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*Research Article*

# Village-Scale Evaluation of PermaNet 3.0: an Enhanced Efficacy Combination Long-Lasting Insecticidal Net Against Resistant Populations of *Anopheles gambiae s.s.*

**A. O. Adeogun,[1,2] J. B. Olojede,[1] A. O. Oduola,[1] and T. S. Awolola[1]**

[1]*Molecular Entomology and Vector Control Research Laboratory, Nigerian Institute of Medical Research, PMB 2013, Yaba Lagos, Nigeria*
[2]*Department of Zoology, University of Ibadan, Ibadan, Nigeria*
*Address correspondence to T. S. Awolola, awololas@hotmail.com*

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**Abstract** *Background.* PermaNet® 3.0 (PN 3.0) is a combination long-lasting insecticidal net (LLIN) designed to have increased efficacy against pyrethroid-resistant malaria vectors. Field testing of this new tool under normal use has been limited. Here we report on a small-scale village trial carried out at two localities where malaria vectors were resistant to pyrethroid insecticides. *Methods.* Nets were distributed to cover all sleeping spaces and evaluated for insecticidal activity. Households were visited to assess net usage and reported side effects. Entomological data were collected on a monthly basis for 12 months. *Results.* Bioassays repeated on domestically used PN 3.0 over 12 months showed persistent bioefficacy although bioefficacy of Olyset decreased over this period ($< 80\%$ mortality). The overall results demonstrated that PN 3.0 was well accepted by nets users and resulted in 8–11% and 34–37% reductions in blood feeding relative to the Olyset and the untreated control respectively. *Anopheles gambiae s.s.* mortality was also greater for PN 3.0 ($> 65\%$ mortality) compared to the Olyset nets ($< 45\%$). *Conclusion.* This study provides persuasive evidence on the increased efficacy of PN 3.0 against malaria vectors with *kdr* only and *kdr* plus metabolic-based pyrethroid resistance mechanisms under realistic LLIN use scenarios.

**Keywords** PermaNet 3.0; village trial; efficacy; resistance; *Anopheles gambiae*

## 1 Background

The use of insecticide-treated nets (ITNs) is a key strategy for protection against malaria infection [6,22]. The bio-efficacy of conventionally-treated nets is known to diminish due to repeated washing and handling, necessitating re-treatment at six to twelve month intervals in order to retain bio-efficacy. The development and promotion of long-lasting insecticidal nets (LLINs) has circumvented the problems associated with re-treatment of nets [16]. Long-lasting nets are manufactured with the aim that the net is more resistant to washing than conventionally treated nets, with minimum criteria of withstanding 20 standard washes under laboratory conditions and 3 years of recommended usage under field conditions. The production of LLINs employs two main technologies. The first involves incorporation of the insecticide into the mixture prior to extrusion of the fibre, such as for Olyset Net® which incorporates permethrin into polyethylene [34]. A second strategy is by coating a resin containing insecticide onto the pre-extruded fibre, such as employed in the development of PermaNet® which uses deltamethrin mixed in a resin and bound around polyester fibres [35].

Pyrethroids are currently the only class of insecticides recommended for use in LLINs. Resistance to pyrethroids has become widespread and is a threat to the success of malaria control programs [12,13,24,31]. Pyrethroid resistance in African malaria vectors is normally associated with two major mechanisms: target site insensitivity and metabolic-based resistance [18,27]. Target site insensitivity to pyrethroid is due to a single point mutation commonly referred to as knock down resistance (*kdr*) leading to modification of the voltage-gated sodium channel making it less susceptible to the binding of pyrethroids [27]. Metabolic-based resistance mechanisms are principally associated with three enzymes families: the cytochrome P450 monooxygenases, carboxylesterases and glutathione-S-transferases [18,27].

Synergists have been used commercially for over 50 years and have contributed significantly to improve the efficacy of insecticides [8,9,19]. This can be attributed to their enzyme-inhibiting action, restoring the susceptibility of insects to the chemical which would otherwise require higher levels of the toxicant for their control [11]. Synergists are also useful for laboratory investigation of resistance mechanisms through their ability to inhibit specific metabolic pathways [11]. PermaNet® 3.0 (PN 3.0)

is a mosaic LLIN which combines deltamethrin-coated polyester side panels and deltamethrin with the synergist piperonyl butoxide (PBO) incorporated in the polyethylene roof [35]. PBO is an inhibitor of mixed function oxidases with potential to reduce activity of enzymes associated with resistance [11] as enhancing penetration of deltamethrin across the insect cuticle [1]. Data from experimental hut trials in West and East Africa have shown the potential of PN 3.0 in controlling resistant malaria mosquitoes when compared to standard LLINs (PermaNet 2.0 and Olyset) that received full WHOPES recommendation [7,21,23,30,32], but there is a paucity of field testing under normal use conditions. The present study was conducted in areas where the malaria vector *Anopheles gambiae sensu stricto* is resistant to pyrethroids. Product acceptance, perceived side effects and user perception of effectiveness were also investigated.

