STUDY PROTOCOL

Dual-active-ingredient, insecticidal nets for preventing malaria: a systematic review protocol [version 1; peer review: awaiting peer review]

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Abstract

Background: Malaria is caused by the Plasmodium parasite and is a highly transmissible disease representing a significant global public health burden. The provision of insecticide-treated mosquito nets (ITNs) has contributed to the reduction of malaria across endemic countries. However, the detection of insecticide resistance in many mosquito vector species potentially threatens the long-term effectiveness of ITNs. A novel method to reduce the impact of insecticide resistance is to treat mosquito nets with multiple active ingredients.

Methods and analysis: This review will comprehensively search the literature (both published and unpublished) for any studies investigating the effectiveness of mosquito nets treated with multiple active ingredients, known henceforth as dual-active-ingredient (DAI) ITNs. The DAI ITNs of interest include those treated with a pyrethroid and non-pyrethroid insecticide (review question 1) or with a pyrethroid and an insect growth regulator (review question 2). Studies will be screened to meet the inclusion criteria by a minimum of two authors, followed by assessment of risk of bias (using appropriate risk of bias tools for randomised and non-randomised studies) and extraction of relevant information using structured forms by two independent authors. Meta-analyses will be carried out where possible for epidemiological outcomes and subgrouping will be considered. Certainty in the evidence will be established with GRADE assessments.

Ethics and dissemination: A full review report will be submitted to the Vector Control and Insecticide Resistance Unit, Global Malaria Program, WHO. A version of this report will be submitted for publication in an open access peer-reviewed journal. The report will
inform the development of WHO recommendations regarding the use of DAI ITNs for the prevention of malaria. This systematic review does not require ethics approval as it is a review of primary studies.

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**Keywords**
Malaria, dual-active-ingredient net, insecticide-treated-nets, ITN, systematic review

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**Competing interests:** Timothy Hugh Barker, Jennifer C. Stone, Sabira Hasanoff, and Zachary Munn are employed by JBI, an evidence-based healthcare research and development organisation situated within the University of Adelaide. Jennifer C. Stevenson is a Technical Officer working in the Global Malaria Program, WHO.

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Introduction

Description of the condition

Malaria is a highly transmissible, parasitic, mosquito-borne disease representing a significant global public health burden. Malaria is caused by the Plasmodium parasite and spreads through the bites of infected female Anopheles mosquitoes. Two species of malaria - P. falciparum and P. vivax - are the most virulent for human beings.

There were an estimated 627,000 deaths due to malaria in 2020. Children under the age of five, pregnant women, and travellers from non-endemic countries are at a greater risk of the disease. The sub-Saharan African region bears the heaviest burden of malaria morbidity and mortality, accounting for 95% of global malaria cases and 96% of global malaria deaths. 80% of these malaria-related deaths were attributed to children under the age of five. There were an estimated 241 million global cases of malaria in 2020, which is an increase from the 227 million cases in 2019. The WHO has approximated that almost half the world’s population is at risk of contracting malaria.

Preventative strategies that have demonstrated success often include targeting of the mosquito vector itself through insecticide-treated net (ITN) distribution and indoor residual spraying (IRS) of insecticides. Substantial progress has been made since 2000 in reducing global malaria cases from 80 cases per 1000 to 57 per 1000 in 2019. This reduction in malaria cases can be attributed to the increased application of malaria control strategies, particularly ITNs, which contributed 68% to the reduction of the malaria burden, and IRS, which contributed 11%, between 2000 and 2015.

Description of the intervention

The provision of insecticide-treated-nets (ITNs) has contributed to the reduction of malaria across endemic countries. Three main ITN classes are recognized by the WHO as given below. The first of these classes includes pyrethroid-only nets prequalified by WHO, and conventionally treated nets. These nets undergo periodic re-treatment of the active ingredient using a WHO prequalified self-treatment kit. Clear and demonstrable value has been shown for products within this class. The WHO has therefore recommended use of pyrethroid-only nets for large-scale deployment due to their enhanced safety profile. However, the identification of insecticide resistance in mosquito vectors may compromise the long-term effectiveness of pyrethroid-treated ITNs. In response to the evolution and spread of pyrethroid resistance, WHO voiced the need for new types of ITNs designed to be effective against insecticide-resistant (primarily pyrethroids) vectors. The WHO has identified two further classes of ITNs, those designed to kill host-seeking insecticide-resistant mosquitoes and those designed to sterilize and/or reduce their fecundity.

