Behavioral responses of pyrethroid-resistant Anopheles gambiae mosquitoes to insecticide-treated bed net

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Abstract

Long-lasting insecticidal nets are an effective tool in reducing malaria transmission. However, with increasing insecticide resistance little is known about how physiologically resistant malaria vectors behave around a human-occupied bed net, despite their importance in malaria transmission. This study assessed the host-seeking behavior of the major malaria vector Anopheles gambiae s.s, when an intact human-occupied treated bed net is in place, with respect to their insecticide resistance status under semi-field conditions. Pyrethroid resistant and susceptible females of Anopheles gambiae s.s were released inside a semi-field environment housing a hut which was occupied by a human host sleeping under treated or untreated bed net trap. Mosquitoes resting inside the hut or exiting and resting outside were collected using a prokopack aspirator, window exit trap and clay pots. The proportion of resistant females caught in the treated bed net trap was higher compared to the susceptible females (OR=1.445; P<0.00019). Resistant mosquitoes were less likely to exit the house when a treated bed net was present compared to the susceptible mosquitoes. The susceptible females were 2.3 times more likely to stay outdoors away from the treated bed net (OR=2.25; P<0.0001).The resistant mosquitoes showed significantly reduced avoidance behavior compared to the susceptible mosquitoes that were observed to exit the house and remained outdoors when a treated bed net was used. However, further investigations of the behavior of resistant mosquitoes under natural conditions should be undertaken to confirm these observations and improve the current intervention which are threatened by insecticide resistance and altered vector behavior.

Introduction

Reduction in malaria morbidity and mortality over the past few years in sub-Saharan Africa is largely attributed to the effectiveness of long-lasting insecticidal nets (LLINs)(Bhatt *et al.* , 2015).This has been possible because the main malaria vectors primarily feed indoors at night, a behavioral pattern that coincides with the period when human hosts are indoors and asleep (Gillies & Coetzee, 1987; Killeen *et al.* , 2006; Bayoh *et al.* , 2014). However, extensive use of insecticides has subjected mosquitoes to intensive selection pressure, resulting in the development of physiological and behavioral resistance, threatening the future of existing tools, hence the need for continuous monitoring of their efficacy and development of novel LLINs(Ranson *et al.* , 2011).

The continued success of the current vector control interventions is dependent on the susceptibility of the target mosquito population to the insecticides used. Pyrethroids are one of the insecticide classes recommended for treating mosquito nets owing to their low mammalian toxicity, unique modes of action such as fast knockdown, and high insecticidal potency, although currently assessment of some innovative nets treated with a combination of a pyrethroid and either a non pyrethroid compound is ongoing(WHO, 2019)(Zaim *et al.* , 2000).Over the past two decades, the use of insecticide-treated nets has increased, exerting greater selection pressure on the malaria vector populations resulting in higher incidences of pyrethroid insecticide resistance that is likely to affect the effectiveness of vector control(Ranson & Lissenden, 2016).Some studies have reported the spread of pyrethroid resistance and the mechanisms involved including target site insensitivity caused by kdr mutations(Ranson *et al.* , 2011; Ochomo *et al.* , 2012) and detoxification enzymes that metabolize the insecticide before reaching its target site(Hemingway & Ranson, 2000). However, it is less clear how the observed resistance affects current control measures. Recent field observations in Africa suggests changes such as increased outdoor host-seeking within the principal malaria vectors, *An. gambiae* and *An. funestus* species complexes(Russell *et al.* , 2011; Derua *et al.* , 2012; Gatton *et al.* , 2013; Bayoh *et al.* , 2014). These behavioral shifts have been largely linked to the increased use of vector control measures selecting for vector species with more exophilic behavior (Bayoh *et al.* , 2010; Mwangangi *et al.* , 2013).

Existing literature on behavioral changes comes mainly from pyrethroid susceptible mosquitoes but the data on the behaviorof pyrethroid-resistant malaria vectors is sparse and, at times conflicting, highlighting the need for additional research. It is suggested that avoidance behavior in mosquitoes that have become insensitive to pyrethroids may weaken due to increased selection pressure exerted by the insecticides used(He *et al.* , 2019).Some authors assert that physiologically resistant mosquitoes may use the recognition of insecticides as a proxy for host presence(Ngufor *et al.* , 2016; Porciani *et al.* , 2017; Carrasco *et al.* , 2019). It is unclear if mechanisms related to insecticide resistance may influence the behavior of malaria vectors, as any molecular change in the insect nervous system, may have a pleiotropic effect on nerve function and insect behavior(Kliot & Ghanim, 2012).

