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Field efficacy and acceptability of PermaNet® 3.0 and OlysetNet® in Kinshasa, Democratic Republic of the Congo

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ABSTRACT

Background & objectives: Insecticide resistance in mosquitoes at Kinshasa may jeopardize the efficacy and usage of long-lasting insecticidal nets (LLINs). Entomological impact, user acceptance and bioefficacy of a combination LLIN (PermaNet® 3.0) and a standard LLIN (OlysetNet®) were evaluated at two sites in Kinshasa characterized by high densities of either *Anopheles gambiae s.s.* (Kindele) or *Culex* spp (Kimbangu).

Methods: Insecticide susceptibility (permethrin, deltamethrin, bendiocarb, propoxur and DDT) was determined via tube tests and bottle assays. Entomological impact of unwashed and washed LLINs and untreated nets was assessed via Latin square, based on rotation of nets and their users through selected houses at each site. User acceptability was evaluated through interviews using a questionnaire and net bioefficacy was measured via cone bioassays with field-derived *An. gambiae s.s.*

Results: The *An. gambiae s.s.* population from Kindele was resistant to DDT and permethrin with mortality rate of 27.3 and 75.8%, respectively, and *kdr* mutations (L1014F) plus suspected metabolic resistance. The *Culex* spp population was resistant to all five insecticides tested. No differences in entomological indices were observed for the five net treatments, but bioefficacy against *An. gambiae* was significantly higher for unwashed and washed PermaNet 3.0 (100 and 71% mortality) than for OlysetNet (56 and 36%). Householders reported a good sleep most often when using unwashed and washed PermaNet (94 and 88%) and least often with unwashed OlysetNet (46%).

Interpretation & conclusion: High bioefficacy via cone bioassays against an *An. gambiae s.s.* population with *kdr* and suspected metabolic resistance was observed with PermaNet 3.0 . Lower biting rates and a higher chance of a good night of sleep were reported when using PermaNet 3.0 compared to OlysetNet.

Key words Democratic Republic of the Congo; insecticide resistance; OlysetNet; PermaNet 3.0

INTRODUCTION

Although long-lasting insecticidal nets (LLINs) adequately circumvent the need for retreatment, insecticide resistance may be a major challenge to sustain their impact in certain areas. As the problem of insecticide resistance grows[1] and examples of reduced efficacy of control interventions are presented[2–4], there is increasing concern over preserving the effectiveness of insecticide-based vector control tools. New generation combination nets that utilise alternative or multiple classes of insecticides or other chemical synergists have or are being developed to address this problem.

One combination LLIN currently recommended by the World Health Organization (WHO) is PermaNet® 3.0. This net combines a pyrethroid (deltamethrin) with a synergist (piperonyl butoxide) in the roof structure to enhance bioefficacy against pyrethroid-resistant malaria vectors. Experimental hut trials in Vietnam, Burkina Faso, Benin, Cote d'Ivoire and Nigeria have indicated increased bioefficacy against pyrethroid-resistant malaria vectors relative to mono-treated deltamethrin or permethrin nets[5–8]. As with other insecticidal interventions, evaluations of PermaNet 3.0 to date have indicated that bioefficacy under field conditions will depend not only on the level of resistance and its underlying mechanisms but also on the behaviour of the specific vector population.

There is also evidence for increased personal protection of PermaNet 3.0 against nuisance mosquitoes, *Culex* spp. In experimental hut studies in Togo and Vietnam, a significant reduction in blood feeding was observed relative to a standard LLIN[9]. However, studies in Tanzania failed to detect an impact on *Culex* populations[10], although this could be a result of low *Culex* densities.

In the Democratic Republic of the Congo (DRC), malaria parasite transmission is maintained mainly by *Anopheles gambiae s.s.* and *An. funestus*[11–13] and is sea-

sonal with peaks during the rainy periods which differ depending upon the locations. In urban areas, the main nuisance mosquito problem is due to *Culex quinquefasciatus* while in rural areas the low mosquito nuisance observed is almost entirely due to two main *Anopheles* species. Published reports on insecticide susceptibility of mosquito species in DRC are scarce. Mulumba *et al*[14–15] confirmed the susceptibility of *An. gambiae s.l.* in Kinshasa to insecticides from all the four classes of insecticides recommended by the WHO for adult mosquito control. Although DDT resistance is reported, Webster *et al*[16] argued that both *An. gambiae* and *An. funestus* were thought to be sensitive to deltamethrin. More recently, evaluations of *An. gambiae s.s.* from four sites in DRC detected resistance to DDT at all sites and to pyrethroids (deltamethrin, permethrin and lambda-cyhalothrin) at three sites with resistance to an organophosphate (malathion) at one site[17]. The L1014F *kdr* allele, often associated with resistance to DDT and pyrethroids, was detected at all the sites albeit with various frequencies. This is of major concern for currently available control approaches which mainly use pyrethroids on nets or DDT, pyrethroids, carbamates or organophosphates sprayed onto the interior walls of houses.

