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Insecticide-treated nets provide protection against malaria to children in an area of insecticide resistance in Southern Benin

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Abstract
Background: Malaria control is heavily reliant on insecticides, especially pyrethroids. Resistance of mosquitoes to insecticides may threaten the effectiveness of insecticide-based vector control and lead to a resurgence of malaria in Africa.

Methods: In 21 villages in Southern Benin with high levels of insecticide resistance, the resistance status of local vectors was measured at the same time as the prevalence of malaria infection in resident children.

Results: Children who used LLINs had lower levels of malaria infection [odds ratio = 0.76 (95% CI 0.59, 0.98, \( p = 0.033 \)]. There was no evidence that the effectiveness of nets was different in high and low resistance locations (\( p = 0.513 \)). There was no association between village level resistance and village level malaria prevalence (\( p = 0.999 \)).

Conclusions: LLINs continue to offer individual protection against malaria infection in an area of high resistance. Insecticide resistance is not a reason to stop efforts to increase coverage of LLINs in Africa.

Keywords: Malaria, Insecticide, Pyrethroid, Resistance, Nets
impact of resistance on prevalence. Because of the lack of
evidence on this important issue, a study to measure
the impact of insecticide resistance on epidemiological
outcomes was launched in five countries; this paper
reports on the results of the assessment conducted in
Benin, West Africa [9]. In Southern Benin resistance to
pyrethroids is high [10] and LLINs are the primary form
of vector control, with two pyrethroid-treated nets (del-
tamethrin and permethrin) in widespread use. In a sam-
ples of 21 villages, standard WHO susceptibility tests [11]
were used to measure mortality of mosquitoes exposed
to these insecticides; and this was compared to prev-
ance of malaria infection in children.

The study addressed two questions:
1. Does resistance to pyrethroids reduce the effective-
ness of LLINs for malaria prevention?
2. Are higher levels of resistance to pyrethroids associ-
ated with a greater prevalence of malaria?

Methods
The study was conducted in 21 villages (subsequently
referred to as clusters) in four rural districts in the Plateau
Department of Benin: Ifangni, Sakete, Ketou and Pobe.
The total area is 3264 km² and the population is approxi-
mately 400,000. There are two rainy seasons: April–July
and September–October. The main malaria vectors are
Anopheles gambiae s.s. and Anopheles coluzzii; both
species are found in most clusters [12]. In West Africa,
there is extensive introgression of resistance mutations
between these species [13] and in Benin in particular lev-
eels of resistance are very similar and, therefore, they are
treated as a single entity here [14]. The National Malaria
Control Programme has distributed LLINs treated
with deltamethrin (PermaNet® 2.0 [Vestergaard] and
DawaPlus® 2.0 [Tana Netting]) and permethrin (Olyset®
Net [Sumitomo]) in the Plateau Department.

Mosquito larvae were collected from breeding habitats
once in each cluster. The sampling took place between
June and August 2015. Whenever possible, more than
6 larval habitats were examined in each cluster. Lar-
vae were reared in an insectary with relative humidity
80 ± 10% and temperature 25 ± 2 °C. Adult mosquitoes
were maintained with 10% honey water after emergence.

Bioassays were performed on 2–5 day old females using
either deltamethrin or permethrin at the standard WHO
diagnostic concentrations of 0.05 and 0.75% respectively
gambiae (Kisumu) was used to check the quality of the
impregnated paper. One hour after exposure, mosqui-
toes were transferred to holding tubes and fed with 10%
honey water. Mortality was recorded 24 h post-exposure.

Results
Mortality of mosquitoes exposed to deltamethrin was
measured in all 21 clusters. The median number of mos-
quitos exposed per cluster was 81 [inter quartile range
(IQR) 53–101]. Median mosquito mortality was 55.2%
(IQR 47.4–68.5%). Mortality to permethrin was meas-
ured in 20 of the 21 clusters. The median number of mos-
quitos exposed was 25 (IQR 22–35). Median mortality
was 18.2% (IQR 8.1–32.2%). In all assays there was 0%
mortality in the control group of mosquitoes. There was
poor correlation between cluster specific deltamethrin
and permethrin mortality (correlation coefficient = 0.20, \( p = 0.407 \)) and therefore analyses were conducted separately for each active ingredient.

In the 21 study clusters, 1621 children from 813 households had blood taken for microscopy. Of these 836 (51.6%) had a malaria infection. Nets were used by 1231 (75.9%) of the children the night before the survey. Net use was associated with lower risk of malaria infection, OR 0.76 (95% CI 0.59, 0.98, \( p = 0.033 \)) (Table 1). Prevalence was not significantly different in children who used a deltamethrin compared to a permethrin net: OR 1.13 (95% CI 0.76, 1.69, \( p = 0.543 \)).

