RESEARCH ARTICLE

Variation in the effectiveness of insecticide treated nets against malaria and outdoor biting by vectors in Kilifi, Kenya [version 1; referees: 4 approved with reservations]

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Abstract

Background: Insecticide treated nets (ITNs) protect humans against bites from the Anopheles mosquito vectors that transmit malaria, thereby reducing malaria morbidity and mortality. It has been noted that ITN use leads to a switch from indoor to outdoor feeding among these vectors. It might be expected that outdoor feeding would undermine the effectiveness of ITNs that target indoors vectors, but data are limited. Methods: We linked homestead level geospatial data to clinical surveillance data at a primary healthcare facility in Kilifi County in order to map geographical heterogeneity in ITN effectiveness and observed vector feeding behaviour using landing catches and CDC light traps in seven selected areas of high and low ITN effectiveness. Results: We observed 33% and 39% visits associated with positive malaria slides among ITN users and non-ITN-users, respectively; ITN use was associated with 22% protection from malaria (crude OR = 0.78, 95% CI: 0.72, 0.84). We obtained significant modification of ITN effectiveness by geographical area (p=0.022), and identified significant hotspots using the spatial scan statistic. Most biting occurred outdoors (62%) and was by An. funestus (76%), and appeared to be more frequent in low ITN effectiveness areas compared with high ITN effectiveness areas (69% vs. 26%, p<0.001), but this was due to a single outlying area. After excluding this outlying area, outdoor biting was similar in low vs. high ITN effectiveness area (69% vs. 75%, p=0.76). Conclusion: Our data therefore do not support the hypothesis that outdoor biting undermines the effectiveness of ITNs in our study area.

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Introduction

Despite the recent scale-up effort to achieve control, malaria continues to cause morbidity and mortality, especially in sub-Saharan Africa. There are uncertainties in global estimates\(^6\); however in 2015, the World Health Organization estimated global deaths due to malaria to be 438,000 (range: 236,000–635,000) and the burden of febrile illness at 214 million cases (range: 149–303 million)\(^7\). Modelling studies suggest that approximately 1.4 billion of the world’s population live at risk of stable malaria and ~1.1 billion at risk of unstable malaria\(^8\).

The frontline tools for malaria control in sub-Saharan Africa, insecticide treated nets (ITNs) and indoor residual spray, are optimally effective if baseline transmission occurs indoors\(^9\). The major vectors of human malaria mostly feed indoors, and transmission can therefore be substantially reduced by these tools\(^9\). The proportion of the at-risk population who have access to ITNs was modeled to have increased from 4% to 67% between 2004 and 2015\(^10\). ITNs operate in three ways: deterrence, excito-repulsion and killing, thereby reducing the density, feeding frequency, feeding success, and survival of *Anopheles* mosquitoes\(^1\). By reducing vector densities and vector survival, ITNs not only directly protect the individual ITN user, but also reduce the overall transmission intensity and protect the whole community when a particular threshold of bed net coverage is reached\(^10\). The evidence base supports ITN use over a range of transmission intensities\(^11\) and protective efficacy has been demonstrated against infection, clinical disease and mortality\(^12,13,14\). However, residual malaria transmission is well described even after optimal ITN use, which could be caused by changes in the behaviour of the mosquito vector that allows them to evade fatal contact with these frontline tools of intervention\(^15\). The most obvious behavioural change is the mosquito vector exhibiting exophilic tendencies – i.e. the vector feeds outdoors on humans.

Among malaria vectors in Africa, the two principal species complexes are: *Anopheles gambiae sensu lato* (s.l.) and *Anopheles funestus* group. Both species complexes feed primarily indoors; however both have exhibited behavioral shifts to outdoor biting or feeding in the early part of the evening following prolonged use of ITNs in some areas\(^6,19,20\). This behavioral change might have resulted from one of three processes: (i) selection, either for species that more readily engages in outdoor feeding, for instance in favour of *An. arabiensis* rather than *An. gambiae sensu stricto* (s.s.); (ii) by selecting for evolutionary change within a species; or (iii) a response to inability to feed during the night in the absence of genetic variation\(^15\). In Western Kenya and South-eastern Tanzania there have been reports of a reduction in indoor feeding by *A. gambiae sensu stricto* (s.s.) and an increase in the relative abundance of *A. arabiensis*. The latter has a broader range of feeding times and biting behavior, including outdoor feeding\(^21-23\). In northern Tanzania, where ITNs have been used for several years, the mosquitoes are biting more frequently during the hours of the early evening and early morning when people are more likely to be awake and vulnerable outside of their nets\(^24,25\). The potential for ITNs to result in species switches was appreciated in earlier controlled trials\(^11,12,26\), and is now reported more widely as ITN use is scaled up in Western Kenya and on the East African coast\(^12,27\).

