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Efficacy of PermaNet® Dual compared to Interceptor® G2 and PermaNet 3.0 in experimental huts in Siaya County, western Kenya

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Abstract

Background Pyrethroid-chlorfenapyr nets have shown significant epidemiological impact over pyrethroid-only and pyrethroid plus piperonyl-butoxide (PBO) in Africa. A non-inferiority evaluation of PermaNet® Dual, a new chlorfenapyr plus deltamethrin net, compared to Interceptor® G2, was conducted in experimental huts in Siaya, Kenya against free-flying pyrethroid-resistant *Anopheles funestus*.

Methods This study was an experimental hut trial, following a 7 by 7 Latin Square design. Seven treatments and seven sleepers were deployed in the experimental huts daily and rotated weekly and daily, respectively. Mosquitoes were collected every morning between 06:30 h and 08:30 h and were assessed for blood feeding and then monitored for immediate knockdown 1-h post collection and delayed mortality after 72 h. Differences in proportional outcomes were analysed using the blocked logistic regression model, while differences in numerical outcomes were analysed using the negative binomial regression model. Non-inferiority determination was performed based on World Health Organization (WHO) protocol.

Results Mortality at 72 h was 30.2% for PermaNet 3.0, 44.4% for the Interceptor® G2 and 49.2% for the PermaNet® Dual. Blood feeding was highest with PermaNet® Dual at 15%, and least with PermaNet® 3.0 at 10%. PermaNet® Dual and Interceptor® G2 had no significant differences in mortality (OR = 1.10, 95% CI 1.00–1.20) or blood feeding (OR = 1.18, 95% CI 1.04–1.33) and the lower confidence bounds were within the non-inferiority margins but for blood feeding, non-inferiority was relatively high to the upper 95% confidence bound. PermaNet® Dual was non-inferior to the Interceptor® G2 and superior to the PermaNet® 3.0 nets in causing mortality but inferior to PermaNet ®3.0 in blood feeding inhibition of the vectors.

Conclusion PermaNet® Dual met the WHO criteria for non-inferiority to Interceptor® G2 and may be considered for deployment for public health use against pyrethroid-resistant *Anopheles* vectors of malaria.

Keywords *Anopheles funestus*, PermaNet® Dual, PermaNet® 3.0, Interceptor® G2, Non-inferiority, Pyrethroid-resistance, Kenya

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Background

Long-lasting insecticidal nets (LLINs) have contributed significantly to the decline in malaria transmission over the past two decades and remain the most widely used malaria vector control tool [1, 2]. LLINs provide a physical barrier against mosquito bites in addition to a toxic dose of insecticides which irritate, repel, knockdown and kill the mosquito resulting in reductions in blood feeding and reducing mosquito's longevity overall [3, 4]. These chemical properties are beneficial as the LLIN ages and becomes holed [5]. Insecticide resistance threatens the effectiveness of these vector control tools [6, 7] and for this reason, there is a need for continuous innovation to ensure LLIN products remain effective against pyrethroid-resistant mosquitoes.

Some of the approaches to mitigate insecticide resistance include the use of LLINs treated with a pyrethroid plus a synergist which is not directly toxic to mosquitoes but inhibits detoxification enzymes and restores susceptibility to insecticides. In September 2017, the World Health Organization (WHO) Global Malaria Programme released updated policy recommendations on the deployment of pyrethroid-PBO (piperonyl butoxide) LLINs [6] followed by the recommendation for deployment of PBO LLINs in areas of ongoing malaria transmission where the principal malaria vector(s) have developed pyrethroid resistance [7]. This recommendation was based on epidemiological data from cluster randomized control trials conducted with Olyset™ Plus in Tanzania which demonstrated that PBO LLINs have additional public health value [8]. Additional evaluations of pyrethroid PBO LLINs contributed further evidence of efficacy in the following years [9–12]. Since the recommendation of the first-in-class dual-active (dual-A.I.) LLINs after the demonstration of public health value in a community-based cluster randomized controlled trial (cRCT) [8, 13], many dual-A.I. LLINs such as Royal Guard® and Interceptor® G2 have been evaluated in WHO Phase I and II trials [14] and have shown promise compared to standard LLINs against pyrethroid-resistant vectors.

Pyrethroid-PBO LLINs have faced three main challenges: First, PBO is a synergist for P450 monooxygenases, but insecticide resistance is often a result of additional mechanisms including metabolic, target-site [15], cuticular [16] and microbial factors [17]. Second, the PBO incorporated in some of these LLINs was observed to wane in concentration by 18–24 months, well before the expected lifetime of an LLIN which is assumed to be 36 months [18]. Third, the deployment of pyrethroid-PBO LLINs alongside IRS with organophosphates is potentially counterproductive as the P450 monooxygenases also serve to activate organophosphates into their toxic metabolites [19].

More recently, studies have demonstrated the additional benefits of dual active LLINs which incorporate pyrethroid and non-pyrethroid insecticides in the same net. The WHO has recently recommended two new classes of LLINs which combine pyrethroids and pyrroles, such as chlorfenapyr, and pyrethroids and insect growth regulators, such as pyriproxyfen (PPF) (https://www.who.int) [20] based on epidemiological impact. Chlorfenapyr acts by disrupting cellular respiration and oxidative respiration phosphorylation in mitochondria [21]. Its unique mode of action has potential for the control of pyrethroid-resistant mosquitoes [22, 23]. The Interceptor® G2 is a pyrethroid-chlorfenapyr LLIN developed by BASF, which has demonstrated improved control of pyrethroid-resistant malaria vectors in experimental hut trials in Benin, Burkina Faso and Tanzania [24–26]. Large-scale trials have also provided further evidence of epidemiological impact [27, 28]. Data from experimental hut studies are useful in comparing new products to first-in-class products that have epidemiological data supporting their use in non-inferiority trials [29]. This study evaluated the non-inferiority of PermaNet® Dual, a new pyrethroid-chlorfenapyr LLIN containing deltamethrin and chlorfenapyr against Interceptor® G2, a pyrethroid-chlorfenapyr LLINs containing alphacypermethrin and chlorfenapyr as well as the superiority of PermaNet® Dual over the PermaNet® 3.0 which contains deltamethrin and PBO.

Methods

Study site and experimental huts

Experimental hut trials (EHTs) were conducted at the *Dala Suna* experimental hut site on the shores of Lake Kanyaboli (0° 02′ 08.5″ N, 34° 11′ 05.0″ E) in Alego Usonga sub-County, Siaya County, western Kenya. The huts are located close to the swamps that provide conducive breeding habitats for malaria vectors and are characterized by a high year-round abundance of *Anopheles funestus* and seasonal peaks of *Anopheles arabiensis*, with average household densities > 300 and > 20 per night, respectively [30]. The area experiences two rainy seasons, one from March to May and the other from October to November, with high malaria transmission throughout the year [31]. The primary economic activities of the local population are subsistence farming, livestock keeping, fishing and small-scale trading [30]. The experimental huts are designed to resemble a typical Kenyan household in structure and mosquito exit/entry points (eaves, windows and doors) (Fig. 1A). Mosquito exit traps were fitted to all four windows of the experimental huts, two windows on the front face and two on the backside of the huts. The walls of the huts are made of blocks and lined with mud on the inside. The floors are tiled with white

Fig. 1 Experimental hut design: **A** front view of the hut fitted with window exit traps, **B** showing the tiled floor and the hut interior walls and C showing the wood baffles

tiles for easy collection of knocked-down and dead mosquitoes (Fig. 1B). The huts have corrugated iron roofs and a 10-cm eave gap. To prevent mosquitoes from exiting the huts, wood baffles are installed at the eave gaps, allowing easy entry for mosquitoes (Fig. 1C). Additionally, the huts are elevated above the ground on a concrete base surrounded by a water-filled moat to keep ants away [29].

