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Antimalarial drug resistance markers in southeast Asia (2000–2025) - a systematic review protocol for surveillance and spatial mapping

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ABSTRACT

Introduction and aim. Southeast Asia has historically been the epicenter of drug resistance against *Plasmodium falciparum*. The resistance has markedly reduced the effectiveness of key antimalarial drugs, such as chloroquine, sulfadoxine-pyrimethamine, and artemisinin-based combination therapy (ACTs). This systematic review protocol aims to characterize the spatio-temporal distribution and trends of drug resistance markers, both molecular and phenotypic, in *P. falciparum* malaria across Southeast Asian countries from 2000 to 2025 and to identify regional research and surveillance gaps.

Material and methods. This study will follow a systematic search of literature published in English with full text from January 2000 to December 2025, including grey literature from five electronic databases. Data extraction and analysis will strictly adhere to the PRISMA-P guidelines and PECO/PICO framework. Risk of bias will be assessed independently by two reviewers using the Joanna Briggs Institute (JBI) critical appraisal tools matched to study design, including the JBI Analytical Cross-Sectional Checklist for molecular surveillance studies and the JBI Prevalence Checklist for epidemiological surveys.

Expected outcomes. This systematic protocol is expected to synthesize evidence on the prevalence, geographical distribution, and temporal trends of resistance markers and treatment failure associated with drug-resistant *P. falciparum* in Southeast Asia.

Keywords. drug-resistant malaria, Greater Mekong sub-region, *Plasmodium falciparum*, southeast Asia, systematic review protocol

Introduction

Plasmodium falciparum malaria remains a critical public health challenge in Southeast Asia, and the Greater Mekong Sub-region (GMS)^{1,2} has historically been recognized as a global epicenter for the emergence and spread of antimalarial drug resistance. Resistance to chloroquine and sulfadoxine-pyrimethamine previously led to its major treatment failure across the region by the late 1990s.¹ Resistance to sulfadoxine-pyrimethamine, which was used as a replacement, emerged within a decade of its widespread appearance.^{3,4} The region now confronts its most consequential drug resistance challenge: partial resistance to artemisinin, the pharmacological foundation of all current WHO-recommended first-line Artemisinin-based combination therapy (ACT).⁵ Molecular markers such as mutations in the *kelch13* (*K13*) propeller domain, *pfprt*, *pfmdr1*, *pfdhfr*, and *pfdhps* genes have become important indicators for monitoring resistance patterns and therapeutic efficacy.^{2,4}

Preliminary clinical observations suggesting unusually prolonged clearance were recorded from approximately 2003 onwards.^{6,7} The first formal peer-reviewed publications confirming artemisinin partial resistance in GMS appeared in 2008 and 2009.^{6,8} These resistant parasites are characterized primarily by single-nucleotide polymorphisms in the *kelch13* (*K13*) propeller domain of *P. falciparum*.^{9,10,11} Since initial documentation, *K13*-mutant parasite lineages have been confirmed in Cambodia, Myanmar, Thailand, Laos PDR, and Vietnam and have remained in sustained circulation across the GMS.^{12,13} Despite substantial reductions in overall malaria incidence, including declines exceeding 70% in Myanmar and 90% in Vietnam,^{14,15} artemisinin-resistant lineages have not been eliminated. Reported increases in cases in certain areas of Myanmar and Cambodia demonstrate that the region has not yet achieved consistent parasite suppression,^{14,16} and this persistence directly threatens the WHO 2030 malaria elimination targets for the GMS.¹⁵

The diverse data can facilitate a precise understanding of the comparative geographical mapping of multi-drug resistance malaria within Southeast Asia. Global and regional environmental pressures challenge the organization of human populations due to administrative boundaries.¹⁷⁻²⁰ Although most published studies have focused majorly on resistance markers, therapeutic outcomes, and drug efficacy, the existing body of evidence remains fragmented across geography, time, and methodology.²¹