Given the important role of the current vector control interventions as a means of reducing the burden of malaria transmission and increasing insecticide resistance, the behaviorof physiologically resistant malaria vectors should be well defined. The host-seeking behavior of the major malaria vector, *Anopheles gambiae s.s* . (hereafter*An.gambiae*) was assessed when an intact human-occupied insecticide-treated and untreated bed net is in place, with respect to their insecticide resistance status under semi-field conditions. We hypothesize that pyrethroid-resistant mosquitoes will seek and bite human hosts indoors despite the presence of indoor-based vector control interventions while the susceptible mosquitoes will leave the house through the windows or eaves and seek blood meal elsewhere. This study provides more information on how the behavior of physiologically resistant vectors might differ in comparison to their susceptible counterparts, an aspect that is poorly understood. There is an urgent need for evidence-based studies on the behavior of malaria vectors in the presence of vector control interventions, given the rapid development of insecticide resistance in a large number of malaria vectors, if the significant gains made in reducing malaria morbidity and mortality is to be maintained.

Methods

Mosquitoes strain used in the experiments

Mosquito strains used in this study consisted of a deltamethrin selected resistant colony and an unselected susceptible colony (hereafter referred to as resistant and susceptible mosquitoes) that were collected from Bungoma in western Kenya. These colonies were selected and maintained at the Centre for Global Health Research, Kenya Medical Research Institute (KEMRI) in Kisumu (Machani *et al.* , 2020), Western Kenya, under standard rearing conditions of 27 ± 2 °C and relative humidity (RH) of 80 ± 10% °C under a L12: D12 h light: dark cycle. During the process, each colonized strain had three independent lineages that started with 200-250 females at every new generation to limit bottleneck effects. The progeny of F1 wild-caught mosquitoes from the same site were also used to undertake these experiments.

Resistant strain: This colony underwent deltamethrin selection after each generation. The 6th generation used was highly resistant with 20% mortality according to the WHO criteria (WHO, 2016). Resistance in this colony was mainly mediated by cytochrome P450 detoxification enzyme. The two kdr mutations 1014S and 1014F were present and at high frequencies (Machani *et al.* , 2020).

Susceptible strain: This strain shares the same genetic background with the resistant colony, however, it was reared in the absence of insecticide selection pressure. After 9 generations without selection pressure, the population had almost lost resistance to deltamethrin (92%) and after 13 generations the population showed increased mortality (97.3%). The vgsc1014S was at a high frequency given that the allele was already fixed in the parent population(Machani *et al.* , 2020). The 14th generation was used in this study.

Wild population : F1 progeny obtained from wild-caught *An. gambiae* female mosquitoes from the same area where the resistant and susceptible colonies were selected were used for validation. Each female(mother) was identified by PCR as *An. gambiae s.s* according to the methods of Scott *et al.*(1993).The wild population had 56% resistance to deltamethrin. The observed resistance is mediated by a mix of metabolic and kdr(Bonizzoni *et al.* , 2012; Ochomo *et al.* , 2012; Wanjala *et al.* , 2015; Machani *et al.* , 2019; Machani *et al.* , 2020).

Semi-field set up

The study was carried out in Western Kenya at the Centre for Global Health Research, Kenya Medical Research Institute, Kisumu. The release and recapture studies were conducted in a MalariaSphere a closed system 20m long x 8m wide(Knols *et al.* , 2002)with slanted roofing (3m in the sides and 4.5m in the middle). The entire structure is covered with insect-proof netting screen that prevents mosquitoes inside the system from escaping into the environment, or vice versa (Fig. 1 A). The system is also double doored for the same reason. Inside the systema3m x 3m mud-walled hut is erected resembling a typical house in the study village in terms of size, structure and mosquito exit/entry points (eaves, window,and door) (Fig. 1B). The structure has local vegetation and grass floor to mimic the natural vegetation and provide shelter for mosquitoes in the outdoor environment (Fig. 1B). Two round clay pots are installed in the enclosure but outside the hut to act as outdoor resting sites (Fig 1F).Inside the hut either a treated LLIN inside a bed-net trap (Mbita trap)or an untreated net inside a bed-net trap as control was hanged (Mathenge *et al.* , 2002). Treated and untreated nets were used at different nights in the same hut. For each night, a consented and remunerated human volunteer slept under the bed-net trap in the hut. To offset any personal bias due to differential sleeping habits or relative attractiveness to mosquitoes, two sleepers were recruited for this experiment and took turns to sleep under the bed net. They were instructed not to consume alcohol or smoke and avoid deodorants during the study period. The volunteer who slept under the bed net served as a bait to attract the mosquitoes into the hut but was not bitten because of the net shield.

