



An experimental hut evaluation of PermaNet® 3.0, a deltamethrin–piperonyl butoxide combination net, against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes in southern Benin

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ABSTRACT

PermaNet 3.0 is a long-lasting combination net with deltamethrin present on the sides and a mixture of deltamethrin and piperonyl butoxide (PBO), an oxidase synergist, on the top panel. An experimental hut trial comparing unwashed and 20 times washed PermaNet 3.0 and PermaNet 2.0, Olyset Net and a conventional deltamethrin-treated net washed three times was conducted in southern Benin. *Anopheles gambiae* and *Culex quinquefasciatus* from this area are highly resistant to pyrethroids through *kdr* and cytochrome P450 mechanisms. The unwashed PermaNet 3.0 killed slightly more *A. gambiae* (52%) than the unwashed PermaNet 2.0 (44%) ($P=0.036$), indicating only partial synergism of resistance. After washing there was significant loss of activity to a similar level, with PermaNet 3.0 killing 31%, PermaNet 2.0 killing 29% and the conventional net killing 26%. Blood-feeding rates were partially inhibited for unwashed PermaNet 3.0 and Olyset Net (27% inhibition). Personal protection against *A. gambiae* derived from PermaNet 3.0 was similar to that from PermaNet 2.0 before washing (50% vs. 47%), and after 20 washes it decreased to 30%. Against *C. quinquefasciatus*, no treatment killed >24% entering the huts. The synergism from unwashed PermaNet 3.0 was lower than expected, probably due to an unidentified resistance mechanism unaffected by PBO.

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1. Introduction

In areas of pyrethroid susceptibility, insecticide-treated nets (ITN) based on pyrethroids confer protection against bites of malaria vectors even when they have holes in

them.^{1,2} Such ITNs also reduce malaria morbidity among users in locations where certain types of pyrethroid resistance have developed, e.g. in Kenya and Côte d'Ivoire.^{3,4} A recent hut trial to examine the efficacy of holed ITNs at a site in southern Benin indicated an outright failure to kill or reduce blood-feeding by the malaria vector *Anopheles gambiae*.⁵ *Anopheles gambiae* in southern Benin is of the M molecular form and is highly resistant owing to knockdown resistance (*kdr*) site insensitivity and elevated oxidase and esterase metabolic mechanisms.⁶

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¹ <http://www.pamverc.org>.

In West Africa, *Culex quinquefasciatus* has always shown less responsiveness to pyrethroid-treated nets than *A. gambiae* owing to multiple resistance, including *kdr* and mixed-function oxidases, more generally referred to as cytochrome P450s or microsomal oxidases.⁷ Consequently, long-lasting insecticidal nets (LLIN) such as PermaNet 2.0 or Olyset Net do not kill this species readily and blood-feeding inhibition is not reduced⁸ to the level normally observed with *A. gambiae*.^{2,9}

PermaNet 3.0 is a new generation of long-lasting combination net developed by Vestergaard Frandsen that incorporates the pyrethroid deltamethrin and the synergist piperonyl butoxide (PBO) on the roof of the net. PBO is generally used in commercial aerosols for potentiating pyrethroid activity against P450s and esterases in flying or domestic insect pests.¹⁰ Combination nets may have utility against pyrethroid-resistant mosquitoes, particularly those whose resistance is based fully or in part on metabolic mechanisms such as non-specific esterases (NSE) and/or cytochrome P450s.

A combination net incorporating PBO is a potential solution to the control of pyrethroid-resistant mosquitoes in Benin as it may synergise against NSE and/or cytochrome P450-based resistance mechanisms. Previously, trials of pyrethroid-treated nets showed high mosquito survival and uninhibited blood-feeding rates through holed netting.⁵ We therefore evaluated PermaNet 3.0 (before and after washing) in experimental huts in southern Benin during a season where insecticide-resistant *A. gambiae* and *C. quinquefasciatus* occur concurrently. PermaNet 2.0, an earlier-generation LLIN containing deltamethrin as a single active ingredient, the LLIN Olyset Net containing permethrin as the active ingredient, and a net conventionally treated with deltamethrin washed to just before exhaustion were tested alongside PermaNet 3.0.