Baseline evaluation of insecticide resistance profile

Larvae of *Anopheles gambiae* sensu lato (*s.l.*) were collected using the standard dipper (Model 320) from their natural breeding sites around the experimental hut site. Adult blood-fed *An. funestus* were collected indoors using the Prokopack (model 1419) from the houses surrounding the experimental hut site, after obtaining consent from household heads. The mosquito collection took place between August and October 2022. The collected mosquitoes were transported to the KEMRI-CGHR insectary, where *An. gambiae s.l.* larvae were raised into adults and blood-fed *An. funestus* were allowed to lay eggs, and their first filial (F_1) generation reared to 3–5 days old adults for insecticide resistance testing. Larvae were reared in rainwater and fed on fine powder of Koi premium fish food under standard controlled conditions (27 ± 2 °C, 80 ± 10% RH and 12:12 light-darkness). Upon emergence, adults were maintained on a 10% sugar solution until bioassay. The Kisumu strain of *An. gambiae*, an insecticide-susceptible strain, was also reared simultaneously under the same conditions and used as a bioassay control.

To assess the susceptibility of *An. gambiae s.l.* and *An. funestus* from the Lake Kanyaboli experimental hut site to the active ingredients of insecticides in the LLINs to be tested, namely PermaNet 3.0 (deltamethrin + PBO) and Interceptor® G2(alpha-cypermethrin + chlorfenapyr) and PermaNet® Dual (deltamethrin + chlorfenapyr) LLINs, WHO tube assay and Center for Disease Control (CDC) bottle tests were conducted. WHO tube tests were carried out on 3 to 5-day-old F0 *An. gambiae* s.l. and F1 *An. funestus* adults according to the WHO protocol [32]. In brief, mosquitoes were exposed to filter papers impregnated with 0.05% alpha-cypermethrin, 0.75% permethrin or 0.05% deltamethrin for 1 h, during which knockdown was recorded every 10 min and mortality was recorded 24 h post-exposure. The intensity of insecticide resistance to pyrethroids was determined by increasing the diagnostic concentrations to 5X and 10X.

non-insecticide-impregnated filter paper was also used as the control. The insecticide-treated filter papers were obtained from the WHO via the Kenya Medical Research Institute (KEMRI), and their quality was assessed against susceptible *An. gambiae* sensu stricto (*s.s.*) mosquitoes (Kisumu strain), as control. Pyperonyl butoxide (PBO) synergist test was also performed: mosquitoes were pre-exposed to 4% PBO for one hour and then exposed to 0.05% deltamethrin, 0.05% alpha-cypermethrin and 0.75% permethrin [32].

CDC Bottle bioassays were performed using the discriminating concentration of chlorfenapyr (100 µg/bottle) and clothianidin (4 µg/bottle + Mero) using both *An. gambiae s.l* and *An. funestus* following the WHO protocol [33]. Each Wheaton 250 ml bottle and its cap was coated with 1 ml of insecticide solution by rolling and inverting the bottles. In parallel, a control bottle was coated with 1 ml of acetone, followed by all bottles being covered with a sheet and left to dry overnight in the dark. Mosquitoes were exposed to chlorfenapyr and clothianidin for 60 min. Following exposure, mosquitoes were transferred to a netted paper cup, provided with lightly moistened cotton wool containing 10% sugar solution (changed daily) for chlorfenapyr-exposed mosquitoes and monitored at 24 h, 48 h, and 72 h. In contrast, clothianidin-exposed mosquitoes were monitored for 24 h only.

Net treatments and treatment arms

Both PermaNet® 3.0 and PermaNet® Dual were supplied by Vestergaard Sarl (Lausanne, Switzerland). Interceptor® G2 was supplied by BASF (Ludwigshafen, Germany*)*. PermaNet® 3.0 was used in this evaluation as a comparator because it is the first dual-active insecticide-treated bed net Vestergaard S.a.r.l incorporating PBO on the top panel and deltamethrin on the side panels. It is also currently the standard of care being deployed in the area to combat pyrethroid resistance. The untreated nets were made of polyester fabric without any insecticide treatment. The Interceptor® G2 was made of polyester fabric coated with 2.4 g/kg (100 mg/m^2) of alpha-cypermethrin and 4.8 g/kg (200 mg/m^2) of chlorfenapyr. PermaNet® 3.0 was made of polyester fabric coated with 2.1 g/kg (84 mg/m^2) of deltamethrin on the sides, and polyethylene incorporated with 4.0 g/kg (120 mg/m^2) of deltamethrin and 25.0 g/kg (800 mg/m^2) of PBO on the roof. PermaNet® Dual was made of polyester fabric coated with chlorfenapyr at 5.0 g/kg (200 mg/m^2), and deltamethrin at 2.1 kg (84 mg/m^2).

Net washing

For each study arm, Seven nets were randomly selected from a cohort of 21 nets of each production batch and subjected to twenty washes following the WHO washing criteria [14]. To prevent contamination between different nets, each LLIN type was washed separately in its washing station, which was equipped with separate assortments. The washing process involved immersing each net individually in a 16-L aluminium basin filled with 10 L of clean groundwater (pH of 7.0 and a hardness of 5 degrees), to which 20 g of soap was added and fully dissolved just before washing. Each net was washed for 10 min with agitation for 3 min, then soaked for 4 min and stirred again for 3 min. The net samples were rinsed twice in 10 L of clean groundwater using the same washing procedure, then dried under shade and stored at ambient temperature between washes. To simulate the wear-and-tear of the nets during use, all the LLINs intended for the hut trial of both treatment wash points and control nets were given 6 holes measuring 4×4 cm. Two holes were created on each of the long side panels and one hole on each of the short side panels, as per WHO guidelines [14].

Hut trial procedure

Experimental hut trials used a 7 by 7 Latin square design (LSD) to evaluate the entomological efficacy of PermaNet® Daul, Interceptor® G2 and PermaNet® 3.0 LLINs washed 20 times and unwashed against free flying pyrethroid-resistant *An. funestus*. At each wash point, the efficacy of these LLINs was compared to an untreated net as a negative control. The trial used 49 nets, fourteen nets of each LLIN type (7 replicates of unwashed and seven replicates of washed), except for the untreated/control net, which had seven nets. Seven consenting human volunteer sleepers slept in the huts from 8:30 PM to 6:30 AM daily throughout the trial period, and to account for individual attractiveness to mosquitoes, they were rotated daily between the huts using a simple 7*7 LSD. The nets were erected inside the experimental huts by tying the edges of the roof panel to nails fixed at the upper corners of the hut wall using string. Treatments were rotated between experimental huts weekly according to a Latin square design to control the hut position effect. In contrast, volunteers were rotated daily to control differences in individual host attractiveness to mosquitoes. Mosquito collections were performed for 7 days in each collection round; on the 8th day, the huts were cleaned and aired to prevent contamination and carry-over effects before the next rotation cycle.

The following treatment arms were evaluated in each experimental hut trial:

1. Untreated net (control)—7 replicates of nets unwashed.
2. PermaNet® Daul—7 replicates of nets washed 20 times.

3. PermaNet® Daul—7 replicates of unwashed nets.
4. Interceptor® G2—7 replicates of nets washed 20 times.
5. Interceptor® G2—7 replicates of washed nets.
6. PermaNet® 3.0—7 replicates of nets washed 20 times.
7. PermaNet® 3.0—7 replicates of unwashed nets.

Mosquito collections and processing

Seven consenting human volunteers slept in experimental huts from 8:30 PM to 06:30 AM during each trial to attract wild, free-flying mosquitoes. All the sleepers were provided with weekly prophylaxis (mefloquine) and instructed to record any side effects experienced during the evaluation period. From 6:30 AM, mosquito collections were conducted using mouth aspirators until 08:00 each morning. The sleepers collected all the dead and alive mosquitoes inside the huts and window exit traps using mouth aspiration. The mosquitoes were scored based on their point of collection, such as wall, roof, floor, net, and under-bed, as well as from the window exit traps. Once collected, the mosquitoes were transferred into clean, netted paper cups and provided with access to a 10% sugar solution. The samples were arranged in cooler boxes and transported to the field insectary laboratory. In the laboratory, the mosquitoes were sorted by status (alive or dead: blood-fed or unfed: gravid or half-gravid) and identified morphologically to species following taxonomical key [34]. All the live mosquitoes were observed for knockdown one-hour post collection, and mortality was recorded every 24 h for 72-h.