Bed net trap for the collection of host-seeking mosquitoes

The Mbita trap which is a bed-net trap described by Mathenge *et al.* (2002) was used to capture host-seeking mosquitoes. This is a modified conical bed-net made of light white cotton cloth instead of netting (Fig.2). The trap has two chambers; the upper trap chamber and the lower bait chamber. The upper chamber contained a netting panel fixed halfway (Fig. 2A) to prevent mosquitoes from reaching the human bait sleeping in the lower chamber (Fig. 2B). For this experiment, the netting panels were either treated or untreated. The treated netting panels were cut from DawaPlus 2.0 a long-lasting insecticidal net (LLIN) containing 80 mg/m^2 deltamethrin. The nets were selected for this experiment based on the fact that they were distributed in the largest proportion in the study site by the National Malaria Control Programme in Kenya during the 2017 mass net campaign. The untreated bed net was obtained from the local market in Kisumu, Kenya.

Mosquito release and recapture

Batches of 200 uninfected and unfed female mosquitoes aged 3-5 days from the resistant or susceptible colonies were gently mouth-aspirated into a clean paper cup. The mosquitoes were sugar starved for 6 hours before releases and color-marked with green (for the susceptible colony) and pink (for resistant colony) fluorescent powder to distinguish them after simultaneous release in the semi-field environment. Mosquito releases were done outside the hut and at the same time of day (1840hrs) to avoid circadian cycle effects. The volunteer entered the bed net 30 mins after the release of the mosquitoes. Fifteen (15) tests were conducted with each net (treated or untreated net). The release was done every 3 days to allow for the wash-out period. Windows of huts were fitted with exit traps to catch exiting mosquitoes (Fig. 1E). The floor of the hut was covered by white sheets to ease the collection of knocked-down mosquitoes. Host seeking mosquitoes trapped in the bed net trap were collected and recorded (Fig. 1D). Validation of these behaviors was done using the F1 progeny obtained from *An. gambiae s.s* caught from the field.

Indoor and outdoor resting mosquito recapture

Mosquitoes that were not caught in the bed net trap or window exit trap were collected from inside the hut and outside at 0700HRS using Prokopack aspirators (John W Hock, Gainesville, FL, USA). For mosquitoes resting indoors, walls and ceilings were systematically aspirated using progressive down and upward movements along its entire length. For outdoor resting mosquitoes, the collection was done from the clay pots (Fig. 1 F) by placing a white mesh from a mosquito cage over the mouth and agitating the mosquitoes inside the pot, causing them to fly and move to the cage (van den Bijllaardt*et al.* , 2009). The corners of the screen house and the vegetation cover were also scanned for resting mosquitoes using the Prokopack aspirator.

WHO cone bio-assay to determine bed-net efficacy

The treated net insecticidal efficacy was confirmed by exposing mosquitoes for 3 mins according to the standard WHO cone bioassay procedure. This was done with 4–5day old, non-blood fed, *An. gambiae s.s* . The bioassays included 5 replicates from both the resistant and susceptible colony with an average of five mosquitoes per tube. The cone bioassays were conducted using DawaPlus 2.0 long-lasting insecticidal net treated with deltamethrin. The F1 progeny of wild-caught mosquitoes were also used for this experiment for validation. After exposure, the groups of mosquitoes were placed in a single 1 L paper cup and provided with cotton wool soaked with 10% sugar solution for 24 hrs. Their knock-down status was measured 60 min post-exposure and mortality were recorded after 24 h. An untreated net was used as a negative control for the assay.

Scientific and Ethical clearance

This study was approved by the Ethical Review Board of the Kenya Medical Research Institute (KEMRI) under the scientific steering committee (SSC 3434). Prior to commencement of the study, volunteers were given an information sheet describing the aims, study procedures, risks and benefits of their participation in this study. Written informed consent was obtained from individual volunteer before the experiments.

