Malaria Journal

RESEARCH

Open Access

The non-inferiority of piperonyl-butoxide Yorkool® G3 insecticide-treated nets compared to Olyset®Plus measured by *Anopheles arabiensis* mortality in experimental huts in Tanzania

Olukayode G. Odufuwa[1,2,3,4*], Masudi Suleiman Maasayi[1,5], Emmanuel Mbuba[1,2,3], Watson Ntabaliba[1], Rose Philipo[1], Safina Ngonyani[1], Ahmadi Bakari Mpelepele[1,5], Isaya Matanila[1], Hassan Ngonyani[1], Jason Moore[1,2,3], Yeromin P. Mlacha[1], Jennifer C. Stevenson[1,2,3] and Sarah Jane Moore[1,2,3,5]

Abstract

Background Non-inferiority trials are recommended by the World Health Organization (WHO) to demonstrate that health products show comparable efficacy to that of existing standard of care. As part of the WHO Global Malaria Programme (GMP) process of assessment of malaria vector control products, a second-in-class insecticide-treated net (ITN) must be shown to be non-inferior to a first-in-class product based on mosquito mortality. The public health impact of the first-in-class pyrethroid-piperonyl butoxide (PBO) ITN, Olyset® Plus, has been demonstrated in epidemiological trials in areas with insecticide-resistant mosquitoes, but there is a need to determine the efficacy of other pyrethroid-PBO nets to ensure timely market availability of nets in order to increase access to ITNs. The non-inferiority of a deltamethrin-PBO ITN Yorkool® G3 was evaluated entomologically against Olyset® Plus in experimental huts in Tanzania, following WHO guidelines for non-inferiority trials.

Methods The trial of the two pyrethroid-PBO ITNs was conducted in experimental huts in Lupiro, Tanzania, using a randomized 7 × 7 Latin square block design. The study ran for 49 nights in 14 huts assessing the mosquito mortality and blood-feeding of wild, free-flying, pyrethroid-resistant *Anopheles arabiensis*. Using the non-inferiority approach, the comparative efficacy (primary endpoint was mosquito mortality at 24 h and secondary endpoint was blood-feeding) of unwashed and 20 times field-washed pyrethroid-PBO Yorkool® G3 ITNs, were compared with the first-in-class product Olyset® Plus and against a pyrethroid-only ITN, PermaNet® 2.0 ITNs, as a standard comparator.

Results The experimental hut trial demonstrated non-inferiority and superiority of Yorkool® G3 to Olyset® Plus based on mosquito mortality [51% vs. 39%, OR 1.68 (95% CI 1.50–1.88)], given that lower 95% CI exceeded 0.74 (delta of 39%) and the margin of no difference (1). Blood-feeding inhibition was high for all treated ITNs (> 90%) and Yorkool® G3 was non-inferior to Olyset® Plus [4% vs. 2%, OR 1.81 (95% CI 1.46–2.39)], given that upper 95% CI was less than 4.85 (delta of 4%). The pyrethroid-PBO ITNs were superior to the pyrethroid-only net, PermaNet® 2.0, as determined by both the proportion of mortality and blood-feeding of mosquitoes (p-value < 0.05).

*Correspondence:
Olukayode G. Odufuwa
oodufuwa@ihi.or.tz
Full list of author information is available at the end of the article

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Conclusion Yorkool® G3 ITNs demonstrated non-inferiority to the first-in-class Olyset® Plus and superiority over the standard pyrethroid-only ITN, PermaNet® 2.0 as measured by mortality and blood-feeding inhibition of wild pyrethroid-resistant *An. arabiensis* mosquitoes. Yorkool® G3 ITNs are potential tools for the control of metabolic insecticide-resistant malaria vectors, and their market availability will contribute to the cost-effective selection of ITNs by malaria control programmes to improve population access to ITNs.

Keywords ITN, Non-inferiority, Insecticide resistance, Pyrethroid, PBO, Yorkool® G3, Mosquito, Malaria, Experimental hut, Tanzania

Background

Vector control, primarily through the use of insecticide-treated nets (ITNs) is the cornerstone in the fight against malaria transmission [1]. However, due to the extensive use of insecticides for mosquito control and agricultural practices [2, 3], mosquitoes have developed resistance to the pyrethroids used on ITNs [4, 5]. Nets treated with both a pyrethroid and a synergist—piperonyl butoxide (PBO) have been developed to counteract metabolic resistance in mosquitoes [6]. The PBO inhibits the action of the metabolic enzymes that detoxify pyrethroids [7]. Epidemiological data from a cluster-randomized trial conducted on the pyrethroid-PBO net (Olyset® Plus) in Tanzania demonstrated a 33% reduction in malaria prevalence over 21 months relative to a standard pyrethroid-only net (Olyset®), in an area with pyrethroid-resistant mosquitoes [8]. Therefore, in 2017 the World Health Organization (WHO) identified PBO-incorporated nets as a new class of ITNs [9], with Olyset® Plus being the first-in-class (FIC) product prequalified by the WHO [9].

The WHO recommends that for new pyrethroid-PBO ITNs to be listed as second-in-class (SIC) products, non-inferiority trials using experimental huts may be used as a means of evaluation [10]. Mathematical modelling of entomological data from experimental hut trials has demonstrated that such studies can be used to predict the epidemiological effect of ITNs, thus supporting non-inferiority trials for the evaluation of novel ITNs [11]. Given that the FIC pyrethroid-PBO ITN (positive control) has been demonstrated to have an impact on malaria prevalence [12, 13], non-inferiority entomological trials that follow established guidelines and procedures [14, 15], can be used as a proxy (similar to clinical surrogates) to determine whether a new product would produce similar epidemiological results. Such entomological trials can be conducted in a shorter timeframe compared to epidemiological trials, thereby bringing new products to market sooner and so increasing access to ITNs.

In a WHO-recommended experimental hut non-inferiority trial, new vector control products should demonstrate comparable entomological performance to the WHO-prequalified FIC or SIC product with the same entomological mode of action as that of the product that is under investigation [14, 15]. Performance is primarily assessed by mortality of malaria vectors with blood-feeding as an additional endpoint [14]. In the assessment, it is also necessary to determine the susceptibility of the wild test mosquitoes to this class of insecticides through the exposure of mosquitoes to a standard comparator. For the assessment of pyrethroid-PBO products, a WHO-prequalified pyrethroid-only ITN is used to demonstrate the additional benefit of PBO. In addition, an untreated net (negative control) should be included in the study to assess the quality of the experiment. The candidate product should demonstrate superior efficacy over the standard comparator and untreated net [14–16].

A new ITN incorporated with pyrethroid insecticide (deltamethrin) and the synergist PBO named Yorkool® G3, has been developed by Yorkool International Trading Co. Ltd, China, as an additional product in the pyrethroid-PBO ITN class. This paper presents experimental hut entomological studies to assess the non-inferiority of Yorkool® G3 pyrethroid-PBO nets to the FIC product, Olyset® Plus, and superiority compared to a standard pyrethroid-only net, PermaNet® 2.0 following WHO procedures [14–16].

Methods

Study area

The experimental hut trial of Yorkool® G3 nets was carried out in Lupiro village (8.385°S and 36.670°E), which is located in Ulanga district, Morogoro region in the south-eastern part of Tanzania (Fig. 1, in grey). The yearly rainfall in the Morogoro region ranges from 1200 to 1800 mm, with temperatures varying between 20 °C and 34 °C. The most abundant malaria vector in the area is *Anopheles (An.) arabiensis* which is resistant to pyrethroids, while pre-exposure to PBO restores susceptibility to pyrethroids and bendiocarb [17]. The main malaria control intervention in the area is ITNs [18].

Quality assessment of Yorkool® G3 nets was performed at the Vector Control Product Testing Unit (VCPTU) facility that is accredited (SANAS GLP0003) for Good Laboratory Practice, and situated at the Bagamoyo branch of Ifakara Health Institute (IHI) in Bagamoyo district of Tanzania (Fig. 1, in green).