In Kilifi, Kenya, a switch in the most common vector, from *An. gambiae sensu stricto* (s.s.) to *An. arabiensis*, occurred during the period of ITN scale-up\(^19\). The increased ability of *An. arabiensis* to feed outdoors might be expected to result in a decrease in ITN effectiveness. However, there is little data to support this contention, and some data and models that are available suggest that ITNs continue to be effective despite outdoor feeding\(^26,27\). The objectives of this study were (i) to examine whether there has been a shift in vector biting patterns and/or vector behaviour, during the period of intense ITN use along the Kenyan coast; (ii) to test for geographical heterogeneity in ITN effectiveness within the surveillance area of a primary healthcare facility in Kilifi County; and (iii) to assess whether outdoor vector biting is a potential explanation for the variation in ITN effectiveness.

Methods

Study area

The clinical surveillance study was conducted between January 2009 and December 2014 within a 6km radius of Pingilikani dispensary in Kilifi County on the Kenyan Coast (Figure 1): within the Kilifi Health and Demographic Surveillance System (KHDSS). All children under 13 years presenting for medical assessment to Pingilikani dispensary (except those with trauma as their only concern) were assessed by research staff and had finger-prick blood samples examined for malaria parasites. Thick and thin blood smears were stained with 10% Giemsa and examined at 1000X magnification for asexual *Plasmodium falciparum* parasites. Before slides could be considered negative, 100 fields were examined. Children with malaria positive slides were treated with co-artemether.

Transmission of malaria peaks after the long rains from April to June and the short rains from October to November each year, although transmission has been declining\(^24-31\). The surveillance area was divided into 2.5x2.5 km regular polygons resulting in 21 geographical areas (Figure 2). As part of KHDSS, four-monthly enumeration rounds were conducted to identify births, deaths and migration events. Each inhabitant was described by their family relationships and their homestead of residence, with geospatial coordinates, and assigned a unique personal identifier\(^32\). These details were used to link children visiting Pingilikani dispensary to geospatial coordinates for the homestead of residence. Data on ITN use was collected once yearly during cross-sectional surveys integrated into the regular KHDSS enumeration since 2008. Questionnaires were used to collect household data on ITN ownership and use on the night prior to enumeration. Seven geographical areas were selected for mosquito sampling out of 21 areas for which clinical effectiveness estimates were determined (Figure 2). The basis of selecting the seven areas was (i) geographical areas with >60 homesteads available for randomization; (ii) areas representative of highest and lowest ITN effectiveness.

Mosquito sampling

Indoor and outdoor biting profiles of *An. gambiae s.l.* and the *An. funestus* group were estimated using human landing catches (HLC) and CDC-light traps (CDC-LT) by visiting randomly selected houses (random selection done by stratified sampling) between July and August 2016. For both indoor and outdoor...
Figure 1. Situation of Kilifi County in Kenya and the map of Kilifi County showing the boundaries of the KHDSS. The map of KHDSS shows the locations and the situation of homesteads and Pingilikani dispensary where the study was conducted. The brown plotted point on the KHDSS map represents homesteads.

Figure 2. Map of the 2.5X2.5 km geographical areas (grids in light gray), the geographical areas where mosquito sampling was conducted (grids in dark gray) and the homesteads where mosquito sampling was done. Each plotted point represents an individual homestead, where color shading indicates ITN effectiveness, with red shading indicating low effectiveness and blue shading indicating high effectiveness.
mosquito collection, HLC was conducted by two pairs of trained male volunteers (one pair was located indoors and the other pair outdoors, but at the same homestead), who sat with their legs exposed and caught mosquitoes that attempted to bite them using an aspirator. HLC was conducted between 18:00:00 hours and 06:00:00 hours for 45 minutes each hour, allowing 15 minutes break for rest. The catches for each hourly interval were stored in separate collection cups. CDC-light traps were also set indoor and outdoor between 18:00:00 hours and 06:00:00 hours. The HLC and the CDC-LT collections took place in different houses. In each geographical area, sampling was conducted for at least 3 days in at least 16 houses; 8 houses for HLC and 8 houses for CDC-LT. In total, 26 days of sampling were conducted across 115 houses in the seven selected geographical areas within the surveillance area.

Mosquito processing
The mosquito samples were morphologically separated for sex and identified for species. The female *Anopheles* mosquitoes were tested for falciparum infection using a sandwich circumsporozoite protein (CSP) enzyme linked immunosorbent assay (ELISA) (anti-CSP capture: P2A10-28 and conjugate: P2A10-CDC antibodies; KPL, Gaithersburg, MD, USA). Individual mosquitoes were stored at -20°C in micro-centrifuge tubes containing a small amount of desiccant (silica gel) separated from the mosquito by a thin layer of cotton prior to ELISA and molecular analysis for sibling species by polymerase chain reaction.