Statistical analysis

The proportions of mosquitoes caught in the bed net trap were interpreted as host-seeking mosquitoes. The proportions were calculated by dividing the number of mosquitoes caught in the bed net trap/exit trap/resting with the total number of mosquitoes recaptured for each phenotype (resistant and susceptible mosquitoes) respectively. Observations of host seeking behavior and exit behavior of resistant and susceptible phenotypes were compared between treatments using generalized linear model (GLM) with binomial distribution and logit link function. The LLINs were considered bio-effective when the percentage of mosquitoes knocked down after 60 min post-exposure was above 95% or mortality after 24 h was above 80% in the WHO cone bioassays(WHO, 2006).Statistical analysis was done using statistical program Stata (Version 14, StataCorp, College Station, Texas).

Results

Responses of mosquitoes to insecticide-treated and untreated bed-net trap

We tested the individual response of resistant and susceptible mosquitoes to a human host sleeping under either an insecticide-treated or untreated net in the hut in the MalariaSphere. In 30 experimental nights (i.e. 15 treated and 15 untreated test repeats) out of 12,000 female *An. gambiae s.s* mosquitoes released, 55.5% (95% CI= [54.7-56.4]) were recovered. A total of 2879 (43.2%) female mosquitoes were caught in the bed net traps out of 6665 mosquitoes recaptured. The proportion of resistant females caught in the treated bed net trap was higher than that of the susceptible females(43 vs 28.3% OR=1.445; 95% CI=[1.25-1.68]; P<0.0001, Fig.3), indicating that the resistant population was 1.4 times more likely to seek a host in the presence of a treated net than the susceptible population. When an untreated bed net trap was erected, the proportion of resistant females (51.1% ,95% CI= [48.7-53.5]) caught in the bed net trap did not differ with that of the susceptible population 51.3% (95% CI=[48.8-53.6]) (OR=0.95; 95% CI=[0.86-1.04]; P>0.05).When the untreated net was present the susceptible mosquitoes were 2.7 times more likely to search for a host than when a treated bed net was present (OR=2.65; 95% CI=[2.29-3.05]; P<0.0001, Fig 3).Overall, there was a significant effect of treatment type on mosquito host-seeking behavior, with more mosquitoes caught in the bed net trap when untreated net was present than when there was a treated bed net trap (OR=1.908; 95% CI=[1.73-2.12]; P< 0.0001,Fig. 3).

For the wild population, a total of 1013 (50.6%) mosquitoes were recaptured out of 2000 F1 females released. The proportion of the wild population caught in the untreated bed net trap was slightly higher 40% compared to treated bed net trap 33.5% (Fig. 3). Although, this was not statistically significant (OR=0.773; 95% CI=[0.598-0.999]; P=0.489). The mortality of the resistant population trapped in the treated bed net trap was 77.7 % (549/706) and 85. 2% (144/169) for the wild population. All the susceptible mosquitoes trapped in the insecticide-treated bed net were dead.

Insecticide induced exophily of resistant and susceptible populations

The proportion of mosquitoes that were caught in the window exit trap when treated bed net trap was present was 5.2% (95% CI= [4.2-6.4]) for the resistant colony and 11.5% (95% CI=[9.6-12.6]) for the susceptible colony. When the untreated bed net was present, the number of mosquitoes exiting reduced for both groups; 2.4% (95% CI=[1.8-3.3]) of resistant population exited and 3% (95% CI=[1.8-3.5]) for the susceptible population (Table 1). Overall, the resistant population was less likely to exit the house if any of the treatment was present compared to the susceptible population (GLM, OR=0.54; 95% CI=[0.432-0.674]; P<0.0001). The susceptible females were 4.6-fold more likely to exit the house when treated bed net (11.5%) was present than when untreated bed net (3%) was used (GLM, OR=4.64; 95% CI=[3.3-6.5]; P<0.0001). For the wild field population, 4% (95% CI= [2.2-5.6]) of the recovered mosquitoes were caught in the exit trap when the treated bed net trap was present, while 3.4% (95% CI=[1.9-5.1]) when the untreated net was used. Even though the field population was likely to exit the house when a treated bed net was present, this was not statistically significant (GLM, OR=1.12; 95% CI=[0.58-2.15]; P=0.719).