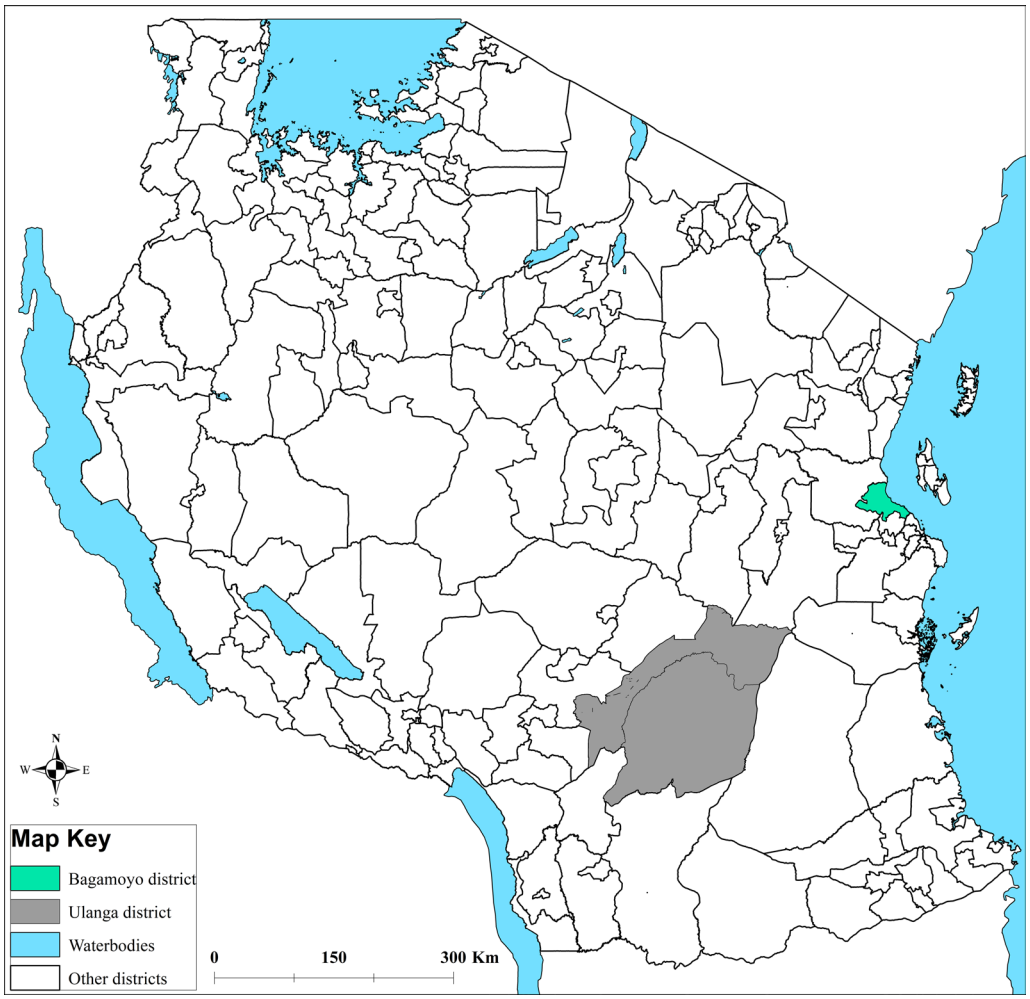

Fig. 1 Geographical location of study area

Study design

The experimental hut trial was executed between September and October 2020. The trial implemented two contiguous 7×7 Latin Squares designs in fourteen huts, commonly referred to as the "Williams Design" [19]. Both nets and volunteers were randomly assigned to huts on the first day, with a sequential rotation strategy employed thereafter, with a daily rotation of volunteers and a rotation of nets at the end of each round (7 nights). Each treatment condition was tested 98 times over the course of 49 nights, following WHO guidelines [14, 15], as depicted in Fig. 2.

Description of test items

Yorkool® G3 ITNs are made of 130 denier yarn, polyethylene fibres coated with 120 milligrams per square metre (mg/m^2) deltamethrin and 440 mg/m^2 piperonyl butoxide (PBO). It was developed by Yorkool International Trading Co. Ltd, China. Olyset® Plus is a knitted monofilament polyethylene net of 150 denier yarn, treated with 20 grams of Active Ingredient per kilogram (g AI/kg), equivalent to 800 mg AI/m^2 permethrin and PBO content of 10 g PBO/kg (400 mg PBO/m^2). The net is manufactured by Sumitomo Chemical Co., Ltd, Japan [20]. The standard comparator ITN used was PermaNet® 2.0, a 55 mg/m^2 deltamethrin-coated ITN manufactured by Vestergaard Frandsen SA [21]. To assess the quality of the experiment, Safi Net was used as a negative control, an untreated polyester net manufactured by A to Z Textiles Mills Ltd, Tanzania.

Mosquitoes

The experimental hut trial used free-flying wild pyrethroid-resistant *An. arabiensis* (Lupiro strain) mosquitoes. The resistance of these mosquitoes is attributed to the overexpression of CYP450 enzymes [22]. Sugar-fed 2–5 day old, insecticide-susceptible *An. gambiae* sensu stricto (*s.s.*) (Ifakara strain) and pyrethroid-resistant *An.*

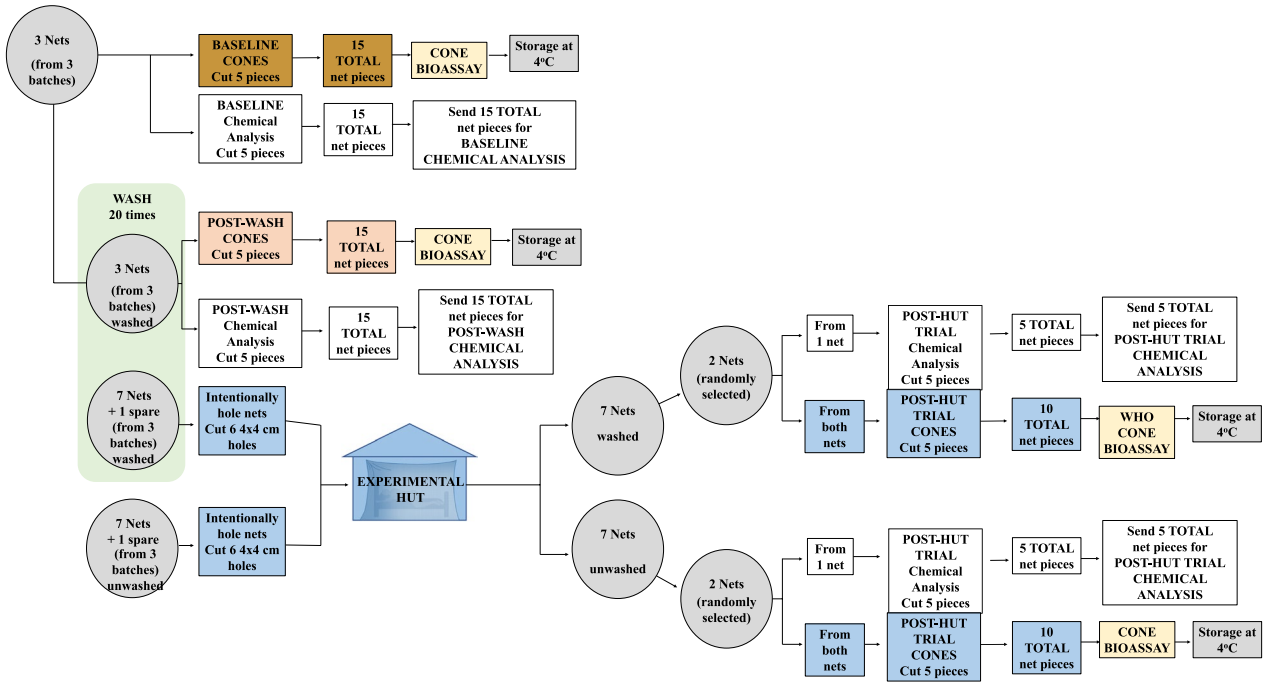

Fig. 2 Experimental hut study design

arabiensis (Kingani strain) mosquitoes were used for the quality of nets' bioefficacy before and after the experimental hut trial (supplementary file 1 and 2 for insecticide susceptibility tests conducted at the time of the study). All mosquito colonies are maintained in insectaries at the VCPTU, Bagamoyo, following standard operating procedures (SOPs) adapted from the MR4 guidelines [23].

Net preparation

Nineteen Yorkool® G3 ITNs from three production batches were prepared for the experimental hut trial (Fig. 2) as follows:

- Three nets (one per production batch), samples (25 cm x 25 cm) were removed when nets were unwashed and after 20x-washed for baseline quality checks of bioefficacy.
- Sixteen nets (from across the three production batches), eight of which remained unwashed, and eight were washed 20 times. Per wash status, seven nets were used each for the experimental hut trials with a spare in case of need of replacement.

For the pyrethroid-PBO positive control net and the standard positive control pyrethroid-only net, an equivalent number of nets from one production batch was used.

Nets selected for washing, including those selected for quality checks, were field-washed at their appropriate wash interval day (one day for Yorkool® G3 and PermaNet® 2.0 and two days for Olyset® Plus [20]) in the facilities in Bagamoyo. Nets were washed in aluminium bowls containing 10 litres (L) of filtered well water with a maximum hardness of 5dH and containing 2 gram per litre of soap ("Jamaa palm oil" soap flakes), using manual agitation of 20 rotations per minute (min) for 10 min. Rinsing was done twice using filtered clean well water. Nets were dried horizontally in the shade then wrapped in foil and stored in labelled plastic bags at 21.4–30.9 °C in the IHI store between washes. The procedure was repeated twenty times for all ITNs.

Each net was assigned a unique four-digit code generated by personnel not involved in the study, ensuring that investigators, technicians, and participants were blinded to the type of ITN. Before testing in the huts, all nets (unwashed and 20×washed) were deliberately holed. Holes each measuring 4 cm×4 cm were made on each of the four sides of the net: two holes at the centre of each of the long side panels, and one hole at the centre of the short panels. Seven nets per arm, plus a spare, were transferred to the net storage facilities in Lupiro where the experimental hut trial was conducted.

For chemical analysis, five samples of netting measuring 25 cm×25 cm were cut from the three unwashed and washed nets used for bioefficacy, to give 15 netting

samples for each wash status to be studied before the experimental hut trial began. On completion of the hut trial, five samples cut from two unwashed and two washed nets were used. Net samples for chemical analyses and bioassays were cut adjacent to one another [24]. All net samples intended for chemical testing were promptly wrapped in aluminium foil after cutting and stored in a refrigerator at 4 ± 2 °C for two weeks before they were shipped to the International Institute of Biotechnology and Toxicology (IIBAT) in India for analysis.

Procedures for experimental hut trial

The experiments were carried out in 14 Ifakara experimental huts as detailed in a previous study [25]. The trial enrolled 14 consenting adult male volunteers to occupy the huts at night. The trial had seven arms: (i) Yorkool® G3, unwashed, (ii) Yorkool® G3, washed 20× at a one-day wash interval, (iii) Olyset® Plus, unwashed, (iv) Olyset® Plus, washed 20× at a two days wash interval, (v) PermaNet® 2.0, unwashed, (vi) PermaNet® 2.0, washed 20× at a one-day wash interval, and (vii) untreated Safi Net as negative control. The primary outcome was the proportion of 24 hour(h) mortality (M24) of mosquitoes and the secondary outcome was the proportion of mosquito blood-feeding. Data were collected for seven nights, with a one-night airing period between rounds to reduce any chance of any residual effects before introducing the next treatment. During the study, technicians hung nets in the experimental huts before 19:00 h and removed nets after the collection of mosquitoes in the morning at 6:00 h. The volunteers adhered to a predetermined roster as they sequentially rotated among the huts. They entered their assigned huts at 19:00 h and slept under the nets until 06:00 h the following morning. At 06:00 h, mosquitoes were collected from (1) inside the nets, (2) the floor, walls, and ceiling, and (3) exit traps, using aspirators. Subsequently, the collected mosquitoes were sorted and categorized based on their location, and status (dead and fed, dead and unfed, alive and fed, and alive and unfed). These mosquitoes were then held in a temperature-controlled room for 24-h with access to a 10% sugar solution at 27.3–28 °C temperature and 58.7–64.9% relative humidity, to assess mortality at 24 h after capture.

