.0 ± 0.0	8.0	6.0 ± 1.0	12.0
1 year	2	50	3.5 ± 0.0	7.0	4.5 ± 0.5	9.0
2 years	2	50	2.5 ± 0.0	5.0	3.0 ± 0.0	6.0
3 years	2	50	2.5 ± 0.0	5.0	2.5 ± 0.5	5.0
4 years	2	50	1.0 ± 0.0	2.0	1.5 ± 0.5	3.0

A total of 60 Perma Net® 2.0 was assessed for physical integrity. There was at least one hole in 10%, 30% and 40% of LLINs with 2 years, 3years and 4 years duration of use respectively Perma Net® 2.0 of less than 1 year duration of use had no holes (Table 2). Significant differences were found in the physical integrity of the nets (P = 0.044).

Table 2: Physical integrity of Perma Net® 2.0 of various duration of usage

Duration	Number with at least 1 holes (%)	Number without any hole (%)	Total number examined.
0 month (control)	0 (0.0)	10 (100.0)	10
6 months	0 (0.0)	10 (100.0)	10
1 year	0 (0.0)	10 (100.0)	10
2 years	1 (10.0)	9 (90.0)	10
3 years	3 (30.0)	7 (70.0)	10
4 years	4 (40.0)	6 (60.0)	10

Discussion

The study recorded high abundance of *Culex quinquefasciatus* and *Aedes aegypti* than other mosquito species in indoor locations where Perma Net®2.0 are used. This is similar to the findings of Egbuche *et al.* (2017) who reported that *Culex quinquefasciatus* and *Aedes aegypti* could be found indoor irrespective of the housing condition. It is an indication that Perma Net®2.0 may have reduced repellency effect which was assessed by using indoor abundance of mosquito species as a metric. Depending on the duration of usage, Perma Net®2.0 showed varied repellency effects. The new Perma Net® 2.0 used as control recorded significantly lower abundance of mosquitoes than Perma Net® of 4 years duration. However, the abundance of mosquitoes in Perma Net® 2.0 of 6 months, 1 year, 2 years and 3 years duration of use fluctuates. It could be as low as that of a new Perma Net® 2.0 or as high as Perma Net® 2.0 of 4 years duration. When former is the case, it shows that Perma Net®2.0 as old as 3 years may still be very effective in producing repellency effects. This is supported by Graham *et al*. (2005) and Atieli *et al.* (2010) who stated that the efficacy of PermaNet® 2.0 against some of the most important disease vectors around the world was maintained even after 20 washes under laboratory and field conditions. The retention of this biological activity, through 20 washes or 3 years of field use without need for re-treatment, is ultimately what defines and distinguishes a long-lasting insecticidal net (LLIN) from a conventionally treated net (WHO, 2013). In a case where Perma Net® 2.0 of less than 3 years duration of use produce repellency effect as low as that of 4 years, it could be attributed to the quality of the Perma Net® 2.0 or the number of times/ conditions in which a Perma Net® 2.0 of a particular duration of use has been washed. This later case is supported by Ochomo *et al*. (2013) who also collected *Culex quinquefasciatus* indoor from LLINs of less than 3 years duration of use (Ochomo *et al*., 2013).

It may also be that the repellency effect of LLINs in general is a function of distance between resting surfaces of mosquitoes and where the net is hung. It is possible that the distance of the resting surfaces of *Culex quinquefasciatus* and *Aedes aegypti* from the position of the Perma Net® 2.0 is shorter than that kept by other endophagic and endophilic mosquitoes. This possibly could be the reason why larger population of them was caught than other mosquitoes in the same house with Perma Net® 2.0. The distance is likely to decrease as the duration of use increases because increasing number of mosquitoes were collected from Perma Net® 2.0 of increasing duration of usage. This keeps them in greater proximity with persons sleeping under the net.

