Pyrethroid resistance in *Anopheles gambiae* not associated with insecticide-treated mosquito net effectiveness across sub-Saharan Africa.

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Abstract

Background

Pyrethroid resistance is a major concern for malaria vector control programs that predominantly rely on insecticide-treated mosquito nets (ITN). Contradictory results of the impact of resistance have been observed in field studies.

Methods

We combined continent-wide estimates of pyrethroid resistance in *Anopheles gambiae* from 2006-2017 with continent-wide survey data to assess the effect of increasing pyrethroid resistance on the effectiveness of ITNs to prevent malaria infections in sub-Saharan Africa. We utilized both a pooled-data approach and meta-regression of survey regions to assess how pyrethroid resistance affects the association between ITN ownership and malaria outcomes in children aged 6-59 months.

Findings

ITN ownership reduced the risk of malaria outcomes in both pooled and meta-regression approaches. In the pooled analysis, there was no observed interaction between ITN ownership and estimated level of pyrethroid resistance (Likelihood ratio [LR] test = 1.127 for the outcome of rapid diagnostic test confirmed malaria infection, p = 0.2885; LR test = 0.161 for the outcome of microscopy confirmed malaria infection, p = 0.161; LR test = 0.646 for the outcome of moderate or severe anemia, p = 0.4215). In the meta-regression approach the level of pyrethroid resistance did not explain any of the variance in subnational estimates of ITN effectiveness for any of the outcomes.

Interpretation

ITNs decreased risk of malaria outcomes independent of the levels of pyrethroid resistance in the malaria vector populations.

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
Funding
DAL did not receive funding and RC received a SOURCE grant from Syracuse University for this project.

Introduction
Insecticide-treated mosquito nets (ITN) have been one of the most effective public health interventions of the 21st century, preventing millions of deaths from the malaria parasite [1]. Previous to their wide-scale deployment, more than 1 million people died annually across sub-Saharan Africa from malaria infections [2], particularly the Plasmodium falciparum parasite, and the parasite caused a further 500 million cases. Broad scale-up of ITNs and across the continent in the early and mid-2000’s [3] contributed to this annual burden being cut in half by 2015 [4]. The 2018 World Malaria Report found that all species of malaria were responsible for 435,000 global deaths and 219 million global cases in 2017 [5], a huge reduction from estimates earlier this century. It is worrisome, however, that the 2018 World Malaria Report found the first annual increase in global malaria cases since 2005.

Mosquito nets provide a personal barrier to prevent infective bites from malaria vectors that predominantly bite at night [6]. An untreated mosquito net will protect the person sleeping beneath from an infective bite of an infected mosquito. However, that infective malaria vector will likely seek a blood meal elsewhere and thus continue malaria transmission. When the mosquito net is treated with insecticide and the mosquito is susceptible, contact with the insecticide kills the mosquito and ends that mosquito’s capacity to continue transmitting malaria [7]. The subsequent effect of ITNs on malaria transmission is profound [8]. When a high number of ITNs are used within a community, a mass killing effect from the insecticide provides a community-level protection, sufficiently affecting the malaria vector populations such that people who do not have an ITN are protected in similar ways to those that do have an ITN [9,10].

Currently, pyrethroids are the only class of insecticides used on ITNs [11], although combination nets treated with multiple insecticides are in various stages of testing [12–14]. Pyrethroids target the central nervous system to kill mosquitoes and reduce the transmission of malaria by incapacitating the vector [15]. Fears about malaria vectors developing pyrethroid resistance were raised at the inception of ITNs [15], and early studies demonstrated widespread reports of resistance [16,17]. Although alarming, it remains to be seen just how pyrethroid resistance will affect malaria control and malaria trends [18–20].