Aim

This review will examine molecular marker data and phenotypic outcomes, specifically, parasite clearance half-lives and treatment failure rates, as complementary or distinct lines of evidence. Understanding the treatment failure issues, particularly the response rates for each anti-malarial drug, is regarded as critical for addressing this global health threat.^{4,15} The PRISMA-P guided systematic review protocol may address these methodological gaps by synthesizing diverse evidence to map the emergence of drug resistance.² The geographic distribution and spatio-temporal evolution in resistance patterns across Southeast Asia have not

been comprehensively addressed within a single surveillance framework. Therefore, this protocol is novel in integration of molecular surveillance data, treatment outcomes, and geospatial evidence to identify regional resistance trends and surveillance gaps. The findings may also reveal methodological limitations and less explored regions, supporting future surveillance strategies and malaria elimination efforts.

Material and methods

Protocol design

This systematic review protocol will follow the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines and the Population, Exposure, Comparator, Outcome (PECO/PICO) framework, and the updated guidance of the Joanna Briggs Institute's (JBI's) critical appraisal checklist for systematic reviews and research syntheses.²² This review is expected to systematically synthesize evidence on molecular and phenotypic antimalarial drug resistance in *P. falciparum* across Southeast Asia between January 2000 and December 2025. The protocol was not prospectively registered in PROSPERO or the Open Science Framework (OSF).

The review process will include five gradual stages: (1) identification of research questions, (2) literature search and retrieval, (3) study screening and selection, (4) data extraction and charting, and (5) evidence synthesis and reporting. A preliminary literature search was conducted between August 2025 and December 2025 to refine the search strategy, eligibility criteria, and extraction framework.

The PECO/PICO framework applied in this review is as follows:

Population: Individuals of any age or gender with confirmed *Plasmodium falciparum* infection in Southeast Asia.

Exposure/Intervention: Exposure to antimalarial drugs, including chloroquine, sulfadoxine-pyrimethamine, and ACTs.

Comparison: Geographic regions, temporal trends, and resistance profiles across Southeast Asian countries.

Outcome: Molecular resistance markers, therapeutic efficacy outcomes, treatment failure rates, and parasite clearance patterns.

Stage 1: research questions

The primary research question is, "What is the spatio-temporal distribution and prevalence of drug resistance markers, molecular and phenotypic, in *P. falciparum* malaria across Southeast Asia from 2000 to 2025?"

The following secondary questions will guide the review:

1. How many peer-reviewed articles and grey literature (e.g., WHO reports, Ministry of Health bulletins, etc.) documents on drug-resistant *P. falciparum* malaria in Southeast Asia were published between 2000 and 2025?

2. What is the geographic distribution and temporal trend of key molecular resistance markers (e.g., *K13*, *pfprt*, *pfdhfr*, *pfdhps*, *pfmdr1*) across Southeast Asian countries?
3. What are the reported therapeutic efficacy outcomes, defined as Day 28/42 treatment failure rates and parasite clearance half-lives associated with CQ, SP, and ACTs use in *P. falciparum* infections across Southeast Asia?
4. Which geographic areas demonstrate consistently elevated resistance marker prevalence or treatment failure rates, and how have these areas changed over the 25-year study period?
5. What are the documented methodological limitations in existing drug resistance surveillance literature, including limitations in geographic coverage, study design, marker selection, sample size, or outcome reporting standards that future research and surveillance programs need to address?

Search strategy

A comprehensive literature search will be conducted across five electronic databases: PubMed, Scopus, ScienceDirect, EBSCO, and Google Scholar. The search strategy will combine Medical Subject Headings (MeSH), free-text keywords, Boolean operators, and synonyms related to *Plasmodium falciparum*, antimalarial drug resistance, molecular markers, and Southeast Asia.

The PubMed search strategy will include:

("Plasmodium falciparum" [MeSH] OR "falciparum malaria")

AND

("Drug Resistance" [MeSH] OR "antimalarial resistance" OR "treatment failure")

AND

("artemisinin" OR "ACT" OR "chloroquine" OR "sulfadoxine-pyrimethamine")

AND

("molecular markers" OR "K13" OR "pfprt" OR "pfmdr1" OR "pfdhfr" OR "pfdhps")

AND

("Southeast Asia" OR Cambodia OR Myanmar OR Thailand OR Vietnam OR Laos).