The efficacy of LLINs against local mosquito populations is most commonly assessed in experimental hut trials as recommended by the WHO Pesticide Evaluation Scheme[18]. These follow a standard protocol using specific replicate housing structures in a latin square design, to allow for comparison of a candidate LLIN with a positive and negative control to determine the effect on deterrence, house entry, mortality and blood feeding of target vectors. In localities where such a testing facility does not exist, LLIN efficacy needs to be tested via an alternative protocol. This study was designed to investigate if an adapted latin square design could be applied in normal village households to evaluate comparative LLIN efficacy and acceptability. PermaNet 3.0, designed for increased bioefficacy against pyrethroid-resistant anopheline vectors, was evaluated against a standard LLIN (OlysetNet®) and an untreated net.

MATERIAL & METHODS

Study sites

The assessment was conducted at two sites in Kinshasa. Kindele in the peri-urban area (approx. at 20 km southeast of Kinshasa City Centre), with high densities of *An. gambiae s.s.* and Kimbangu (three in urban Kinshasa) with *Culex* spp nuisance. The study was conducted from January to May 2010 to coincide with the peak in the rainy season.

Study design

A baseline survey was carried out at each site to determine householder willingness to be included and to measure the relative density of mosquitoes in the selected households. Collections were done via overnight CDC light-traps. Based on the results, 20 households were selected randomly at each location with similar housing construction and approximately similar mosquito densities.

Treatment arms

The treated nets tested were: (a) PermaNet® 3.0 unwashed; (b) PermaNet® 3.0 washed 20 times; (c) Olyset Net® unwashed; (d) OlysetNet® washed 20 times; and (e) untreated polyester net. Each net type was assigned to four households per week at each of the sites for a total of 20 households per site. Sufficient nets of the specific type were provided to cover all persons in the household. At the end of each week, householders were asked to complete a simple questionnaire and existing nets were replaced with a net of a different treatment. Net types were coded such that householders and surveyors were not aware of treatment was being evaluated at each household.

Long-lasting insecticidal nets

PermaNet® 3.0 LLIN (Vestergaard Frandsen SA, Switzerland) and OlysetNet®LLIN (Sumitomo Chemical, Japan), have been approved by WHOPES[19]. The untreated net was a multifilament polyester (75 denier) fabric. The manufacturer-specified size of all nets was 160 cm wide × 180 cm long × 150 cm high. A standard procedure was used for washing nets (b) and (d) as per WHOPES Phase-II testing guidelines[18]. Nets were washed in clean water in aluminium bowls containing 10 L of well water with a small quantity of local soap. Nets were agitated for 3 min, left to soak for 4 min and re-agitated for 3 min. Agitation was conducted by hand at approx. 20 rotations per min. Nets were then dried vertically in the shade. For Olyset only, nets were then heated to 60°C for four hours in a regulated heater based on local regeneration time observations (F. Watsenga, Personal Communication). The subsequent wash for all the nets was then performed the following day.

Insecticide resistance testing

The insecticide susceptibility status of *An. gambiae s.s.* mosquitoes from Kindele and *Culex* spp from Kimbangu was determined using WHO discriminating doses and standard insecticide susceptibility kits[19]. CDC

bottle assays without and with synergists were also used for assessing *An. gambiae s.s.* susceptibility to selected insecticides as per the standard procedures[20]. Mosquitoes for assays were derived from larvae collected at each site which were reared to adults under standard conditions at the insectary of the University of Kinshasa. Unfed adult 2–3 day-old females were used in both WHO susceptibility tests and CDC bottle assays.