Their presence indoors would increase their chances of disease transmission especially of persons sleeping outside the net. It shows that these mosquito species have behavioural adaptation to bite and /or rest inside houses with Perma Net® 2.0. Even if they only rest indoor, they may utilize any breeding site especially the closed larvae habitat (Egbuche *et al.,* 2016) within the house to breed and increase their abundance. This also keeps them close to human habitation for disease transmission. With lymphatic filariasis still endemic in some parts of Nigeria (Dogara *et al*., 2012; Iboh *et al.,* 2012; Okonofua *et al*., 2014; Okorie *et al*., 2015), increase in the population of *Culex quinquefasciatus* will increase chance of *Wuchereria bancrofti* being transmitted from one person to another. *Aedes aegypti* is the main vector of pathogens that cause dengue fever, yellow fever and other arboviral diseases. Their presence is also of public health importance as high rate of unrecognized dengue virus infection has been reported in parts of the rainforest region of Nigeria (Onoja *et al.,* 2016).

Perma Net® 2.0 recorded significantly very low mortality effects of 16% and 23% on *Culex quinquefasciatus* and *Aedes aegypti* respectively. It shows that it failed the WHO efficacy requirement of ≥ 80% mortality. It could be due to increased level of insecticide resistance by some members of the local populations of *Culex quinquefasciatus* and *Aedes aegypti*. Similar finding has been reported by Chandre (1998). Nevertheless, new Perma Net® 2.0 had significantly highest mortality effect on both species of mosquitoes, followed by Perma Net® 2.0 of 6 months and 1 year duration of use. Against *Aedes aegypti*, Perma Net® 2.0 of 1 year fluctuates in its mortality effect. It may be due to gradual development of resistance in *Aedes aegypti* population. What it implies is that *Culex quinquefasciatus* and *Aedes aegypti* attracted by body gases emitted by humans sleeping under Perma Net® 2.0 (Lindblade *et al*., 2015) may escape being killed even though it may not be able to feed. Contrary to the findings of this research, Sreehari *et al*. (2009) and Atieli *et al*. (2010) observed increased efficacy of PermaNet® in producing > 80% mortality in *Anopheles culicifacies* and *Anopheles stephensi* mosquitoes after up to 20 hand washes and up to 10 machine washes. The difference here is however on the mosquito species exposed to Perma Net® 2.0. It is possible that the *Culex quinquefasciatus* and *Aedes aegypti* subjected to cone bioassay did not

have much contact with the net pieces within the three minutes of exposure due to the repellency effect of the nets. This repellence may decrease the time the mosquitoes are exposed to the net during the 3-min time period, due to the fact that the mosquitoes may be resting on the cone rather than on the net surface. This is likely going to be true for the new Perma Net® 2.0 that showed the greatest repellency effect on *Culex quinquefasciatus* and *Aedes aegypti*. Reduced mortality effect with Perma Net® 2.0 of increasing duration of use may be attributed to reduction in the quality of the pyrethroid used, procedure used in treating the net, loss of the insecticide due to washing of the net or loss of the insecticide due to increased environmental temperature. High environmental temperature of long duration may result in quick loss in the bioavailability of the insecticide. This is because LLINs retain more insecticide when dried under the shade with room temperature (Atieli *et al*., 2010). Also accelerated diffusion of the insecticides due to heat exposure has been confirmed by Gimnig *et al*. (2005) and Sreehari *et al*. (2009).

With poor repellency and failed mortality effect, physical integrity of the net may be the last option for protection. LLINs with hole(s) permit mosquito entry, thereby providing little or no protection against the vectors. From the study, Perma Net® 2.0 can develop holes at 2 years duration of use thereby reducing its ability to provide physical barrier. This is in line with the finding of Ochomo *et al*., (2013) where he reported that LLINs develop holes within three years of use. Hakizimana *et al.,* (2014) also reported that the serviceable life of LLINs is approximately two, rather than three years, which suggests that the impact of the LLINs intervention during year three could be well below that seen in years one and two. Presence of holes may be attributed to the frequency of wash, frequency of use or pressure of handling while the net is being used.

In conclusion, Perma Net® 2.0 has physical integrity of less than 2 years, repellency effect of between 6 months to 3 years and very low mortality effects of <80%. It shows that the physical integrity, repellency and mortality effects of Perma Net® 2.0 in the control of *Culex quinquefasciatus* and *Aedes aegypti* is less than 3 years and reduces as the duration of use increases.

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