Statistical analysis
Statistical analyses were performed using STATA v13.1 (StataCorp, College Station, TX, USA). To assess for geographical heterogeneity, we used the logistic regression model to analyze data on over 20,000 visits from children attending Pingilikani dispensary. The outcome of interest was presence of malaria by microscopy on presentation to the dispensary. The potential risk factors included: ITN use, age of the child, year of presentation to the dispensary and the geographical area, as defined by the 2.5×2.5 km regular polygons. We assessed whether the effect of ITN use on malaria was altered by geographical area by including an interaction term between geographical area and ITN use. We also assessed whether the effect of ITN use was altered by the age of the child and whether geographical areas altered the effect of age. To assess the nonlinear effect of age in the regression models, multiple fractional polynomial transformation was used. Given that the hospital malaria episodes were clustered within patients, we allowed for clustering by using a logistic regression model with robust standard errors. The robust standard errors were used to account for the clustering effect in the estimation of the standard errors. The ratio of malaria in the non-ITN users that to in the ITN users was expressed as an odds ratio (OR) as determined by logistic regression. ITN effectiveness was calculated as (1 – OR) × 100. Model fit was assessed by examining residuals against covariates. Spearman’s rank correlation was used to assess the association between ITN effectiveness and prevalence of malaria. SaTScan software (version 9.4; https://www.satscan.org/), a spatial scan statistic developed by Kulldorff, was used to detect potential spatial variations of ITN effectiveness by identifying statistically significant geographical clustering of ITN effectiveness.

In order to compare counts of female *Anopheles* captured, we determined the relative proportion of each mosquito species in each geographical area and ITN effectiveness levels (ITN effectiveness was divided into 2 levels based on the estimates obtained from the logistic regression above – i.e. high and low ITN effectiveness). Three areas with high ITN effectiveness and four areas with low ITN effectiveness were selected based on the findings of the scan statistic. We compared the proportion of vectors biting outdoors in each geographical area. We estimated the confidence intervals of these proportions using the binomial distributions, and tested for an association between biting preference and ITN effectiveness (at the level of geographical area).

Results
Geographical variations in ITN effectiveness
Between 2009 and 2014, there were 20,827 visits to Pingilikani dispensary made by 4,992 children aged between 3 months to 12 years (Supplementary Table 1). Of these visits, 7,220 (35%) were classified as episodes of malaria, with a median number of 7 (IQR: 4, 12) episodes per child during this time period. The number of children, cases of malaria and ITN use in the 21 geographical areas examined is summarized in detail in Supplementary Table 1. ITN use was consistently >50% in all geographical areas and the prevalence of ITN use in non-malaria cases was 74.9% (95% CI: 74.2, 75.6).

Among children who were ITN users, 33% (5045/15234) of the visits were associated with positive malaria slides, whereas among non-ITN-users 39% (2175/5593) of the visits were associated with positive malaria slides. ITN use was associated with a 22% protection from malaria; crude OR = 0.78, 95% CI: 0.72, 0.84 (p<0.001). When geographical area was added to the model as an interaction term with ITN use, we obtained a statistically significant variation in ITN effectiveness between the geographical areas (p=0.014). Geographical variation in ITN effectiveness remained robust (p=0.022) even after adjusting for the year of visitation to the dispensary and plausible interactions (i.e. interactions between ITN use and nonlinear age, and between geographical area and nonlinear age). The stratum specific adjusted OR for the association of ITN use on malaria in the geographical areas was calculated and shown in the order of decreasing effectiveness (Figure 3 & Supplementary Table 1). Previous data have shown that ITN effectiveness is lower in areas of high malaria transmission. This did not appear to be the explanation for variation in effectiveness in this data (Supplementary Figure 1); the Spearman rho coefficient value for the association of ITN effectiveness and prevalence of malaria was 0.308, p=0.331.

Hotspots
Using the logistic regression model, we estimated ITN effectiveness for each individual homestead where there was sufficient data to calculate a point estimate (i.e. >30 observations from homestead aggregated at a 2.5 km smoothing). Using SaTScan software, we identified 6 significant hotspots of low ITN effectiveness: p=0.001 for 4 hotspots, p=0.002 and p=0.014 for a 5th and 6th hotspot (Figure 4). We concluded that spatial variation in ITN effectiveness was not due to random noise based on the 95% confidence intervals obtained from the logistic regression.
Figure 3. Scatter plot of stratum specific adjusted Odds Ratio and 95% confidence intervals of 12 geographical areas in order of decreasing effectiveness.