2. Methods and materials

2.1. Long-lasting insecticidal nets

PermaNet 3.0 (Vestergaard Frandsen SA, Aarhus, Denmark) is a LLIN consisting of a top panel made of monofilament polyethylene (100 denier) fabric incorporating deltamethrin at 4 g/kg (121 mg/m²) and PBO at 25 g/kg (759 mg/m²) plus side panels made of multifilament polyester (75 denier) fabric with a strengthened border coated with a wash-resistant formulation of deltamethrin with a target dose of 2.8 g/kg (85 mg/m²).¹¹

PermaNet 2.0 (Vestergaard Frandsen SA) is a LLIN made of multifilament polyester (75–100 denier) fabric, factory-coated with a wash-resistant formulation of deltamethrin at a target dose of 1.8 g/kg (55 mg/m²).¹¹

Olyset Net (Sumitomo Chemicals, Osaka, Japan) is a LLIN made of knitted polyethylene thread with permethrin at 20 g/kg (2% w/w) incorporated during fibre extrusion.¹²

The conventionally treated net was a white polyester multifilament net (SiamDutch Mosquito Netting Co., Bangkok, Thailand) treated by hand on site in an aqueous solution of deltamethrin formulation (K-Othrine SC; Bayer, Leverkusen, Germany) to give an application rate of 25 mg/m².

2.2. Washing of the nets

The conventionally treated deltamethrin ITN was washed to just before the point of insecticide exhaustion (cut-off) as defined by the WHO.¹³ This treatment served as a positive control against which to judge LLIN performance. The point of exhaustion was determined by washing the ITN according to the Phase II washing protocol and performing WHO cone bioassays after each wash to establish the point at which the net reaches the cut-off criteria of $\geq 80\%$ mortality and/or $\geq 95\%$ knockdown.¹³ The standardised washing protocol required nets to be washed in 10 l of 2 g/l soap solution ('Savon de Marseille') using manual agitation for 6 min during a 10-min washing/soaking period. Washing was done every 2 days and nets were rinsed twice and dried between washes.

2.3. Mosquito strains

Mosquitoes used for the laboratory experiment in tunnel tests were: (i) *A. gambiae* Kisumu, a pyrethroid-susceptible reference strain originally from Kenya; (ii) *A. gambiae* VKPER, a pyrethroid-resistant strain, fixed for the *kdr* gene, originally from the Kou Valley in Burkina Faso; and (iii) *C. quinquefasciatus* from Cotonou City, Benin, containing multiple resistance mechanisms to pyrethroids, organophosphates and carbamates. Underlying mechanisms include *kdr*, elevated esterases and oxidases.⁶

2.4. Tunnel tests

Tunnel tests were carried out on samples of PermaNet 3.0 netting cut from the roof and lower sides of the net after 0, 10 and 20 standardised washes. The tunnel test is described by the WHO.¹⁴ Briefly, a 60-cm long glass tunnel is divided into two chambers by a transverse netting insert, fitted onto a frame that slots across the tunnel. Nine 1-cm diameter holes cut into the netting allow passage of mosquitoes. Unfed female mosquitoes are released into one chamber at dusk and left overnight in the dark to pass through the holed netting and feed on a guinea pig restrained on the other side of the chamber. The following morning the numbers of mosquitoes found alive or dead and fed or unfed in each compartment are recorded. Delayed mortality is recorded after 24 h.

2.5. Experimental hut trial

The evaluation was carried out in experimental huts situated in Akron, a village on the periphery of Porto Novo, the administrative capital of Benin. The site is a horticultural area covering approximately 20 ha. Pest control in this area included use of carbamates, pyrethroids and the organophosphate malathion.¹⁵ The site and surrounding marshes provide a long breeding season for mosquitoes. The local population of *A. gambiae* is comprised entirely of the M taxon and is resistant to pyrethroids and DDT, with *kdr* at high frequency and metabolic resistance also present.⁶ The nuisance mosquito *C. quinquefasciatus* is present year round and is resistant to pyrethroid, carbamate and organophosphate insecticides.⁶

Seven experimental huts at the site were selected for the present study. The design of the huts is similar to those used in Côte d'Ivoire.^{2,8} Each hut is made from concrete bricks with a roof of corrugated iron and a ceiling of thick polyethylene sheeting covered with palm thatch on the interior surface. Each hut stands on a concrete base surrounded by a water-filled moat to exclude ants that would otherwise carry off dead mosquitoes. Entry of mosquitoes occurs via four slits, 1 cm wide, located on three sides of the hut. Mosquitoes are able to egress into a veranda trap projecting from the fourth side.