The second class of ITNs, designed to kill resistant mosquitoes, includes those that combine pyrethroid insecticides with other ingredients. One such type of nets in this class includes ITNs treated with a pyrethroid and piperonyl butoxide (PBO). PBO is a synergist that inhibits metabolic enzymes within the mosquito that work to detoxify (and therefore reduce effectiveness of) insecticides. The public health value of these pyrethroid-PBO ITNs has been demonstrated and the WHO have therefore formally recommended the use of these nets in areas where pyrethroid-resistant mosquitoes are present. This class also provisionally includes nets that combine pyrethroids with other active ingredients (henceforth referred to as dual active ingredient nets, DAI). However, public health value has yet to be determined for a DAI ITN treated with pyrethroid and non-pyrethroid formulations. Studies on one type of DAI ITN treated with alphacypermethrin (a pyrethroid) and the pyrrole chlorfenapyr have recently demonstrated both entomological and epidemiological benefits.

Finally, the third class of ITNs, those designed to sterilize and/or reduce the fecundity of host-seeking insecticide-resistant mosquitoes, provisionally includes DAI ITNs treated with a pyrethroid insecticide and an insect growth regulator such as pyriproxyfen. Pyriproxyfen is an insecticide that interferes with the reproduction and development of female mosquitoes, effectively sterilising them. The public health value has yet to be determined for a DAI ITN treated with a pyrethroid and an insect growth regulator.

This systematic review is specifically interested in two interventions. These interventions will be considered as separate review questions in the one review. The first intervention includes DAI ITNs treated with a pyrethroid and non-pyrethroid insecticide. The second intervention includes DAI ITNs treated with a pyrethroid and an insect growth regulator.

Why it is important to do this review

Anopheles gambiae (s.s.) and An. funestus (s.s.), mosquitoes found in Africa, are the most efficient vectors for the malaria parasite P. falciparum. These vectors have recently demonstrated widespread resistance to pyrethroid insecticides and this presents a significant concern for the long-term efficacy of these insecticides for use in vector control programmes including insecticide residual spraying and in the treatment of mosquito nets. DAI ITNs may provide a solution to address vector pyrethroid resistance and so be used by malaria control programmes. There is an urgent need to systematically...
review the evidence on the effectiveness of DAI ITNs as tools for the control and prevention of malaria. There have been no previous systematic reviews on this topic.

**Protocol**

**Review questions**

1. In areas with ongoing malaria transmission, should nets treated with a pyrethroid and non-pyrethroid insecticide versus either nets treated with pyrethroid insecticide alone or with pyrethroid insecticide in combination with PBO be used to prevent malaria in adults and children?

2. In areas with ongoing malaria transmission, should long-lasting insecticidal nets treated with a pyrethroid and an insect growth regulator versus either nets treated with pyrethroid insecticide alone or with pyrethroid insecticide in combination with PBO be used to prevent malaria in adults and children?

**Main objective**

1. To assess the benefits (on malaria transmission or burden) and harms (adverse effects and unintended consequences) of insecticidal nets treated with a pyrethroid and a second active ingredient (either non-pyrethroid insecticide or insect growth regulator).

**Secondary objectives**

1. To retrieve studies on contextual factors relating to dual-active-ingredient insecticide-treated-nets where epidemiological outcomes have been reported for these interventions.

2. To retrieve entomological studies that have provided supportive data from studies where epidemiological outcomes have been reported for these interventions.

**Methods**

This review will be conducted in line with guidance from JBI, Cochrane, and GRADE (Grading of Recommendations, Assessment, Development and Evaluation). It will be reported in line with PRISMA 2020 and this protocol is reported in line with PRISMA-P. The methods for the two review questions are presented together below. Where the methods differ based on the question they are presented separately. This review has been registered within PROSPERO (ID: CRD42022333044).