Procedures for experimental hut trial quality checks: bioefficacy and chemical analysis

Prior to the experimental hut trial, pre-test quality checks were conducted to assess the bioefficacy [≥ 95% knockdown at 60 minutes (KD60)/≥ 80% M24)] using cone bioassay, and nets were stored for chemical analysis of the insecticide on the net at time 0, the day before the first wash (Fig. 2). Bioefficacy and storage of samples for chemical analysis tests were again carried out after completing the necessary 20 field washes. Post-experimental checks were conducted at the end of the hut trial using the nets evaluated in the huts (both unwashed and 20× washed) against susceptible *An. gambiae s.s.* (Ifakara strain), and strongly pyrethroid-resistant *An. arabiensis* (Kingani strain) was exposed to nets using cone bioassay according to the WHO procedures [15], while samples for chemical content were shipped to IIBAT for insecticide chemical analysis.

Data analysis

Before the experimental hut trial, the power of the study was estimated using estimates from an earlier trial conducted in the same site following the previous WHO non-inferiority guidelines [26]. Using R software [27], a generalized linear mixed model (GLMM) simulated 1000 times was used to determine the study power for a 7×7 arm trial in 14 huts for 49 nights. The following parameters were accounted for in the model: an estimated median number of 28 mosquitoes per night per hut, 22% mortality for the unwashed pyrethroid-PBO products and 14% mortality for the washed pyrethroid-PBO products, overall study variation of log of 1.034 with treatment, volunteer, and hut adjusted for fixed effects, and non-inferiority margin of 0.7 OR (a margin selected to detect the smallest effect size of 5% difference between products, and maintaining a balance between study feasibility and public health implication of failing to detect an inferior product [26]).

Statistical analysis was conducted using STATA 16 software [28]. Descriptive analysis was conducted for cone bioassays for the primary outcomes, KD60, and M24 for quality checks. Results were presented as arithmetic mean percentages with 95% Confidence Intervals (CI).

Outcomes (both proportion of 24-h mortality and blood-feeding) in the experimental hut trial were analysed using binomial logistic regression. For the non-inferiority analyses, 1) unwashed Yorkool® G3 nets were compared with unwashed Olyset® Plus nets; 2) 20× washed Yorkool® G3 nets were compared with 20× washed Olyset® Plus nets; and 3) a separate comparison of the average value of the outcomes for unwashed and 20× washed nets was conducted. The delta to assess the non-inferiority for 24-h mortality was estimated using: $\frac{x-7}{100-(x-7)} / \frac{x}{100-x}$, where x was the 24-h mortality of mosquitoes captured in the huts with the comparator pyrethroid-PBO nets, Olyset® Plus. The delta for blood-feeding was estimated using: $\frac{x+7}{100-(x+7)} / \frac{x}{100-x}$, where x was the proportion of blood-feeding of mosquitoes captured from the huts with Olyset® Plus net. Non-inferiority was established when the lower confidence interval model-estimated odds ratio (OR) of Yorkool®

G3 nets compared to Olyset® Plus nets was not lower than the delta of the proportion of mosquito mortality, and when the upper confidence interval of the OR of the Yorkool® G3 nets in comparison to Olyset® Plus nets was not higher than the delta of the proportion of mosquito blood-feeding.

For the superiority analysis, the following comparisons were made for each of the study outcomes: 1) unwashed Yorkool® G3 and unwashed Olyset® Plus nets compared to unwashed PermaNet® 2.0 nets; 2) 20×washed Yorkool® G3 and 20×washed Olyset® Plus nets compared to 20×washed PermaNet® 2.0 nets; and 3) the average value of the study outcomes for unwashed and 20×washed pyrethroid-PBO nets against pyrethroid nets. The pyrethroid-PBO products were deemed superior when the OR for the proportion of 24-h mortality was higher than 1.00 and the p-value < 0.05, and the OR was lower than 1.00 and the p-value < 0.05 for the proportion of mosquitoes' blood-feeding.

In these model analyses, fixed effects included the treatment, hut, volunteer, and day of sleeping in the huts following the WHO guidelines [14]. For the analysis performed on the average of the outcomes, the wash status (unwashed and 20×washed) of the net was added as a fixed effect.

A post hoc simulation was conducted to check the power of the study following the new guideline [14]. The new guidelines detailed the change of the threshold of non-inferiority from a fixed 0.7 OR to a fixed 7% difference in the proportion of mosquito mortality. This was done by simulating a generalized linear mixed model a thousand times, with treatment, volunteer, hut, and day adjusted as fixed effects using R software [27]. Actual estimates of the study were used in the simulation: a median number of 26 mosquitoes per hut, overall study variation of 93%, and 40% dispersion of mosquitoes following a negative binomial distribution. For unwashed condition, 53% mortality from Yorkool® G3 and 44% for Olyset® Plus, and for 20×washes, 49% mortality from Yorkool® G3 and 35% mortality for Olyset® Plus nets were used in the simulation. Data were interpreted following CONSORT guidance [Piaggio 2012] and figures were drawn using watermelon plots following WHO Guidance [14].

Results

Experimental hut study power

Before the experimental hut trial, the power of the study was estimated to be 97% for unwashed nets and 82% for 20×washed nets. Following the latest guideline, the post hoc study power was 98.4% for unwashed nets and 99.9% for 20×washed nets.

Experimental hut trial

Baseline information

Night-time temperature ranged between 21.0 and 32.3 °C and relative humidity was between 45 and 85% in Lupiro during the study period. Nets were stored at room temperature (< 32 °C) throughout the trial. The experimental hut trial started four days after the last wash (washing ended on the 29th of August 2020 and the trial started on the 2nd of September 2020). A total number of 21,974 *An. arabiensis* mosquitoes were collected over 49 nights of data collection. The median number of mosquitoes captured per night per hut was 26 (Interquartile range (IQR):14–43) but was heterogeneous and varied by product (Fig. 3).

Mosquito Mortality at 24 hours

Yorkool® G3 nets induced significantly higher mosquito mortality at 24-h [50.9% (95% confidence interval (CI): 47.5–54.3)] than all other ITNs: Olyset® Plus [39.2% (95% CI 36.1–42.3)], PermaNet® 2.0 [27.2% (95% CI 24.7–29.7)] and untreated net [11.1% (95% CI 8.1–14.1)] (Fig. 4). Based on the WHO non-inferiority margin of 7% difference in mosquito mortality, Yorkool® G3 was non-inferior and superior to Olyset® Plus nets [odds ratio (OR): 1.78 (95% CI 1.65–1.92)], as the lower confidence interval value was greater than delta ($\delta = 0.74$), with the direction of effect being higher for both the unwashed and 20×washed Yorkool® G3 nets over Olyset® Plus nets (Fig. 5 and Table 1). Furthermore, a superiority analysis demonstrated that pyrethroid-PBO ITNs had a higher killing effect than pyrethroid-only ITNs of all wash conditions (Table 2). This was also seen for ITNs over untreated nets (the negative control) (Table 2).

Mosquito Blood-feeding

The proportions of mosquito blood-feeding were substantially lower for all treated nets than those of the untreated nets, with all ITNs inducing more than 90% feeding inhibition (Fig. 6). The lowest proportions of blood-feeding were seen for Olyset® Plus nets (Fig. 6). Yorkool® G3 nets were non-inferior [OR 1.87 (95% CI 1.46–**2.39**)] to Olyset® Plus nets; as the upper confidence interval was below the non-inferiority margin. but the difference was statistically significant as the lower confidence interval was above 1 (Table 1, Fig. 7). As was seen for mosquito mortality in the superiority analysis, pyrethroid-PBO ITNs reduced significantly more blood-feeding than the pyrethroid-only ITNs (Table 2), indicating the additional benefit of the PBO synergist. Likewise, all ITNs significantly reduced *An. arabiensis* blood-feeding compared to untreated net (Table 3).

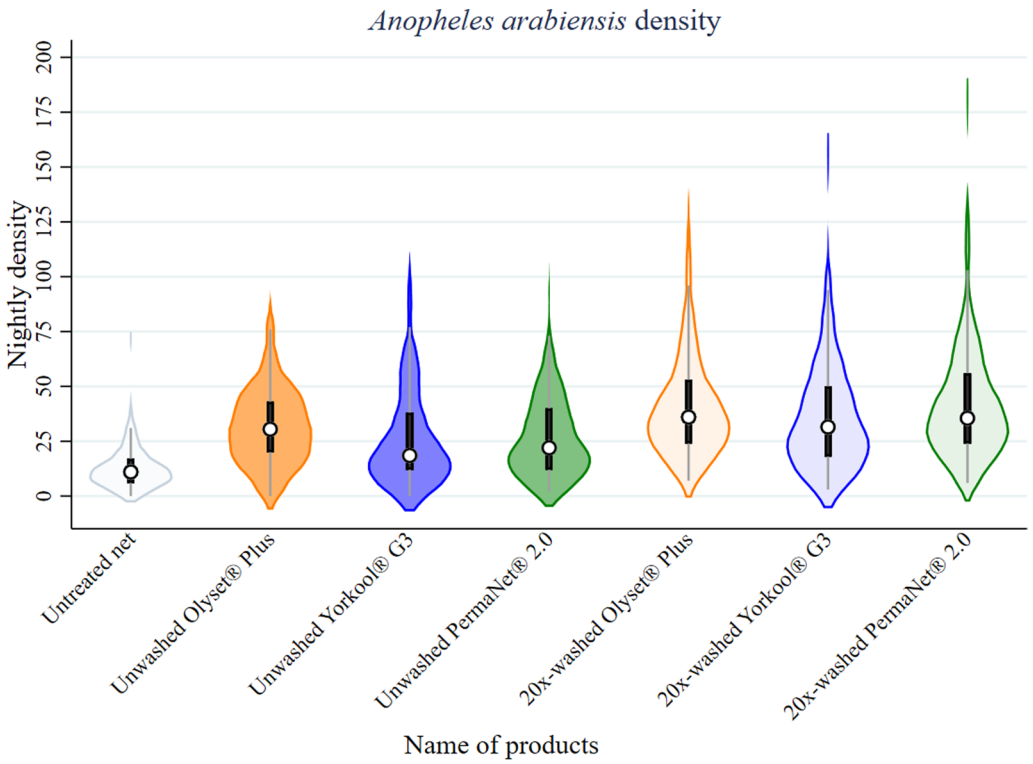

Fig. 3 Violin plot of the distribution of numbers of female *Anopheles arabiensis* mosquitoes collected per hut-night. Filled violins: Unwashed nets; unfilled violins: 20 × washed nets. Green: pyrethroid-only nets, orange: first-in-class pyrethroid-PBO nets, blue: pyrethroid-PBO candidate nets