Figure 4. Scatter plot of estimated insecticide treated net (ITN) effectiveness for individual homesteads aggregated at a 2.5km smoothing. Each plotted point represents an individual homestead, where color shading indicates ITN effectiveness, with red shading indicating low effectiveness and blue shading indicating high effectiveness. The large black circles indicate the significant hotspots (analyzed without smoothing).
analysis for geographical areas and the existence of significant hotspots by SaTScan, and selected seven geographical areas for further entomological studies to represent a range of ITN effectiveness estimates.

**Vector abundance**

Over 26 nights, 411 female *Anopheles* mosquitoes were collected by both methods (i.e. 259 by HLC and 152 by CDC-LT), representing a mean of 15.8 mosquitoes per night. 63% of mosquitoes were collected using HLC. Of the 411 mosquitoes, 314 (76%, 95% CIs 72%, 80%) were *An. funestus* group, which was significantly greater than *An. gambiae s.l.* (p<0.001). The proportion of *Anopheles* mosquitoes caught outdoors (62%; 95% CI: 57,67) was significantly greater than the proportion caught indoors (p<0.001). There were more *Anopheles* mosquitoes collected outdoors in all geographical areas except area 6, where most of the mosquitoes were collected indoors (Table 1). The frequencies of species collected in each geographical area are summarized in Supplementary Table 2. *An. funestus* group was the most prevalent species in all areas.

The species and proportion of mosquitoes collected in areas of high vs. low ITN effectiveness are summarised in Table 2 and Supplementary Figure 2. Overall, the proportion of outdoor biting was higher in the low ITN effectiveness areas (69% vs. 27%, p <0.001), but this apparent significance was due to a single area (labelled area 6), which was an outlier for indoor biting (Figure 5). When we excluded area 6, the proportion of outdoor biting in the low vs. high ITN effectiveness areas was non-significant (69% vs. 75%, p=0.76). Moreover, when analysed by individual geographical area there was not a visually obvious trend associating increasing outdoor biting with decreasing ITN effectiveness in the seven geographical areas (Figure 5). The Spearman rho coefficient value for the association of ITN effectiveness and proportion of mosquitoes collected outdoors was -0.464, p=0.302.

### Table 2. Composition of the mosquito species in areas of high and low ITN effectiveness.

<table>
<thead>
<tr>
<th>Trap type</th>
<th>Species</th>
<th>Low ITN effectiveness areas</th>
<th>High ITN effectiveness area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total (N)</td>
<td>Outdoor (n)</td>
</tr>
<tr>
<td>HLC</td>
<td><em>An. gambiae</em></td>
<td>57</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td><em>An. funestus</em></td>
<td>152</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>Other Anopheles</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>230</td>
<td>135</td>
</tr>
<tr>
<td>CDC-LT</td>
<td><em>An. gambiae</em></td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><em>An. funestus</em></td>
<td>96</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Other Anopheles</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>110</td>
<td>100</td>
</tr>
</tbody>
</table>

*HLC: Human landing catches, CDC-LT: CDC light trap, %: Proportion per 100, N & n: number of mosquitoes collected.

### Table 1. Proportion of *Anopheles* mosquitoes collected indoors and outdoors.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number collected</th>
<th>% Indoor (CI)</th>
<th>% Outdoor (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>411</td>
<td>38 [33, 43]</td>
<td>62 [57, 67]</td>
</tr>
<tr>
<td>Species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>An. gambiae s.l.</em></td>
<td>76</td>
<td>17 [9, 27]</td>
<td>83 [73, 91]</td>
</tr>
<tr>
<td><em>An. funestus</em></td>
<td>314</td>
<td>46 [40, 52]</td>
<td>54 [48, 60]</td>
</tr>
<tr>
<td>Other anopheles</td>
<td>21</td>
<td>0</td>
<td>100 [84, 100]</td>
</tr>
<tr>
<td>Geographical area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>171</td>
<td>46 [38, 53]</td>
<td>54 [47, 62]</td>
</tr>
<tr>
<td>6</td>
<td>59</td>
<td>83 [71, 92]</td>
<td>17 [8, 29]</td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>44 [14, 79]</td>
<td>56 [21, 86]</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>33 [4, 78]</td>
<td>67 [22, 96]</td>
</tr>
<tr>
<td>16</td>
<td>6</td>
<td>17 [0.4, 64]</td>
<td>83 [36, 99]</td>
</tr>
<tr>
<td>19</td>
<td>113</td>
<td>12 [7, 20]</td>
<td>88 [80, 93]</td>
</tr>
<tr>
<td>20</td>
<td>47</td>
<td>19 [9, 33]</td>
<td>81 [67, 91]</td>
</tr>
</tbody>
</table>

*CI: Confidence Interval, %: Proportion per 100

**Discussion**

Malaria is an important public health problem in sub-Saharan Africa, and many countries, including Kenya, have attempted to reduce this burden by increasing ITN ownership and usage. However, previous reports have shown that prolonged ITN use leads to behavioral shifts in the mosquito vector from indoor to outdoor biting or feeding in the early part of the evening. This shift in mosquito feeding behavior might be expected to result in a decrease in ITN effectiveness. We identified statistically significant
geographical variation in the effectiveness of ITN and identified areas where ITN effectiveness was found to be consistent with the 50% estimate reported in the literature\textsuperscript{[1,39,40]}, and other areas where ITNs were less effective (Figure 3). This variation could conceivably have arisen as a result of variations in quality of ITNs, patterns of use, host resistance, insecticide resistance or other factors, including random variation. We investigated whether variations in outdoor vector biting was a potential explanation.