Supplementary laboratory assays

Cone test

Cone testing was performed with net pieces (25 cm × 25 cm) drawn from before and after the field trial of all wash points of all LLINs used in this evaluation. Four cones were attached to each net piece, and five non-blood-fed female mosquitoes were aspirated into each of the four cones and exposed for 3 min [14]. Both *An. gambiae,* Kisumu strain and *An. funestus* F1 of 3–5 days were introduced in each cone. In total, 100 mosquitoes were used per net/species. After exposure, the mosquitoes were transferred into clean paper cups, provided with a 10% sugar solution, and knockdown was recorded 60 min post-exposure, with mortality recorded at 24 h, 48 h, and 72 h. Mosquitoes were kept under the same laboratory conditions described above. The insecticide-susceptible strain of *An. gambiae* Kisumu was used as control.

Tunnel test

The tunnel test measures host-seeking mosquitoes' mortality and blood-feeding success in an experimental chamber. This experiment was designed to provide further insight and explain the toxicity of unwashed and washed nets used in the huts. Tunnel assays were conducted against the pyrethroid-resistant *An. funestus* F1 from the experimental hut site with the same net pieces of Interceptor® G2 and PermaNet® Dual tested in the cone assay. The tunnel test chamber mimics the behavioural interactions between free-flying mosquitoes and nets during host-seeking. It consists of a square glass tunnel divided one-third (20 cm) of its length by a box frame fitted with a net sample Fig. 2. In the short section of the tunnel, a rabbit bait was held in a cage with its back sheared and exposed for easy accessibility and feeding by mosquitoes [14]. In contrast, in the long sections (40 cm), 100 5–8-day-old mosquitoes were released at 6:00 PM and left until 7:00 AM under standard controlled conditions (27 ± 2 °C temperature, 80 ± 10% RH). The net pieces used in the experiment had nine small holes, each measuring 1 cm in diameter, which allowed mosquitoes to enter the baited chamber. The mosquitoes were collected from the tunnel in the morning and examined for mortality and blood-feeding success. The surviving mosquitoes were placed in clean paper cups with a label and given access to a 10% sugar solution. Delayed mortality of the live mosquitoes was recorded every 24 h, up to a maximum of 72 h.

Chemical assays

Two nets were randomly selected from all the wash points in every arm, before and after the hut trials, and five pieces were obtained from each net apart from PermaNet 3.0, from which 3 pieces were obtained from the top and 1 from each side (7 pieces total) following WHO guidelines on net cutting. The cut net pieces were shipped wrapped in aluminium foil to the Vestergaard

Fig. 2 A Tunnel assay set up in the laboratory to assess mosquito mortality and blood feeding success with dual active LLINs

ISO/IEC 17025 accredited Vector Control Laboratories in Vietnam for testing to determine the wash retention of active ingredients in the net pieces using analytical methods validated and published by the Collaborative International Pesticides Analytical Council (CIPAC). Briefly, deltamethrin in the roof of PermaNet 3.0 (roof) was extracted from net samples by heating under reflux for 30 min with xylene using dicyclohexyl phthalate as internal standard. The solvent was evaporated, and the residue dissolved in hexane. Deltamethrin was extracted from the nets, including PermaNet® Dual and PermaNet 3.0 sides using dicyclohexyl phthalate and the concentration was determined by normal phase high-performance liquid chromatography with UV diode array detection (HPLC–DAD). Alpha-cypermethrin in Interceptor® G2 as well as chlorfenapyr in Interceptor® G2 and PermaNet® Dual were sonicated with heptane using dicyclohexyl phthalate as internal standard and determined by gas chromatography with flame ionisation detection (GC-FID). Lastly, PBO in PermaNet 3.0 roof was extracted from net samples by heating under reflux for 30 min with xylene using octadecane as internal standard and determined by GC-FID.

Data analysis

The primary outcomes measured by comparing the treatments and control experimental huts were blood-feeding inhibition (the reduction in blood feeding in treatments compared with that in the control huts), immediate and delayed mortality (the proportion of mosquitoes that are dead in the morning of collection and the cumulative proportion dead at 24, 48 or 72 h). In addition, induced exophily (the proportion of mosquitoes that are found in the exit traps) and deterrence (proportional reduction in the number of mosquitoes collected in the treated huts relative to the number collected in the control huts with untreated nets) were evaluated.

The difference in proportional outcomes (mortality, blood feeding and exophily) between treatments and control at all wash points were analysed using a blocked logistic regression model, while differences in numerical outcomes (entry) were analysed using a negative binomial regression model. Tests of non-inferiority between PermaNet® Dual and Interceptor® G2 for both mortality and blood feeding were performed according to the WHO protocol [35]. The analysis included both washed and unwashed nets with an independent variable in the washing model. A candidate product is considered non-inferior to the active comparator product if: (a) the lower 95% confidence interval of the odds ratio describing the difference in mortality between the candidate and comparator product is > 0.7 and/or, (b) the upper 95% confidence interval of the odds ratio describing the difference in blood feeding between the candidate and comparator product is < 1.43. The superiority between PermaNet® Dual and PermaNet 3.0 was also assessed based on whether mortality rates were higher and blood feeding rates lower at a 5% significance level (i.e. $p < 0.05$). All analyses were done using R Statistical Software (v4.2.2; R Core Team 2021).

Ethical considerations and compliance with GLP

Ethical approval for the trial was issued by the Scientific and Ethical Review Unit of KEMRI (SERU 4536) for involving humans and animals. This study was also reviewed by the CDC and was determined to meet the definition of research involving human subjects. Still, the CDC's involvement was not considered to constitute an engagement in human subjects research. Prior to recruitment into the study, formal informed consent was obtained from the volunteer sleepers. The participants were each given a weekly course on malaria prophylaxis (Mefloquine) to protect them from contracting malaria. This site is accredited by the Kenya Pest Control Products Board (PCPB) for the national evaluation of vector control products for registration purposes. The study was conducted in strict conformance with WHO non-inferiority guidelines for the evaluation of second-in-class LLINs [35]. Additionally, the site has begun the process towards GLP accreditation and conducts all study procedures in strict conformance with GLP requirements.

Results

Insecticide resistance profile of the local mosquito populations

No mortality was recorded in the controls. Therefore, Abbott's formula was not used to correct the mortality rates. Pyrethroid resistance was detected in all species (Table 1). *An. gambiae s.l.* and *An. funestus* from the study area showed resistance to the diagnostic dose of deltamethrin (1X), with only 45% and 72% mortality observed, respectively. Although there was an increase in mortality when exposed to higher doses of deltamethrin (5X), the mortality rates increased from 45 and 72% (for the 1X dose) to 84% and 77% for *An. gambiae s.l.* and *An. funestus*, respectively. Exposure to the highest 10X diagnostic dose resulted in 100% mortality for *An. gambiae* and 92% for *An. funestus*. The results were comparable for both species when testing permethrin and alpha-cypermethrin insecticides, with none achieving 100% mortality even after increasing the diagnostic doses to 10 times the standard dose. Pre-exposure to PBO restored full susceptibility to deltamethrin and partial restoration of susceptibility to permethrin and alpha-cypermethrin in the *An. gambiae* population, but susceptibility was partially restored in *An. funestus* to all tested pyrethroids.