Linking pyrethroid resistance in malaria vectors to ITN effectiveness to prevent malaria infections and reduce malaria transmission has been more challenging than documenting the spread of resistance. Some studies suggest that in areas with pyrethroid resistance, ITNs are less effective [21,22]. Others find that ITNs are still associated with reduced risk of malaria despite high levels of pyrethroid
resistance [23–26]. These studies generally struggle with four separate issues. First, due to the inability to randomly allocate ITN access, these studies suffer from selection bias wherein households with ITN access are more likely to be predisposed to lower risk of malaria transmission than households without ITN access independent of the effect of ITNs [27]. Second, some of these studies do not actually include estimates of ITN effectiveness, and the studies that do utilize ITN use as their measure of effectiveness. ITN use may not be the best measure to understand the impact of pyrethroid resistance on ITN effectiveness. Even if malaria vectors are resistant to the insecticide in the ITN, the mosquito netting still provides a physical barrier and consistent use will decrease mosquito exposure and thus malaria risk [28]. This is evident where in areas of pyrethroid resistance ITNs with holes lose their effectiveness [29]. Third, each of the studies is confined to a particular study site. Investigating phenomena at particular study sites limits the variability of resistance available, as the broad spectrum of resistance prevalence is not incorporated within the study. And fourth, the studies utilize an overly simplistic assessment of insecticide resistance, typically with dichotomized low resistance and high resistance areas that prevent an examination of a dose-response relationship between ITN effectiveness and pyrethroid resistance.

Herein we attempt to address these four challenges and better understand how widespread pyrethroid resistance is affecting the ability of ITNs to reduce malaria transmission. We leverage nationally representative surveys across sub-Saharan Africa and recently published near continent-wide estimates of pyrethroid resistance of Anopheles gambiae in sub-Saharan Africa[30] to address the issues of geographic limitation, a limited resistance spectrum, and oversimplification in the statistical measure of insecticide resistance. We further utilize exact matching to minimize the influence of selection bias, and a measure of ITN ownership rather than ITN use to reduce the residual confounding of the effect of mosquito netting as a barrier.

**Methods**

**Search strategy and selection criteria**

We utilized data from nationally representative Demographic and Health Surveys (DHS) to assess the relationship between insecticide resistance and the effectiveness of ITNs. All DHS surveys conducted in sub-Saharan Africa between 2006-2017 that were publicly available as of January 15, 2020 were considered for inclusion in the analyses with the following criteria: the survey contained information on an outcome (malaria infection status in children either as measured through rapid-diagnostic test or through microscopy, or anemia status as measured through a hemocue rapid hemoglobin assessment), and the survey contained information on household ITN ownership. We further limited these surveys to those that were conducted in areas with estimated prevalence of pyrethroid resistance [30]. The DHS program is
funded by USAID and assists lower income countries in conducting nationally-representative surveys primarily aimed at measuring trends in child mortality and women’s fertility with statistical power at the regional (sub-national) level. Additionally, these surveys gather a host of information on numerous factors associated with health, including malaria indicators when the survey is conducted in a malaria-endemic country.

**Outcome measures**

We considered three separate outcome measures in children aged 7-49 months: confirmation of a malaria infection through rapid diagnostic test, confirmation of a malaria infection through microscopy, or hemoglobin levels < 10 g/dl (moderate to severe anemia). Anemia is an historical indicator of malaria transmission, and is also known to be associated with effective malaria control such as ITNs [31].

**ITN ownership measure**

We defined the primary exposure of ITN ownership as the child living in a household that owned at least one ITN.

**Exact matching of observations**

Selection bias presents the largest challenge to validity in assessing ITN effectiveness in observational studies. We followed an exact matching approach similar to the analysis of ITN effectiveness conducted by Lim et al. to minimize this selection bias [32], wherein we matched households on the probability of owning an ITN. Specifically, we matched households within survey datasets on the following covariate pattern: region of the country as per the DHS, wealth categorized as rich or poor, mother’s education categorized as any or none, and the house being in an urban or rural location. Matching was done using the MatchIt package [33,34], in R version 3.6.2 [35].