For the WHO susceptibility tests, DDT (4%), permethrin (0.75%), deltamethrin (0.05%), bendiocarb (0.1%) and propoxur (0.1%) were tested, for *Anopheles*[20] and *Culex*[21]. For CDC bottle assays, permethrin (21.5 µg/bottle) and deltamethrin (12.5 µg/bottle) were tested for *An. gambiae* only using standard procedures (CDC 2009). Assays were also conducted for permethrin following pre-exposure to piperonyl butoxide (PBO), s,s,s-tributyl phosphorotrithioate (DEF) or ethacrynic acid (ETAA) using standard dosages (CDC 2009). Negative controls without insecticide were assessed concurrently.

Specimens used in WHO susceptibility tests were assayed to determine species via polymerase chain reaction (PCR)[22], M and S molecular forms via restriction fragment length polymorphism PCR[23] and to detect *kdr* mutations via hot ligation oligonucleotide assay[24] as per standard procedures.

Entomological indices

CDC light-trapping[25] was conducted in selected households in both the study areas once per week from 1800 to 0600 hrs the following day. Standard procedures were followed with traps placed approximately 1.5 m from the ground, next to the mosquito net at the foot end of the bed. Specimens from each household were placed in labelled collection cups and transferred to the laboratory for sorting, species identification using keys[26], and enumeration.

User questionnaire

At the end of each week, the head of the household was issued a questionnaire to investigate for the net issued during the previous week: whether it was used, any observed health side effects, perceived benefits, and comparison to previously issued nets.

Net bioavailability

Standard WHO cone bioassays[18] were performed at the end of the field assessment on four nets from each of PermaNet 3.0 unwashed and washed, and OlysetNet unwashed and washed, using adults reared from *An. gambiae* larvae collected from Kindele site. For each net, subsamples (30 × 30 cm) were taken from the roof, lower side and upper side for PermaNet 3.0 or the roof and side for OlysetNet. Four cones were placed on each subsample and five non-blood fed, 2–3 day-old females were introduced and exposed for 3 min before being held for 60 min and observed for knock down then held for 24 h and observed for mortality. Mean knock down (KD_{60}) and mortality (MT_{24}) were calculated for each treatment group. Subsamples of untreated nets were assessed concurrently as negative controls.

Statistical analysis

For WHO susceptibility tests, CDC bottle assays and WHO cone bioassays, Abbott's adjustment was applied when the control mortality was >5% with assay results discarded if control mortality was >20%[19]. WHO susceptibility test and CDC bottle assay mortality data were used to define the resistance status of *Anopheles* and *Culex* for each insecticide using the standard criteria[20]. *kdr* allelic frequency was determined using genotyping calculation expressed by the formula: $F_{kdr} = 2N_{RR} + N_{RS} / 2(N_{SS} + N_{RS} + N_{RR})$.

Statistical software used for analyses of entomological impact, user acceptance and net bioefficacy data were Excel, SPSS and StatsDirect, with chi-square test and Fisher's Exact test used for assessing relationships resulting from contingency table. In addition, the Standard Normal Deviate (SND) test was used to compare the proportions between groups.

Ethical clearance and consent

Approval was obtained from the Ethics Review Committee of the University of Kinshasa. Informed and free consent was obtained from all the study participants. All the participants were offered chemoprophylaxis during and for one month after the study.

RESULTS

Insecticide resistance status

All the *Anopheles* spp specimens from Kindele and Kimbangu were identified as *An. gambiae s.s.* of M molecular form (n = 53). *Anopheles gambiae* from Kindele were found to be resistant to DDT and permethrin via WHO susceptibility tests, with low knock down rates and mortality < 80% (MT_{24} of 27.3 and 75.8%, respectively) (Fig. 1). Full susceptibility to deltamethrin, bendiocarb and propoxur was identified due to rapid knock down (KT_{50} of 17.2, 17.4, and 12.3 min and KT_{95} of 31.6, 28.9, and 18.4 min, respectively) and high mortality (MT_{24} of 100% for all). CDC bottle assays also indicated some resistance to permethrin but not to deltamethrin, with a maxi-