2.6. Study design

The following seven treatment arms were compared: (i) PermaNet 3.0 unwashed; (ii) PermaNet 3.0 washed 20 times; (iii) PermaNet 2.0 unwashed; (iv) PermaNet 2.0 washed 20 times; (v) polyester net, conventionally treated with deltamethrin at 25 mg/m², washed until just before exhaustion (three times); (vi) Olyset Net unwashed; and (vii) polyester net untreated (control).

The trial took place between 22 October 2008 and 6 January 2009. Treatment arms were rotated once through the huts according to a Latin Square design. A treatment was assigned at random to a particular hut for 6 nights of observation before being rotated to the next hut. Seven sleepers were rotated through the seven huts on consecutive nights except Sundays and holidays. Data were collected for 56 nights. Three nets were available per treatment arm and each net was tested twice in each hut during the 6-night rotation. At the end of each rotation the huts were cleaned and aired for 1 day and the treatments moved to the next hut.

Each net was deliberately holed with six 4 cm × 4 cm holes to simulate a worn net. Mosquitoes were collected from the floor, walls, exit traps and inside the nets and were scored as dead or alive and as fed or unfed. Live mosquitoes were held for 24 h to determine delayed mortality.

The primary outcomes were: (i) deterrence, i.e. reduction in hut entry relative to the control huts fitted with untreated nets; (ii) insecticide-induced exiting, i.e. the proportion of mosquitoes found in exit traps relative to control huts; (iii) blood-feeding inhibition, i.e. the proportional reduction in blood-feeding relative to untreated nets; and (iv) mortality, i.e. the proportion of mosquitoes killed (immediate plus delayed).

Assuming a treatment deters a considerable number of mosquitoes from entering the hut, then the values given by proportion blood-feeding or proportion killed in the treatment hut may, respectively, underestimate the full personal protective effect and overestimate the full insecticidal efficacy of the treatment.

The personal protective effect of a treatment is best described by the reduction in the number of blood-fed mosquitoes in the treatment hut relative to the number blood-fed in the control hut:

$$\% \text{Personal protection} = 100(B_u - B_t)/B_u$$

where B_u is the total number of blood-fed mosquitoes in the untreated control hut and B_t is the total number

of blood-fed mosquitoes in the huts with insecticide treatment.

The overall insecticidal effect of a treatment takes into account the possibility that many mosquitoes might be deterred from entering the hut through repellency and hence not killed by the treatment. A mass killing effect is desirable to reduce transmission. The overall insecticidal effect of a treatment relative to the number of mosquitoes that would ordinarily enter an untreated hut can be estimated as a percentage using the following formula:

$$\text{Overall insecticidal effect (\%)} = 100(K_t - K_u)/(T_u - K_u)$$

where K_t is the number killed in the treated hut, K_u is the number dying in the untreated control hut and T_u is the total number collected from the control hut.

Where control mortality is >5% the induced treatment mortality was corrected for control.

2.7. Chemical analysis of nets used in the experimental hut trial

Chemical analysis was conducted on samples of PermaNet 3.0, PermaNet 2.0 and the ITN cut from the four side panels and the one top panel pre washing (25 samples), post washing (25 samples) and post hut trial (25 samples). From each sample, pieces were taken to determine density or were homogenised and an analytical portion of 300 mg was taken for determination of deltamethrin, deltamethrin α R-isomer and/or PBO.

Deltamethrin, deltamethrin α R-isomer and PBO were extracted by heating under reflux for 60 min with xylene and were determined by gas chromatography with flame ionisation detection (GC-FID) using the internal standard calibration¹⁶.

Before the analysis of net samples, the analytical method was successfully validated for its specificity, linearity of detector response, accuracy, repeatability and reproducibility.

2.8. Data analysis

Analysis of experimental hut data was carried out using logistic regression for proportional data (proportions blood-feeding, dying and exiting each night) and negative binomial regression for numeric data [numbers collected (deterrence), dying (overall insecticidal effect) and feeding (personal protection) each night] after adjusting for the effects of individual huts and sleepers. Data were analysed using Stata 9 software (StataCorp LP, College Station, TX, USA).

Proportional data from laboratory tunnel tests were normalised using arcsine square root transformation and data were analysed using ANOVA.