Table 1 Insecticide resistance status of malaria vectors of Lake Kanyaboli, western Kenya

Assays	Insecticide	Dose	Concentration	Sample size	% Mortality	
					An. gambiae	*An. funestus*
WHO tube	Alphacypermethrin	1X	0.05%	100	82	45
		5X	0.25%	100	88	60
		10X	0.50%	100	93	94
	PBO + Alphacypermethrin	1X	0.05%	100	95	97
	Deltamethrin	1X	0.05%	100	45	77
		5X	0.25%	100	84	72
		10X	0.50%	100	100	92
	PBO + deltamethrin	1X	0.05%	100	100	97
	Permethrin	1X	0.75%	100	82	64
		5X	3.75%	100	98	94
		10X	7.50%	100	100	86
	PBO + permethrin	1X	0.75%	100	99	95
	Pirimiphos-methyl	1X	0.25%	100	100	100
WHO bottle	Clothianidin		4 µg/ml	100	100	100
	Chlorfenapyr		100 µg/ml	100	100	100

The non-pyrethroids insecticides (pirimiphos methyl, clothianidin and chlorfenapyr) tested using CDC bottle bioassay resulted in 100% mortality when exposed to diagnostic doses.

Mosquito entry and exit rates in experimental huts

A total of 15,114 pyrethroid-resistant female *An. funestus* were collected during the experimental hut evaluation. More mosquitoes were collected in huts with the unwashed PermaNet® 3.0 compared to the washed PermaNet® 3.0 and the washed and unwashed Interceptor® G2. Exit rates were significantly higher for the washed and unwashed PermaNet® 3.0 compared to all other treatments, while the exit rates for the unwashed Interceptor® G2 were significantly lower than the untreated net. No other significant differences in exit rates were observed.

Non-inferiority assessment from the experimental hut

According to the recent provisional WHO guidelines, for a candidate LLIN to be included in an established intervention class, it must demonstrate non-inferiority to the first-in-class product which has already demonstrated public health value (Interceptor® G2, for pyrethroid-chlorfenapyr ITN class) and superiority to pyrethroid only LLIN in experimental hut trial [35].

The non-inferiority margin is set at 0.7 for mortality and 1.43 for blood feeding. The odds ratio for the difference in mosquito mortality between PermaNet® Dual and Interceptor® G2 was 1.21 (95% confidence interval 1.093587–1.337), while the odds ratio for the difference in mosquito blood feeding was 1.18 (95% confidence interval 1.04–1.33) in mosquitoes. Following the WHO criteria described above, PermaNet® Dual is non-inferior to Interceptor® G2 based on the mortality (49% vs 44%, $p < 0.047$) induced in pyrethroid-resistant *An. funestus* in the experimental hut trial in Lake Kanyaboli, Kenya, while the PermaNet® Dual is both inferior and non-inferior to the Interceptor® G2 based on blood-feeding inhibition (85% vs 87%, $p < 0.001$) (Table 2). For the superiority assessment, PermaNet® Dual was superior to PermaNet® 3.0 in mortality induced (49% vs 30%, $p < 0.001$) but was inferior to PermaNet® 3.0 in blood feeding (10% vs 15%, $p < 0.001$) (Table 2). Due to the high control mortality (37%), Abbott's correction was applied to all mortality data (Table 3). After correction, the mortality rates were 30.2%, for PermaNet® 3.0, 49.2% for PermaNet® Dual, and 44.4% for Interceptor® G2. Despite the reduction in absolute mortality rates, the relative performance of the nets and the conclusions regarding non-inferiority and superiority remained consistent with the uncorrected data.

Supplementary assay results

Both washed and unwashed PermaNet® Dual and Interceptor® G2 pieces tested induced low mortality in cone bioassays (< 73% for all tests, Fig. 3) against susceptible *An. gambiae s.s,* Kisumu strain, indicates that the cone bioassay is unsuitable for testing slow-acting actives even when combined with pyrethroids, a fast-acting active ingredient. PermaNet 3.0 roof net pieces induced

Table 2 Results from the non-inferiority assessment of PermaNet® Dual to Interceptor® G2 against wild pyrethroid-resistant An. funestus in experimental huts in Siaya, western Kenya

Primary indicators	Variables	PermaNet® 3.0	PermaNet® Dual	Interceptor® G2	PermaNet® Dual
	Total collected	4390	4481	4036	4481
Mortality	Total dead	2441	3029	2629	3029
	Observed mortality (%)	56	68	65	68
	Corrected mortality (%)	30.2	49.2	44.4	49.2
	Odds ratio	–	2.246	–	1.211
	Std. error (on log odds scale)	–	0.080	–	0.050
	P-value	–	< 0.001	–	0.047
	95% CIs	–	1.920–2.627	–	1.097–1.337
	WHO efficacy criteria	–	Significantly higher ($p < 0.05$)	–	Lower 95% CI > 0.7
	Conclusion	–	Superior	–	Non-inferior
Blood feeding	Total blood-fed	423	671	517	671
	Blood-feeding (%)	10	15	13	15
	Odds ratio	–	1.627	–	1.176
	Std. error (on log odds scale)	–	0.110	–	0.076
	P-value	–	< 0.001	–	0.012
	95% CIs	–	1.425–1.856	–	1.037–1.334
	WHO efficacy criteria	–	Significantly lower ($p < 0.05$)	–	Upper 95% CI < 1.43
	Conclusion	–	Inferior	–	Inferior and non-inferior

Table 3 Results from the non-inferiority assessment of PermaNet® Dual to Interceptor® G2 and superiority to PermaNet® 3.0 against wild pyrethroid-resistant *An. funestus* in experimental huts in Siaya, western Kenya (with Abbott's correction applied to mortality data and regression analysis results included)

Outcome	Treatment	Total collected	Event count	%	Corrected %	Regression coefficient (SE)	Odds ratio (95% CI)	P-value
Mortality	Control	2207	817	37.0	–	Reference	–	–
	PermaNet® 3.0	4390	2441	55.6	30.2	0.809 (0.054)	2.246 (2.021–2.495)	< 0.001
	PermaNet® Dual	4481	3029	67.6	49.2	1.304 (0.053)	3.684 (3.320–4.088)	< 0.001
	Interceptor® G2	4036	2629	65.1	44.4	1.167 (0.054)	3.212 (2.889–3.572)	< 0.001
Blood feeding	Control	2207	926	42.0	–	Reference	–	–
	PermaNet® 3.0	4390	423	9.6	–	− 1.869 (0.068)	0.154 (0.135–0.176)	< 0.001
	PermaNet® Dual	4481	671	15.0	–	− 1.407 (0.062)	0.245 (0.217–0.276)	< 0.001
	Interceptor® G2	4036	517	12.8	–	− 1.573 (0.065)	0.207 (0.183–0.235)	< 0.001

Control mortality was 37%

Abbott's correction was applied to mortality data

Regression coefficients and odds ratios are from blocked logistic regression models adjusting for hut, sleeper, day, and wash status

the highest mortality rates (100%) for all the wash points, with sides-inducing mortality rates of > 92% (Fig. 4).

Tunnel assays results

Mortality rates of *An. funestus* in tunnel tests against the Interceptor® G2 and the PermaNet® Dual were high at all wash points (> 96.6%). Interceptor® G2 induced the highest mortality rate with 20 washes after the hut trial at 99.1% while PermaNet® Dual had the highest mortality rate of 98.2% with unwashed net pieces obtained from LLINs pieces after the hut trial. However, there was no significant difference in mortality between the two pyrethroid–chlorfenapyr LLINs (Fig. 5).

High blood-feeding inhibition of 96% was witnessed with samples of unwashed PermaNet® Dual after the hut trial whereas Interceptor® G2washed 20 times pieces after the hut trial induced the lowest blood-feeding inhibition of 80% (Fig. 6).