## 2 Methods

### 2.1 Study sites

Three villages at Ikorodu (Igbokuta, Agundun, and Lantoro) in south-western Nigeria and three others at Kainji (Monai, Dongogari, and Sabogari) in north-central Nigeria were selected for the study based on available pyrethroids resistance data [2,3,10,25]. The study area at Ikorodu is at the outskirts of Lagos. The three villages have a combined population of 500 people and similar sleeping pattern with an average of three persons per room. The area is usually flooded during the rainy season and provides mosquito breeding sites year round. Previous studies have shown that the main malaria vector in this area, *Anopheles gambiae sensu stricto*, is resistant to pyrethroids by the *kdr*-based resistance mechanism [25]. The study area at Kainji, with a population of 950 people, is located around the Kainji Dam. The three villages have similar housing structures (mainly traditional houses built with mud and a thatched roof) and sleeping pattern with an average of four persons per room. *Anopheles gambiae sensu stricto* and *An. arabiensis* are the predominant malaria vectors at the site. *Anopheles gambiae* is resistant to pyrethroids by the *kdr* and metabolic P450-based resistance mechanisms [2,25].

### 2.2 Insecticide susceptibility test and synergist study

Insecticide susceptibility tests were conducted on mosquitoes collected from the 6 villages in April 2010. Two to three day old adult *An. gambiae s.l.* reared from larval collection in each village were identified morphologically [14,15] and were exposed to permethrin (0.75%) and deltamethrin (0.05%). The 1 h insecticide exposure followed the standard WHO protocol and test kits [33]. For each village, the population of *An. gambiae s.l.* that survived the insecticide exposure was divided into two: (1) the first subset was analyzed together with dead mosquito to species level using PCR [28] and also for the presence of the *kdr* mutation using allele-specific PCR diagnostic tests designed for the West and East African *kdr* mutation [13,26]; (2) the second subset was induced to lay eggs in the insectary and F1 progeny were used for synergist and biochemical analyses as previously described [5]. In brief, PBO was tested for synergistic activity with permethrin or deltamethrin; mortality was compared between mosquitoes exposed and unexposed to PBO to determine the role of metabolic degradation as a mechanism for pyrethroid resistance. To investigate the relative role of specific metabolic pathways inhibited by this synergist, enzyme assays were carried out on live mosquitoes to measure esterase, glutathione S-transferase (GST) and cytochrome P450 monooxygenase activity [4,5]. All mosquitoes tested were identified to species level by PCR [28].

### 2.3 Mosquito nets

PermaNet® 3.0 nets were provided by Vestergaard Frandsen, Switzerland. Olyset® nets (Sumitomo, Japan) were procured from a local market in Kampala, Uganda with a production date of October 2009. Untreated polyester nets were procured from a local market in Lagos, Nigeria. Before the commencement of the study, village group meetings were held and volunteers were educated on the objectives of the study. Householders were provided with basic information on correct net usage. A survey of sleeping patterns was then carried out and used to estimate the total number of existing nets for each village. Existing nets were collected except in the control village where they were retained. Study nets were given a unique code by sewing a label onto them. A "net master list" was then developed for each village for follow-up. Net distributions were conducted on 1st May 2010. At Kainji, the village of Monai was randomly assigned to PN 3.0 and 125 nets were distributed to cover all sleeping spaces. 50 Olyset nets were distributed at Dongogari and 50 untreated polyester nets in the control village (Sabogari). At Ikorodu, the village of Igbokuta was randomly assigned to PN 3.0 with 50 nets; 50 Olyset nets were distributed at Agundun and 50 untreated polyester nets in the control village (Lantoro). In each case, nets were distributed to cover all sleeping spaces. The nets were washed on April 28th 2010 prior to the initial distribution and every three months following distribution, nets were collected and washed (July 2010, October 2010, January 2011, and April 2010). Net washing was carried out at a central location using the standard WHOPES washing guideline 33. Nets were then dried in the shade and returned to the same households.

### 2.4 Bioassays on nets

Before each washing round, the same 10 randomly-selected nets from each village were used in bioassay. Bio-efficacy was assessed first using the reference Kisumu susceptible laboratory strain of *An. gambiae s.s.* in a standard WHO

conical exposure chamber [36]. Additional bioassays were then carried out with a laboratory resistant strain of *An. gambiae s.s.* from Nigeria named "AGN." This strain was colonised in 2005 from larvae collected from "Ipokia" near Lagos in South Western Nigeria and exhibited resistance to deltamethrin (72% mortality) and permethrin (58% mortality) in WHO susceptibility tests [2]. For all net types, four side panels and the roof panel of each net were tested [36]. One cone test was conducted per side panel, with five (2–3 day old non-bloodfed) female mosquitoes used per cone for a total of 25 mosquitoes of each strain tested on each net. In all, 500 mosquitoes (250 *An. gambiae* Kisumu strain and 250 AGN strain) were used per village in each bioassay round. Mosquitoes concurrently exposed to an untreated net were used as the control.