3. Results

3.1. Experimental hut trials

Table 1 shows the experimental hut trial results of treatments for *A. gambiae* and *C. quinquefasciatus*.

Table 1
Summary of experimental hut trial results for *Anopheles gambiae* and *Culex quinquefasciatus* at Akron field station^a

Net treatment	Total collected	% caught in veranda (95% CI)	% blood-fed (95% CI)	% feeding inhibition (95% CI)	% personal protection (no. fed)	% mortality (95% CI)	% control-corrected mortality (95% CI)	% insecticidal effect (no. dead)
<i>Anopheles gambiae</i>								
Control	185 ^a	22 (16–28) ^a	65 (58–72) ^a	–	–	16 (10–21) ^a	–	–
PermaNet 3.0, 0 washes	128 ^b	59 (50–67) ^b	48 (39–56) ^b	27 (24–33)	50 (61) ^a	52 (44–61) ^b	44 (35–52)	24 (67) ^a
PermaNet 3.0, 20 washes	155 ^a	50 (42–58) ^b	54 (47–63) ^a	16 (10–21)	30 (15) ^b	31 (24–38) ^c	18 (12–24)	12 (48) ^b
PermaNet 2.0, 0 washes	114 ^b	63 (54–72) ^b	56 (47–65) ^a	14 (9–20)	47 (64) ^a	44 (35–53) ^d	33 (25–42)	13 (50) ^b
PermaNet 2.0, 20 washes	174 ^a	51 (43–58) ^b	59 (50–67) ^a	10 (7–16)	33 (81) ^b	29 (22–36) ^c	16 (11–22)	14 (51) ^b
Olyset Net, 0 washes	197 ^a	63 (56–69) ^b	48 (41–55) ^b	27 (21–32)	22 (94) ^c	27 (21–34) ^c	14 (9–19)	16 (54) ^b
Deltamethrin ITN, 3 washes	170 ^a	41 (33–49) ^c	62 (54–69) ^a	6 (3–10)	13 (105) ^d	26 (20–33) ^c	13 (8–18)	10 (45) ^b
<i>Culex quinquefasciatus</i>								
Control	628 ^a	35 (31–39) ^a	32 (28–35) ^a	–	–	13 (11–16) ^a	–	–
PermaNet 3.0, 0 washes	653 ^a	73 (69–76) ^b	14 (11–16) ^b	57 (54–60)	55 (89) ^a	20 (17–22) ^b	7 (5–9)	8 (129) ^a
PermaNet 3.0, 20 washes	671 ^a	63 (59–67) ^b	24 (21–27) ^c	23 (19–27)	18 (162) ^b	19 (16–22) ^b	6 (4–8)	7 (125) ^a
PermaNet 2.0, 0 washes	596 ^a	67 (63–71) ^b	23 (20–27) ^c	26 (23–29)	54 (91) ^a	24 (21–28) ^b	12 (10–15)	11 (145) ^a
PermaNet 2.0, 20 washes	725 ^a	47 (43–50) ^c	27 (21–30) ^c	15 (11–19)	21 (156) ^b	22 (19–25) ^b	10 (9–12)	14 (161) ^a
Olyset Net, 0 washes	647 ^a	69 (65–72) ^b	9 (7–11) ^{b,d}	71 (69–74)	70 (59) ^c	20 (17–24) ^b	8 (6–10)	8 (132) ^a
Deltamethrin ITN, 3 washes	671 ^a	42 (38–46) ^a	29 (25–32) ^a	9 (6–12)	2 (193) ^d	21 (18–24) ^b	8 (6–10)	10 (139) ^a

ITN: insecticide-treated net.

^a For each species, values in columns not sharing the same superscript letter are significantly different at the 5% level.

3.1.1. Untreated control nets

Over the 56 nights, 185 *A. gambiae* females (3.3 per night) and 628 *C. quinquefasciatus* (11.2 per night) were collected in the huts containing untreated nets; 65% of *A. gambiae* (121/185) and 32% of *C. quinquefasciatus* (198/628) were blood-fed. This corresponds to 2.2 anopheline and 3.5 culicine bites per person per night. Exiting rates into the verandas were 22% for *A. gambiae* and 35% for *C. quinquefasciatus*. Natural mortality remained relatively low at approximately 14% for both species (Table 1).