There was no evidence that the effect of nets was different in clusters with lower resistance to deltamethrin compared to those with higher resistance OR 1.11 (95% CI 0.65, 1.90, \( p = 0.698 \)) (Table 2); this was true even when restricted just to those children who slept under deltamethrin treated nets OR 0.82 (95% CI 0.44, 1.53, \( p = 0.532 \)). The estimated effect of a 10% increase in mosquito mortality was negligible: OR 1.00 (95% CI 0.85, 1.17, \( p = 0.999 \)). There was also little association between malaria prevalence in clusters with lower resistance to permethrin compared to clusters with higher resistance OR 0.88 (95% CI 0.50, 1.53, \( p = 0.640 \)) (Table 2), even when restricted to children who slept under permethrin treated nets OR 0.66 (95% CI 0.28, 1.54, \( p = 0.336 \)). The estimated effect of a 10% increase in mosquito mortality was negligible: OR 0.99 (95% CI 0.90, 1.09, \( p = 0.893 \)).

**Discussion**

While insecticide resistance in malaria vectors is widespread [2], there is little evidence of its impact on the effectiveness of nets and how in turn this influences epidemiological outcomes [2, 9, 15]. This study found that use of insecticide treated nets was associated with lower risk of malaria infection in an area of high pyrethroid resistance. Furthermore, there was no evidence that the effect of nets differed in villages stratified by vector resistance. The study also found no association between pyrethroid resistance and malaria prevalence. The fact that no impact of pyrethroid resistance on net effectiveness and malaria prevalence was detected in this study is both an interesting and comforting result. It suggests that whilst insecticide resistance is a grave threat to the long-term sustainability of malaria control it has not apparently reached a level, at least in Benin, where it would render LLINs ineffective. Control programmes should continue to strive to attain high LLIN coverage at the same time researchers and industry seek to develop alternative control options.

Nets present a physical barrier to the mosquito as well as the protection offered by the repellent and killing effect of insecticide. In addition to the physical barrier, a recent meta-analysis found that insecticide treated nets still offered greater protection than untreated nets when local vectors are resistant to pyrethroids [16]. Furthermore, even if insecticides no longer kill mosquitoes, there could still be an excito-repellent effect. There are, therefore, plausible mechanisms through which LLINs continue to provide personal protection in the face of pyrethroid resistance.

On top of personal protection offered by LLINs, if the coverage of nets is high enough there is a mass effect [17] which benefits both users and non-users by reducing the lifespan of mosquitoes. If resistance attenuated the mass effect of LLINs without affecting personal protection, one would expect to see a greater difference in malaria risk between users and non-users in areas of high resistance than in areas of low resistance; a difference which could be further exacerbated by diverting mosquitoes from users to non-users without killing them; no such difference was observed in this study.

It would, however, be reckless to conclude from this study that insecticide resistance has no impact on malaria transmission. There are a number of reasons why an association between resistance and malaria prevalence might not have been observed in this study, even if there is an impact of resistance on malaria control. Resistance to pyrethroids was observed in all study clusters [11] and, therefore, there may be an impact of resistance on malaria prevalence across all study clusters but the absence of truly susceptible mosquito populations and a relatively insensitive resistance definition prevents us detecting an effect.

While mortality 24 h post exposure is a pragmatic test for the presence of resistance, it does not necessarily mean it is a good measure of the strength of resistance in resistant mosquito populations. Moreover, recent data suggests that even in mosquitoes classified as resistant by discriminant dose tests there are likely to be epidemiologically important sub-lethal effects [18]. Molecular screening of resistance mechanisms or measures of resistance based on a dose response relationship may be a more informative method of characterization although only the former is likely to be able to be pushed to the scale that studies of this nature require [19].

Since it is not possible to randomize villages to different levels of insecticide resistance and not ethical to
randomize children to not using nets, all studies of this issue must be observational—and, therefore, subject to confounding. In this study, two important confounders (age and SES) were adjusted for which made little impact on the analysis, but some residual confounding may remain [9]. Nonetheless, this study found that LLINs continued to offer individual protection in an area of high insecticide resistance and found no association between resistance and malaria prevalence where LLIN use was high. Insecticide resistance threatens the major gains that have been made in reducing malaria disease burden, but whilst for alternative control approaches are searched for efforts should be redoubled on to increase access to LLINs in Africa.