**Eligibility criteria**

**Participants**

Studies conducted in adults and children who are residents of a region with ongoing malaria transmission and have been provided with an insecticide-treated-net are eligible for this review. Where studies have included travellers, attempts will be made to extract only information on residents (i.e., those people currently living and sleeping in the study area), such as by contacting the authors or attempts to identify subgroups in the report.

**Interventions**

The interventions of interest are dual-active-ingredient (DAI) insecticidal treated nets (ITNs). DAI ITNs will be eligible where they have been treated with a pyrethroid and non-pyrethroid insecticide (review question 1) or with a pyrethroid and an insect growth regulator (review question 2). The level of ITN distribution (per household or per individual) will not impact the eligibility of studies into the review. However, different levels of coverage can be investigated through subgroup analyses where the data permit.

**Background interventions**

Background interventions (co-interventions) will likely be encountered and information on these will be extracted. These are interventions other than the interventions under consideration or any other malaria or vector-specific control intervention. Studies conducted where background interventions are present will be included if these background interventions were balanced between intervention and control arms.
Comparators
This systematic review will consider studies that have compared the interventions of interest against nets treated with pyrethroid insecticide alone or with pyrethroid insecticide in combination with PBO. The same comparator will be used for both review questions specified above.

Outcomes
The following outcomes will be considered for inclusion and are grouped into epidemiological outcomes, entomological outcomes, unintended benefits, and harms/unintended consequences.

Epidemiological

• Malaria case incidence rate – Defined as symptoms plus parasitaemia, over a population at risk or person-time. Detected either through passive or active surveillance.

• Malaria infection incidence – Defined as parasitaemia with or without symptoms, over a population at risk of person-time. Detected through passive or active surveillance.

• Incidence of severe disease – Defined as hospitalization with parasitaemia, over a population at risk or person-time.

• Parasite prevalence – Parasitaemia with or without symptoms, over the population sampled. Detected through cross-sectional surveys.

• All-cause mortality – Number of deaths over the population at risk or person-time.

• Malaria mortality – Number of deaths attributed to malaria over the population at risk or person-time.

• Prevalence of anaemia – Defined by study thresholds of anaemia.

Entomological

Entomological outcomes will only be included in this review where epidemiological outcomes were also considered by the primary study.

• Entomological inoculation rate (EIR) – Defined as the number of infective bites received per person per unit of time.

• Sporozoite rate – Percentage of female Anopheles mosquitoes with sporozoites in the salivary glands.

• Anopheline density – Number of female anopheline mosquitoes in relation to the number of specified shelters or hosts or to a given period sampled, specifying the methods of collection.

• Biting rate – Average number of mosquito bites received by a host in a unit of time, specified according to the host and mosquito species.

• Mortality of adult female Anopheles – Defined as the mosquito being knocked down, immobile or unable to stand or take off for 24 hours after exposure to a discriminating concentration of an insecticide (or as reported in the primary evidence).

Contextual factors

Outcomes related to contextual factors will only be included in this review where epidemiological outcomes were also considered by the primary study.

• Values and preferences – The values and preferences of the individuals and populations receiving the intervention.
• Acceptability – Extent to which those receiving the intervention consider the intervention to be appropriate, based on anticipated or experienced cognitive and emotional responses to the intervention. Includes willingness to participate in the intervention.

• Health equity – Extent to which the intervention benefits all populations and the potential to discriminate based on sex, age, ethnicity, culture, language, sexual orientation or gender identity, disability status, education, socioeconomic status, residence or any other characteristic.

• Financial and economic considerations – Costs, resource intensiveness, overall economic impact, cost-benefit.

• Feasibility considerations – legal barriers to implementation, programmatic considerations, timeliness (the ability to reach all targeted households/household members in a timely manner) among others.

Unintended benefits

• Epidemiological impact on other vector-borne diseases

Harms and/or unintended consequences of interventions

• Adverse effects known to be associated with insecticides, including skin irritation, irritation of upper airways, nausea, and headache.