### 2.5 Monthly entomological evaluation

Adult mosquitoes were collected in a total of 10 randomly-selected houses (one room per house) in each village once prior to net distribution, a month following distributions and thereafter once per month for 12 months. The same houses were used for the duration of the study. Mosquito densities were measured in the trial and control villages by the following methods:

#### *2.5.1 Floor sheet collection*

White floor sheets were placed in the 10 randomly selected rooms per village each evening preceding collections. In the morning, the floor sheets were carefully removed and all dead or moribund mosquitoes were collected and counted [29].

#### *2.5.2 Indoor resting collection*

A 10 minute search using a flash light was conducted in the same room used for the floor sheet collection and all mosquitoes found were collected with a suction tube.

#### *2.5.3 Window exit trap collection*

A square exit trap ($50 \times 50$ cm) with a conical aperture [29] was mounted on a window of each selected room at 18.00 h the day preceding the evaluation. The next morning, all mosquitoes in the exit trap were collected.

All collected *Anopheles* spp. were numbered by house and their status (i.e., dead/alive, blood fed/unfed) was recorded. Live mosquitoes from indoor resting catches and exit trap collections were transferred to paper cups, provided sucrose solution (10%), and were kept for 24 h in the laboratory to measure delayed mortality. Samples were identified using morphological keys [14, 15]. Those belonging to the *An. gambiae* complex were further analyzed for species using PCR [28].

### 2.6 Net tracking and household questionnaires

Two methods were used to collect data. Initially, house-to-house surveys for net usage and physical status of nets were conducted monthly. Using the net master list, all self-identified heads of households were interviewed. The questionnaires were used to determine people's perception of the benefits and/or side effects during use of nets. Where nets were no longer available, interviews were conducted once to determine reasons for halted usage. Focus group discussion were conducted after the 12th month to obtain descriptive information on volunteers' perception on the use of LLINs. Two focus group discussion guided by a member of the research team were held in each village, with one each with the households heads and individuals sleeping under the nets.

### 2.7 Data analysis

Data collected were analyzed using the STATA statistical package (STATA Corp LP, USA, version 9.1). Results from the insecticide susceptibility tests were analyzed according to the recommendations of WHO [33]. Four parameters were compared amongst PN 3.0, Olyset nets and the untreated nets: (i) percentage of house entering, (ii) mosquito densities over the period, (iii) blood feeding rate and (iv) mortality rate. For each entomological parameter, comparisons amongst treatment groups were made by ANOVA and a chi square tests with the significance level set to $p$-value $< 0.05$.

## 3 Results

### 3.1 Insecticide resistance and synergist analysis

Species composition varied by field site, with mosquitoes tested identified as a mix of 65% *Anopheles gambiae s.s.* and 35% *An. arabiensis* (Kainji) or as pure collection of *An. gambiae s.s.* (Ikorodu). Insecticide susceptibility tests carried out on wild-caught *An. gambiae s.l.* from the three villages in Kainji showed that *An. gambiae s.s.* exhibited possible or confirmed resistance to permethrin (62–75% mortality) and deltamethrin (77–81% mortality) (Table 1). *Anopheles gambiae s.s.* from the three villages at Ikorodu showed possible or confirmed resistance to permethrin (69–82% mortality) and confirmed resistance to deltamethrin (75–79% mortality) (Table 1).

The *kdr* assays detected the West African *kdr* mutation (*kdr-w*) while the East African (*kdr-e*) was not found in any specimens tested. The overall *kdr* frequency was 26–40% at Kainji without significant variation ($p > 0.05$) amongst the three villages (Table 1). In contrast, the *kdr* frequency at Ikorodu was 61–78% and was similar for the three villages ($p > 0.05$). Progeny of surviving mosquitoes from Kainji exposed to PBO followed by permethrin or deltamethrin exposure showed a significant increase in mortality (87–94%) compared to those exposed to permethrin ($p = 0.026$) or deltamethrin ($p = 0.023$) only (Table 2), indicating the likely presence of monooxygenase-mediated metabolic resistance. However, surviving mosquitoes from the three villages at

**Table 1:** Final 24 h mortality of *Anopheles gambiae s.s.* following exposure to permethrin and deltamethrin for 1 h, and the corresponding knock down resistance (*kdr*) allelic frequencies in populations from the study sites at Kainji and Ikorodu in Nigeria.