Quality assurance bioefficacy

All the samples (25 cm x 25 cm) of both unwashed and 20x-washed Yorkool® G3 nets which were tested before (15 out of 15 samples) and after (10 out of 10 samples) the experimental hut trial met acceptable quality performances of ≥ 95% mosquito knockdown after 60 min or ≥ 80% mosquito mortality at 24 h after exposure (WHO criterion for bioefficacy [16]) of the laboratory-reared resistant *An. arabiensis* and susceptible *An. gambiae* mosquitoes. This was also seen for the pyrethroid-only ITNs before and after the trial on susceptible *An. gambiae* mosquitoes. However, for the pyrethroid-PBO positive control, Olyset® Plus nets, quality assurance was met based on the proportion of knockdown (KD60) for unwashed condition only, and not on 20x-washed condition, indicating that Olyset® Plus net was not as wash resistant, Table 4.

Chemical content

The chemical content of all the nets tested before washing was within 25% ± of the target dose as per WHO guidelines [16]. However, after washing, only Yorkool® G3 nets, both the deltamethrin and PBO content, were within the target dose, while the pyrethroid and PBO AI target doses of none of the other positive controls were within the range. Although all the nets had high (> 95%) wash resistance indices, washing resulted in a lower concentration of the active ingredients with Yorkool® G3 nets retaining 94% and 76% of deltamethrin and PBO, respectively, Olyset® Plus 69% of permethrin and 57% of PBO, and PermaNet® 2.0 61% of deltamethrin. The greater retention of the pyrethroid compared to PBO indicates that the PBO is lost more quickly after 20 washes (Table 5).

Discussion

Yorkool® G3 nets, treated with deltamethrin and PBO are considered a new product under the second class of nets described by the WHO, 'ITNs designed to kill host-seeking insecticide-resistant mosquitoes', and that provides greater protection than pyrethroid-only nets [15]. For prequalification of this product by the WHO, the study was conducted to investigate the entomological efficacy of Yorkool® G3 nets in experimental huts in Tanzania according to WHO prequalification guidance [15], and the non-inferiority guidelines of Global Malaria Programme (GMP) at the WHO [14] with a conservative budget to fulfil both of their requirements.

Yorkool® G3 nets were found to be non-inferior and superior to the first-in-class pyrethroid-PBO ITNs,

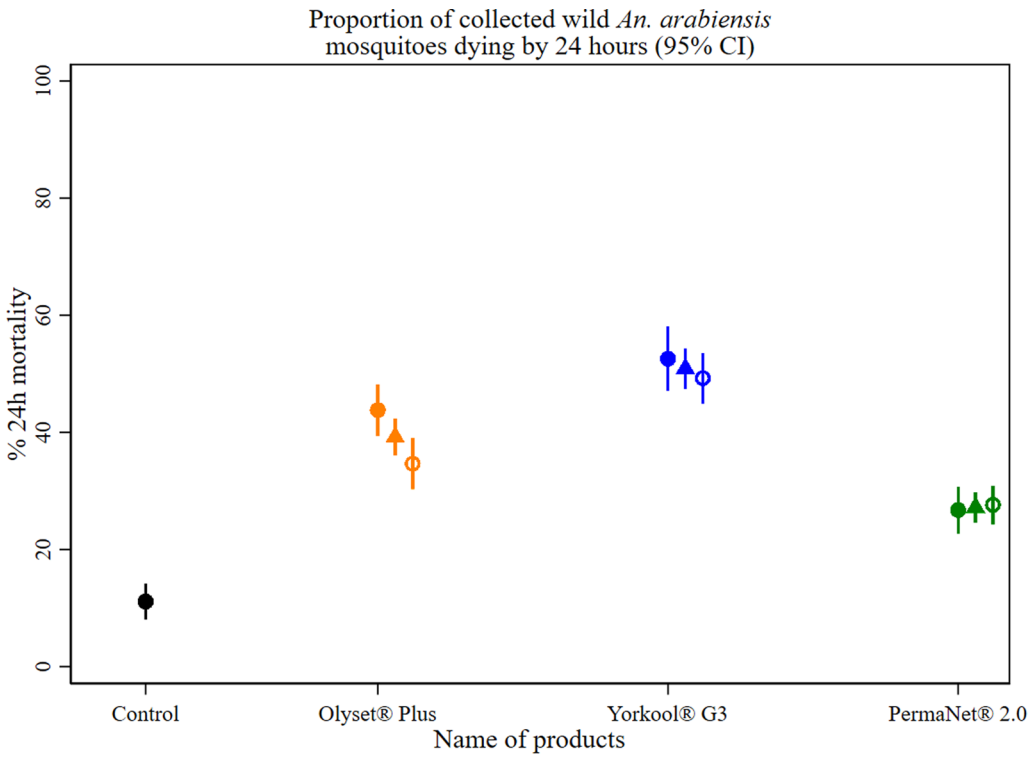

Fig. 4 The proportion of female *Anopheles arabiensis* mosquitoes dying at 24 h (95% CI) after collection from experimental huts. Circles represent unwashed, hollow circles 20x-washed and triangles unwashed and 20x-washed combined

Olyset® Plus, in terms of inducing additional mosquito mortality. Reasons for this finding could be attributed to the different pyrethroids used in each net. Olyset® Plus nets are treated with permethrin which is known to be irritant [29]. This may result in less contact with the net and, therefore, less time to pick up a lethal dose, compared to deltamethrin that is a contact toxicant and less irritant than permethrin. The lower blood-feeding inhibition of Yorkool® G3 nets compared to Olyset® Plus nets is consistent with a recent study comparing Olyset® Plus with another deltamethrin-PBO net, PermaNet® 3.0 [30], which was also attributed to the effect of the different pyrethroids used. Although, the WHO makes its recommendations primarily on the basis of mosquito mortality. For an ideal controlled experiment, products with identical chemicals should be used, however, the availability of such products may be limited particularly if manufacturers are reluctant to provide their net for testing. For quality purposes, nets used in non-inferiority analyses should be sourced from the manufacturers directly.

Entomological efficacy of enhanced mortality and reduced blood-feeding induced by the addition of PBO to pyrethroid ITNs to metabolic pyrethroid-resistant malaria vectors was demonstrated in this study for both PBO products, confirming the utility of such nets for malaria control. Similar effects have been reported in previous experimental hut trials in northern Tanzania [31], Benin [13, 30, 32], Burkina Faso [33], and Côte d'Ivoire [34]. However, the magnitude of the observed effects can vary depending on factors such as hut design, location, and the resistance intensity and mechanism of the vector in different areas [35, 36]. The current study was conducted in an area where *An. arabiensis* is the predominant vector with metabolic resistance to pyrethroid insecticides [17].

The epidemiological efficacy of pyrethroid-PBO nets has been confirmed in large-scale cluster randomized controlled trials in Tanzania and Uganda [8, 37]. Given the comparable performance of Yorkool® G3 nets to the first-in-class pyrethroid-PBO ITN, Olyset® Plus, the net evaluated in these trials, it could be assumed that Yorkool® G3 nets would also be of public health value, however community durability studies are needed to ensure that the ITNs provide sustained protection and remain cost-effective over three years, usually the interval of ITN campaigns [38]. Durability in the field (the strength of the fabric and insecticide retention) of an ITN is not equal to the simulated 'aging' of the nets through washing (20x-washes). Factors that affect net durability when used in the community, and not accounted for