We found that \textit{An. funestus} was more prevalent than \textit{An. gambiae s.l.} species complex, consistent with a previous report\textsuperscript{19}. We observed small-scale spatial variability in vector abundance (Table 1), which is consistent with previous reports on the Kenyan Coast\textsuperscript{20,41}. We also observed a higher proportion of mosquito vectors collected outdoors than indoors, in areas of both high and low ITN effectiveness (Figure 5). On first principles one would expect that outdoor biting would lead to ITNs becoming ineffective. However, despite seeing consistent outdoor biting throughout the study area this did not appear to be associated with an overall reduction in ITN effectiveness. We may have observed an apparently statistically significant increase in the prevalence of outdoor biting in areas of low ITN effectiveness. However, this was due to a single outlying geographical area and there was no variation in prevalence of outdoor biting after this area was excluded. This suggests the statistical significance of the initial comparison may have been due to ecological confounding, where a geographical area with high ITN effectiveness happened to have more indoor mosquitoes, but this relationship was not confirmed in other areas (Figure 5).

How should we interpret the finding that outdoor feeding does not consistently lead to a reduction in ITN effectiveness? It is possible that the higher proportion of mosquitoes biting outdoors represents a behavioral response to unsuccessful feeding attempts made indoors during the night, and therefore it may simply be a marker of successful ITN use. This avoidance behavior may exert a cost on the vector, and so ITNs may in fact still be protective in areas where outdoor biting is observed, as has been suggested previously\textsuperscript{27}.

Spatial heterogeneity in malaria exposure has been described at micro-epidemiological level at varying transmission settings\textsuperscript{42} and is responsible for variations in disease risk within small geographical areas and is evidenced by local clustering of malaria infections. Within the 2.5 km squared geographical areas, ITN effectiveness

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{scatter_plot.png}
\caption{Scatter plot of estimated insecticide treated net (ITN) effectiveness and the proportion of \textit{Anopheles} mosquitoes collected outdoors and 95% confidence intervals of these estimates.}
\end{figure}
appears to have been spatially heterogeneous (Figure 4); however, we were unable to demonstrate a significant association between ITN effectiveness and outdoor biting at the level of seven small geographical areas. The observed geographical variation in ITN effectiveness therefore remains unexplained. Possibilities include insecticide resistance, or geographical variations in human behavior in terms of ITN use.

Our study has some limitations. Data on ITN use may have been incorrectly reported, as we did not require each resident to be present to respond to the ITN ownership and use questions. We attempted to minimize this by instructing data collecting teams to interview only residents of the same homestead regarding ITN ownership and usage. There may have been some misclassification as we did not ascertain ITN use during hospital visitation but instead used the yearly ITN data collected by the KHDSS. The results may also be biased and confounded by other unmeasured factors (e.g., variation in the quality and type of ITN, urbanization, socio-economic status and mother’s education). Therefore, the estimates obtained could be an overestimation or underestimation of the true effectiveness. It is likely that we underestimated the protection afforded by the use of high-quality ITN because we included all ITNs, regardless of quality. The vast majority of ITNs in the area are long-lasting insecticidal nets, hence we do not expect substantial variation in insecticidal efficacy. The accuracy of the human landing catches may be affected by the inter-individual differences in attracting mosquitoes. The size of our study limits power: with a sample size of 411, and the proportion of mosquitoes biting outdoors at 69% in low ITN effectiveness areas we therefore had >90% power to detect a reduction to 27% or lower in high ITN effectiveness areas. Our study was therefore powered to detect only a large difference in the proportion of vectors caught outdoors. However, we reasoned that reductions of ITN effectiveness to less than half of the previously documented efficacy of 50% would require a doubling of the proportion of mosquitoes feeding outdoors. Hence our study was powered to detect large variations in the frequency of outdoor biting. Furthermore, since the proportion of vectors collected outdoors was high throughout the study area despite preserved ITN effectiveness in many areas, we conclude that the pattern of outdoor feeding identified in our site does not undermine ITN effectiveness.