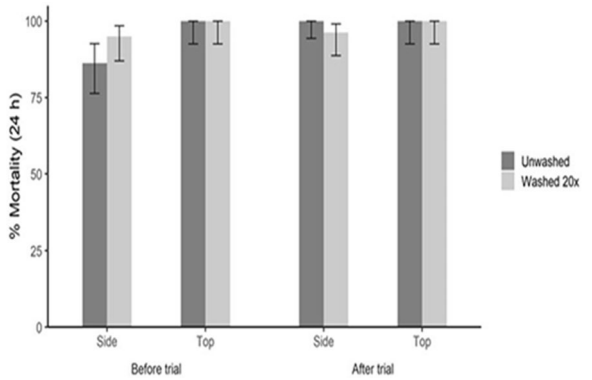

Fig. 3 Cone assay mortality results of *An. gambiae,* Kisumu strain when exposed to PermaNet® 3.0 net for 3 min. Error bars represent 95% confidence intervals

Chemical assays

All the unwashed LLINs had AI content within the manufacturer-specified range. Retention of AI was lowest in the net pieces cut from the PermaNet® Dual washed 20 times (43% deltamethrin and 47% chlorfenapyr) and highest in the net pieces cut from the Interceptor® G2 washed 20 times (83.5% alpha-cypermethrin and 81% chlorfenapyr). Net pieces obtained from the PermaNet® 3.0 had retention of 64, 92.8 and 82% for deltamethrin on the sides, deltamethrin on the roof and PBO, respectively (Table 4).

Discussion

This study evaluated the efficacy (mortality and blood feeding inhibition) and wash resistance of PermaNet® Dual (Vestergaard) in comparison to Interceptor® G2 (BASF) and PermaNet® 3.0. (Vestergaard) against pyrethroid-resistant free-flying *An. funestus* mosquitoes in experimental huts on the shores of Lake Kanyaboli in Siaya County, western Kenya. This locality has a year-round abundance of *An. funestus* and seasonal abundance of *An. arabiensis*. This trial was conducted in the dry season and therefore only *An. funestus* had adequate numbers for statistical comparisons, averaging 44 female mosquitoes per hut per night. The Lake Kanyaboli area is mostly swampy with permanent stagnated pools of water conducive to the development of *An. funestus s.s.* with peak numbers > 300 mosquitoes per structure per night in the rainy seasons [30].

All three LLINs evaluated here had significantly higher mortality rates on the free-flying *An. funestus* mosquitoes relative to the control in the experimental huts. PermaNet® Dual induced the highest mortality rates which was not significantly different from Interceptor® G2 but was significantly higher than PermaNet® 3.0 at corroborating results from previous hut trials in Benin [36]. Similar observations have been made in experimental hut trials evaluating PermaNet® Dual and Interceptor® G2 where in each instance, the pyrethroid-chlorfenapyr LLIN induced higher mortality than the pyrethroid-PBO or pyrethroid-only LLINs [25, 36, 37]. The application of Abbott's correction to account for high control mortality resulted in lower absolute mortality

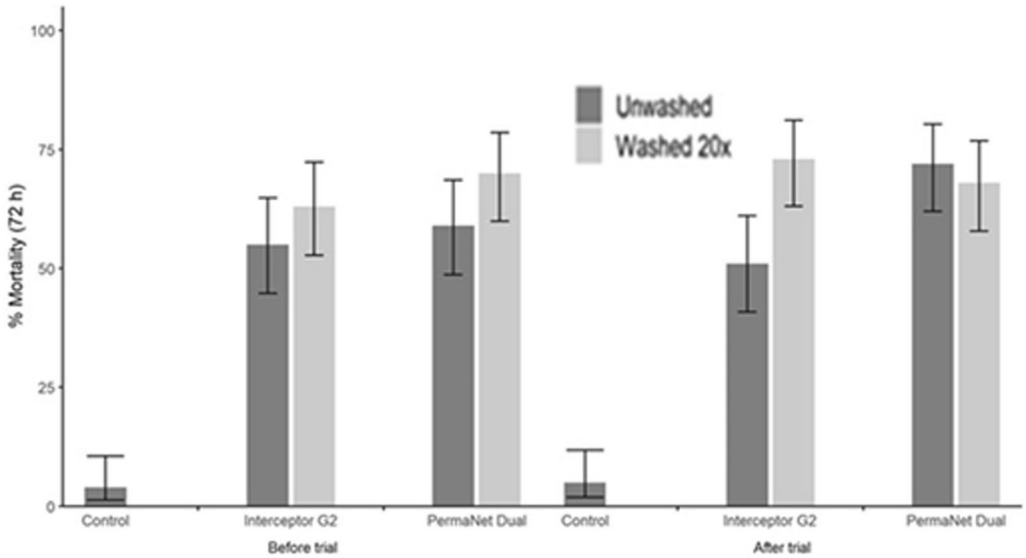

Fig. 4 Cone assays mortality result of *An. gambiae,* Kisumu strain when exposed to dual actives ITNs following WHO guidelines. Error bars represent 95% confidence intervals

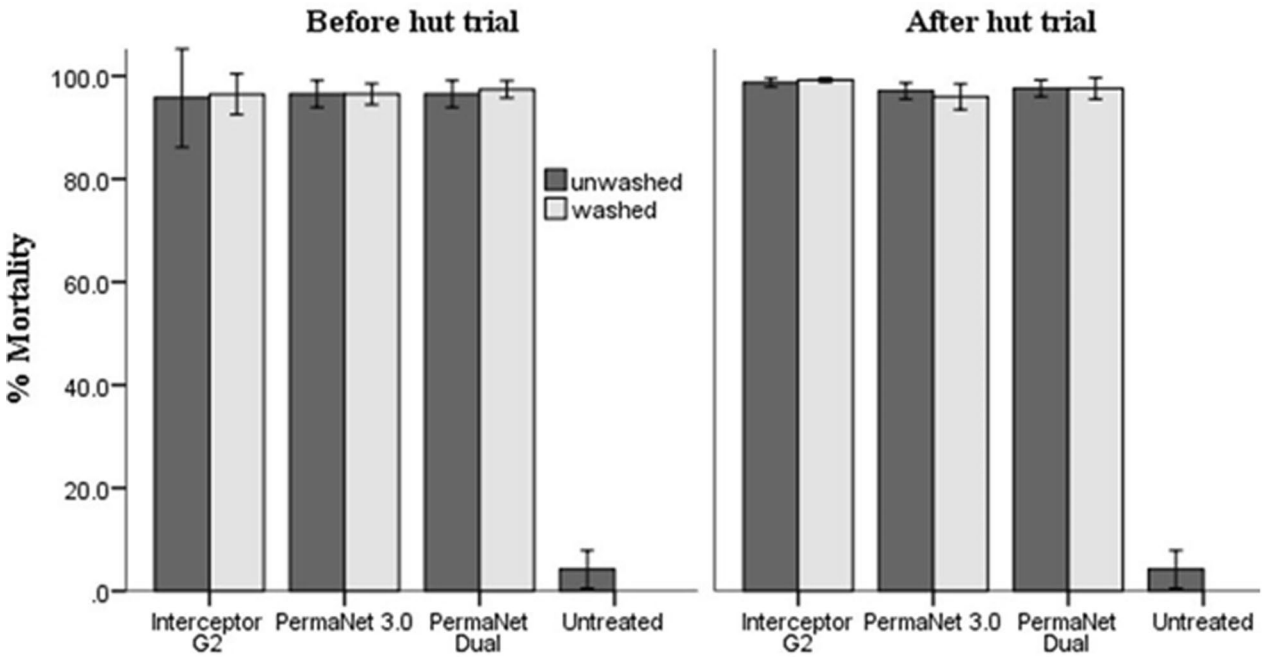

Fig. 5 Mortality rate of pyrethroid-resistant *An. funestus* F1 mosquitoes exposed to Interceptor® G2, PermaNet® Dual and PermaNet® 3.0 in tunnel tests. Error bars represent 95% confidence intervals