3.1.2. Treated nets

3.1.2.1. *Anopheles gambiae*. On average, 2.8 *A. gambiae* were collected per day from treated huts. The only significant reduction in entry rate was observed with the unwashed PermaNet 3.0 (31% reduction) and unwashed PermaNet 2.0 (38% reduction) (Table 1). All ITNs except the ITN washed to cut-off stimulated higher exiting (range 51–63%) compared with the untreated net (22%) ($P < 0.001$), but there were no significant differences between treatments.

No treatment inhibited blood-feeding by much (Table 1). Blood-feeding rates were similar for the unwashed PermaNet 3.0 and Olyset Net but the level of inhibition, though significant, was only 27% compared with the untreated net ($P < 0.001$). Feeding inhibition with PermaNet 3.0 was lost after washing and was not evident at all with PermaNet 2.0 either before or after washing.

All treatments induced higher mortality among *A. gambiae* than the untreated net ($P < 0.05$). Across all treatment types, the insecticidal impact against *A. gambiae* was limited (average mortality ranged from 26% to 52%). The unwashed PermaNet 3.0 killed significantly more *A. gambiae* (52%) than the unwashed PermaNet 2.0 (44%) ($P = 0.036$), indicating a partial synergism of the pyrethroid resistance occurring in the *A. gambiae* Akron population. After 20 washes, however, there was a significant loss of insecticidal activity from PermaNet 3.0 (31% mortality), which was similar to that of PermaNet 2.0 after 20 washes (29% mortality) and to deltamethrin ITN washed to cut-off (27% mortality) ($P < 0.001$). The unwashed PermaNet 3.0 and unwashed PermaNet 2.0 killed significantly more *A. gambiae* than the unwashed Olyset Net ($P < 0.001$).

The level of personal protection against *A. gambiae* biting from PermaNet 3.0 (50%) was similar to that from PermaNet 2.0 (47%) but protection fell significantly after 20 washes to 30% for PermaNet 3.0 and 33% for PermaNet 2.0 ($P < 0.001$).

The Olyset Net and the deltamethrin ITN washed to cut-off provided lower personal protection (22% and 13% respectively) than the PermaNet treatments. The overall insecticidal effect was rather low across all treatments, but was slightly higher for the unwashed PermaNet 3.0 (24%) (Table 1).

3.1.2.2. *Culex quinquefasciatus*. The average number of *C. quinquefasciatus* collected per day from treated huts was 11.8. There was no evidence of deterrence from any of the treatments ($P > 0.05$) (Table 1). All treatments stimulated an increased exiting of mosquitoes into the veranda, with the possible exception of the ITN washed to

cut-off which did not differ significantly from the control ($P = 0.12$).

Inhibition of blood-feeding was apparent for all treatments except for the ITN washed to cut-off. The percentage blood-fed was significantly lower for unwashed PermaNet 3.0 (14%) than for unwashed PermaNet 2.0 (23%) ($P < 0.01$). The unwashed Olyset Net showed the highest rates of blood-feeding inhibition.

Mortality rates across treatments were consistently lower among *C. quinquefasciatus* than among *A. gambiae*. None of the treatments killed >24% of *C. quinquefasciatus* that entered the huts.

The trend in personal protection against *C. quinquefasciatus* mirrored that of *A. gambiae*, with the exception of Olyset Net which gave the highest protection of all (70%) against this species. The overall insecticidal effect never exceeded 14% for any treatment (Table 1).

No complaint or adverse effect from any treatment was reported by the sleepers during the course of the study.

3.2. Chemical analysis of nets used in the experimental hut trials

The results of chemical analysis are presented in Figure 1.

3.2.1. PermaNet 2.0

The mean loading dose of deltamethrin on PermaNet 2.0 was observed to be in the range 64–66 mg/m² (sides–top). This fell to 8.8–14.0 mg/m² (sides–top) after 20 washes and remained at that level post trial. Deltamethrin retention after 20 washes was 13% on the sides and 19% on the roof (Figure 1).

3.2.2. PermaNet 3.0

The mean loading dosage of deltamethrin was in the range 94–98 mg/m² on the sides (two nets tested) and 119–129 mg/m² on the roof of the net (two nets tested).