| Study area/villages | No. exposed (24 hrs % mortality) | Genotype and frequency of the *kdr* alleles (%) | | | | No. exposed (24 hrs % mortality) | Genotype and frequency of the *kdr* alleles (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.75% Permethrin | | | | | 0.05% Deltamethrin | | | | |
| **Kainji** | | RR | RS | SS | F(R) | | RR | RS | SS | F(R) |
| Monai | 156 (62.2) | 28.8 | 3.8 | 67.4 | 32.6 | 130 (76.9) | 19.2 | 12.9 | 67.9 | 26.1 |
| Dongogari | 130 (68.5) | 21.5 | 7.7 | 70.8 | 29.2 | 118 (80.5) | 17.8 | 16.9 | 65.3 | 34.7 |
| Sabogari | 104 (75.0) | 17.3 | 23.1 | 59.6 | 40.4 | 101 (77.2) | 22.8 | 15.8 | 61.4 | 38.6 |
| **Ikorodu** | | | | | | | | | | |
| Igbokuta | 130 (73.8) | 35.4 | 26.9 | 37.7 | 62.3 | 140 (75.0) | 24.3 | 36.4 | 39.3 | 60.7 |
| Agundun | 150 (69.3) | 35.3 | 42.7 | 22.0 | 78.0 | 140 (79.3) | 20.0 | 44.3 | 35.7 | 64.3 |
| Lantoro | 125 (82.4) | 17.6 | 47.2 | 35.2 | 64.8 | 120 (79.2) | 20.0 | 42.5 | 37.5 | 62.5 |

F(R): frequency of the *kdr* alleles.

**Table 2:** Bioassay results comparing 24 h mortality of pyrethroid-resistant populations of *Anopheles gambiae s.s.* from six villages in Nigeria following exposure to permethrin and deltamethrin in the presence and absence of pre-exposure to piperonyl butoxide.

| | No. exposed (24 h % mortality)[a] | | | | | |
|---|---|---|---|---|---|---|
| | 0.75% Permethrin | 4% PBO + 0.75% permethrin | $p$-value | 0.05% Deltamethrin | 4% PBO + 0.05% deltamethrin | $p$-value |
| **Kainji** | | | | | | |
| Monai | 108 (65.7) | 115 (94.8) | 0.026 | 122 (76.2) | 120 (87.5) | 0.023 |
| Dongogari | 120 (70.0) | 108 (91.7) | | 114 (78.1) | 114 (89.5) | |
| Sabogari | 110 (71.8) | 112 (88.4) | | 116 (75.0) | 118 (92.4) | |
| **Ikorodu** | | | | | | |
| Igbokuta | 112 (72.3) | 116 (76.7) | 0.062 | 120 (77.5) | 115 (79.2) | 0.072 |
| Agundun | 118 (65.2) | 112 (70.5) | | 116 (81.9) | 118 (83.1) | |
| Lantoro | 110 (79.1) | 115 (81.7) | | 118 (82.2) | 112 (83.0) | |

PBO: piperonyl butoxide.
[a]Figures in parentheses denote % mortality of the mosquitoes exposed.

Ikorodu exposed to permethrin or deltamethrin after PBO exposure did not show a significant increase in mortality when compared to those exposed to permethrin and deltamethrin only ($p > 0.05$ for both insecticide) (Table 2). Biochemical analysis revealed a significant increased level ($p = 0.022$) of monooxygenase in the resistant mosquito population from Kainji compared to either the Kisumu or Ikorodu strain (Figure 1), further suggesting monooxygenase involvement in pyrethroid metabolism in the Kainji population. The difference in the mean GST or esterase activity between the Kainji and Kisumu or Ikorodu strains was not significant ($p > 0.05$ for both GST and Esterase).

### 3.2 Bioassays

Bioassays conducted on PN 3.0 at baseline (April 2010) and during quarterly evaluations showed that all PN 3.0 produced 100% knockdown and 100% mortality against the reference Kisumu susceptible strain and also the resistant strain of *Anopheles gambiae s.s.* The Olyset nets also produced 100% knockdown and 100% mortality against the Kisumu susceptible strain during the same period, but the mean knock down rate against the resistant strain of *An. gambiae s.s.* during the period of the study at both Kainji and Ikorodu was $< 90$ (Figure 2). Similarly, mortality in the Olyset net against the resistant strain of *An. gambiae s.s.* showed greater than 90% mortality only for the first quarter, declining to78% and 72% mortality at the end of the study in Kainji and Ikorodu, respectively (Figure 3).