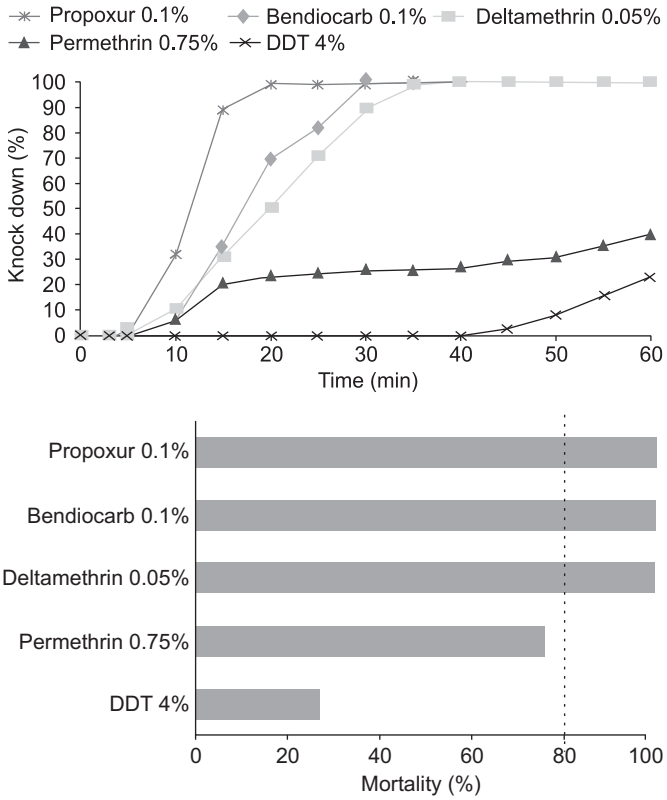

Fig. 1: Overview of the resistance status of *An. gambiae s.s.* from Kindele site. Lines represent mean percent knock down over 60 min of exposure to insecticide-impregnated papers in WHO susceptibility tests. Bars represent mean percent mortality after 24 h post-exposure for the same test mosquitoes. Dotted line (80%) is the WHO resistance threshold[19].

mum mortality of 93.9% reached after 75 min exposure to permethrin, whereas 100% mortality was observed after 30 min exposure to deltamethrin (Fig. 2). Pre-exposure to DEF and PBO did not significantly increase mortality due to permethrin (97.9 and 95.9% mortality after 120 min exposure, respectively). However, pre-exposure to ETAA yielded 100% mortality by 60 min post-exposure to permethrin, indicating the possible presence of elevated glutathione transferase activity in the *An. gambiae* population. *kdr* alleles were also identified in some specimens from both Kindele and Kimbangu, representing the first reports of the *kdr* mutation in *An. gambiae s.s.* from DRC. Very few specimens were available for processing (n = 7), with one homozygous and heterozygous each detected from Kindele and one homozygous from Kimbangu for overall allelic frequencies of 0.38 and 0.33, respectively.

Culex spp from Kimbangu were identified as resistant to all the five insecticides via WHO susceptibility tests, with low knock down rates over the duration of exposure and delayed mortality of <80%. Mortality was similarly recorded low against bendiocarb, DDT and

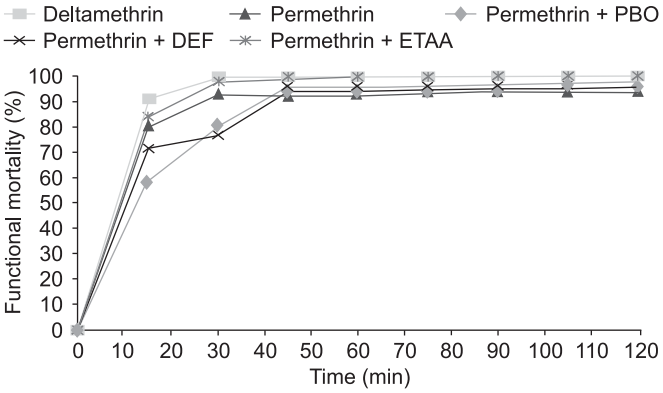

Fig. 2: Additional information on resistance status of *An. gambiae s.s.* from Kindele site. Lines represent mean percent functional mortality (as indicated by mosquitoes unable to rest) over 120 min exposure to deltamethrin- or permethrin-coated bottles in CDC bottle assays. 60 min pre-exposure to the synergists PBO, DEF or ETAA was also conducted prior to permethrin exposure.