After 20 washes the amount on the sides had fallen to 17 mg/m² and post trial the amount had fallen further to 8.4 mg/m². By contrast, deltamethrin on the roof showed only a slight decrease after 20 washes from 119 mg/m² to 101 mg/m². This is due to the deltamethrin on the roof being incorporated into the polyethylene and thus less tractable to washing.

PBO on the roof of the net was recorded at 731 mg/m² and 856 mg/m² before washing (two nets tested), at 435 mg/m² after washing and at 258 mg/m² by the end of the trial.

Deltamethrin retention after 20 washes was 18% on the sides and 84% on the roof (Figure 1).

3.3. Tunnel tests on PermaNet 3.0 netting washed 0, 10 and 20 times

The effect of PermaNet 3.0 netting against *A. gambiae* Kisumu and VKPER strains and against *C. quinquefasciatus* Kpankpan strain are shown in Figure 2. Passage of mosquitoes through PermaNet 3.0 netting was highly inhibited for each species and strain ($P < 0.001$). Passage inhibition was still apparent after 20 washes whether from

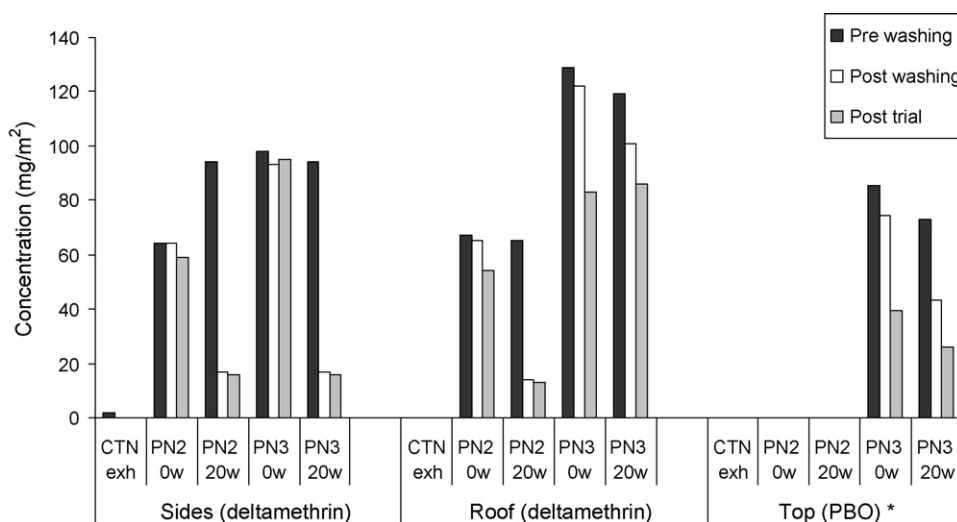


Figure 1. Chemical analysis of deltamethrin and piperonyl butoxide (PBO) content of PermaNets used in the hut trial. The content of samples was analysed pre washing and re-analysed post washing but pre trial as well as post trial. Number of washes is recorded on the X-axis below treatments. * PBO content on top was 10 times the values recorded on the Y-axis. CTN exh: conventionally treated deltamethrin net washed to just before the point of insecticide exhaustion; PN2: PermaNet 2.0; PN3: PermaNet 3.0; W: number of washes.

the sides or roof of PermaNet 3.0. The presence of PBO induced no significant repellent effect (Figure 2A).

The trends in blood-feeding inhibition mirrored that of passage inhibition. Blood-feeding inhibition induced by the side and roof netting was almost 100% both in *A. gambiae* Kisumu and VKPER strains even after 20 washes (Figure 2B). Blood-feeding inhibition was less in *C. quinquefasciatus* Kpankpan than in *A. gambiae*.

Control mortality recorded in all three strains during the tunnel tests never exceeded 5%. Roof or sides washed up to 20 times gave 100% mortality of susceptible *A. gambiae* Kisumu. Against the *kdr* strain of *A. gambiae* VKPER, the roof netting (deltamethrin + PBO) killed almost 100% and showed no decline in activity after washing. By contrast, activity of the side netting showed a progressive decline in activity over 20 washes (Figure 2C). These results provide evidence of synergism against *kdr*, perhaps linked to enhanced penetration.

In the local *C. quinquefasciatus* Kpankpan strain bearing multiple resistance mechanisms, PBO gave partial synergism, with 57.5% being killed by the unwashed roof netting compared with just 14% by the unwashed side netting (Figure 2C). After 10 washes the roof netting induced only 15% mortality.