Equivalent search syntax adapted to indexing systems and operators will be applied for Scopus, ScienceDirect, EBSCO, and Google Scholar databases. Grey literature sources, including WHO reports, governmental publications, and institutional surveillance reports, will also be searched. For Google Scholar, the first 200 results with 100 pages sorted by relevance will be screened to improve feasibility and reproducibility. Only studies published in English between January 2000 and December 2025 will be considered eligible (Fig. 1).

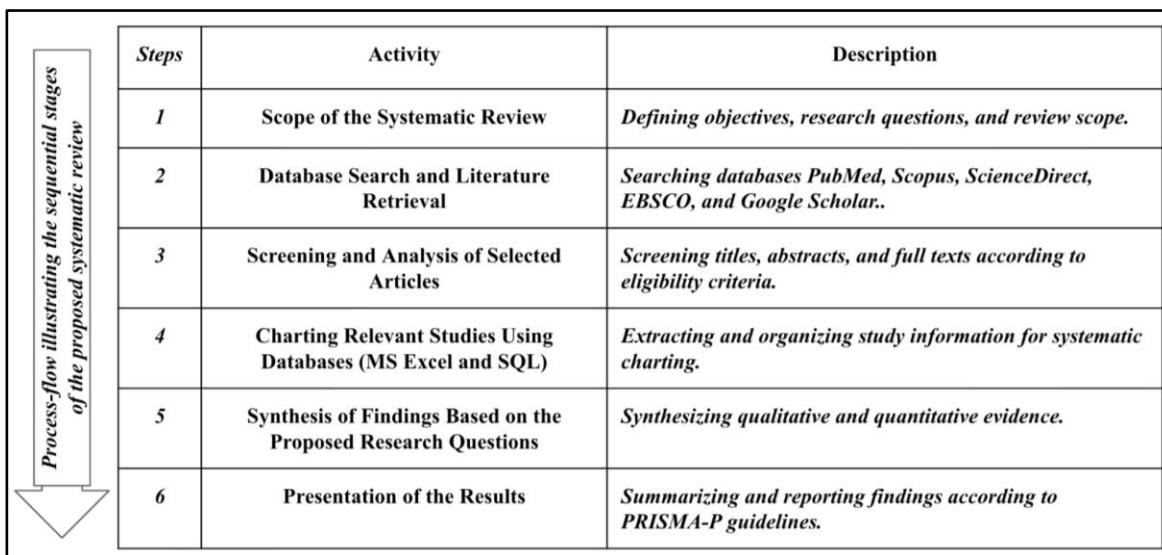


Fig. 1. Conceptual workflow of the proposed systematic review protocol for surveillance and spatial mapping of antimalarial drug resistance in Southeast Asia (2000–2025)

Specific variables used for the study

The variables will include study identification details (authors and year of publication), methodological characteristics (design type: randomized control trial, observational, molecular surveillance, and in vitro studies), geographic locations of study sites (countries/regions in Southeast Asia), demographic profiles (age groups, gender, clinical setting (outpatient/inpatient/community-based), pharmacological intervention (chloroquine, sulfadoxine-pyrimethamine, and artemisinin-based combination therapies), molecular resistance markers studied (e.g., molecular markers like *pfprt*, *pfdhfr*, *pfmdr1*, and *K13* mutations), therapeutic efficacy outcomes, and epidemiological patterns (incidence and distribution) (Table 1).

Table 1. Specific variables used for the study selection

No	Variables	Identification details
1	Study identification details	Authors, year of publication
2	Methodological characteristics	Design type: randomized control trial, observational, molecular surveillance, in vitro studies
3	Geographic locations of study sites	Countries/regions in Southeast Asia
4	Demographic profiles	Age groups, gender, clinical setting (outpatient/inpatient/community-based)
5	Pharmacological intervention	Chloroquine, sulfadoxine-pyrimethamine, and ACTs
6	Molecular resistance markers studied	<i>pfprt</i> , <i>pfdhfr</i> , <i>pfmdr1</i> , and <i>K13</i> mutations
7	Therapeutic efficacy outcomes, and epidemiological patterns	Incidence, and distribution

Stage 2: retrieving relevant studies

Authors will apply a systematic search strategy to identify scientific journals, grey literature, and peer-reviewed publications aligned with our objectives. This protocol will use a systematic approach: titles, abstracts, and keywords for searching, screening, and reviewing the studies. Data will be extracted from the studies published between January 2000 and December 2025. In the second phase, we will import the selected list to a systematically featured database (MS Excel or Amazon Aurora DSQL) for further categorizing the references.