• Human behaviour changes e.g. change in sleeping location

• Any influence on neighbouring houses e.g., increased vector abundance/biting in houses without nets

• Environmental impacts such as biodiversity and ecosystem changes.

• Entomological impacts e.g., mosquito behaviour changes such as changes in outdoor biting rate, biting times, feeding preference, development of insecticide resistance, change in vector composition.

Setting

Studies conducted in countries with ongoing malaria transmission will be considered for this review. The presence of other background interventions will not impact on study eligibility as long as they are present in both arms equally. Studies where additional malaria interventions are considered standard of care were implemented will be included as long as interventions (both malaria and non-malaria) were balanced between intervention and control arms.

Study design

Only randomised and non-randomised controlled studies, that have included at least more than one cluster per arm will be considered for this review. Non-randomised controlled study designs will only be included when there is a comparison/control group present. This can include historical controls. There will be no exclusions regarding any buffer period (i.e. when participants act as their own controls) or length of intervention or timing of measurement of outcomes (these details will be extracted). All observational studies and modelling studies will be excluded.

There will be no exclusions based on language or publication status (i.e. published, unpublished, in press, in progress, pre-print). There are no date limitations. For studies published in languages other than English, Google Translate will be used to determine whether the study meets inclusion criteria. Where studies are published in a language other than English and meet inclusion criteria, Google Translate translations will be reviewed by a person fluent in the language.

Search strategy

The search strategy aims to locate both published and unpublished studies and was developed with the input of a health librarian.

An initial limited search of PubMed via NCBI was undertaken to identify relevant articles on this topic. The terminology contained in the titles and abstracts of relevant articles, including related subject headings, were used to develop a full search strategy for malaria and insecticidal nets. The search strategy, including all identified keywords and subject headings, will be adapted for each included database and/or information source, by using Polyglot and with the aid of a
medical librarian. The reference list and citations of all studies undergoing extraction will be screened for additional studies using CitationChaser and/or a related citations search. The full search strategy for major databases is available in an online repository.

The databases to be searched include Cochrane Central Register of Controlled Trials (CENTRAL), published in The Cochrane Library and including the Cochrane Infectious Diseases Group Specialized Register; PubMed (NCBI); Embase (Ovid); CINAHL with full text (EBSCO), US National Institute of Health Ongoing Trials Register; ISRCTN registry; The WHO’s International Clinical Trials Registry Platform (WHO ICTRP).

Additionally, experts in the field and relevant organisations will be asked whether they know of any studies (completed or ongoing) that are relevant to this review topic.

**Study selection and screening**
Following the search, all identified citations will be collated and uploaded into EndNote. Duplicates will be removed using Screenatron. The studies will then be imported into Covidence where they will be screened on their titles and abstracts by two or more independent reviewers for assessment against the eligibility criteria for the review. Potentially relevant studies will be retrieved in full. The full text of selected citations will be assessed in detail against the eligibility criteria by two or more independent reviewers. Studies that have been excluded at full text screening that do not meet the eligibility criteria will be recorded and the reasons for exclusion will be reported. Any disagreements that arise between the reviewers at each stage of the selection process will be resolved through discussion, or with an additional reviewer/s. The results of the search and the study inclusion process will be reported in full in the final review and presented in a PRISMA 2020 flow diagram. Where relevant systematic reviews are identified in the search, we will review the list of included and excluded studies (from that review) for consideration, however the systematic reviews themselves will not be included.

Where members of the author team are named as authors on primary studies identified through the search, they will be excluded from any decision-making regarding the inclusion of the study or the assessment of the study risk of bias.

**Contextual factors and entomological studies**
Studies that potentially provide important information regarding the contextual factors and entomological outcomes will only be included in the review if they also provide data relating to the epidemiological outcomes of interest in this review.

**Data extraction**
Data will be extracted from papers included in the review by two or more independent reviewers using a tailored data extraction tool developed by the reviewers available as an Appendix. The type of data to be extracted includes characteristics of participants, study methods and the key findings. This tool will first be piloted on two studies included in the review following study selection (as such, the final extraction tool may be subject to revision). Any disagreements that arise between the reviewers will be resolved through discussion, or with an additional reviewer/s. If appropriate, authors of papers will be contacted to request missing or additional data where required.