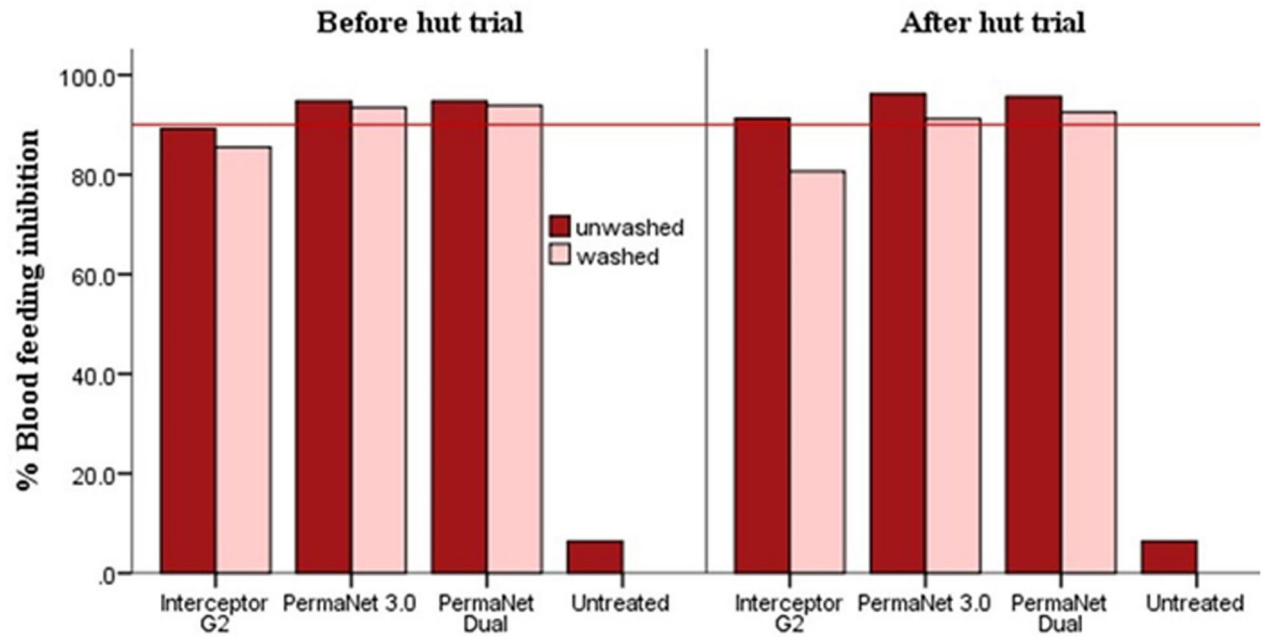

Fig. 6 Blood-feeding inhibition of pyrethroid-resistant *An. funestus,* Siaya strain against new generation nets in tunnel tests. The red lines indicate WHO cut-off criteria for efficacy in tunnels

rates for all treatments. However, the relative efficacy of the nets remained consistent, with PermaNet® Dual still demonstrating non-inferiority to Interceptor® G2 and superiority to PermaNet® 3.0 in terms of mosquito mortality. This suggests that while environmental factors may have influenced overall mosquito survival in the experimental huts, they did not substantially alter the comparative performance of the different net types.

Table 4 The content of active ingredients contained in unwashed and washed net pieces before and after the experimental hut trial in Siaya, Kenya

ITN Brand	Active ingredient (s)	AI content (g/kg)		AI retention (%)
		Unwashed	Washed 20X	
PermaNet® 3.0	Deltamethrin (sides)	1.75	1.12	64.0
	Deltamethrin (roof)	3.61	3.35	92.8
	PBO (roof)	19.11	15.69	82.1
Interceptor® G2	Alpha-cypermethrin	2.85	2.38	83.5
	Chlorfenapyr	5.56	4.51	81.1
PermaNet® Dual	Deltamethrin	2.09	0.90	43.1
	Chlorfenapyr	5.00	2.38	47.6

Despite the lower mortality observed in this and other experimental hut studies, pyrethroid-PBO LLINs have been shown to offer up to 2 years better protection, with reduced parasite prevalence and vector densities than pyrethroid-only LLINs in Uganda and Tanzania [8, 9]. However, the rapid loss of PBO is a concern. A study in Tanzania noted that the PBO content of the nets was significantly reduced at 12 months and was almost lost by 24 months, a risk for sustained efficacy against pyrethroid-resistant malaria mosquitoes over the expected three-year lifetime [10]. For this reason, dual active nets with three years of effectiveness are urgently needed to complement vector control efforts in areas of high pyrethroid resistance.

High resistance to alphacypermethrin, deltamethrin and permethrin was observed in both *An. funestus* and *An. arabiensis* which coincides with earlier reports [38, 39]. Higher concentrations of deltamethrin and permethrin in WHO tube assays (0.50% and 7.5%, respectively) and deltamethrin and alpha-cypermethrin (5X and 10X, respectively) in bottle assays were effective against *An. gambiae*, but not against *An. funestus,* indicating a higher intensity of resistance in *An. funestus* relative to sympatric vectors. Full susceptibility of both malaria vectors from the area to non-pyrethroids insecticides at standard doses: neonicotinoids (clothianidin), pyrrole (chlorfenapyr) and organophosphate (pirimiphos-methyl) was observed, despite high resistance to pyrethroids indicating that these classes could be effective for rotation or use of mixture formulations for malaria control in the region. The above finding was also an indication that there was no cross-resistance between pyrethroids and these other classes of insecticides. The addition of PBO as a synergist was observed to partially restore the observed susceptibility in both *An. arabiensis* and *An. funestus* indicating the involvement of P450 monooxygenases in the resistant phenotypes as has been reported elsewhere [40–42]. However, it partially restored susceptibility to > 95% mortality, which is close to full susceptibility, which suggests the involvement of other resistance mechanisms.

PermaNet® Dual was non-inferior to Interceptor® G2 (the first in class), with an odds ratio of 1.21 (1.10–1.34, P > 0.05) at a non-inferiority margin of 0.7 according to the WHO guidelines for evaluation of non-inferiority to first in class products [43]. Following this criterion, PermaNet® Dual does not need to undergo evaluation for epidemiological impact but is available for recommendation as a second product in the same class. The PermaNet® Dual has since been prequalified by the WHO (https://extranet.who.int/pqweb/vector-control-product/PermaNet-dual) and is therefore available for immediate deployment to contribute to insecticide resistance management (IRM). Additionally, PermaNet® Dual was superior in inducing mortality relative to PermaNet® 3.0 with an odds ratio of 2.25 (1.92–2.63, P > 0.001). This shows the contribution of chlorfenapyr to the control of resistant mosquitoes where mechanisms other than P450 monooxygenases are active such as in this population. Similar findings have been documented in Tanzania [10], where there was a higher impact on entomological outcomes in clusters with Interceptor® G2 than those with PermaNet® 3.0, and in another experimental hut trial evaluating the non-inferiority of PermaNet® Dual to Interceptor® G2 [36].

Blood-feeding inhibition was significantly higher with PermaNet® 3.0 compared to both Interceptor® G2 and PermaNet® Dual but was not significantly different between the two pyrethroid-chlorfenapyr nets. Results from a separate study comparing ® G2 and chlorfenapyr-only control showed higher blood-feeding rates in the chlorfenapyr-only arm indicating that pyrethroids contribute the most to blood-feeding inhibition [37]. The current study indicates that PBO in PermaNet® 3.0 synergized the blood-feeding inhibition and, therefore, lower blood-feeding rates were achieved compared to the pyrethroid-chlorfenapyr

nets. PermaNet® 3.0 was superior to PermaNet® Dual in blood-feeding inhibition. PermaNet® Dual was non-inferior to Interceptor® G2 in blood feeding inhibition possibly due to the higher irritability of alpha-cypermethrin.