Stage 3: study selection

Eligibility criteria

The inclusion and exclusion criteria will be created based on the PECO/PICO framework.

Inclusion criteria

Studies will be included if they satisfy all of the following criteria:

1. This systematic review will include studies from January 2000 to December 2025 in the English language strictly. Reporting at least one of the following: (i) molecular resistance marker data (*K13* codon-specific mutations; *pfprt* K76T; *pfdhfr/pfdhps*; *pfmdr1*); (ii) therapeutic efficacy outcomes (Day 28/42 treatment failure rates, parasite clearance half-lives, or cure rates); or (iii) in vitro drug susceptibility data.
2. Participants of any age or gender with confirmed *P. falciparum* infections (microscopy, RDT, or PCR) will be included from outpatient, inpatient, or community-based clinical settings.
3. Conducted in one or more Southeast Asian countries, including the GMS region (Cambodia, Laos, Myanmar, Thailand, and Vietnam) and other Southeast Asian nations (Indonesia, Malaysia, the Philippines, Singapore, Timor-Leste, and Brunei).
4. Eligible study designs, which include cases with nonrandomized and randomized controlled trials and observational studies (cohort, cross-sectional, and case-control); reports of molecular surveillance, and in vitro susceptibility studies will be taken into account.

Exclusion criteria

1. Studies that focus on other *Plasmodium* species (*P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*) will be excluded. Studies outside Southeast Asia will be removed.
2. Studies that are focused on pathogen biology, diagnostics, or unrelated treatments related to drug-resistant malaria.

Study procedure and selection of the studies

Stage 4: charting the data

The systematic review will follow a standardized variable list using a spreadsheet. Extracted variables will include study identification details, names of the authors' details, publication year, study design, demographic details, drugs investigated, molecular resistance markers, participant characteristics, and therapeutic efficacy outcomes. All retrieved references will be imported into reference management software (i.e., Zotero), and duplicate records will be removed prior to screening. Two independent reviewers will conduct title and abstract screening followed by full-text assessment according to predefined inclusion and exclusion criteria. Disagreements between reviewers will be resolved through discussion and, where necessary, consultation with a third reviewer. The final systematic review will present the study selection process using a PRISMA flow diagram.

Spatial mapping

Resistance distribution maps will be produced as evidence synthesis products, generated from geospatial data extracted from the included literature and not from primary field data collection. Geospatial visualization will be conducted using QGIS (v.2025) to map study site locations and resistance marker prevalence by geographic administrative unit.¹²

Risk of bias assessment

Risk of bias will be assessed independently by two reviewers for each included study, using JBI critical appraisal tools matched to study design.²² The JBI analytical cross-sectional checklist will be applied for molecular surveillance and cross-sectional studies, the JBI prevalence checklist for epidemiological prevalence studies, and the JBI critical appraisal checklist for randomized controlled trials for interventional studies. Disagreement in risk of bias findings will be considered during evidence synthesis and interpretation of study findings.

Stage 5: collating, summarizing, and reporting the results

The purpose of the proposed systematic review is to collect existing information on the pattern and trends, summarize the findings from the included studies, and identify endemic regions that need further research. This systematic review will involve the integration of findings from the included studies and the identification of research gaps for future investigation. These will be collected and synthesized using both descriptive statistics and thematic analysis. The authors will arrange all the processed evidence after tabulation to summarize, analyze, and make a final report on the antimalarial evidence. The reporting of this systematic review will strictly follow PRISMA-P guidelines to ensure the transparency and eligibility of the study.