**Statistical analysis**

We utilized two separate approaches to assess the influence of pyrethroid resistance on the effectiveness of insecticide-treated mosquito nets.

**Pooled analysis approach.**

We combined all survey data meeting inclusion criteria and exactly matched into a single dataset. We extracted the estimates of resistance, seasonality of malaria transmission from the Mapping Malaria Risk in Africa (MARA) maps [36], estimates of the *Plasmodium falciparum* parasite prevalence rate in children aged 2-10 \( (PfPR) \) from the Malaria Atlas Project [37], and the relative abundance of the *Anopheles gambiae* vector [38] to the cluster geocoordinates using the Raster package [39] in R version 3.6.2 [35]. \( PfPR \) estimates were only available for the years 2000-2015 from the MalariaAtlas package, and so we assigned surveys conducted 2016-2017 \( PfPR \) levels of 2015 (n= 14 surveys).
We then estimated the relationship between ITN effectiveness and the prevalence of resistance using an interaction term predicting the outcome using a logistic regression approach with the matched group as a random intercept (Equation 1). We adjusted analyses for child’s age categorized as years (<1, 1, 2, 3, 4), household wealth quintile, mother’s education (none, some primary, completed primary or higher), whether the data were collected during the transmission season or not, household location (urban or rural), and PfPR levels (continuous). The three separate outcomes were modeled in the same fashion, one for the outcome of RDT-confirmed malaria infection and the other for the outcome of microscopy-confirmed malaria infection. We conducted this analysis for all observations in the dataset, and then limited the analysis to those observations where the relative abundance of *An. gambiae* was > 0.5. The analyses were conducted using Stata version 15.1.

Equation 1: Representation of pooled analysis wherein $\pi_{ijklm}$ is a dichotomous outcome for child $i$ in household $j$ in cluster $k$ in survey $l$ in matched group $m$, ITN$_j$ is whether the household has access to any ITN or not, Res$_k$ is the level of pyrethroid resistance access in the community, $C_{ij}$ is a vector of child characteristics, $H_j$ is a vector of household characteristics, $S_k$ is a vector of cluster characteristics, and $\zeta_m$ is a random intercept for matched group $m$ that is assumed to be normally distributed with a mean of zero.

$\gamma_{ijklm} | \pi_{ijklm} \sim Binomial(1, \pi_{ijklm})$

$logit(\pi_{ijklm}) = B_1 ITN_j \times Res_k + \chi C_{ijk} + \delta H_j + \kappa S_k$

$\zeta_m \sim N(0, \varphi)$

Meta-regression approach

We parsed all survey data meeting inclusion criteria to DHS region level. We extracted the estimates of pyrethroid resistance, the *Plasmodium falciparum* parasite prevalence rate in children aged 2-10 (PfPR) from the Malaria Atlas Project [37], and the relative abundance of the *Anopheles gambiae* vector[38] to the DHS region level using the Raster package [39] in R version 3.6.2 [35]. PfPR estimates were only available for the years 2000-2015 from the MalariaAtlas package, and so we assigned surveys conducted 2016-2017 PfPR levels of 2015 (n= 14 surveys).

We then estimated the effectiveness of ITNs for each DHS region as described previously using equation 1. We conducted a meta-regression of ITN effectiveness within DHS regions using the metafor package [40] in R version 3.6.2 [35] with the prevalence of pyrethroid resistance at the region level serving as the predictor. We also tested for interactions between pyrethroid resistance and both PfPR as well as the abundance of *Anopheles gambiae*. 
Role of the funding source

DAL was unfunded for this work. RC received a SOURCE grant from Syracuse University. The sponsors had no role in the study design; collection, analysis, and interpretation of the data; in the writing of the report; and in the decision to submit the paper for publication. DAL had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Data available

We identified 92 survey datasets conducted in sub-Saharan Africa between 2006-2017 publicly available as of January 15, 2020 with information on ITN ownership, malaria infection in children, or child’s anemia status. Of these 92 surveys, 30 were excluded from any analysis due to the following reasons: 12 surveys contained no information on malaria infection or anemia status, 5 surveys contained no information on household ITN ownership, and 8 surveys could not be matched to resistance estimates (the surveys were conducted in Angola, Sao Tome and Principe, or Madagascar where no resistance estimates were available). A further seven surveys had no cluster geocoordinates and so were excluded from the pooled analysis but included in the meta-regression approach. Figure 1 shows the geographical distribution of 67 surveys included in the analyses.