### 3.3 Mosquito room entry rate

Entry rates of mosquitoes per room were calculated by pooling all mosquitoes collected using floor sheets, hand catches, and window exit traps in the ten randomly selected rooms for each village (Table 3). Before net distributions, there was no significant difference in entry rates for the three villages at either Kainji and Ikorodu ($p > 0.05$ at both). The impact of the introduction of PN 3.0 and Olyset nets on the entry rate was noticeable with a significant decrease in entry rates observed for villages with LLINs while an increase was observed for those with untreated nets at both Kainji

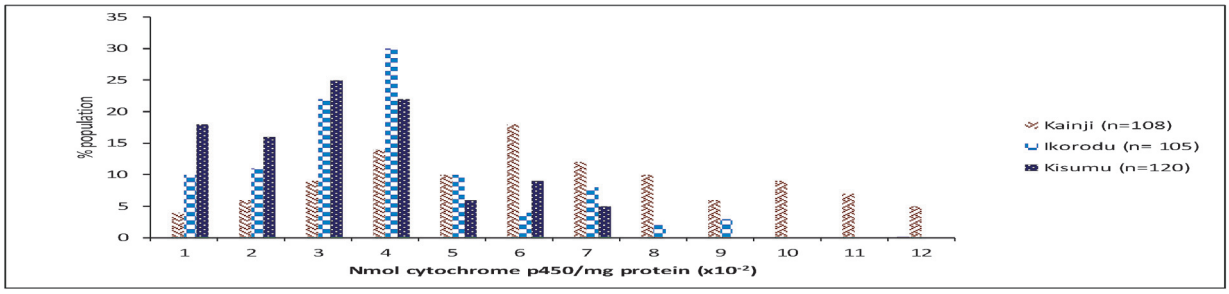


**Figure 1:** Frequency distribution of monooxygenase level detected in pyrethroid-resistant *An. gambiae s.s.* populations from Kainji and Ikorodu and in the susceptible *An. gambiae s.s.* Kisumu strain via biochemical assays.

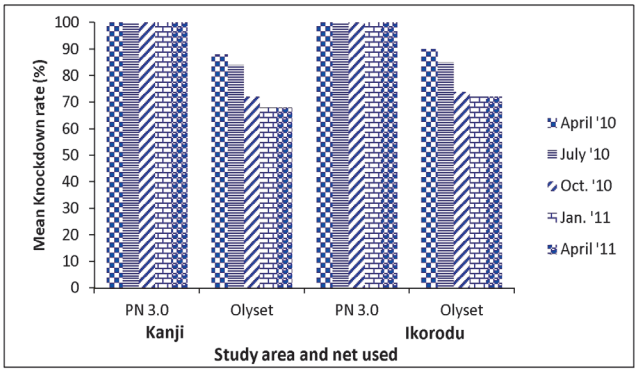


**Figure 2:** Mean knock down rates (KD) of pyrethroid-resistant laboratory strain of *Anopheles gambiae s.s.* (AGN) based on 3-minutes exposure to PermaNet 3.0 and Olyset nets in WHO cone bioassays prior to (April 2010) and following field usage for 3, 6, 9 and 12-months.

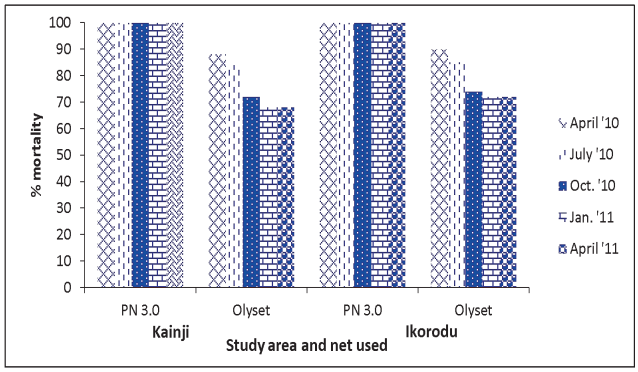


**Figure 3:** Bio-efficacy of PermaNet 3.0 and Olyset nets prior to and following field usage for 3, 6, 9 and 12-months based on % mortality in 3-minutes exposure in WHO cone bioassays using a pyrethroid-resistant laboratory strain of *Anopheles gambiae s.s.* (AGN).

and Ikorodu. There was no difference in mean monthly entry rates of *An. gambiae s.l.* in villages with PN 3.0 compared to Olyset at either Kainji and Ikorodu ($p > 0.05$ at both).

### 3.4 Impact of intervention on *Anopheles* densities

Before intervention in April 2010, there was no significant difference in the room density for the three villages at either Kainji and Ikorodu ($p > 0.05$ at both) showing that all three villages at each location were similar in relation to *Anopheles* productivity (Figures 4 and 5). However, following LLIN distribution in May 2010, there was a sharp decline ($> 50\%$) in the density *An. gambiae* in the PN 3.0 village in Kainji compared to the untreated net, and this remained significant for 12 months ($p = 0.006$). The impact of the introduction of the PN 3.0 was also noticeable compared to the untreated net at Ikorodu (Figure 5). A similar trend was observed with the introduction of the Olyset net at Kainji (Figure 4) and Ikorodu (Figure 5) when compared to the villages with the untreated nets. However, there was no significant difference in the density of *An. gambiae s.l.* in PN 3.0 and the village with the Olyset net at Ikorodu ($p=0.17$) or Kainji ($p=0.56$).