In summary, we found no evidence that the currently observed switch from indoor to outdoor biting leads to reduced ITN effectiveness. The outdoor biting observed may therefore have been the result of high levels of ITN use leading to unsuccessful attempts at indoor feeding. It remains possible that selection pressures might lead to the emergence of populations of mosquitoes that are better adapted to outdoor feeding. Outdoor feeding is becoming more common in parts of Africa and may represent evolutionary change in some areas, with a potential to undermine ITN effectiveness. Therefore, malaria control programs require monitoring to assess the impact of ITNs on vector populations and vector behavioral change as well as monitoring ITN effectiveness as vectors evolve. Detailed studies of vector bionomics, continuous monitoring and malaria transmission dynamics are essential for predicting disease outbreaks and vector control in the region.

Ethical approval
This study was approved by the Kenya Medical Research Institute Scientific Ethics Review Unit (KEMRI/ERU/CGMER/C/024/3148). Written informed consent was obtained from the parents/guardians of the children attending the dispensary.

Data availability
Data that support the findings of this study (hospital surveillance, ITN community surveys and mosquito collection) are available from the KEMRI Institutional Data Access/Ethics Committee, for researchers who meet the criteria for access to confidential data. Details of the criteria can be found in the KEMRI-Wellcome data sharing guidelines. The data includes homestead level coordinates as an essential component and these are personally identifiable data. Access to data is provided via the KEMRI-Wellcome Data Governance Committee: Data_Governance_Coordinator@kemriwellcome.org; Tel, +254708 587 210; Contact person, Marianne Munene (Secretary; Tel, +254709 983 436).

Author contributions
AK oversaw field implementation of the study, analyzed and interpreted the data and drafted the manuscript. JMM, MKR and PB conceived the study, helped with the field implementation of the study, and reviewed and revised the manuscript. PM, IO, JM, and JAGS reviewed and revised the manuscript. All authors approved the final manuscript, as submitted.

Competing interests
The authors declare that they have no competing interests.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Supplementary material

Supplementary Table 1: Description of insecticide treated net (ITN) use, cases of malaria and ITN effectiveness in the 2.5x2.5 km geographical areas. Data includes the number of children observed, number visits made to Pingilikani hospital by the children, the number and proportion of malaria among ITN use or non-ITN-users in the 21 geographical areas, the stratum specific adjusted Odds Ratio (aOR) and the Confidence Interval (95% CI); ‡ areas with fewer than 35 observations were excluded from the logistic regression due to perfect prediction and/or collinearity.

Click here to access the data.

Supplementary Table 2: Composition of the mosquito species in seven geographical areas. Data includes number of Anopheles mosquitoes collected by human landing catches (HLC) and CDC light trap (CDC-LT) indoor or outdoor in the seven geographical areas, and the overall proportion.

Click here to access the data.

Supplementary Figure 1: Scatter plot of the log odds ratio of insecticide treated net (ITN) effect and 95% confidence interval of the overall proportion.

Click here to access the data.

Supplementary Figure 2: Bar graph of the proportion of Anopheles mosquito species collected in areas of low and high insecticide treated net (ITN) effectiveness.

Click here to access the data.

References


Open Peer Review

Current Referee Status: ? ? ? ?

Version 1

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The authors have conducted a study addressing the hypothesis that outdoor feeding of mosquitoes undermines the effectiveness of ITNs.

The entomology presented in the paper is inadequate to answer the hypothesis presented for the following reasons:

1. 5 years of clinical data are presented (2009-2014) but only one month of mosquito sampling is conducted in 2016, two years after the last piece of clinical data was collected.

2. No PCR speciation was reported. In this area there are a number of cryptic species that look the same but differ in both their behaviour and their ability to transmit malaria. No molecular techniques were used to test the mosquito species. So you could have a switch from An. gambiae s.s. that bites indoors and has high vectorial competence to An. arabiensis that bites outdoors and has lower vectorial competence. The same is true in the An. funestus complex that is comprised of a number of outdoor biting species like An. leesoni or An. rivulorum.

3. The authors reported that PCR (polymerase chain reaction) was done on the mosquitoes yet I cannot find data in the paper reporting the outcome of the PCR. All data reports An. gambiae s.l. and An. funestus group.

The paper explores changing mosquito behaviour with lowered effectiveness of nets but only used one month of vector collections two years after the clinical data was collected to test this link and the actual species present are not reported. I therefore find this a big stretch of the data. Vector density, composition and behaviour varies throughout the year and these collections were made for a short time. I therefore don't think the data are sufficient to accept or reject the hypothesis.

That being said the rest of the data is very useful and nicely presented. The data do demonstrate that there is substantial outdoor biting in June/July, and I should like to see the species composition in the area seeing as the authors report that the PCR was done. Outdoor biting may not increase malaria if the vectors doing the outdoor biting are not very competent for malaria.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Referee Expertise: Medical entomology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Gerry F. Killeen

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Apart from some unfortunately important exceptions, the data for this study are meticulously collected and analysed. However, many of the most important results are either over-interpreted or misinterpreted so these exceptions are substantive. In fact, the correct interpretation may well be almost the exact opposite of that presented here: That LLINs are consistently effective across a landscape where transmission is dominated by a vector that primarily attacks people indoors at night while they are asleep.