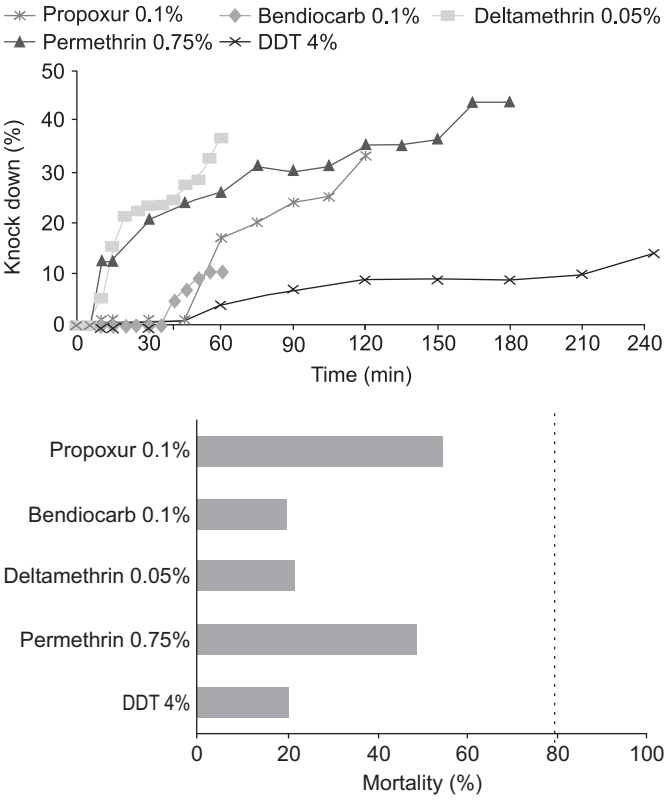

Fig. 3: Overview of the resistance status of *Culex* spp from Kimbangu site. Lines represent mean percent knock down over specified times of exposure to insecticide-impregnated papers in WHO susceptibility tests. Bars represent mean % mortality at 24 h post-exposure for the same test mosquitoes. Dotted line (80%) is the WHO resistance threshold.

deltamethrin (MT_{24} of 19.3, 20 and 21.2%, respectively), and was higher for permethrin (48.5%) and propoxur (54%) (Fig. 3).

Entomological impact

Entomological data were unavailable in cases of householders absence (n = 2) and were removed if householders had sprayed with insecticidal repellent within the previous week (n = 3). Remaining data were divided by site, and due to higher densities, detailed analyses were carried out for *An. gambiae* at Kindele and *Culex* spp at Kimbangu in order to determine the influence of household, week and net type on entomological parameters.

The number of *An. gambiae* and *Culex* spp differed significantly by site ($p = 0.0003$ and 0.0009, respectively). The total number of anophelines collected at Kindele was 681 and at Kimbangu was 125. A total of 99% of the collected anophelines were females, and of these, 5.5% were identified as blood-fed. The total number of culicines collected at Kindele was 188 and at Kimbangu was 19,501. Overall, 67.7% of the collected culicines were females, and of these, 9.5% were identified as blood-fed.

Anophelines at Kindele

The number of *An. gambiae* at Kindele did not vary between baseline and subsequent weeks ($p = 0.7442$) but did vary between households ($p < 0.0001$), ranging from 0 to 40 anophelines captured per house for a single sampling period after intervention (mean = 5.6, median 3). The net type was not found to influence the number of Anophelines for different weeks or households ($p = 0.3073$ and 0.0634, respectively). Similar findings were observed for females and the proportion of blood-feds. Therefore, the type of net did not have any significant influence on these parameters at Kindele ($p > 0.05$ for all).

Culicines at Kimbangu

Similar findings on relationships that were observed in Kindele for anophelines were also observed for culicines in Kimbangu except that the number of *Culex* spp at Kimbangu did vary significantly between weeks ($p = 0.0171$) with an increase from baseline (n = 2602) and a peak at Week 1 and 2 (n = 4739 and 4701, respectively) followed by a decrease in subsequent weeks ($n = \leq 2548$). There was significant variation in the number of culicines between households ($p = 0.0017$), with the number captured per household for a single sampling period after intervention ranging from 0 to 726 (mean = 174.2, median = 137). The type of net did not influence the number of culicines for different weeks or among households ($p = 0.4465$ and 0.3095, respectively). Similar relationships held for females and the proportion of blood-feds, such that the net type did not have a significant influence on these parameters at Kimbangu ($p > 0.05$ for all).