Indoor and outdoor resting behavior in response to insecticide-treated and untreated bed net.

The proportion of resistant females caught resting indoors in the hut when a volunteer slept under a treated bed net trap was lower 46.2%compared to susceptible females 53.8% (OR=1.4; 95% CI= [1.23-1.62]; P<0.0001). The susceptible females were 1.4 more likely to avoid biting a host sleeping under a treated bed net and resort to search for a host elsewhere or rest away from the intervention, unlike the resistant females. When untreated bed net was in place, there was no difference observed between the proportions of resistant females and susceptible females caught resting indoors in the hut (OR=1.1; 95% CI=[0.97-1.28]; P=0.121).The proportion of susceptible females caught resting outdoors when a treated net was used, was higher 64.5% compared to resistant females 35.4%. The susceptible population was 2.3 times more likely to stay outdoors away from the treated bed net (OR=2.25; 95% CI= [1.7-2.9]; P<0.0001; Fig. 4).

For wild population the proportion of females caught resting indoor or outdoor when a treated bed net was present was 52.03% vs 52.2% respectively compared to the untreated bed net trap (47.9% vs 47.8% respectively, Fig. 4). Even though the proportion resting indoor or outdoor was high when the treated bed net was present, there was no significant difference (Indoor: OR=1.2; 95% CI=[0.94-1.54]; P=0.139; outdoor: OR=1.11; 95% CI=[0.72-1.71])

LLIN bioassay and knockdown rates against resistant and susceptible colonies

Prior to the semi field trials, the efficacy of the treated bed net was evaluated. The knockdown response of the resistant females exposed to DawaPlus2.0 for 60 minutes was 7% whilst 83% for the susceptible population. The mortality rate for the resistant colony was 13% (95% CI=[9.1-15.9]) whilst 92% (95% CI=[89.4-94.9]) for susceptible population . The mortality rate for the F1 progeny of the wild population was 59% (95% CI=[50.3-67.9]) when exposed to DawaPlus 2.0.

Discussion

Physiological resistance in mosquito populations to common public health insecticides across Africa is widely reported but evidence of the actual impact of this, the functionality and efficacy of LLINs is scarcely discussed or documented(Ranson *et al.* , 2011). Monitoring the host-seeking behavior of physiologically resistant mosquitoes in the presence of indoor vector control tools is necessary to determine whether the efficacy of the tools could be compromised with the resistance profiles or whether they can be optimized. This study provides insights into the behavior of pyrethroid-resistant *An. gambiae* when they encounter pyrethroid-based LLIN in a free flight environment similar to the field settings. The results demonstrate that in the presence of a treated net, the host-seeking performance was not altered for resistant females, unlike the susceptible females that were observed to exit the house and remained outdoors when a treated net was used.

One of the consequences of the massive roll out of LLINs is the change in mosquito behaviour where the interventions may select vectors with increased exophily (feeding outdoors early in the evening or morning hours when LLINs are not in use) because of the exposure to insecticides (Gatton *et al.* , 2013). This study observed large proportion of host seeking susceptible females exiting the house and resting outdoors than resistant females when treated net was present. The observed behaviour suggests that in the presence of insecticides, susceptible mosquitoes may be pushed from indoor treated environment and resort to search blood meal outdoors or rest outdoors and initiate their search for a host soon after dusk, leading to increased outdoor transmission. Examples of spatial avoidance of insecticide-treated environment has been observed in malaria vectors in the field, displaying increased outdoor host-seeking and resting outdoors following the implementation of IRS and ITNs (Moiroux *et al.* , 2012;Parker *et al.* , 2015; Spitzen *et al.* , 2017). This indicates that, substantial part of residual malaria transmission is occurring outdoors, raising the questions on the effectiveness of LLINs in reducing malaria infections when susceptible indoor feeding mosquitoes are diverted to feed outdoors when people are outside LLINs. On the other hand, the findings suggest, physiologically resistant malaria vectors that have developed the capacity of blood-feeding or resting indoors in the presence of LLINs, may compromise the effectiveness of LLINs, maintaining the indoor malaria transmission.