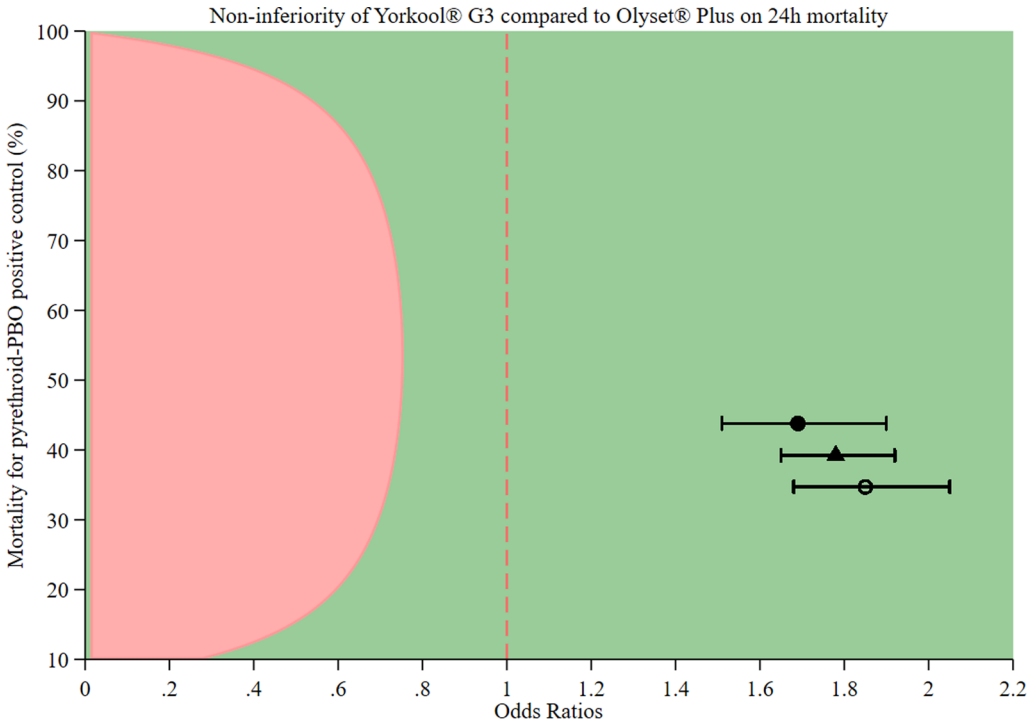

Fig. 5 Mortality (95% CI) of female *Anopheles arabiensis* mosquitoes at 24 h after collection from experimental huts demonstrating non-inferiority and superiority of Yorkool® G3 compared to Olyset® Plus nets. Circles represent unwashed, hollow circles 20x-washed and triangles unwashed and 20x-washed combined. The pink semi-circle represents the non-inferiority margin (delta). Lower CI must fall above delta to be deemed non-inferior. Lower CI must be above both delta and 1 to be superior. If the product is inferior upper CI must fall below delta

Table 1 Non-inferiority analysis of pyrethroid-PBO nets: Yorkool® G3 compared to Olyset® Plus

Outcomes	Products	Condition	Total recaptured	Total outcome	% Arithmetic mean (95% CI)	Delta for 7% difference	OR (95%CI)	Test outcome
Mortality at 24 h. (Primary Outcome)	Olyset® Plus	Pooled*	7356	2754	39.2 (36.1–42.3)	0.74	1	
	Yorkool® G3		6340	3243	50.9 (47.5–54.3)		1.78 (**1.65**–1.92)	Non-inferior and superior
	Olyset® Plus	Unwashed	3154	1395	43.8 (39.5–48.1)	0.75	1	
	Yorkool® G3		2756	1570	52.6 (47.2–57.9)		1.69 (**1.51**–1.90)	Non-inferior and superior
	Olyset® Plus	20×washed	4202	1359	34.7 (30.4–38.9)	0.72	1	
	Yorkool® G3		3584	1673	49.3 (45.0–53.5)		1.85 (**1.68**–2.05)	Non-inferior and superior
Blood-feeding (Secondary outcome)	Olyset® Plus	Pooled*	7356	117	2.0 (1.4–2.6)	4.85	1	
	Yorkool® G3		6340	168	4.1 (2.9–5.3)		1.87 (1.46–**2.39**)	Non-inferior
	Olyset® Plus	Unwashed	3154	43	1.7 (0.9–2.4)	4.85	1	
	Yorkool® G3		2756	77	5.5 (3.2–7.7)		2.55 (1.73–**3.75**)	Non-inferior
	Olyset® Plus	20×washed	4202	74	2.3 (1.4–3.2)	4.85	1	
	Yorkool® G3		3584	91	2.8 (2.0–3.6)		1.49 (1.08–**2.05**)	Non-inferior

*unwashed and washed nets combined

Odds ratio for the effect of Yorkool® G3 ITNs compared to the pyrethroid-PBO positive control, Olyset® Plus, estimated using logistic regression adjusting for the effect of volunteer, day, and hut as fixed effects. For the pooled analysis, wash condition was adjusted for as a fixed effect. For 24-h mortality, lower margin (presented in bold) of the confidence interval must not be lower than the delta. For blood-feeding, upper margin (presented in bold) of the confidence interval must not be higher than the delta

Table 2 Superiority analysis of pyrethroid-PBO products: pyrethroid-PBO nets compared to PermaNet® 2.0 nets

Outcomes	Product	Condition	Total recaptured	Total outcome	% Arithmetic mean (95%CI)	OR (95%CI)	*p*-value	Test outcome
Mortality at 24 h (Primary Outcome)	PermaNet® 2.0	Pooled*	7001	1992	27.2 (24.7–29.7)	1		
	Olyset® Plus		7356	2754	39.2 (36.1–42.3)	1.63 (1.52–1.76)	< 0.0001	Superior
	Yorkool® G3		6340	3243	50.9 (47.5–54.3)	2.91 (2.69–3.14)	< 0.0001	Superior
	PermaNet® 2.0	Unwashed	2730	770	26.7 (22.9–30.6)	1		
	Olyset® Plus		3154	1395	43.8 (39.5–48.1)	2.16 (1.93–2.43)	< 0.0001	Superior
	Yorkool® G3		2756	1570	52.6 (47.2–57.9)	3.66 (3.24–4.13)	< 0.0001	Superior
	PermaNet® 2.0	20 × washed	4271	1222	27.6 (24.4–30.8)	1		
	Olyset® Plus		4202	1359	34.7 (30.4–38.9)	1.33 (1.20–1.47)	< 0.0001	Superior
	Yorkool® G3		3584	1673	49.3 (45.0–53.5)	2.46 (2.23–2.72)	< 0.0001	Superior
Blood-feeding (Secondary outcome)	PermaNet® 2.0	Pooled*	7001	282	5.2 (4.0–6.3)	1		
	Olyset® Plus		7356	117	2.0 (1.4–2.6)	0.36 (0.29–0.45)	< 0.0001	Superior
	Yorkool® G3		6340	168	4.1 (2.9–5.3)	0.68 (0.55–0.83)	< 0.0001	Superior
	PermaNet® 2.0	Unwashed	2730	117	6.2 (4.2–8.3)	1		
	Olyset® Plus		3154	43	1.7 (0.9–2.4)	0.28 (0.19–0.40)	< 0.0001	Superior
	Yorkool® G3		2756	77	5.5 (3.2–7.7)	0.71 (0.52–0.96)	0.027	Superior
	PermaNet® 2.0	20 × washed	4271	165	4.2 (3.2–5.2)	1		
	Olyset® Plus		4202	74	2.3 (1.4–3.2)	0.44 (0.33–0.58)	< 0.0001	Superior
	Yorkool® G3		3584	91	2.8 (2.0–3.6)	0.65 (0.50–0.86)	0.002	Superior

*unwashed and washed nets combined

Estimations were done using logistic regression adjusting for the effect of volunteer, day, and hut as fixed effects. For the pooled analysis, wash condition was adjusted for fixed effect. For superiority on mortality, OR must be higher than 1.00 and *p*-value < 0.05, and for blood-feeding, OR must be lower than 1.00 and *p*-value < 0.05

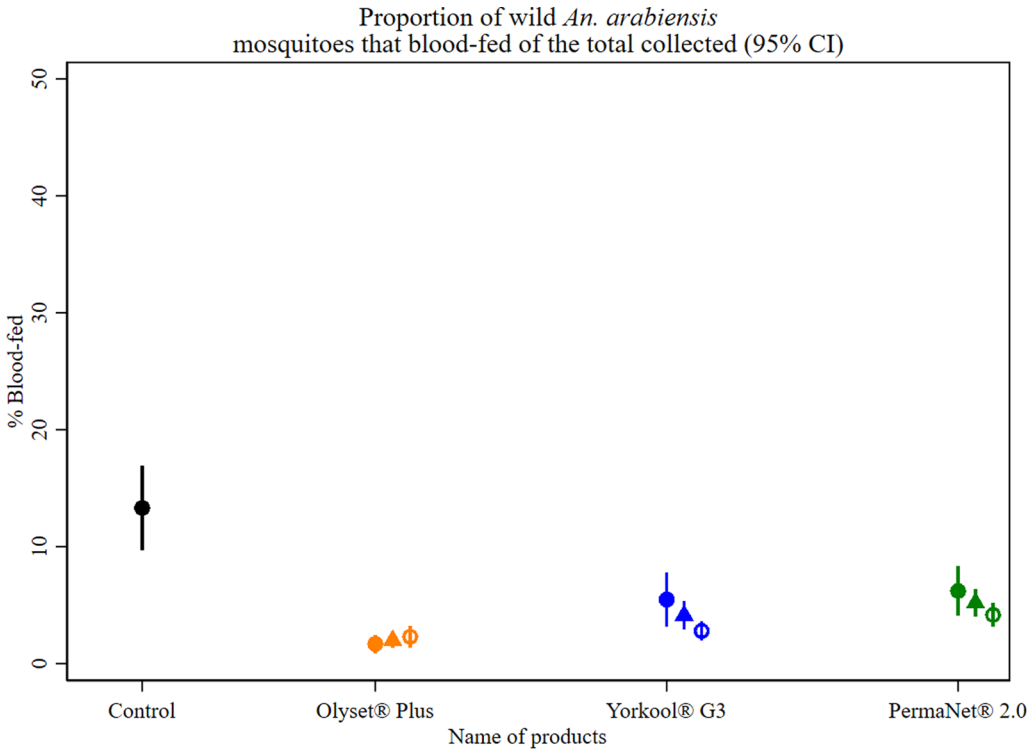

Fig. 6 The proportion of female *Anopheles arabiensis* mosquitoes that were blood-fed (95%) after collection