Net bioefficacy

Overall bioefficacy as measured via cone tests using wild-caught *An. gambiae s.s.* was significantly higher for PermaNet 3.0 than for OlysetNet (Table 1). Unwashed PermaNet 3.0 induced a significantly higher knock down and mortality than washed PermaNet 3.0 ($Z = 4.197$, $p < 0.001$ and $Z = 4.547$, $p < 0.001$, respectively). Similarly, unwashed OlysetNet had a higher bioefficacy than the same net washed 20 times ($Z = 2.27$, $p = 0.012$ and $Z = 2.153$, $p = 0.016$, respectively). However, even PermaNet 3.0 that had been washed 20 times had a significantly higher overall bioefficacy than unwashed OlysetNet (17.4% higher knock down and 15.2% higher mortality). Furthermore, approximately double the knock down and mortality was observed for washed PermaNet 3.0 relative to washed OlysetNet. While all the sections of unwashed PermaNet 3.0 induced 100% knock down and mortality for washed PermaNet 3.0, the roof had the highest bioefficacy followed by the lower sides and then the upper sides. There was no significant difference in bioefficacy between the roof and sides of unwashed and washed OlysetNet ($p < 0.05$ for all).

Table 1. Bioefficacy of PermaNet® 3.0 and OlysetNet® after field usage

Section	Unwashed (%)		Washed 20 × (%)	
	knock down (60 min)	mortality (24 h)	knock down (60 min)	mortality (24 h)
PermaNet® 3.0				
Roof	100 ± 0	100 ± 0	91.3 ± 6.3	88.6 ± 2.4
Side upper	100 ± 0	100 ± 0	63.3 ± 6.9	55.2 ± 3.5
Side lower	100 ± 0	100 ± 0	68.8 ± 3.8	68.8 ± 3.8
Total	100 ± 0	100 ± 0	74.4 ± 13.7	70.9 ± 14.6
OlysetNet®				
Roof	60.8 ± 4.4	55.7 ± 4.3	39.8 ± 3.4	38.5 ± 6.6
Side	53.2 ± 3.9	55.7 ± 3.1	23.8 ± 16	34 ± 2.7
Total	57 ± 5.6	55.7 ± 3.5	31.8 ± 13.7	36.2 ± 5.2

Mean (± standard deviation) knock down at 60 min and mortality at 24 h of *An. gambiae s.s.* from Kindele site after exposure in 3 min WHO cone bioassays on roof and side sections of unwashed and 20-times washed PermaNet® 3.0 and OlysetNet® LLINs.

User acceptance

Reported net usage did not differ significantly between the two sites ($p = 0.157$), with 84.3% of householders interviewed indicating that they slept under a net every night during the study (Table 2). However, householders were more likely to report mosquito bites in Kimbangu (19.8%) than in Kindele (5%) ($p = 0.004$). There was a significant association between net usage and lack of reported biting at each site ($p = 0.001$ for Kindele and $p = 0.004$ for Kimbangu), with nightly net

Table 2. Summary of entomological impact and user acceptance data for 20 houses each at Kindele and Kimbangu

Site/Mosquito species	Kindele *An. gambiae s.s.*	Kimbangu *Culex* spp
Entomological impact		
Total number collected	681	19,501
Percent females	99.8	67.4
Percent females blood-fed	2.5	9.6
Mean number per household	5.9	171.1
PermaNet 3.0 unwashed	4.6	132.6
PermaNet 3.0 washed 20×	4.7	216.9
Untreated net	8.6	202.1
OlysetNet unwashed	6.6	130.1
OlysetNet washed 20×	3.4	203.9
User acceptance		
Percent reporting net usage all nights	25	45
Percent reporting side effects	43.8	31.6

usage associated with low biting (reported by 15.1% of householders) and non-nightly usage associated with higher biting (reported by 60% of householders).

For the different net types, there was a significant difference in reported usage for both the sites ($p = 0.002$ at Kindele and $p < 0.001$ at Kimbangu). While >80% of the householders reported sleeping under PermaNet, unwashed OlysetNet or untreated nets every night, nightly usage was less common for washed OlysetNet at both Kindele (43.8%) and Kimbangu (45%). Furthermore, at Kimbangu biting was more commonly reported by the householders issued OlysetNet either washed (42.1%) or unwashed (27.8%) than for those issued an untreated net (15%) or PermaNet unwashed (5%) or washed (10.5%) ($p = 0.029$).

In terms of reported health side effects, a running nose and unpleasant odour were more commonly reported in Kimbangu (6.3 and 16.7% of householders, respectively) compared to Kindele (no reports of either). However, there was no noted difference between the sites in reports of other side effects such as sneezing, headache, nausea, burning sensation, and watery eyes (all $p > 0.05$). Overall, there was no significant difference in reported health-related concerns between net types (all $p > 0.05$).