These results were not significantly different between unwashed nets and nets washed 20X, although the trends were towards higher mortality in the 20X washed nets. PermaNet® Dual and Interceptor® G2 did not have reductions in induced mortality or blood feeding inhibition after 20 washes, indicating good wash resistance, which is the current standard WHO proxy for an LLIN giving good performance for up to three years of use, despite less than 50% AI retention in the PermaNet® Dual. Previous studies have reported similar results [36, 37].

Standard laboratory cone bioassays with PermaNet® Dual and Interceptor® G2 failed to predict their efficacy against pyrethroid-resistant *An. funestus s.l.* in experimental huts. Cone bioassays with pyrethroid-chlorfenapyr nets did not meet the WHO criteria for the susceptible *An. Gambiae*, Kisumu strain PermaNet® 3.0 while tunnel tests with the PermaNet® Dual resulted in > 95% mortality against F1 progeny of wild *An. funestus*, affirming the unsuitability of cone bioassays for the evaluation of chlorfenapyr LLINs. These findings are similar to earlier ones reported in Benin and Cote d'Ivoire [36, 44] and indicate that tunnel tests are required as a laboratory assay of pyrethroid plus chlorfenapyr nets.

The primary limitation of the study is the high mosquito mortality rates (37%) observed in the control huts. There was a significant difference in mosquito mortality rates between mosquitoes collected from the control exit trap (63%) and the control indoor (11%), averaging 37%. This suggests that the high mortality in the control hut could be attributed to strong and swift winds around the lake where the experimental hut is located. In addition, there was an unexpectedly high rate of exophily in the control arm which was higher than PermaNet® Dual and Interceptor® G2 at 28%, and which could not be explained. Mortality at 72 h in the control arm was 37% which was higher than most other hut studies including another pyrethroid-chlorfenapyr net experimental hut study with *An. funestus* in Tanzania [30]. This was likely due to excess mortality in the exit traps as the experimental hut sites are located on the shores of Lake Kanyaboli and receive strong winds through the night which desiccated the mosquitoes which escaped into the exit traps leading to increased mortality. However, given the high densities of *An. funestus* per hut per day (44), this did not affect the statistical power of the study.

Conclusions

PermaNet® Dual, the candidate product (deltamethrin + chlorfenapyr), was non-inferior to Interceptor® G2, the reference product (alphacypermethrin + chlorfenapyr) in causing mortality and inducing blood-feeding inhibition of free-flying wild pyrethroid-resistant *An. funestus* in this experiment. PermaNet® Dual was superior to PermaNet® 3.0, the positive control (deltamethrin + PBO) in causing mortality but inferior in the blood-feeding inhibition of wild pyrethroid-resistant *An. funestus* in this experiment. Overall, PermaNet® Dual met the WHO efficacy criteria in relation to non-inferiority to Interceptor® G2 and can be deployed in areas of high pyrethroid resistance.

Abbreviations

AI	Active ingredient
CDC:	Centers for Disease Control and Prevention
CI	Confidence interval
CIPAC	Collaborative International Pesticides Analytical Council
cRCT	Cluster randomized controlled trial
DNA	Deoxyribonucleic acid
EHT	Experimental hut trial
F1	First filial (first generation offspring)
GC-FID	Gas chromatography with flame ionisation detection
GLP	Good laboratory practice
HPLC–DAD	High-performance liquid chromatography with UV diode array detection
IRM	Insecticide resistance management
IRS	Indoor residual spraying
ISO/IEC	International organization for standardization/international electrotechnical commission
KEMRI	Kenya medical research institute
KEMRI-CGHR	Kenya medical research institute center for global health research
LLIN	Long-lasting insecticidal net
LSD	Latin square design
P450	Cytochrome P450 (enzyme)
PBO	Piperonyl butoxide
PCPB	Pest control products board
PPF	Pyriproxyfen
RH	Relative humidity
s.l.	Sensu lato (in the broad sense)
s.s.	Sensu stricto (in the strict sense)
SE	Standard error
SERU	Scientific and ethical review unit
SVG	Scalable vector graphics
WHO	World Health Organization

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views, decisions, or policies of the U.S. Centers for Disease Control and Prevention.

Author contributions

EO and BA conceived the study. NO, SA and EO designed the study and excuted the trial. VM and BA analysed the data. NO, SA wrote the manuscript. CO, PO, ER, LK, JG, and EO revised the manuscript. All authors reviewed and approved the final manuscript for publication.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Consent for publication
This manuscript has been published with the permission of the KEMRI Director General.

Competing interests
All the authors declared that they have no competing interests.