1. The biggest single problem with this paper is that the indoor and outdoor biting rate estimates come from stationary, fully exposed human volunteers exhibiting artificial experimental behaviours, without adjusting them for normal human behaviours that mean most of us are indoors asleep during the peak biting hours of nocturnal African malaria vector mosquitoes. This is an understandable and common mistake, but a very important one. Like Anopheles funestus in most locations across Africa, the 55-45 distribution of biting location preference for this population is essentially indiscriminate, so it is the behaviour of humans that determines where exposure actually occurs. So unless everyone in coastal Kenya sleeps half indoors and half outdoors throughout the night, simply comparing indoor versus outdoor HLCs is misrepresentative and greatly exaggerates the contribution of outdoor biting to transmission by this species. Once adjusted for human behaviour patterns, >90% of human biting exposure to this key vector species
1. The most important data clearly missing from the characterization of the study scenario are (a) sporozoite rates (mentioned in the methods but not the results) and EIR estimates, to confirm that Anopheles funestus group mosquitoes are the most important vectors of malaria in this area, (b) quantitative estimates of where and when humans are exposed to these two major vector taxa (not species unless PCR data are added) that weight the biting estimates by surveys of human behaviour [2-5]. These are increasingly common calculations applied to data from all over the tropics [6-13], and vitally important to conduct before making any quantitative statements about proportional contributions of outdoor biting exposure.

2. There is no evidence of any “shift” in behaviours over time presented here, so the term “undermines” is unjustified and seems to create an argument that hasn’t been made. Most behaviours that enable residual malaria transmission despite LLIN use are pre-existing, although plastic, and often it’s just the vector population composition that shifts 14, so the term “limits” is more appropriate.

3. While indeed there is no evidence here that outdoor transmission contributes to ongoing transmission, there is also no evidence that it does not. Such outdoor fractions of transmission can only be expected to become epidemiologically detectable once larger quantities of indoor transmission (which I’m convinced is the case here as explained above) have been tackled. So the phraseology of conclusions needs to be tempered using words like “yet”, and explain how these currently minor fractions of transmission may emerge as important contributors to sustained endemicity once further progress has been made with indoor control [14,15].

4. In any case, LLINs clearly fall a long way short of being 100% efficacious with 22% personal protection estimated here, so there clearly are considerable limitations to this technology that need explanation. To get a better handle on whether outdoor exposure does contribute to residual transmission, in our experience it’s necessary to test as a function of individual human behavioural profiles weighted by activity patterns for the most dominant local vectors [13]. Indeed human behaviour is the primary driver of where and when exposure occurs [1] and is far more variable than the mosquito behaviours that matter within a single vector species [15].

5. In any case, very few mosquitoes were caught (Supplementary Table 2) and CDC light traps catches indoors and outdoors are not comparable, so reporting these data as indicators of the degree of exophagy or endophagy is going too far and overstretching very little entomological data.

6. The fact that these are not differentiated to species (again, though this is mentioned in the methods but no results are presented) also means that areas with apparently different mosquito behaviours are probably areas that simply have different relative abundances of primary vector, secondary vector and non-vector species within the Anopheles funestus group and within the Anopheles
gambiae complex. For example, greater outdoor feeding at dawn and dusk is a known characteristic of Anopheles rivulorum and Anopheles parensis, originally discovered in this region on the basis of their distinctive behaviours and much weaker vectorial capacities.

8. The term “species” is used very loosely and interchangeably with other taxonomic classification levels, resulting in some misleading over-interpretation. While Anopheles gambiae sensu lato is indeed a complex, Anopheles funestus sensu lato is a group (not a complex, as stated in the introduction) and neither can be described as a species, unless one is talking about unambiguously identified individual specimens of the nominate species, which are by far the most efficient species within each taxon.

9. All of these most important limitations seem to be missing from the paragraph opening with the sentence “Our study has some limitations”.

10. What is called “effectiveness” here refers only to the relatively minor personal protection effect of bednets, and does not capture any variations in community-level impact. All fine but please explain this study limitation clearly.

11. Correspondingly, doesn’t capture how big a change this transmission picture is relative to the same setting 10 to 15 years ago when nominate Anopheles gambiae were still quite abundant. The explanations about the relative abundance of vector taxa (not species) is accurate but rather static and lacking in long term context, demonstrating the much bigger overall impact on vector populations and endemicity. This is a pity when this contemporary study has been conducted in an area with so much historical entomological literature, so please enrich the narrative.