4. Discussion

The rationale behind combining PBO with a pyrethroid is that pyrethroid efficacy increases via PBO acting both as a synergist through its action as a metabolic enzyme inhibitor and as an adjuvant through its effect on enhanced cuticular penetration of deltamethrin.¹⁷ We demonstrated under laboratory conditions that PBO in PermaNet 3.0 exerts a synergistic effect, which was particularly marked against the pyrethroid-resistant *A. gambiae* strain in which resistance was mediated by *kdr* only; this synergy was still evident after 20 washes. Synergism was also evident in

laboratory tests on multiresistant *C. quinquefasciatus* but not after washing the nets several times.

Although PermaNet 3.0 in the experimental hut trial induced higher levels of mortality among *A. gambiae* than either PermaNet 2.0, Olyset Net or the conventional ITN, mortality with all types of ITN was still rather low (corrected mortality 13–44%) in Southern Benin compared with other locations.^{2,8,9} These results confirm the earlier finding from the same area of Benin that vector control with ITNs is being undermined by pyrethroid resistance.⁵

PermaNet 3.0 goes some way to controlling resistant mosquitoes when unwashed. It showed greater insecticidal efficacy than unwashed PermaNet 2.0 or unwashed Olyset Net against *A. gambiae*, but the difference after 20 washes was not significant. The difference between PermaNet 3.0 and PermaNet 2.0 is likely to be attributable to PBO synergy, but the contribution of the higher deltamethrin content of PermaNet 3.0 relative to PermaNet 2.0 should not be ruled out. To prove this would require a control with the same dosage of deltamethrin as in PermaNet 3.0 but minus the PBO.

Under the present experimental conditions, PermaNet 3.0 outperformed the ITN washed to cut-off and hence fulfils the WHO criteria for LLINs.¹³ The WHO has not yet set criteria for LLIN performance in resistance management. Proof would, in any event, require testing against a population heterogeneous for insecticide resistance and susceptibility with demonstration of differential survival or reduced selection of resistance. Experimental hut trials can provide insight on this, but the evidence from the present trial is that none of the LLINs would delay or overcome selection of the pyrethroid resistance type that exists in southern Benin.

Whilst in the overnight tunnel tests a relatively high synergy occurred against *C. quinquefasciatus*, there was no evidence from the hut data that mosquitoes were