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References

1. World Health Organization. World malaria report 2022. Geneva: World Health Organization; 2022.
2. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature. 2015;526:207–11.
3. Gimnig JE, Vulule JM, Lo TQ, Kamau L, Kolczak MS, Phillips-Howard PA, et al. Impact of permethrin-treated bed nets on entomologic indices in an area of intense year-round malaria transmission. Am J Trop Med Hyg. 2003;68(4 Suppl):16–22.
4. Hawley WA, Phillips-Howard PA, ter Kuile FO, Terlouw DJ, Vulule JM, Ombok M, et al. Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in western Kenya. Am J Trop Med Hyg. 2003;68(4 Suppl):121–7.
5. Shah MP, Steinhardt LC, Mwandama D, Mzilahowa T, Gimnig JE, Bauleni A, et al. The effectiveness of older insecticide-treated bed nets (ITNs) to prevent malaria infection in an area of moderate pyrethroid resistance: results from a cohort study in Malawi. Malar J. 2020;19:24.
6. Ochomo E, Bayoh NM, Kamau L, Atieli F, Vulule J, Ouma C, et al. Pyrethroid susceptibility of malaria vectors in four districts of western Kenya. Parasit Vectors. 2014;7:310.
7. Gnanguenon V, Azondekon R, Oke-Agbo F, Sovi A, Ossè R, Padonou G, et al. Evidence of man-vector contact in torn long-lasting insecticide-treated nets. BMC Public Health. 2013;13:751.
8. Protopopoff N, Mosha JF, Lukole E, Charlwood JD, Wright A, Mwalimu CD, et al. Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled, two-by-two factorial design trial. Lancet. 2018;391:1577–88.
9. Staedke SG, Gonahasa S, Dorsey G, Kamya MR, Maiteki-Sebuguzi C, Lynd A, et al. Effect of long-lasting insecticidal nets with and without piperonyl butoxide on malaria indicators in Uganda (LLINEUP): a pragmatic, cluster-randomised trial embedded in a national LLIN distribution campaign. Lancet. 2020;395:1292–303.
10. Lukole E, Protopopoff N, Massue DJ, Agaba BB, Gonahasa S, Mosha JF, et al. Protective efficacy of holed and aging PBO-pyrethroid synergist-treated nets on malaria infection prevalence in north-western Tanzania. PLoS Glob Public Health. 2022;2: e0000453.
11. Minakawa N, Kongere JO, Sonye GO, Awuor B, Hu J, Lutiali PA, et al. Long-lasting insecticidal nets incorporating piperonyl butoxide reduce the risk of malaria in children in Western Kenya: a cluster randomized controlled trial. Am J Trop Med Hyg. 2021;105:461–71.
12. Pennetier C, Bouraima A, Chandre F, Piameu M, Etang J, Rossignol M, et al. Efficacy of Olyset® plus, a new long-lasting insecticidal net incorporating permethrin and piperonyl-butoxide against multi-resistant malaria vectors [corrected]. PLoS ONE. 2013;8: e75134.
13. Chaplin M, Choi L, Ranson H. Piperonyl butoxide (PBO) combined with pyrethroids in insecticide-treated nets to prevent malaria in Africa. Cochrane Database Syst Rev. 2021;5:12776.
14. WHO. Guidelines for laboratory and field-testing of long-lasting insecticidal nets. Geneva: World Health Organization; 2013.
15. Clarkson CS, Miles A, Harding NJ, Weetman D, Kwiatkowski D, Donnelly M, et al. The genetic architecture of target-site resistance to pyrethroid insecticides in the African malaria vectors *Anopheles gambiae* and *Anopheles coluzzii*. Mol Ecol. 2021;30:5303–17.
16. Yahouédo GA, Chandre F, Rossignol M, Ginibre C, Balabanidou V, Mendez NGA, et al. Contributions of cuticle permeability and enzyme detoxification to pyrethroid resistance in the major malaria vector *Anopheles gambiae*. Sci Rep. 2017;7:11091.
17. Dada N, Sheth M, Leibman K, Puschhof J, Duguma D, Meisel JS, et al. Whole metagenome sequencing reveals links between mosquito microbiota and insecticide resistance in malaria vectors. Sci Rep. 2018;8:2084.
18. Mechan F, Nabie S, Amoah B, Lynd A, Yewhalaw D, Dengela D, et al. LLIN evaluation in Uganda project (LLINEUP): The fabric integrity, chemical content and bioefficacy of long-lasting insecticidal nets treated with and without piperonyl butoxide across two years of operational use in Uganda. Curr Res Parasitol Vector Borne Dis. 2022;2: 100092.
19. Syme T, Ranson H, Dugassa S, Sanou A, Davies T, Coleman M, et al. Pyrethroid-piperonyl butoxide (PBO) nets reduce the efficacy of indoor residual spraying with pirimiphos-methyl against pyrethroid-resistant malaria vectors. Sci Rep. 2022;12:6857.
20. WHO. Guidelines for malaria 14 March 2023. Geneva: World Health Organization; 2023.
21. Raghavendra K, Barik TK, Sharma P, Bhatt RM, Srivastava HC, Sreehari U, et al. Chlorfenapyr: a new insecticide with novel mode of action can control pyrethroid resistant malaria vectors. Malar J. 2011;10:16.
22. Agumba S, Gimnig JE, Ogonda L, Ombok M, Kosgei J, Munga S, et al. Diagnostic dose determination and efficacy of chlorfenapyr and clothianidin insecticides against *Anopheles* malaria vector populations of western Kenya. Malar J. 2019;18:243.
23. Oxborough RM, Seyoum A, Yihdego Y, Chabi J, Wat'senga F, Agossa FR, et al. Determination of the discriminating concentration of chlorfenapyr (pyrrole) and *Anopheles gambiae sensu lato* susceptibility testing in preparation for distribution of Interceptor® G2 insecticide-treated nets. Malar J. 2021;20:316.
24. N'Guessan R, Odjo A, Ngufor C, Malone D, Rowland M. A chlorfenapyr mixture net Interceptor® G2 shows high efficacy and wash durability against resistant mosquitoes in West Africa. PLoS ONE. 2016;11: e0165925.
25. Bayili K, N'do S, Namountougou M, Sanou R, Ouattara A, Dabiré RK, et al. Evaluation of efficacy of Interceptor® G2, a long-lasting insecticide net coated with a mixture of chlorfenapyr and alpha-cypermethrin, against pyrethroid resistant *Anopheles gambiae* s.l. in Burkina Faso. Malar J. 2017;16:190.
26. Mosha FW, Lyimo IN, Oxborough RM, Matowo J, Malima R, Feston E, et al. Experimental hut evaluation of the pyrrole insecticide chlorfenapyr on bed nets for the control of *Anopheles arabiensis* and *Culex quinquefasciatus*. Trop Med Int Health. 2008;13:644–52.
27. Accrombessi M, Cook J, Ngufor C, Sovi A, Gbénou V, Hounkonnou C, et al. Efficacy of pyriproxyfen-pyrethroid long-lasting insecticidal nets (LLINs) and chlorfenapyr-pyrethroid LLINs compared with pyrethroid-only LLINs for malaria control in Benin: a cluster-randomised, superiority trial. Lancet. 2023;401:435–46.
28. Mosha JF, Kulkarni MA, Lukole E, Matowo NS, Pitt C, Messenger LA, et al. Effectiveness of long-lasting insecticidal nets with pyriproxyfen-pyrethroid, chlorfenapyr-pyrethroid, or piperonyl butoxide-pyrethroid versus pyrethroid only against malaria in Tanzania: final-year results

of a four-arm, single-blind, cluster-randomised trial. Lancet Infect Dis. 2023;23:581–92.
29. WHO. Data requirements and protocol for determining non-inferiority of insecticide-treated net and indoor residual spraying products within an established WHO intervention class. Geneva: World Health Organization; 2019.
30. Agumba S, Onyango SA, Gesicho MB, Ochieng JB, Ogutu N, Owuor K, et al. Experimental hut and field evaluation of a metofluthrin-based spatial repellent against pyrethroid-resistant A*nopheles funestus* in Siaya County, western Kenya. Parasit Vectors. 2024;17:6.
31. Steketee RW, Wirima JJ, Slutsker L, Khoromana CO, Heymann DL, Breman JG. In vivo response of Plasmodium falciparum to chloroquine in pregnant and non-pregnant women in Siaya district, Kenya. Bull World Health Organ. 1987;65:885–90.
32. WHO. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva: World Health Organization; 2016.
33. WHO. Standard operating procedure for testing insecticide susceptibility of adult mosquitoes in WHO bottle bioassays. Geneva: World Health Organization; 2022.
34. Coetzee M. Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). Malar J. 2020;19:70.
35. WHO. Determining non-inferiority of insecticide-treated nets and indoor residual spray products within an established product class. Evidence review group meeting report. Geneva: World Health Organization; 2018.
36. Syme T, Awolola TS, Olayemi IK, Ibeh E, Anogweh I, Egbuche CM, et al. PermaNet Dual, a new deltamethrin-chlorfenapyr mixture net, shows improved efficacy against pyrethroid-*resistant Anopheles gambiae sensu lato* in southern Benin. Sci Rep. 2023;13:12232.
37. Tungu PK, Malima R, Mosha FW, Lyimo I, Maxwell C, Kaur H, et al. Efficacy of interceptor® G2, a long-lasting insecticide mixture net treated with chlorfenapyr and alpha-cypermethrin against *Anopheles funestus*: experimental hut trials in north-eastern Tanzania. Malar J. 2021;20:180.
38. WHO. Determining non-inferiority of insecticide-treated nets and indoor residual spray products within anestablished product class. Evidence Review Group meeting report 5–6 July 2018 Geneva, Switzerland. Geneva: WorldHealth Organization; 2018
39. Ochomo E, Bayoh MN, Brogdon WG, Gimnig JE, Ouma C, Vulule JM, et al. Pyrethroid resistance in *Anopheles gambiae* s.s. and *Anopheles arabiensis* in western Kenya: phenotypic, metabolic and target site characterizations of three populations. Med Vet Entomol. 2013;27:156–64.
40. Morgan JC, Irving H, Okedi LM, Steven A, Wondji CS. Pyrethroid resistance in an *Anopheles funestus* population from Uganda. PLoS ONE. 2010;5: e11872.
41. Djouaka R, Irving H, Tukur Z, Wondji CS. Exploring mechanisms of multiple insecticide resistance in a population of the malaria vector *Anopheles funestus* in Benin. PLoS ONE. 2011;6: e27760.
42. Coetzee M, Koekemoer LL. Molecular systematics and insecticide resistance in the major African malaria vector *Anopheles funestus*. Annu Rev Entomol. 2013;58:393–412.
43. WHO. Data requirements and protocol for determining non-inferiority of insecticide-treated net and indoor residual spraying products within an established WHO policy class. Geneva: World Health Organization; 2019.
44. Zahouli JZB, Koudou BG, Müller P, Malone D, Tano Y, Utzinger J. Small-scale field evaluation of PermaNet® Dual (a long-lasting net coated with a mixture of chlorfenapyr and deltamethrin) against pyrethroid-resistant *Anopheles gambiae* mosquitoes from Tiassale. Cote d'Ivoire Malar J. 2023;22:36.

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