The strategy of LLINs in malaria prevention is to deter mosquitoes from entering houses and reduction in blood feeding rates, achieved as a consequence of excito-repellent effects of the pyrethroids (HODJATI & Curtis, 1997).In this study, a higher proportion of the resistant females were caught in the treated bed net trap compared to the susceptible population. The resistant females were less likely to avoid the search for a host when a treated net was present, unlike the susceptible population that was observed to avoid contact with the treated net. One plausible explanation for the difference in behaviours is the pleiotropic effects on nerve function associated with a point mutation in the voltage-gated sodium channels of resistant mosquitoes, as it interferes with the sensitivity of the sensory nervous system to pyrethroids resulting in reduced avoidance behavior (Lee *et al.* , 1999; Corbel *et al.* , 2004). This implies that in the field, physiologically resistant mosquitoes are likely to spend more time in search of a host in the presence of insecticides increasing their probability of encountering a host unlike their susceptible counterparts. In nature, pyrethroid-resistant mosquitoes have been found resting inside holed LLINs (Ochomo *et al.* , 2013). Such behavior may compromise the efficacy of the current indoor-based vector control tools resulting in increases in malaria transmission indoors (Killeen *et al.* , 2006). Recent studies from western Kenya observed high resistance levels, rates of human blood index and sporozoite rates in the mosquitoes resting indoors compared to the mosquitoes collected resting outdoors (Degefa *et al.* , 2017; Machani *et al.* , 2020). The study findings are in agreement with similar studies that have observed reduced host-seeking performance of susceptible mosquitoes in the presence of LLIN unlike the resistant mosquitoes whose behavior was not altered (Kawada *et al.* , 2014; Diop *et al.* , 2015; Porciani *et al.* , 2017; Zhou *et al.* , 2020).

This findings of this study shows that despite the coverage of the indoor interventions, it is evident that not all malaria transmission can be controlled with the existing tools that are indoor based. The population of vectors that move outdoors are not taken care of, a situation that creates a pressing need for supplementary vector control tools to control residual transmission.

Conclusion

The results show significantly reduced avoidance behavior of the resistant mosquitoes compared with the susceptible population. The susceptible females were more likely to exit and rest outdoors away from the treated environment. This might be a reason for increased outdoor malaria transmission in sub-Saharan Africa. This situation calls for urgent deployment of control tools that can complement the current vector control methods to tackle outdoor malaria transmission.

Abbreviations

F_1**:** first filial generation F_6: sixth filial generation Vgsc: voltage-gated sodium channel ITNs: insecticidal treated nets LLIN: Long lasting insecticidal nets IRS: indoor residual spray KDR: Knockdown resistant gene

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Authors' contribution

MM, EO, GY, and YAA conceived and designed the study. MM performed data collection, MM did data analysis and drafted the manuscript. EO, FA, AKG, GY, and YAA, supervised data collection and manuscript writing. All authors have read and approved the final manuscript.**Competing interest**The authors have declared that no competing interest.

Availability of data and materials

The datasets used for the current study are available at the repository of the Kenya Medical Research Institute.

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Population	**Condition**	**No. released**	**No. recaptured**	**exiting (%)**	**Odds ratio**	**CI 95%**
wild population (F1 progeny)	Treated	1000	504	4.0	1.1	0.59-2.16
	Untreated	1000	509	3.5	0.9	0.46-1.69
Susceptible	Treated	3000	1745	11.1	4.6	3.31-6.51
	Untreated	3000	1648	3.0	0.2	0.15-0.30
Resistant	Treated	3000	1644	5.2	2.1	1.41-2.99
	Untreated	3000	1628	2.6	0.5	0.33-0.70

Table 1. Proportion of mosquitoes exiting following the use of treated and untreated bednet trap

List of figures

Figure1. The semi-field set-up photographs showing (A) The screen house, (B) inside the screen house with a traditional hut and plants, (C,D) erected bed net trap (Mbita trap) with the upper chamber containing trapped mosquitoes as shown with circles, (E) exit trap fitted on the window of the hut, (F) clay pots for collecting outdoor resting mosquitoes.

Figure 2. Bed net trap photographs showing (A) upper chamber contained a netting panel fixed halfway (B) the lower chamber.

Figure 3 . Percentage of host-seeking mosquitoes from the three populations trapped in the treated and untreated Mbita bed net trap. Error bars indicate 95% confidence intervals.

Figure 4 . Percentage of mosquitoes resting indoors and outdoors when a treated and untreated bed net trap was present. Error bars indicate 95% confidence intervals.

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