**Assessment of the risk of bias**
Two review authors will independently assess the risk of bias for each study using the Cochrane Risk of Bias 2 tool for randomised controlled trials (and the Risk of Bias 2 tool for cluster trials where appropriate). The domains of bias to be considered in this tool include bias arising from the randomisation process, bias due to deviations from the intended interventions, missing outcome data, bias in measurement of the outcome and bias in selection of the reported result. The Risk of Bias 2 tool will first be piloted on two studies included in the review following study selection. Risk of bias assessment will be undertaken at the result level. Any disagreements that arise between the reviewers will be resolved through discussion, or with an additional reviewer/s. If appropriate, authors of papers will be contacted to request missing or additional data where required.

**Data synthesis and meta-analysis**
For epidemiological outcomes, where possible, we will pool studies in a meta-analysis using Review Manager 5 (RevMan5). Results from studies will be pooled in this manner when two or more studies report results for the same outcome in a format conducive to meta-analysis. Where there is only one study contributing data to a particular outcome, a forest plot will still be presented for illustrative purposes (without a meta-analysis estimate). A narrative synthesis of the results will accompany any meta-analysis. Where studies cannot be pooled together in a meta-analysis a narrative synthesis will be presented.
For dichotomous data we will calculate effect sizes as relative risks or odds ratios depending on the nature of data collection undertaken in the pooled study. These results will be presented with their 95% confidence intervals (CIs). When there are no events in a treatment arm, RevMan will add a fixed value of 0.5 to the empty cell. If there are no events in the study, the study will not contribute to the pooled relative estimate of effect from the meta-analysis, however we will keep these results to inform baseline risk for absolute as opposed to relative comparisons and use risk difference instead of relative risk. Where incidence rates are reported, we will calculate the incidence rate ratios. Where possible, adjusted estimates will be extracted. When three or more studies contribute to a meta-analysis a random effects model will be used. A fixed-effect model will be used when there are only two studies contributing to a meta-analysis.

Entomological outcomes, cost data and data related to contextual factors will only be narratively synthesised together.

**Assessment of heterogeneity and publication biases**

Heterogeneity (both clinical and methodological) will be assessed by first comparing the included studies against each other in terms of the eligibility criteria specified above. Statistical heterogeneity will be assessed through visual inspection of the forest plot and by the Cochran’s Q (P value 0.05), and I² statistic. Interpretation of the I² statistic will be according to the guidance in the Cochrane Handbook for Systematic Reviews of Interventions.¹⁹

1. 0% to 40%: might not be important;
2. 30% to 60%: may represent moderate heterogeneity;
3. 50% to 90%: may represent substantial heterogeneity; or
4. 75% to 100%: considerable heterogeneity.

If a meta-analysis has been constructed using the random-effects model, the tau² (an estimate of between-study variability) will also be reported. Publication bias will be addressed by first seeking both published and unpublished literature using the comprehensive search strategy discussed above. Additionally, if there is an adequate number of studies (at least 10) included in a meta-analysis, a funnel plot will be created and investigated.²⁰ For continuous data and if 10 or more studies have been included in a meta-analysis, we will also use Egger’s test.²¹ If effect sizes appear to depend on the size of the trial, this association will be further investigated as being due to publication bias or heterogeneity between studies.

**Unit of analysis issues**

We are likely to encounter unit of analysis issues, as randomisation is likely to occur at the cluster level.²² These unit of analysis issues will be addressed by using the generic inverse variance method in RevMan when studies have analysed their data accounting for their cluster design. If authors have not accounted for the effect of clustering in their analysis, we will inflate standard errors for these studies included in any meta-analysis following the methods in the Cochrane Handbook.²² For all studies that have accounted for the effect of clustering then no changes will be made to the data imputed for meta-analysis.