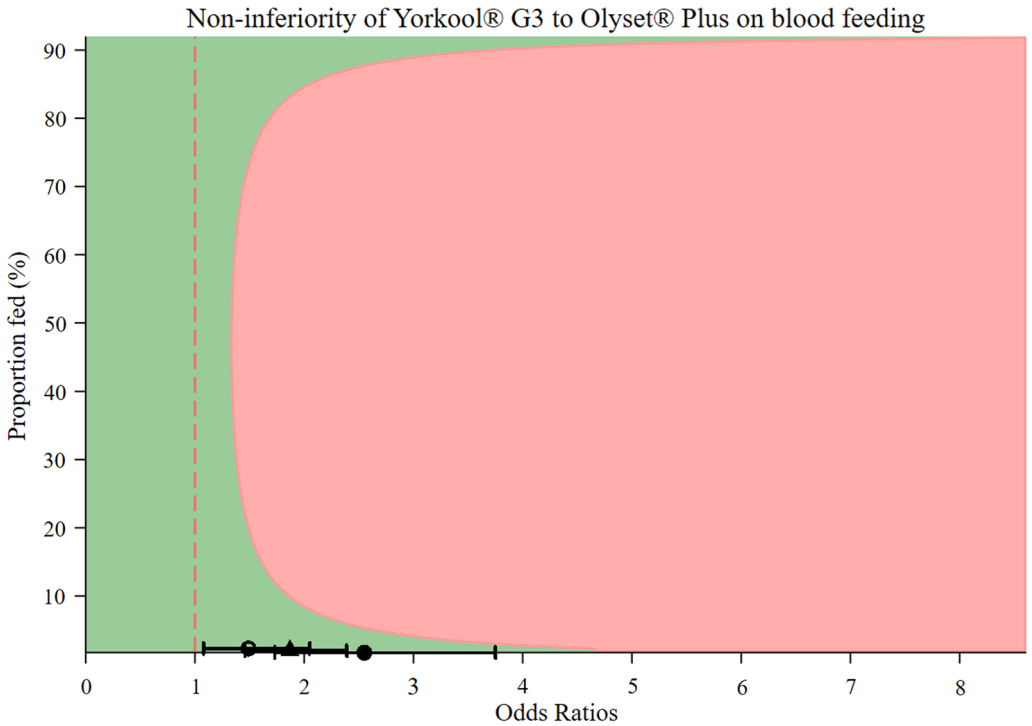

Fig. 7 Blood-feeding (95% CI) of female *Anopheles arabiensis* mosquitoes after collection from experimental huts demonstrating non-inferiority of Yorkool® G3 compared to Olyset® Plus nets. Circles represent unwashed, hollow circles 20x-washed and triangles unwashed and 20x-washed combined. The pink semi-circle represents the non-inferiority margin (delta). The be non-inferior must upper CI must fall below delta.. To be superior the upper confidence interval must be lower than both delta and 1, and to be inferior the lower confidence interval must fall above delta

through laboratory or field washing, include how the nets are used and maintained (human behaviour) and environmental conditions that affect insecticide evaporation loss, washing, and abrasion, among many others [39].The main reason for ITN loss is attrition after nets become too damaged to be considered useful by users [40].

One of the factors that makes a net "long-lasting" is the ability of the net to consistently have sufficient chemical content available at its surface to induce high mortality of mosquitoes following contact. This was seen for Yorkool® G3 nets in the quality checks, 100% of the original chemical content was observed after 20×washes. In bioefficacy evaluations before the hut trials, 67% of exposed insecticide-resistant mosquitoes died, whilst mortality following the studies was 95%. This was not the case for the pyrethroid-PBO comparator net; none of the net samples evaluated were within the target dose range for chemical retention for the pyrethroid-PBO comparator, and poor mortality was recorded in the cone bioassay experiment. One potential limitation of non-inferiority analyses where a first-in-class product has to be the standard comparator, is when the entomological efficacy in terms of mosquito mortality is lower than any other product to be compared, or the mode of action of the pyrethroids used may be different. In this case, second or third-in-class products may always appear non-inferior. Conversely, if the first-in-class product results in very high mosquito mortality, then future products that may still have public health value, may be shown to be inferior in many instances. This was taken into account by WHO and resulted in 1) additional products being acceptable as comparators and 2) the non-inferiority margin being widened from 5 to 7% [14].

Given the current situation of increasing resistance in malaria vectors, which contributes to the stalling or increase in malaria prevalence in certain malaria-endemic regions [41], rotation of pyrethroids has been suggested for the management of insecticide resistance [42], however, studies have shown no significant effect in the restoring of susceptibility of malaria vectors to pyrethroid insecticides [43, 44]. A promising strategy is to explore the rotation of classes of AI at every mass distribution interval of three years [38]. Ideally, the rotation interval should be influenced by evidence of the time it takes for mosquitoes to develop resistance to the distributed class of AI. In context, Yorkool® G3 ITNs being a PBO product could be used in rotation with chlorfenapyr product or other classes of AI at mass campaign intervals. Mosaic nets–treating different panels of a net with different pyrethroids or classes of insecticide such

Table 3 Superiority analysis of insecticide-treated products

Outcomes	Product	Condition	Total recaptured	Total outcome	% Arithmetic mean (95%CI)	OR (95%CI)	*p*-value	Test outcome
Mortality at 24 h (Primary Outcome)	Untreated net		1277	145	11.1 (8.1–14.1)	1		
	PermaNet® 2.0	Pooled*	7001	1992	27.2 (24.7–29.7)	3.80 (3.14–4.60)	< 0.0001	Superior
	Olyset® Plus		7356	2754	39.2 (36.1–42.3)	6.21 (5.15–7.50)	< 0.0001	Superior
	Yorkool® G3		6340	3243	50.9 (47.5–54.3)	11.05 (9.13–13.37)	< 0.0001	Superior
	PermaNet® 2.0	Unwashed	2730	770	26.7 (22.9–30.6)	3.17 (2.60–3.87)	< 0.0001	Superior
	Olyset® Plus		3154	1395	43.8 (39.5–48.1)	6.85 (5.65–8.31)	< 0.0001	Superior
	Yorkool® G3		2756	1570	52.6 (47.2–57.9)	11.59 (9.51–14.13)	< 0.0001	Superior
	PermaNet® 2.0	20 × washed	4271	1222	27.6 (24.4–30.8)	3.12 (2.57–3.78)	< 0.0001	Superior
	Olyset® Plus		4202	1359	34.7 (30.4–38.9)	4.14 (3.42–5.01)	< 0.0001	Superior
	Yorkool® G3		3584	1673	49.3 (45.0–53.5)	7.68 (6.34–9.30)	< 0.0001	Superior
Blood-feeding (Secondary outcome)	Untreated		1277	146	13.3 (9.8–16.8)	1		
	PermaNet® 2.0	Pooled*	7001	282	5.2 (4.0–6.3)	0.38 (0.30–0.49)	< 0.0001	Superior
	Olyset® Plus		7356	117	2.0 (1.4–2.6)	0.14 (0.10–0.18)	< 0.0001	Superior
	Yorkool® G3		6340	168	4.1 (2.9–5.3)	0.26 (0.20–0.34)	< 0.0001	Superior
	PermaNet® 2.0	Unwashed	2730	117	6.2 (4.2–8.3)	0.40 (0.30–0.52)	< 0.0001	Superior
	Olyset® Plus		3154	43	1.7 (0.9–2.4)	0.11 (0.08–0.16)	< 0.0001	Superior
	Yorkool® G3		2756	77	5.5 (3.2–7.7)	0.28 (0.21–0.38)	< 0.0001	Superior
	PermaNet® 2.0	20 × washed	4271	165	4.2 (3.2–5.2)	0.35 (0.27–0.44)	< 0.0001	Superior
	Olyset® Plus		4202	74	2.3 (1.4–3.2)	0.15 (0.11–0.20)	< 0.0001	Superior
	Yorkool® G3		3584	91	2.8 (2.0–3.6)	0.23 (0.17–0.30)	< 0.0001	Superior

*unwashed and washed nets combined

Estimations were done using logistic regression adjusting for the effect of volunteer, day, and hut as fixed effects. For the pooled analysis, wash condition was adjusted for fixed effect. For superiority on mortality, OR must be higher than 1.00 and *p*-value < 0.05, and for blood-feeding, OR must be lower than 1.00 and *p*-value < 0.05

as PermaNet® 3.0 with deltamethrin coated on the side panels and PBO incorporated on the roof panel [30], are efficacious in controlling pyrethroid-resistant mosquitoes. The development of additional AI classes for ITNs is urgently needed to allow for the management of insecticide resistance by malaria control programmes, and continuation of Indoor Residual Spray (IRS) for control of insecticide resistance is needed (REF).

A few limitations can be drawn from the study including (1) no power calculation was done to determine the replicates of the samples tested using cone bioassay before and after the hut trial because the study was implemented before the 2023 WHO guidelines for ITN testing was published [15], (2) at the time of the experiment, the retention of mosquitoes was not measured. However, this was checked in 2023 using untreated nets and found to be 89% (range 50–100%). Therefore some mosquitoes may have escaped. The probability of escape may be lower for products with rapid knockdown and kill than the control. However, when this was tested there was no difference in retention according to product mortality when this relationship was tested in 2021 (Ross, pers. Comm.), and (3) our findings are from one site which may be different from studies performed elsewhere due to the ecological differences, experiment procedures, and hut designs. Despite these limitations, this study fulfilled the WHO recommended requirements for conducting non-inferiority studies in experimental huts [14] as follows: (1) selection of a study site where the primary vectors against which the nets are designed to target dominate, i.e. for pyrethroid-PBO nets the primary malaria vectors resistant to pyrethroids and susceptibility to pyrethroids is restored by PBO. In Lupiro, the predominant malaria vector is *An. arabiensis* and is resistant to several pyrethroids. Studies of insecticide susceptibility at the time of the experiment demonstrated mortalities of 16%, 48%, and 22% against permethrin, deltamethrin, and alpha-cypermethrin, respectively, which was restored by PBO (Supplementary file 2). (2) Using recommended hut designs: this study was conducted in Ifakara experimental huts, one of the listed designs by WHO [15]. (3) Using a study design that reduces variability. Using a 7 × 7 Latin Square design in fourteen huts, this study aimed to minimize variability as far as possible. With the use of many huts, multiple replicates could be undertaken in a short period with minimal budget implications. (4) Ensuring the study is of sufficient power, i.e. > 80%, Using a thousand simulations of the model of the data analysis and accounting for estimates (using data from previous studies, the number of mosquitoes per