**Authors’ contributions**

Designed the study: AOH, SC, JF, YSdT, AA, FT, PA, TH, DKG, MA, AM, TBM, MD, IK. Anlysed the data: JB, TL, MD, IK. Wrote the manuscript: JB, AOH, SC, MD, IK. All authors read and approved the final manuscript.

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### Table 1 Effectiveness of nets

<table>
<thead>
<tr>
<th>Area</th>
<th>Reported use of net the previous night</th>
<th>Malaria prevalence in children aged 6 months to 10 years, % (n/N)</th>
<th>Odds ratio (95% CI)</th>
<th>Adjusted odds ratio* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All clusters</td>
<td>No</td>
<td>54.1% (211/390)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>50.8% (625/1231)</td>
<td>0.76 (0.59, 0.98), p = 0.033</td>
<td>0.77 (0.60, 1.00), p = 0.053</td>
</tr>
<tr>
<td>Lower resistance to deltamethrin (mosquito mortality &gt;55.2%)</td>
<td>No</td>
<td>52.2% (107/205)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>49.6% (306/617)</td>
<td>0.82 (0.58, 1.17), p = 0.282</td>
<td>0.85 (0.59, 1.22), p = 0.381</td>
</tr>
<tr>
<td>Higher resistance to deltamethrin (mosquito mortality &lt;55.2%)</td>
<td>No</td>
<td>56.2% (104/185)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>52.0% (319/614)</td>
<td>0.69 (0.48, 1.00), p = 0.052</td>
<td>0.70 (0.48, 1.02), p = 0.061</td>
</tr>
<tr>
<td>Interaction parameter for the difference in net effectiveness between higher and lower resistance clusters</td>
<td></td>
<td></td>
<td>0.84 (0.51, 1.41) p = 0.513</td>
<td>0.82 (0.49, 1.38) p = 0.457</td>
</tr>
<tr>
<td>Lower resistance to permethrin (mosquito mortality &gt;18.2%)</td>
<td>No</td>
<td>60.1% (113/188)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>51.4% (304/592)</td>
<td>0.73 (0.51, 1.04), p = 0.082</td>
<td>0.75 (0.52, 1.07), p = 0.115</td>
</tr>
<tr>
<td>Higher resistance to permethrin (mosquito mortality &lt;18.2%)</td>
<td>No</td>
<td>49.5% (95/196)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>51.6% (292/566)</td>
<td>0.84 (0.58, 1.20), p = 0.335</td>
<td>0.86 (0.59, 1.24), p = 0.416</td>
</tr>
<tr>
<td>Interaction parameter for the difference in net effectiveness between higher and lower resistance clusters</td>
<td></td>
<td></td>
<td>1.15 (0.69, 1.92) p = 0.595</td>
<td>1.15 (0.68, 1.94) p = 0.600</td>
</tr>
</tbody>
</table>

* Adjusted for age and SES

### Table 2 The association between deltamethrin and permethrin mortality on malaria prevalence

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Effect of</th>
<th>Malaria prevalence in children aged 6 months to 10 years, % (n/N)</th>
<th>Odds ratio (95% CI)</th>
<th>Adjusted odds ratio* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deltamethrin</td>
<td>Dichotomized resistance</td>
<td>Lower resistance (mosquito mortality &gt;55.2%)</td>
<td>50.2% (413/822)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Higher resistance (mosquito mortality &lt;55.2%)</td>
<td>52.9% (423/799)</td>
<td>1.11 (0.65, 1.90), p = 0.698</td>
</tr>
<tr>
<td></td>
<td>Linear increase in mosquito mortality</td>
<td>10% increase</td>
<td>–</td>
<td>1.00 (0.85, 1.17), p = 0.999</td>
</tr>
<tr>
<td>Permethrin</td>
<td>Dichotomized resistance</td>
<td>Lower resistance (mosquito mortality &gt;18.2%)</td>
<td>53.5% (417/780)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Higher resistance (mosquito mortality &lt;18.2%)</td>
<td>50.8% (387/762)</td>
<td>0.88 (0.50, 1.53), p = 0.640</td>
</tr>
<tr>
<td></td>
<td>Linear increase in mosquito mortality</td>
<td>10% increase</td>
<td>–</td>
<td>0.99 (0.90, 1.09), p = 0.893</td>
</tr>
</tbody>
</table>

* Adjusted for age, SES and net use
Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
Study tools and anonymized dataset will be submitted to LSHTM Data Compass (http://datacompass.lshtm.ac.uk/).

Ethics approval and consent to participate
This study was approved by the Benin Ministry of Health, the National Ethics Committee for Health Research at the Ministry of Health. Written consent was obtained from all participants, who were informed of objectives of the study and the advantages and disadvantages of participation.

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References