Ethical considerations

The present study does not require any institutional ethical approval, as it will not involve direct collection of data from human research participants.

Dissemination

This research article containing the outcomes of the systematic review will be submitted to a scientific journal for publication. Study findings will be disseminated via open-access publications in peer-reviewed journals. The findings will be communicated through presentations, relevant meetings, conferences, seminars, and workshops. This review will aim to inform regional and global strategies for the prevention and containment of drug-resistant malaria in endemic regions.

Evidence extraction and analysis

This review will identify studies from January 2000 until December 2025. A search will be conducted for studies that are eligible after retrieving and evaluating the published articles across five major databases: PubMed, Google Scholar, ScienceDirect, EBSCO, and Scopus. The final result with data synthesis and mapping will be presented using PRISMA-P guidelines in the completed systematic review.

Discussion

Resistance to the world's most effective antimalarial drug, artemisinin, and its derivatives (i.e., ACTs) has emerged as a serious threat to global malaria control efforts.^{13,20} This systematic review protocol maps the existing evidence regarding *P. falciparum* resistance to drug patterns across Southeast Asia's GMS using the PICO/PECO framework [Population: Southeast Asian adult population of any age or gender with confirmed *P. falciparum* infection; Exposure/Intervention/Comparison: across Southeast Asia's Greater Mekong Sub-region, 2000-2025; Outcome: Multidrug resistance effect intervention. By employing a comprehensive search strategy across five databases and grey literature sources, this protocol addresses identified gaps in synthesizing heterogeneous evidence from molecular surveillance and epidemiological studies regarding the key resistance markers, including *pfcr*, *pfdhfr*, *pfdhps*, *pfmdr1*, and *Pfkelch13* mutations.^{23,24} Post-pandemic interruptions in malaria surveillance systems, healthcare accessibility, and therapeutic monitoring may have significantly contributed to gaps in resistance surveillance across endemic Southeast Asian regions.²⁵

Expected contribution of the protocol

This systematic review protocol aims to investigate the incidence and distribution of drug resistance as well as challenges related to drug-resistant *P. falciparum* malaria in endemic regions of Southeast Asia. The

severity and mortality of malaria caused by *P. falciparum* are considered crucial public health issues in selected regions of Southeast Asia, including the GMS, despite the rapidly declining rate of malaria cases. As a global epicentre for multi-drug-resistant malaria, especially against artemisinin-based therapies, it requires focused, long-term, sustained attention and effective interventions.

Conclusion

Antimalarial drug resistance in *Plasmodium falciparum* remains a major hindrance to malaria eradication policies in Southeast Asia, particularly within the GMS. This systematic review protocol provides a structured framework to synthesize evidence on the spatial and temporal distribution of molecular resistance markers and therapeutic efficacy outcomes reported between 2000 and 2025. By integrating molecular, phenotypic, and geospatial evidence under PRISMA-P guidelines, the review aims to identify resistance zones, surveillance gaps, and multidrug-resistant malaria trends across the region. The findings are expected to support evidence-based treatment policies, strengthen regional surveillance systems, and contribute to malaria elimination strategies in Southeast Asia.

Declarations

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Author contributions

Conceptualization, S.S.R. and S.D.; Methodology, S.S.R. and S.D.; Software, S.S.R.; Validation, S.S.R., S.D. and N.M.; Formal Analysis, S.S.R.; Investigation, S.S.R.; Resources, N.M.; Data Curation, S.D.; Writing – Original Draft Preparation, S.S.R., S.D. and N.M.; Writing – Review & Editing, S.S.R. and N.M.; Visualization, S.S.R.; Supervision, N.M.; Project Administration, N.M.

Conflicts of interest

The authors declare there is no conflict of interest.

Data availability

The datasets generated and analysed during the current study will be available from the corresponding author on reasonable request.

Ethics approval

This systematic review protocol did not involve direct human participation or interaction; hence, ethics approval and consent to participate were not required. Despite this, the study adhered to all institutional

protocols (both nationally and internationally) and made use of best practices throughout the study selection, review, and reporting processes when conducting this analysis on the studies included in this analysis.

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