Table 4 Cone bioassay test data before washing, after washing 20 times, and after experimental hut trial with pyrethroid-susceptible *Anopheles gambiae* (Ifakara strain) and pyrethroid-resistant *Anopheles arabiensis* (Kingani strain)

			Olyset® Plus				Yorkool® G3				PermaNet® 2.0			
Strain	**Treatment Arm**	**No**	**No. KD60**	**% KD60 (95% CI)**	**No. mortality**	**% 24 h mortality (95% CI)**	**No. KD60**	**% KD60 (95% CI)**	**No. mortality**	**% 24 h mortality (95% CI)**	**No. KD60**	**% KD60 (95% CI)**	**No. mortality**	**% 24 h mortality (95% CI)**
Susceptible *An. gambiae* s.s	Baseline quality check	300	300	100	300	100	300	100	300	100	300	100	206	68.7 (62.2–75.1)
	20 × washed before hut	300	52	17.3 (12.9–21.7)	26	8.7 (5.3–12.0)	300	100	300	100	300	100	300	100
	Unwashed after hut	200	200	100	175	87.5 (79.3–95.7)	200	100	200	100	200	100	186	93.0 (88.3–97.7)
	20 × washed after hut	200	181	90.5 (85.3–95.7)	50	25.0 (16.7–33.3)	200	100	200	100	200	100	200	100
Resistant *An. arabiensis*	Baseline quality check	300	300	100	300	100	300	100	206	68.7 (63.7–73.6)	–	–	–	–
	20 × washed before hut	300	18	6.0 (3.1–8.9)	37	12.3 (7.5–17.2)	300	100	201	67.0 (62.0–72.0)	–	–	–	–
	Unwashed after hut	200	200	100	96	48.0 (37.2–58.8)	200	100	169	84.5 (79.4–89.6)	–	–	–	–
	20 × washed after hut	200	143	71.5 (64.6–78.4)	30	15.0 (9.6–20.4)	200	100	189	94.5 (91.3–97.7)	–	–	–	–

Number of mosquitoes exposed (No.), number (No.), percent (%) knockdown at 60 min (KD60), % 24-h (24 h) mortality (adjusted for control)

Table 5 Insecticidal content of nets by wash: Number of samples tested (N), active ingredient (AI) content (g/kg) with standard deviation (SD), % of nets within 25%± of target dose, AI within net spatial variation (% RSD), and wash retention index of Yorkool® G3, Olyset® Plus and PermaNet® 2.0 net samples before and after 20 washes

Treatment Arm	No. net samples	AI content (g/kg) before trial Arithmetic Mean (SD)		% Within target dose range		AI Within Net Variation (% RSD)		AI retained after 20 washes (%)	Wash Resistance Index (%) (AI retained per wash)	No. net samples	AI Content (g/kg) after trial Arithmetic Mean (SD)	
		0 washes	20 washes	0 washes	20 washes	0 washes	20 washes				0 washes	20 washes
Yorkool® G3 deltamethrin	15	3.08 (0.04)	2.91 (0.02)	100	100	1.30	0.69	94.3	99.7	5	3.07 (0.04)	2.91 (0.02)
Yorkool® G3 PBO		11.26 (0.07)	8.52 (0.06)	100	100	0.62	0.70	75.7	98.6		11.21 (0.07)	8.53 (0.06)
Olyset® Plus permethrin	15	19.26 (0.13)	13.28 (0.2)	100	0	0.67	1.51	69.0	98.2	5	19.35 (0.08)	13.22 (0.08)
Olyset® Plus PBO		9.84 (0.1)	5.58 (0.09)	100	0	1.02	1.61	56.7	97.2		9.87 (0.06)	5.49 (0.06)
PermaNet® 2.0 deltamethrin	15	1.43 (0.03)	0.88 (0.02)	100	0	2.10	2.27	61.2	97.6	5	1.44 (0.03)	0.87 (0.02)

No. Number, *AI* Active Ingredient, *SD* Standard deviation, % percentage, *g* gram, *kg* kilogram

night, % mortality of mosquitoes, and variation between huts, volunteers and day) it was estimated that the study had greater than 98% power. (5) Using mortality as the primary outcome, assess non-inferiority and superiority of products using appropriate statistical analyses where the treatment, hut, volunteer, and day are included in the model as fixed effects [14].

The study suggests a superior performance of Yorkool® G3 nets to the first-in-class Olyset® Plus nets which has evidence of improved performance in reducing malaria relative to pyrethroid nets from randomized control trials [12, 45]. The superior performance of Yorkool® G3 nets relative to Olyset® Plus nets using entomological surrogates of malaria control (vector mortality) provides reassurance that Yorkool® G3 net is a promising tool that can be used by malaria control programmes, increasing the number of products available for the control of pyrethroid-resistant mosquitoes.

Conclusion

Yorkool® G3 ITN was non-inferior and superior in terms of mosquito mortality and non-inferior in terms of blood-feeding inhibition to the first-in-class product within the pyrethroid-PBO ITN intervention class, Olyset® Plus ITN. Our non-inferiority and superiority evaluations were easy to implement and could be feasibly conducted within the recommended time frame and at the same cost as a standard hut trial, following the guidelines set by the prequalification team and GMP at WHO. This implies that new ITNs within this class will be readily available in the market to allow malaria control programmes to choose cost-effective ITNs for malaria control.

Abbreviations

AI	Active Ingredient
An.	Anopheles
C	Centigrade
CI	Confidence Interval
cm	Centimetre
CYP	Cytochrome
FIC	First-in-Class
g	Gram
GLMM	Generalized linear mixed model
GMP	Global Malaria Programme
hrs	Hours
IHI	Ifakara health institute
IIBAT	International Institute of Biotechnology and Toxicology
ITN	Insecticide-treated net
kg	Kilogram
Ltd	Limited
m	Metre
mg	Milligram
MR4	Malaria Research
M24	Mortality at 24 h
OR	Odds ratio
PBO	Piperonyl butoxide
SIC	Second-in-class
SOP	Standard Operating Procedures
VCPTU	Vector Control Product Testing Unit
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-024-05130-7.

Additional file 1
Additional file 2
Additional file 3
Additional file 4
Additional file 5
Additional file 6
Additional file 7

Acknowledgements

This paper is dedicated to the memories of Godfrey Ligema, who contributed to the supervision of the testing of the nets in the experimental hut, hanging and removal of the nets in the huts, and morphologically identifying the wild mosquitoes for data recording. Heartfelt appreciation goes to the entire team of VCPTU both based in Bagamoyo and Lupiro for their dedication and implementation of the study. A huge thanks to the people of Lupiro for their cooperation during the study. We extend our gratitude to Fu Haili, Yin Qing, and the entire team of the Yorkool® International Trading Co., Ltd, China for the funding provided to implement the study.

Author contributions

OGO conceptualized and conducted the experimental hut trial, analysed all data, and drafted the manuscript; MSM co-drafted the manuscript; EM substantially advised on the implementation of the study and reviewed the manuscript; WN supervised the experimental hut trial and reviewed the manuscript; RP provided logistics support and reviewed the manuscript; SN, ABM, IM conducted all bioassays testing and reviewed the manuscript; HN supervised volunteers, record observations and reviewed the manuscripts, JM advised on the implementation of the study designs and reviewed the manuscript; YM and JS substantially reviewed the manuscript; and SJM conceptualized the study and substantially reviewed the manuscript. All authors approved the final draft of the manuscript.

Funding

Tianjin Yorkool International Trading Co., Ltd, China provided funds for the testing of the products. The funders were not involved in the study design, analysis or interpretation.

Availability of data and materials

Data are available in supplementary files. File 3 contains cone bioassay data on unwashed net samples before the experimental hut trial, file 4 contains cone bioassay data for 20× washed net samples before the experimental hut trial, file 5 contains cone bioassay data for unwashed and 20× washed net samples after experimental hut trial, file 6 contains experimental hut trial data and file 7 contains chemical analysis data.

Declarations

Ethics approval and consent to participate

The study was approved by the IHI Review Board with certificate number IHI/IRB/No: 29-2020 and the National Institute for Medical Research-Tanzania (NIMR) with certificate number NIMR/HQ/R.8a/Vol.IX/3521.

Consent for publication

Permission to publish was granted by the National Institute for Medical Research-Tanzania (NIMR) referenced BD.242/437/01B/14.

Competing interests

OGO, MSM, JS, EM, KS, RP, SN, ABM, IM, HN, JM, and SJM test vector control products for a range of manufacturers.

Author details
[1]Vector Control Product Testing Unit (VCPTU) Ifakara Health Institute, Environmental Health, and Ecological Sciences, P.O. Box 74, Bagamoyo, Tanzania. [2]Vector Biology Unit, Department of Epidemiology and Public Health, Swiss Tropical & Public Health Institute, Kreuzstrasse 2, 4123 Allschwill, Basel, Switzerland. [3]Faculty of Science, University of Basel, Petersplatz 1, 4001 Basel, Switzerland. [4]MRC International Statistics and Epidemiology Group, London School of Hygiene and Tropical Medicine (LSHTM), London WC1E 7HT, UK. [5]School of Life Sciences and Bioengineering, The Nelson Mandela African Institution of Science and Technology (NM-AIST), P.O. Box 447, Arusha, Tanzania.