Figure 1: Geographic distribution of data included in the analyses
Variation of pyrethroid resistance

At the survey cluster level, estimates of pyrethroid resistance within this dataset ranged from 10.5% to 99.9% with a median level of 89.1% (Figure 2). Pyrethroid resistance was associated with decreased levels of PfPR within survey clusters (rho = -0.3061, n = 18,927 clusters) and decreased relative abundance of An. gambiae (rho = -0.2321, n = 19,588 clusters).

Figure 2: Distribution of estimated pyrethroid resistance across datasets included in the analyses.

Effect of pyrethroid resistance on association between ITNs and malaria outcomes – pooled analysis approach

After exact matching and adjusting for known predictors but without accounting for resistance, ownership of an ITN was associated with a reduction in the risk of a malaria infection as well as having moderate or severe anemia (Table 1). After accounting for resistance, there was no evidence of an interaction between ITN ownership and pyrethroid resistance levels for any of the outcomes (Table 2). ITN ownership was still associated with a reduction in the risk of a malaria infection as well as having moderate or severe anemia. Limiting the analysis to areas with >50% An. gambiae did not change the results.

Table 1: Association between ITN ownership and various outcomes without taking pyrethroid resistance into account. Children were matched on probability of ITN ownership. Models were adjusted for urban/rural, child's age, wealth quintile, mother's education, PfPR, malaria transmission season, and survey dataset.
Table 2: Likelihood ratio test for including an interaction term for pyrethroid resistance and ITN ownership. Children were matched on probability of ITN ownership. Models were adjusted for urban/rural, child's age, wealth quintile, mother's education, PfPR, malaria transmission season, and survey dataset.

| Outcome                  | Likelihood ratio test for interaction between pyrethroid resistance and ITN ownership | P-value | N children (n matched groups) |
|--------------------------|--------------------------------------------------------------------------------------|---------|------------------------------|
| RDT positivity           | 1.127                                                                                | 0.2885  | 137,943 (4,493)              |
| Microscopy positivity    | 0.161                                                                                | 0.6880  | 116,556 (3,643)              |
| Moderate or severe anemia| 0.646                                                                                | 0.4215  | 163,332 (5,486)              |

Effect of pyrethroid resistance on association between ITNs and malaria outcomes – meta-regression approach

Within-DHS region specific estimates of the effectiveness of ITNs were available for 349 regions for the outcome of RDT-confirmed malaria infection, 243 regions for the outcome of microscopy-confirmed malaria infection, and 611 regions for the outcome of moderate or severe anemia. In unadjusted meta-analyses, ITN ownership was associated with a reduced risk of RDT-confirmed malaria infection and microscopy-confirmed malaria infection, but not moderate or severe anemia (Table 3).

Pyrethroid resistance did not explain any of the regional variance in the effectiveness of ITN ownership against the outcomes (Figure 3), nor did any interaction between pyrethroid resistance and either PfPR or the abundance of *An. gambiae*.