### 3.5 Mosquito mortality

Total mosquito mortality in each village was recorded as a sum of the immediate and delayed mortality divided by the total number of mosquitoes collected. Similarly low mortalities were observed for mosquitoes collected at the three villages in Kainji ($< 1\%$) and Ikorodu ($< 2\%$) prior to net distribution. Following net distribution, virtually all *An. arabiensis* collected in either PN 3.0 or Olyset net villages at Kainji were found dead (98.6% mortality). Overall, mortality of *An. gambiae s.s.* varied between villages at both Kainji and Ikorodu (Figure 6). In villages with PN 3.0, mortality was $> 65\%$, the overall mortality in villages using Olyset nets was $< 45\%$ while in the villages with untreated nets mortality was $< 3\%$.

### 3.6 Mosquito feeding success

Prior to net distribution, there was no significant difference in the proportion of *An. gambiae s.l.* that had bloodfed at the three villages at either Kainji (32–43%) or Ikorodu (37–46%) ($p > 0.05$ for both). Following net distribution, the proportion of blood-fed *An. gambiae s.s.* varied significantly

**Table 3:** Number of *Anopheles* caught monthly (entering rate) by indoor resting catch (by hand), window exit trap and floor sheet collection in 10 randomly selected rooms before and after distribution of PN 3.0, Olyset or untreated nets at three villages each in Kainji and Ikorodu in Nigeria from April 2010 to April 2011.

| Location | Treatment | Total, before net distribution ($n = 1$) | | | | Monthly mean (±SD), after net distribution ($n = 12$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Indoor resting catch | Exit trap | Floor sheet collection | Total* | Indoor resting catch | Exit trap | Floor sheet collection | Total* |
| **Kainji** | | | | | | | | | |
| Monai | PermaNet 3.0 | 18 | 10 | 0 | 28 | 3.2 (±1.11) | 1.2 (±0.79) | 10.8 (±0.18) | 15.3 |
| Dongogari | Olyset | 14 | 11 | 0 | 25 | 5.1 (±1.06) | 6.5 (±0.02) | 11.7 (±1.69) | 23.3 |
| Sabogari | Untreated net (control) | 16 | 11 | 0 | 27 | 28.9 (±5.95) | 8.3 (±0.12) | 1.1 (±0.51) | 38.3 |
| **Ikorodu** | | | | | | | | | |
| Igbokuta | PermaNet 3.0 | 23 | 22 | 1 | 46 | 1.6 (±0.95) | 8.2 (±0.11) | 18.7 (±3.67) | 28.5 |
| Agundun | Olyset | 20 | 23 | 0 | 43 | 7.6 (±2.06) | 11.4 (±0.16) | 12.2 (±2.01) | 31.2 |
| Lantoro | Untreated net (control) | 20 | 25 | 0 | 45 | 30.1 (±4.83) | 15.9 (±0.32) | 1.2 (±0.69) | 47.2 |

Mosquito collections were made in 10 rooms once per month in villages with PN 3.0, Olyset and untreated nets before and after nets distribution.
*Total = Indoor resting catch + exit trap + floor sheet collection.

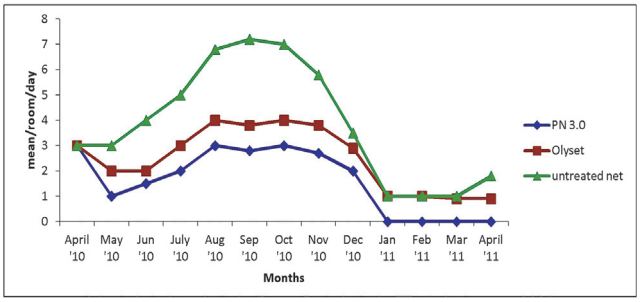


**Figure 4:** Mean number of *Anopheles gambiae s.s.* per room (pooled from monthly indoor resting, exit trap and floor sheet collections) at 10 houses with PN 3.0, Olyset and untreated nets at Kainji during the pre-intervention (April 2010) and intervention period (May 2010–April 2011).

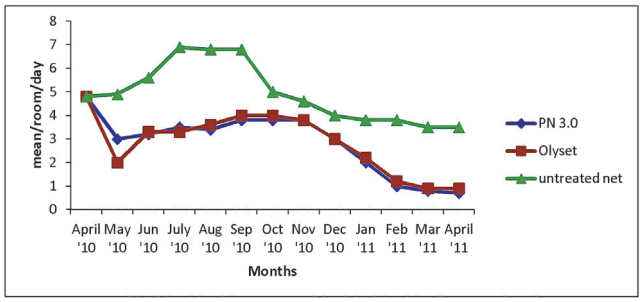


**Figure 5:** Mean number of *Anopheles gambiae s.s.* per room (pooled from monthly indoor resting, exit trap and floor sheet collections) at 10 houses with PN 3.0, Olyset and untreated nets at Ikorodu during the pre-intervention (April 2010) and intervention period (May2010–April 2011).

between villages with PN 3.0, Olyset nets or untreated nets at both Kainji ($p = 0.021$) and Ikorodu ($p = 0.032$) (Figure 7). At Kainji, there were no blood fed *An. arabiensis*; all bloodfed mosquitoes were identified as *An. gambiae s.s.* by PCR. The overall proportion of bloodfed females was $<$ 3.0% for villages with PN 3.0, three times higher (10–13%) in villages with the Olyset nets, and twelve times higher in villages with untreated nets (37–39%). Overall, the use of PN 3.0 resulted in 8–11% and 34–37% reductions in blood feeding relative to the Olyset nets and the untreated controls, respectively.