Received: 31 May 2024 Accepted: 5 October 2024
Published online: 16 October 2024

References

1. 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.
2. Abuelmaali SA, Elaagip AH, Basheer MA, Frah EA, Ahmed FT, Elhaj HF, et al. Impacts of agricultural practices on insecticide resistance in the malaria vector *Anopheles arabiensis* in Khartoum State. Sudan PLoS One. 2013;8:e80549.
3. Matowo NS, Tanner M, Munhenga G, Mapua SA, Finda M, Utzinger J, et al. Patterns of pesticide usage in agriculture in rural Tanzania call for integrating agricultural and public health practices in managing insecticide-resistance in malaria vectors. Malar J. 2020;19:257.
4. Ranson H, Lissenden N. Insecticide resistance in African *Anopheles* mosquitoes: a worsening situation that needs urgent action to maintain malaria control. Trends Parasitol. 2016;32:187–96.
5. WHO. Global report on insecticide resistance in malaria vectors: 2010–2016. Geneva, World Health Organization, 2018.
6. Gleave K, Lissenden N, Chaplin M, Choi L, Ranson H. Piperonyl butoxide (PBO) combined with pyrethroids in insecticidetreated nets to prevent malaria in Africa. Cochrane Database Syst Rev. 2021;5:CD012776.
7. Dadzie SK, Chabi J, Asafu-Adjaye A, Owusu-Akrofi O, Baffoe-Wilmot A, Malm K, et al. Evaluation of piperonyl butoxide in enhancing the efficacy of pyrethroid insecticides against resistant *Anopheles gambiae s.l.* in Ghana. Malar J. 2017;16:342.
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 fact. Lancet. 2018;391:1577–88.
9. WHO. List of WHO prequalifed vector control products. Geneva, World Health Organization, 2023.
10. WHO. Technical consultation on determining non-inferiority of vector control products within an established class. Geneva, World Health Organization, 2021.
11. Sherrard-Smith E, Winskill P, Hamlet A, Ngufor C, N'Guessan R, Guelbeogo MW, et al. Optimising the deployment of vector control tools against malaria: a data-informed modelling study. Lancet Planet Health. 2022;6:e100–9.
12. Martin JL, Mosha FW, Lukole E, Rowland M, Todd J, Charlwood JD, et al. Personal protection with PBO-pyrethroid synergist-treated nets after 2 years of household use against pyrethroid-resistant *Anopheles* in Tanzania. Parasit Vectors. 2021;14:150.
13. Pennetier C, Bouraima A, Chandre F, Piameu M, Etang J, Rossignol M, et al. Efficacy of Olyset(R) Plus, a new long-lasting insecticidal net incorporating permethrin and piperonyl-butoxide against multi-resistant malaria vectors. PLoS ONE. 2013;8:e75134.
14. WHO. Data requirements and protocol for determining comparative efficacy of vector control products, with a focus on insecticide treated nets and indoor residual spraying products. Second edition. Malaria Policy Advisory Group Meeting. Geneva, World Health Organization, 2023.
15. WHO. Guideline for the prequalification assessment of insecticide treated nets. Geneva, World Health Organization, 2023.
16. WHO. Guidelines for laboratory and field-testing of long-lasting insecticidal nets. Geneva, World Health Organization, 2013.
17. Pinda PG, Eichenberger C, Ngowo HS, Msaky DS, Abbasi S, Kihonda J, et al. Comparative assessment of insecticide resistance phenotypes in two major malaria vectors, *Anopheles funestus* and *Anopheles arabiensis* in south-eastern Tanzania. Malar J. 2020;19:408.
18. Okumu F, Finda M. Key Characteristics of residual malaria transmission in two districts in South-Eastern Tanzania-implications for improved control. J Infect Dis. 2021;223:S143–54.
19. Johnson PC, Barry SJ, Ferguson HM, Muller P. Power analysis for generalized linear mixed models in ecology and evolution. Methods Ecol Evol. 2015;6:133–42.
20. WHO. Report of the fifteenth WHOPES working group meeting: review of Olyset plus, Interceptor LN, Malathion 440 EW, Vectobac GR. Geneva, World Health Organization, 2012.
21. WHO. Report of the twelfth WHOPES working group meeting: review of Bioflash GR, PermaNet 2.0, PermaNet 3.0, PermaNet 2.5 and Lambda-cyhalothrin LN. WHO/HTM/NTD/WHOPES/200808. Geneva, World Health Organization, 2008.
22. Matowo J, Kabula B, Kavishe RA, Oxborough RM, Kaaya R, Francis P, et al. Dynamics of insecticide resistance and the frequency of kdr mutation in the primary malaria vector *Anopheles arabiensis* in rural villages of Lower Moshi, North Eastern Tanzania. J Parasitol Vector Biol. 2014;6:31–41.
23. Malaria Research and Reference Reagent Resource Center (MR4). Methods in Anopheles Research. Resources. 4th Edn. 2014.
24. Skovmand O, Dang DM, Tran TQ, Bossellman R, Moore SJ. From the factory to the field. Considerations of product characteristics for insecticide-treated net (ITN) bioefficacy testing. Malar J. 2021;20:363.
25. Swai JK, Soto AC, Ntabaliba WS, Kibondo UA, Ngonyani HA, Mseka AP, et al. Efficacy of the spatial repellent product Mosquito Shield against wild pyrethroid-resistant *Anopheles arabiensis* in south-eastern Tanzania. Malar J. 2023;22:249.
26. WHO. Determining non-inferiority of insecticide-treated nets and indoor residual spray product within an established prodcut class. Evidence Review Group meeting report. Geneva, World Health Organization, 2018.
27. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2021.
28. StataCorp. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC. 2019.
29. Achee NL, Sardelis MR, Dusfour I, Chauhan KR, Grieco JP. Characterization of spatial repellent, contact irritant, and toxicant chemical actions of standard vector control compounds. J Am Mosq Control Assoc. 2009;25:156–67.
30. Ngufor C, Fagbohoun J, Agbevo A, Ismail H, Challenger JD, Churcher TS, et al. Comparative efficacy of two pyrethroid-piperonyl butoxide nets (Olyset Plus and PermaNet 3.0) against pyrethroid resistant malaria vectors: a non-inferiority assessment. Malar J. 2022;21:20.
31. Kweka EJ, Lyaruu LJ, Mahande AM. Efficacy of PermaNet® 3.0 and PermaNet® 2.0 nets against laboratory-reared and wild Anopheles gambiae sensu lato populations in northern Tanzania. Infect Dis Poverty. 2017;6:11.
32. N'Guessan R, Asidi A, Boko P, Odjo A, Akogbeto M, Pigeon O, et al. An experimental hut evaluation of PermaNet((R)) 3.0, a deltamethrin-piperonyl butoxide combination net, against pyrethroid-resistant Anopheles gambiae and Culex quinquefasciatus mosquitoes in southern Benin. Trans R Soc Trop Med Hyg. 2010;104:758–65.
33. Bayili K, N'Do S, Yadav RS, Namountougou M, Ouattara A, Dabiré RK, et al. Experimental hut evaluation of DawaPlus 3.0 LN and DawaPlus 4.0 LN treated with deltamethrin and PBO against free-flying populations of *Anopheles gambiae s.l.* in Vallée du Kou, Burkina Faso. PLoS ONE. 2019;14:e0226191.
34. Oumbouke WA, Rowland M, Koffi AA, Alou LPA, Camara S, N'Guessan R. Evaluation of an alpha-cypermethrin + PBO mixture long-lasting insecticidal net VEERALIN® LN against pyrethroid resistant *Anopheles gambiae s.s.*: an experimental hut trial in M'bé, Central Côte d'Ivoire. Parasit Vectors. 2019;12:544.
35. Churcher TS, Lissenden N, Griffin JT, Worrall E, Ranson H. The impact of pyrethroid resistance on the efficacy and effectiveness of bednets for malaria control in Africa. Elife. 2016;5:e16090.
36. Nash RK, Lambert B, N'Guessan R, Ngufor C, Rowland M, Oxborough R, et al. Systematic review of the entomological impact of insecticide-treated nets evaluated using experimental hut trials in Africa. Curr Res Parasitol Vector Borne Dis. 2021;1:100047.

37. Maiteki-Sebuguzi C, Gonahasa S, Kamya MR, Katureebe A, Bagala I, Lynd A, et al. Effect of long-lasting insecticidal nets with and without piperonyl butoxide on malaria indicators in Uganda (LLINEUP): final results of a cluster-randomised trial embedded in a national distribution campaign. Lancet Infect Dis. 2023;23:247–58.
38. WHO. Achieving and maintaining universal coverage with long-lasting insecticidal nets for malaria control. Geneva, World Health Organization, 2017.
39. Gnanguenon V, Azondekon R, Oke-Agbo F, Beach R, Akogbeto M. Durability assessment results suggest a serviceable life of two, rather than three, years for the current long-lasting insecticidal (mosquito) net (LLIN) intervention in Benin. BMC Infectious Diseases 2014.
40. Briet O, Koenker H, Norris L, Wiegand R, Vanden Eng J, Thackeray A et al (2020) Attrition, physical integrity and insecticidal activity of long-lasting insecticidal nets in sub-Saharan Africa and modelling of their impact on vectorial capacity. Malar J 19:310. https://doi.org/10.1186/s12936-020-03383-6
41. WHO. World malaria report. Geneva, World Health Organization, 2023.
42. WHO. The global plan for insecticide resistance management in malaria vectors. Geneva, World Health Organization, 2012.
43. Lissenden N, Kont MD, Essandoh J, Ismail HM, Churcher TS, Lambert B, et al. Review and meta-analysis of the evidence for choosing between specific pyrethroids for programmatic purposes. Insects. 2021;12:826.
44. Moyes CL, Lees RS, Yunta C, Walker KJ, Hemmings K, Oladepo F, et al. Assessing cross-resistance within the pyrethroids in terms of their interactions with key cytochrome P450 enzymes and resistance in vector populations. Parasit Vectors. 2021;14:115.
45. Shepard DS, Odumah JU, Awolola ST. Cost-effectiveness of PBO versus conventional long-lasting insecticidal bed nets in preventing symptomatic malaria in Nigeria: results of a pragmatic randomized trial. Am J Trop Med Hyg. 2020;104:979–86.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.