Table 3: Results from unadjusted meta-analyses of ITN ownership on the outcomes. DHS data were parsed to region. Children were matched on probability of ITN ownership. Region-specific models were adjusted for urban/rural, child's age, wealth quintile, and mother's education.

| Outcome                  | Odds ratio (95% Confidence Interval) | P-value | N DHS regions | I-squared |
|--------------------------|--------------------------------------|---------|---------------|-----------|
| RDT positivity           | 0.888 (0.833 – 0.947)                | < 0.001 | 349           | 0.00%     |
| Microscopy positivity    | 0.857 (0.795 – 0.925)                | < 0.001 | 243           | 0.00%     |
| Moderate or severe anemia| 0.965 (0.921 – 1.011)                | 0.1330  | 611           | 0.00%     |
Figure 3: Bubble plots showing the relationship between pyrethroid resistance at the region level and effectiveness of ITN ownership against a) RDT-confirmed malaria infection, b) microscopy-confirmed malaria infection, and c) moderate or severe anemia.

Discussion

Key results

These results suggest that the effectiveness of ITNs is not affected by the prevalence of pyrethroid resistance across sub-Saharan Africa. ITN ownership was associated with a reduced risk of a malaria infection (either RDT- or microscopy-confirmed) as well as a reduced risk of moderate or severe anemia. These reductions were modest, just more than 10% for the malaria infection and 5% for moderate severe anemia. Importantly, however, pyrethroid resistance did not modify these reductions in any way. There was also agreement across both the pooled and meta-regression approaches utilized herein.

Limitations

Selection bias is present in the data, and occurs either as houses owning ITNs are predisposed to less malaria transmission independent of ITN ownership or ITN distribution programs are targeted toward areas of higher malaria transmission and therefore children in houses owning ITNs are predisposed to more malaria transmission independent of ITN ownership. We attempted to mitigate the effects of this selection bias on the analysis using exact matching, as we and others have done in previous analyses of ITN ownership using these data [9,32]. It is possible that some residual bias is still present, and if so we would expect that bias to overinflate the effect of ITN ownership. However, should there be a decrease in ITN effectiveness with increasing pyrethroid resistance we would still expect to see that effect given the limitations in the data.
Pyrethroid resistance estimates for these data were quite high, with a median of 89%. Unfortunately, with such high levels of pyrethroid resistance spread across the continent we could not examine the effectiveness of ITNs across the entire range from 0-100% resistance. Given the large volume of data we included in our analyses we still had sufficient power to determine that ITN ownership reduces the risk of malaria infection even when estimated pyrethroid resistance levels approach 100% of the vector population.

**Interpretation**

These results are positive news for malaria control program. ITNs remain the primary vector control strategy throughout sub-Saharan Africa, and they appear to be effective even at high levels of pyrethroid resistance. Others have described in detail this resistance paradox, where in laboratory experiments pyrethroid resistance confers protection for malaria vectors but in epidemiological studies vector control confers protection against malaria infection [41]. In general two separate hypotheses are present as to how ITNs maintain effectiveness in the face of widespread resistance. The first hypothesis suggests that the same genes that confer pyrethroid resistance alter the ability of the vector to transmit the parasite. A fitness cost to pyrethroid resistance has been documented in the *An. gambiae*, as evidenced by studies showing that non-resistant mosquitoes outcompete resistant mosquitoes [42]. The second hypothesis suggests that the insecticide still functions, just with a different type of effect. One example of this second hypothesis is that pyrethroid-resistant mosquitoes lose their irritancy to the insecticide, thus allowing for increased contact and a higher dose of the insecticide than typical mosquitoes [43]. This increased dose then leads to the mosquito’s death. Another example of this second hypothesis is that a dose of pyrethroids results in delayed mortality of pyrethroid-resistant mosquitoes [44], perhaps due to impaired flight or behavioral effects of the insecticide [45]. Whatever the reason, ITNs still appear to be effectively reducing malaria transmission even when pyrethroid resistance approaches 100% in vector populations.

**Conclusion**

Insecticide resistance is a chief concern for malaria control programs. These results confirm the notion that pyrethroid-based ITNs are still working to prevent malaria despite high levels of pyrethroid resistance observed in malaria vector populations.

**Declaration of interests**

The authors have no conflict of interests.
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