### 3.7 Net usage and households perceived effectiveness

Data were analysed separately for each village and pooled when no significant difference was found between villages with the same net at Kainji and Ikorodu. At the commencement of the study, all households in the six villages indicated their willingness to participate and gave consent. However, two months after the study began, 81% of the 100 people with the untreated nets (control villages) said it provided no protection against mosquitoes bites and only 40% of them had the nets by the end of the study. Almost all LLINs were still in use at the end of the study (99% for both PN 3.0 villages and 99% for both Olyset villages). Although a slightly higher proportion of people sleeping under PN 3.0 reported a reduction in the number of mosquito bites (95%) compared to the Olyset nets (92%), the difference was not statistically significant ($p > 0.05$). Sneezing was the main side effect reported by 18.5% of the 173 people that slept under PN 3.0. The proportion of people that reported sneezing for PN 3.0 was significantly lower than for Olyset net ($p = 0.040$). In addition, dizziness (18%) and skin irritation (12%) were also reported as main side effect among the 99 people that slept under Olyset

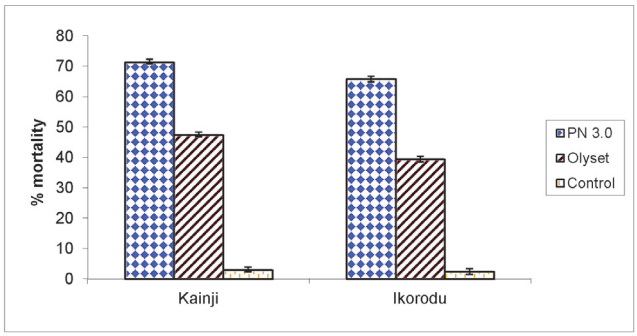


**Figure 6:** Mean mortality rate (%) based on immediate and delayed mortality of wild-caught female *Anopheles gambiae s.s.* (pooled from monthly indoor resting, exit trap and floor sheet collections) from 10 houses each in villages with PermaNet 3.0, Olyset nets or untreated nets at Kianji and Ikorodu from May 2010 to April 2011.

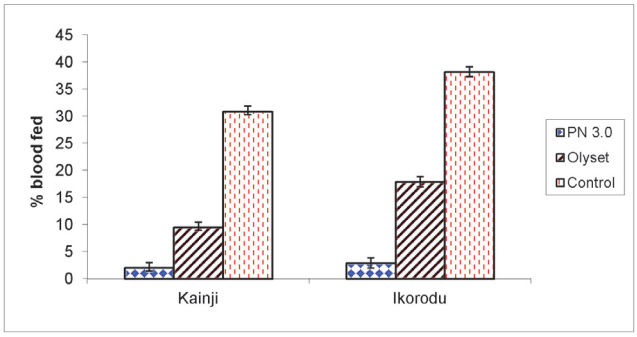


**Figure 7:** Mean proportion bloodfeeding (%) of wild-caught female *Anopheles gambiae s.s.* (pooled from monthly indoor resting, exit trap and floor sheet collections) from 10 houses each in villages with PermaNet 3.0, Olyset nets or untreated nets at Kainji and Ikorodu from May 2010 to April 2011.

(Table 4). Approximately 25% also complained about the smell of the Olyset nets. A significantly higher proportion of people using PN 3.0 (89.6%) versus Olyset (69.7%) indicated that the intervention was beneficial ($p = 0.043$). The descriptive data from the focus group discussion (data not shown) indicated this was because it also reduced the number of mosquitoes, bed bugs and cockroaches during the study. Thus, they indicated a preference for PN 3.0 over nets previously distributed by the Local Authority.