**Subgroup and sensitivity analysis**

Where the data are available, several potential effect modifiers will be assessed using subgroup analyses. These will also be explored as potential contributors to heterogeneity in the overall analysis. These include:

- Level of transmission; (High: incidence of about 450 cases/1000 persons/year or *Plasmodium falciparum* (Pf) /*Plasmodium vivax* (Pv) prevalence of ≥35%; Moderate: incidence of 250-450 per 1000 persons per year and Pf/Pv prevalence of 10-35%; Low: incidence of 100-250 per 1000 persons per year and Pf/Pv prevalence of 1-10%; Very low: incidence of <100 per 1000 persons per year and Pf/Pv prevalence <1%). (The level of transmission will be categorized according to the schema found in the *Framework for malaria elimination*²³; seasonality of transmission.
- Species of parasite
- Coverage of intervention applied
- Insecticides used and class for both active ingredients (i.e., pyrroles such as chlorfenapyr, insect growth regulators such as pyriproxyfen).
• Characteristics of insecticides used, e.g., target sites, modes of action, and duration required to produce such effect(s).

• Durability of net and insecticides used

• Level of net coverage per person or household

• Coverage of other background interventions.

• Vector species characteristics e.g., species, behaviours, insecticide resistance, among others.

• Setting e.g., rural/urban/peri-urban.

• Population demographics e.g., sex/age/SES/ethnicity etc.

• Human behaviour e.g., sleeping behaviour

• Time from implementation.

Sensitivity analyses will be conducted to determine the following:

1. The impact of bias by excluding studies that are at a high risk of bias. If there is no difference between the high risk of bias and low risk of bias studies, the original analysis result will stand. In the case where there are differences between the estimate of the pooled high risk of bias studies as compared to the low risk of bias studies, all results will be presented and the preference of the WHO Guideline Development Group will be followed as to what estimate (i.e., the full analysis or only the low risk of bias studies) should be used for grading and as the basis of recommendations.

2. Where we have inflated standard errors for trials where cluster designs have not been considered, we will analyse trials as if the individual was the unit of randomisation.

**Patient and public involvement**

This protocol for a systematic review is being conducted for the purposes of informing a WHO guideline. Guideline development panel members, which include many diverse stakeholders including the public/patients, end-users, experts and decision makers have shaped the review questions and focus and will guide the interpretation of the results.

**GRADE**

The GRADE approach\(^{10}\) for grading the certainty of evidence will be followed for this review. GRADE Evidence Profiles will be created using GRADEpro GDT for each comparison considered. The evidence profile will present the following information for each outcome where appropriate: absolute risks for the treatment and control, estimates of relative risk, and a rating of the certainty of the evidence base. Certainty of evidence for outcomes synthesised using the data from RCTs start as high, and this rating can be downgraded for five domains. These include risk of bias, indirectness, inconsistency, imprecision, and risk of publication bias of the review results. Where appropriate and possible, the outcomes reported in the evidence profiles will be:

• Malaria case incidence rate

• Malaria infection incidence

• Incidence of severe disease

• Parasite prevalence

• All-cause mortality

• Malaria mortality

• Prevalence of anaemia
Ethics and dissemination
As this systematic review is a review of primary studies it does not require ethics approval. No individual level participant data will be collected. All study files (data extraction forms, risk of bias assessments, RevMan files etc.) will be made available via a publicly available project space using open science platforms. A full review report will be submitted to the Vector Control and Insecticide Resistance Unit, Global Malaria Program, WHO and the Vector Control Guidelines Development Group to inform potential revisions to WHO recommendations for malaria prevention. A version of this report will be submitted for publication in an open access peer-reviewed journal.

Strengths of this study
- This study plans to use a rigorous systematic review methodology including comprehensive searching and study selection, risk of bias assessment, and extraction by multiple reviewers in duplicate.
- This review is taking a broad approach to inclusion in terms of outcomes, interventions, and controlled study designs.

This work will support guideline development in the field of malaria control and prevention.

Data availability

Underling data
No data are associated with this article.

Extended data
Open Science Framework: Dual-active-ingredient, insecticidal nets for preventing malaria: a systematic review protocol, DOI: 10.17605/OSF.IO/ZVGFD.

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

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