The frequency of householders reporting a good sleep differed depending on the net type ($p < 0.001$). This was the highest for PermaNet unwashed and washed (94.1 and 87.5%), followed by unwashed OlysetNet (85.3%), untreated net (80.6%) and was the lowest for washed OlysetNet (45.5%). The nets remained in excellent condition throughout the study period, and were perceived as being new or clean by the householders. Although no significant preference was evident between nets, OlysetNet was reported as being too small or narrow by 27.8% (unwashed) or 66.7% (washed) of the householders.

When two net types were measured following washing, there was an overall shrinkage in the size of OlysetNet (97.5 ± 8.3% of the specified dimensions) and an overall increase in the size of PermaNet 3.0 (110.3 ± 5.3% of the specified dimensions). For separate dimensions, OlysetNet increased in height (108.4 ± 3) but decreased in length (92.4 ± 1.3) and width (91.7 ± 3.5%), whereas PermaNet 3.0 increased in height (112.1 ± 3.8), length (104.7 ± 2.7) and width (114.1 ± 3.6%).

DISCUSSION

This represents the first known study to compare the field efficacy of LLINs in existing housing structures in DRC, and also the first to use local field-derived mosquitoes to assess LLIN bioefficacy via cone tests in DRC. Although there was no difference detected in the impact on field entomological indices by net type, cone bioassays clearly indicated a significantly higher bioefficacy of PermaNet 3.0 compared to OlysetNet even after PermaNet 3.0 had been subjected to 20 washes. User surveys also indicated better performance of PermaNet 3.0, and unwashed OlysetNet were particularly associated with high reported biting rates and low reported frequency of a good night of sleep.

It is highly possible that the failure to detect differences in entomological impact despite significant difference in net bioefficacy may have been due to the study design. Many of the *p*-values observed during data analyses were close to 0.05, indicating that a larger or more robust study structure could potentially have yielded different conclusions. In contrast to the usual approach for such bioefficacy evaluations of LLINs, this study used human populations and local housing structures that were already in existence at the study sites. This would have introduced numerous sources of variation, such as: differences in the number of people under nets and thus acting as either attractants or blood meals for vectors; differences in housing construction such as the quality of material (e.g. metal or thatched roves) and number and size of windows/doors which could influence house attractancy and entry opportunities for vectors; and other human factors which could have influenced vector behaviour (e.g. time of entry and exit of humans from nets, cooking practices, etc). For these reasons, the WHO recommends using standardised experimental huts with a single sleeper per hut following set patterns of LLIN usage and rotation between houses to account for any differences in individual attractancy[18]. This design should

limit the differences between individual households and persons over time whilst revealing differences in mosquito parameters due to each treatment being tested. However, the establishment of such huts was not feasible in this case (nor was larger and longer field study), due to personnel and time limitations.

Differential susceptibility of the local *An. gambiae s.s.* population to deltamethrin versus permethrin would have contributed somewhat to the vast difference in bioefficacy of PermaNet 3.0 versus OlysetNet. WHO tube tests revealed full susceptibility to deltamethrin but confirmed resistance to permethrin (75.8% mortality) while CDC bottle assays also indicated susceptibility to deltamethrin but low level pyrethroid resistance (93.9% mortality) with potential glutathione-s-transferase (GST) activity. However, these levels of resistance translated into significant differences in susceptibility of the population to deltamethrin- versus permethrin-treated LLINs in cone bioassays. This emphasises the fact that insecticide susceptibility data from WHO tube tests cannot be directly interpreted to predict the susceptibility of a population to vector control formulations. Hence, the importance of bioefficacy tests such as cone bioassays using field-derived vectors. However, such bioefficacy evaluations also have limitations in predicting the impact of an intervention on a given vector population as those do not take into account vector behaviour and other extrinsic parameters. In a study in Mali[4], while no difference was detected in susceptibility of two *An. gambiae s.l.* populations to an alpha-cypermethrin LLIN, reduced efficacy was identified at one of the two sites during experimental hut studies. The somewhat tenuous link between insecticide susceptibility status of a population and the anticipated field impact of a particular vector control tool underscores the importance of field-based assessments of vector control candidates under local conditions where feasible.