12. While I agree with the closing statement about enhancing entomological surveillance, in my experience many groups are under-interpreting or misinterpreting the data they already have, so perhaps that capacity limitation merits some emphasis as a priority.

References


*Is the work clearly and accurately presented and does it cite the current literature?*

No

*Is the study design appropriate and is the work technically sound?*

No

*Are sufficient details of methods and analysis provided to allow replication by others?*

Partly

*If applicable, is the statistical analysis and its interpretation appropriate?*

No

*Are all the source data underlying the results available to ensure full reproducibility?*

Partly

*Are the conclusions drawn adequately supported by the results?*

No
**Competing Interests:** No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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**Seynabou Sougoufara**

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The authors present here the study on the variation of the effectiveness of insecticide-treated bed nets against malaria and the outdoor biting by vectors in Kilifi, Kenya.

The manuscript reported the geographical heterogeneity of malaria prevalence according several parameters mainly including the ITN effectiveness and the feeding behaviour of *Anopheles* vectors. The design and method of the study are well presented in the section “Methods” as well as the statistical analysis. Clinical surveillance was analyzed in the study between January 2009 and December 2014 that covers a long period. Thus it will be interesting if authors add in their explanatory factors the dry and wet season. It will be also important to explain the discrepancy between the date of clinical surveillance data collection (January 2009 and December 2014) and the mosquito collection (July and August 2016). We have any informations if the level of ITN use varied or is the same during both periods.

Additionally the main part of the subject underlines the effectiveness of the ITNs. However, authors should describe at first that the effectiveness of ITNs is monitoring taking into account the physical integrity of nets, bioefficacy and the insecticidal compounds even though they focused more their study on feeding place and malaria prevalence. It will be also more appropriate if authors interpreted their result according to level of ITNs use according to areas and discuss though their outcomes the effectiveness of ITN. For instance in the abstract the expression of “*high and low effectiveness*” in the part of method is a hasty affirmation.

In the section of “Results” I think that the Supplementary Table 1 has to be presented in the main manuscript as it present malaria prevalence according to area and the level of ITNs use. Moreover the presentation of results must be more detailed and the effect of each risk factors cited in the part of “Statistical analysis” must be presented. I don’t understand why authors said “*ITN use was consistently >50% in all geographical areas*”, meaning that here we have no information about the difference of level use between areas. The authors have summarized too much the description of the results in this part. Authors presented in the part “Mosquito processing” laboratory works such ELISA-CSP and molecular analysis, however the results of these analysis have not been presented in this study. Regarding the result on vector abundance, authors have to present the results according to absolute densities and less on the proportion of species in the place of mosquito collection.

The relevance of the study will be more remarkable if authors greatly discuss in deep their outcomes by
comparing with other studies. Additionally, the review of the literature has to be strengthened, “33 off the 43 references are more than 5 years old and some newer papers are missing.

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Partly

**Are all the source data underlying the results available to ensure full reproducibility?**
Partly

**Are the conclusions drawn adequately supported by the results?**
Partly

**Competing Interests:** No competing interests were disclosed.

**Referee Expertise:** Entomology, immunology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Referee Report 25 April 2017

doi:10.21956/wellcomeopenres.11943.r22156

Heiko Becher
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This report refers to statistical methods. Other relevant issues (“… and does it cite the current literature?”) are not considered.

The authors investigate the relation between ITN use and malaria prevalence, and secondly spatial variation in the effectiveness of ITN.

Overall, the methods are too briefly described and make a thorough evaluation difficult. Some remarks may help to update the manuscript.

- The authors collected data from 20827 visits of 4992 children, i.e. about 5 visits for each child on average, from 21 areas. For each visit, parasitemia was assessed. The probability of parasitemia was modeled with a logistic regression model with ITN use, age, year and area as covariables, plus interaction terms. To account for correlated observations, a robust estimate of the standard
errors was employed, although the exact method used is not given (reference should be provided). I wonder why season (rainy / dry) was not considered. The full result of the model is not given, and I wonder whether the large number of interaction terms in the model gave in a meaningful result. The Supplementary Table 1 gives the ORs for ITN use by area which is difficult to follow since (i) the numbering of the areas does not give information on spatial distribution (ii) it is not easy to see from the table whether malaria prevalence and ITN use differs between areas (iii) the effect of the other covariables is unknown (is there some confounding? What is the effect of age? Was a full fractional polynomial procedure used?).

- The Kulldorf statistic was used, if I understand correctly, to identify clusters of high or low ITN effectiveness without taking malaria prevalence and ITN use into account. Is that true? This seems not correct to me but maybe I misunderstood the procedure.

- The proportion of vectors biting outdoors was compared for the areas. This would mean ignoring the absolute biting frequency which differs largely between areas.

Overall, the authors have carefully interpreted the results.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

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