## 4 Discussion

This study evaluated the new LLIN, PermaNet 3.0, which consists of a combination of deltamethrin and the synergist PBO to improve bioefficacy against pyrethroid-resistant malaria vectors. A number of experimental hut studies in Africa have evaluated PN 3.0 in comparison to PN 2.0 or Olyset nets with variable reports on the efficacy

**Table 4:** Net users' perceptions of side effects and benefits of PermaNet 3.0 and Olyset nets.

| | Proportion (%) of net owners[†] | |
|---|---|---|
| | PN 3.0 $n = 173$[‡] | Olyset $n = 99$[‡] |
| Unpleasant smell | 3 (1.7) | 25 (25.2) |
| Dizziness | 2 (1.1) | 18 (18.2) |
| Running nose | 5 (2.9) | 8 (8.1) |
| Fever | 2 (1.1) | 2 (2.0) |
| Headache | 3 (1.7) | 1 (1.0) |
| Sore eyes | 0 | 5 (5.0) |
| Skin irritation | 8 (4.6) | 12 (12.1) |
| Coughing | 0 | 0 |
| Vomiting | 0 | 0 |
| Sneezing | 32 (18.5) | 28 (28.3) |
| Sleeplessness | 3 (1.7) | 1 (1.0) |
| Was the net beneficial? | 155 (89.6) | 69 (69.7) |
| Did the use of the net reduced mosquito bites | 164 (94.8) | 91 (91.9) |
| Would you continue sleeping under the net? | 167 (96.5) | 70 (70.7) |

[†]Data were analysed separately for each village and pooled when no significant difference was found between villages with the same type of net.
[‡]Two PN 3.0 and one Olyset net user did not have the nets after 6 months and were excluded from the final analysis.

of PN 3.0 against pyrethroid resistant *Anopheles* and *Culex* species depending on the main vectors and levels and types of resistance mechanisms [7,21,23,30]. Based on modelling of PN 3.0 data from the experimental hut studies in Vietnam, Cameroon, Burkina Faso, and Benin, observed increases in bioefficacy against *Anopheles* vectors (relative to a deltamethrin-only LLIN) were associated with marked decreases in the simulated intensity of malaria transmission [20]. The results of the present study are based on comparative data collected from six different villages using PN 3.0, Olyset nets and untreated nets over a one-year period in areas where the main malaria vector *An. gambiae s.s.* is resistant to permethrin and deltamethrin. The resistance status of the malaria vector to permethrin and deltamethrin as ascertained by WHO susceptibility test remained unchanged and showed comparable results with previous reports from the same area [2,25]. Molecular, synergist, and biochemical analysis provided supporting evidence of *kdr* and metabolic-based resistance in the villages at Kainji. This presents further evidence of multiple pyrethroid resistance mechanisms in *An. gambiae s.s.* reported in our earlier study in Nigeria [2]. Similar findings have been reported in neighboring countries [10,17].

The bioassay data on nets showed that field-used and washed PN 3.0 maintained 100% mortality against a resistant laboratory strain of *An. gambiae s.s.* during the 12 months of the study. In contrast, the Olyset nets showed reduced efficacy over the same period. This is

consistent with results from an earlier experimental hut study comparing PN 3.0 and Olyset nets in Nigeria, in which bioefficacy against resistant mosquitoes was maintained following 20 standard washes for PN 3.0 but not for Olyset [Awolola unpublished].

The results of the monthly mosquito collections showed that although there was a reduction in the entry rate and density of *An. gambiae* following LLIN distribution, there was no difference in these parameters between PN 3.0 and Olyset villages at either Kainji or Ikorodu. However, PN 3.0 caused more than 65% mortality in all *Anopheles gambiae s.s.* entering the houses and provided better protection compared to the Olyset net. This indicated enhanced comparative efficacy of PN 3.0 in areas with *kdr* resistance and kdr plus metabolic resistance in *An. gambiae s.s.* As evident in the synergist analysis of the resistant mosquito populations from Ikorodu, it could be argued that if the rationale behind combining PBO with a pyrethroid is to increase the efficacy of deltamethrin through the synergist's action as a metabolic enzyme inhibitor, then the efficacy of the product in term of mosquito mortality should be less pronounced in an area such as Ikorodu where metabolic resistance was absent. A possible explanation for the improved efficacy in the area with only *kdr* resistance may be connected to the higher deltamethrin content in PN 3.0 in relation to similar nets by the same manufacturer, although this cannot be ascertained as no side-by-side comparison was conducted. Even so, the observed variation in mosquito mortality and feeding success rate between villages with PN 3.0 and Olyset suggests that PN 3.0 may be useful in areas of pyrethroid-resistance.

PN 3.0 was also well accepted by the users. Aside from sneezing, none of the people that used the nets complained of major side effect as a result of sleeping under the nets. Most preferred the nets to those previously distributed in the villages. Among the advantages given were that the use of PN 3.0 reduced mosquito bites in the rooms and that the intervention was beneficial as it killed more bed bugs, cockroaches and spiders compared to nets previously distributed. Further studies should explore this potential advantage, as it may increase user acceptability.

## 5 Conclusion

We demonstrated that the use of PN 3.0 resulted in substantial reductions in blood feeding rates, and increased the mortality of wild populations of pyrethroid-resistant *An. gambiae s.s.* in two areas of Nigeria. It is recommended that this tool be considered for strategic implementation particularly in areas where pyrethroid resistance has been identified or LLINs have shown reduced efficacy.

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