The high level of resistance detected in *Culex* spp to all the five insecticides tested was not unexpected. Resistance to multiple insecticides has been detected previously in *Culex* spp from Kinshasa[16]. Although LLINs are not designed to target *Culex* or other nuisance mosquito populations, correct usage of intact nets with sufficiently small hole size provides protection from *Culex* bites even where insecticide resistance may be high. The importance of assessing the impact of nets on *Culex* populations is related to the perceived benefit of nets by users, rather than actual health benefits in areas where *Culex* are not the vector of any significant diseases. That is, if people perceive that nets are protecting them from mosquito bites (or even malaria), they may be more inclined to use the nets frequently and correctly[27–29], whereas if there is no perceived benefit they may be discouraged from using nets. However, such perception is difficult to document and warrants further investigation under different settings.

Other published semi-field studies for PermaNet 3.0 have compared this net to mono-treated LLINs in experimental hut structures in areas with pyrethroid-resistant malaria vectors. PermaNet 3.0 was shown to have increased bioefficacy relative to deltamethrin only, PermaNet 2.0 in areas with resistant malaria vectors in Kou Valley, Burkina Faso[5] and Akron, Benin[6], and against permethrin only OlysetNet in New Bussa, Nigeria[8]. In other areas, such as in Pitoa, Cameroon[5] and Yaokoffikro, Cote d'Ivoire[7] there was variable difference in bioefficacy compared to a mono-treated LLIN depending on net wash status. This is a clear indication that the relative increase in bioefficacy of this combination net will vary depending on the level and mechanism(s) of insecticide resistance present in the local mosquito population. This emphasises the importance of conducting comparative trials on such new tools designed for increased bioefficacy against pyrethroid-resistant malaria vectors, and defining robust alternative protocols for application in areas, where establishment of experimental huts is not feasible. Ideally, such studies should include an assessment of the age-structure of populations though this would need to be easily implementable in disease-endemic settings.

There has been some discussion in the literature on whether it is the higher dose of deltamethrin or the presence of PBO that increases the bioefficacy of the roof of PermaNet 3.0. The synergistic impact of piperonyl butoxide has been well-documented for various insect species, for which it has been shown to enhance the penetration of insecticide into the insects[30] and inhibit the metabolic enzymes used to sequester or break the insecticide[31]. Bingham *et al*[32] clearly demonstrated the synergistic impact of PBO when coupled with deltamethrin using net samples against a highly pyrethroid-resistant *Ae. aegypti* population from Vietnam. Both low and high dose of deltamethrin had little impact on the population (1 and 5% mortality respectively), whereas there was an increase to 98% mortality when PBO was incorporated into the sample along with a low dose of deltamethrin. However, the issue of whether increased bioefficacy is due to the concentration of deltamethrin or the presence of PBO on the surface of the net roof is less important than how the net is performing as a whole. Modelling of data from independent experimental hut studies with PermaNet 3.0 indicated consistently higher protection conferred versus a deltamethrin-only net when both personal and community protection were considered[33].

For the user acceptance evaluation, although there may have been some self-report bias this would have been minimised since householders were not aware of the particular type of LLIN they had been issued plus over the duration of the study they gave feedback on each net type. Unsurprisingly, nightly net usage was associated with fewer reports of biting than was less frequent net usage. Reported usage of washed OlysetNet (44–45%) was much lower than for all other net types (>80%), likely because of these nets being too small or narrow as reported by 67% of householders and as observed during net measuring. Lower usage rates of washed OlysetNet may have contributed to higher reported biting rates at Kimbangu though biting was also high with unwashed OlysetNet, which may indicate that the large mesh size of this LLIN type allowed access to mosquitoes. Such access would be more likely in the presence of reduced permethrin susceptibility, as was the case for *Culex* spp at Kimbangu (48% mortality). More frequent reports of a good night of sleep as associated with PermaNet 3.0 both unwashed and washed support the use of this LLIN in Kinshasa; such a perceived benefit is likely to be related to more frequent and correct usage which is especially important where reduced susceptibility to pyrethroids has been detected.

CONCLUSION

Anopheles gambiae s.s. (M form) from Kindele was resistant to DDT and permethrin but susceptible to deltamethrin, propoxur and bendiocarb. The west African *kdr* mutation was detected and susceptibility to permethrin was restored with pre-exposure to ETAA in bottle bioassays indicating the likely presence of elevated glutathione transferase enzymes. Although there were no detectable differences in *Anopheles* or *Culex* indices according to the net type or wash status, PermaNet 3.0 both unwashed and washed showed significantly higher bioefficacy against *An. gambiae s.s.* in cone bioassays and was associated with enhanced usage and perceived benefits compared to OlysetNet.

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