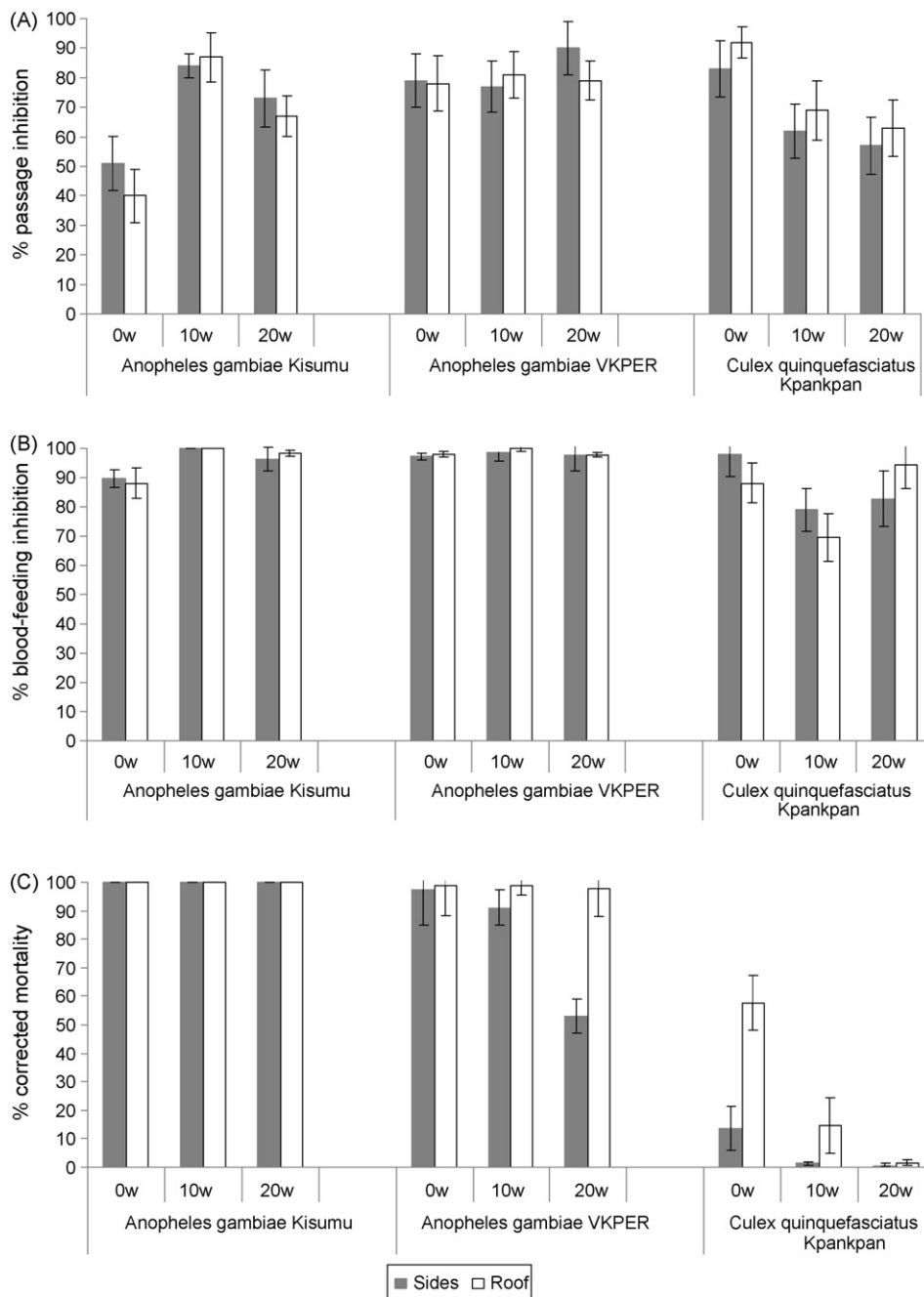


Figure 2. Tunnel tests using PermaNet 3.0 top and side netting before and after washing (10 or 20 times) against pyrethroid-susceptible (*Anopheles gambiae* Kisumu) and pyrethroid-resistant (*A. gambiae* VKPER and *Culex quinquefasciatus* Kpankpan) mosquitoes: (A) % passage inhibition; (B) % blood-feeding inhibition; and (C) % corrected mortality. Error bars indicate the 95% CI around the estimates. W: number of washes.

contacting the PBO on the roof for sufficient time to pick up a full synergistic dose. Failure of *C. quinquefasciatus* to contact the roof may partly explain the difference in synergy between laboratory assays and huts.

The main finding is the evidence of synergy from unwashed PermaNet 3.0 against *A. gambiae*, but this turned out to be lower than expected probably because of unidentified resistance mechanisms that are unaffected by PBO in

Benin. An earlier hut trial comparison in Tanzania against pyrethroid-susceptible *A. gambiae* demonstrated equally high mortality both with PermaNet 3.0 and PermaNet 2.0 and little or no loss of activity after 20 washes.¹⁸

The complexity of pyrethroid resistance in *C. quinquefasciatus*^{6,7} enables survival in the presence of PermaNet 3.0 despite the fact that PBO inhibits some of the enzymes that confer resistance. Similar results

were obtained with PermaNet 3.0 in Tanzania against pyrethroid-resistant *C. quinquefasciatus*.¹⁸

Owing to its moderate performance against pyrethroid-resistant *A. gambiae* M taxon as well as the continuing challenge of pyrethroid-resistant *C. quinquefasciatus*, PermaNet 3.0 does not provide a solution to the problem of pyrethroid resistance in southern Benin. Investigation of metabolic mechanisms other than esterase and oxidases in southern Benin is underway in order to gain further insight into the problem of resistance that is confronting malaria vector control in Africa.

Authors' contributions: RN supervised the trial, analysed the data and drafted the manuscript; AA, PB and AO conducted the trial and laboratory tests, participated in the analysis of the data and revised the manuscript critically for intellectual content; MA contributed to the trial design and revised the manuscript critically for intellectual content; OP conducted the chemical analysis and summarised the results and revised the manuscript critically for intellectual content; MR conceived the trial, interpreted the results and co-wrote the manuscript. All authors read and approved the final manuscript. RN and MR are guarantors of the paper.

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