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Review Article

Genetic Diversity and Multiplicity of *Plasmodium falciparum* Infection in Southeast Asia: New Insights from a Systematic Review

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Abstract
Plasmodium falciparum caused four million new malaria cases in Southeast Asia in 2023, with heterogeneous transmission patterns and the development of artemisinin resistance. The merozoite surface protein genes (msp-1 and msp-2) serve as genetic markers for analyzing the parasite population structure and multiplicity of infection (MOI). However, a comprehensive synthesis of regional data is limited. This study aimed to determine the genetic diversity and MOI of *P. falciparum* in Southeast Asia. This systematic review was guided by PRISMA guidelines with searches in the Scopus, PubMed, ProQuest, and Google Scholar databases (2014–2024). The inclusion criterion was observational studies, analyzing the genetic diversity of *P. falciparum* via msp-1 and msp-2 markers in Southeast Asia. The extracted data included the frequency of msp-1 and msp-2 family alleles, the prevalence of polyclonal infections, and the mean MOI value. Quality assessment was performed via the joanna briggs institute critical appraisal tools with narrative synthesis following the synthesis without meta-analysis (SWiM) guidelines. Fifteen studies; Indonesia (40%), Thailand (26.67%), Myanmar (20%), Vietnam and Malaysia (6.66%) with 1,830 samples successfully genotyped from 2,130 collected samples. The MAD20 (msp-1) allele dominated most locations, with frequencies of up to 100% in Lampung. The distribution of msp-2 alleles showed geographical variation, with FC27 dominating in Papua (96.2%) and 3D7/IC in Vietnam (97.0%). The prevalence of polyclonal infection ranged from 0-84.6%, with MOI values ranging from 1.0-2.93. The hyperendemic areas presented high MOIs (>2.0), whereas the hypoendemic areas presented MOIs close to 1.0, confirming a positive correlation with malaria transmission intensity. The *P. falciparum* population in Southeast Asia shows high genetic diversity, with geographically variable allele distribution patterns, and MOI values are correlated with malaria endemicity levels. These findings support the need for regional molecular surveillance and a polyvalent approach to the development of msp-based vaccines.

Keywords: genetic diversity, malaria surveillance, merozoite surface protein, molecular epidemiology, multiplicity of infection, *Plasmodium falciparum*, Southeast Asia

1. INTRODUCTION

Malaria caused by *Plasmodium falciparum* infection remains a global public health challenge with various complexities [1][2]. Southeast Asia recorded 4 million new malaria cases in 2023, with heterogeneous transmission patterns across the region. The development of artemisinin resistance threatens regional elimination programs, which require a more precise surveillance approach to monitor parasite evolution [3]. Genetic diversity in *P. falciparum* populations is a fundamental factor influencing virulence, the ability of the parasite to evade the host's immune response, and the spread of resistant strains [4]-[6]. Analysis of the genetic structure of parasite populations is an important molecular epidemiological tool for tracking transmission dynamics, evaluating the impact of interventions, and identifying genetic markers potentially associated with clinical phenotypes and/or drug resistance [7]-[11].

Among the many genetic markers, the merozoite surface protein-1 (msp-1) and merozoite surface protein-2 (msp-2) genes are widely used because of their high level of polymorphism, which is caused by point mutations and intragenic recombination [12][13]. Allele variation in both genes, particularly in block 2 of msp-1 (three allele families: MAD20, K1, and RO33 allele family of merozoite surface protein-1 (RO33)) [14][15] and block 3 of msp-2 (two allele families: FC27 and IC1/3D7) [16][17], has proven useful for distinguishing parasite strains and determining the multiplicity of infection (MOI), which is defined as the number of genetically distinct parasite clones in a single infection [4][18].

Several studies have characterized the variation in msp-1 and msp-2 alleles in clinical isolates *of P. falciparum* in various Southeast Asian countries [7][19][20]. These studies show different patterns of

Table 1. Searches with keywords.

Database	Keywords (Term)	Search Results (*n*)
Scopus	TITLE-ABS-KEY (*Plasmodium falciparum* OR *P. falciparum* AND merozoite surface protein OR msp1 OR msp2 AND genetic diversity AND multiplicity of infection OR polyclonal infection AND Southeast Asia OR Brunei Darussalam OR Indonesia OR Cambodia OR Laos OR Malaysia OR Myanmar OR Philippines OR Singapore OR Thailand OR Timor-Leste OR Vietnam)	17
PubMed	Search: ((((((((((((((((((plasmodium falciparum[MeSH Terms]) AND (MSP-1[MeSH Terms])) OR (MSP-1[MeSH Terms])) OR (merozoite surface protein[MeSH Terms])) OR (msp1[MeSH Terms])) AND (genetic diversity[MeSH Terms])) OR (genetic variation[MeSH Terms])) OR (multiplicity infection[MeSH Terms])) OR (polyclonal infection[MeSH Terms])) AND (southeast asia[MeSH Terms])) OR (indonesia[MeSH Terms])) OR (malaysia[MeSH Terms])) OR (thailand[MeSH Terms])) OR (vietnam[MeSH Terms])) OR (myanmar[MeSH Terms])) OR (Philippines[MeSH Terms]) OR (Cambodia[MeSH Terms]) OR (Laos[MeSH Terms]) OR (Singapore[MeSH Terms]) OR (Brunei[MeSH Terms]) AND ((ffrft[Filter]) AND (controlledclinicaltrial[Filter] OR observationalstudy[Filter] OR randomizedcontrolledtrial[Filter]) AND (fft[Filter]) AND (2014/1/1:2024/1/31[pdat]) AND (data[Filter])) AND ((ffrft [Filter]) AND (controlledclinicaltrial[Filter] OR observationalstudy[Filter] OR randomizedcontrolledtrial[Filter]) AND (fft [Filter]) AND (2014:2023[pdat])) AND ((ffrft[Filter]) AND (controlledclinicaltrial[Filter] OR observationalstudy[Filter] OR randomizedcontrolledtrial[Filter]) AND (fft[Filter]) AND (2014:2023[pdat])) Filters: Free full text, Full text, Observational Study, Randomized Controlled Trial, English, Exclude preprints, from 2014 - 2023	758
Google Scholar	("*Plasmodium falciparum*" OR "*P. falciparum*") AND ("MSP-1" OR "MSP-2" OR "merozoite surface protein" OR "msp1" OR "msp2") AND ("genetic diversity" OR "genetic variation" OR "multiplicity of infection" OR "MOI" OR "polyclonal infection") AND Southeast Asia OR Brunei Darussalam OR Indonesia OR Cambodia OR Laos OR Malaysia OR Myanmar OR Philippines OR Singapore OR Thailand OR Timor-Leste OR Vietnam	932
ProQuest	("*Plasmodium falciparum*" OR "*P. falciparum* OR malaria") AND ("merozoite surface protein" OR "msp1" OR "msp2" OR "MSP-1" OR "MSP-2") AND ("genetic diversity" OR "genetic variation" OR "polymorphism" OR "allelic diversity") AND ("multiplicity of infection" OR "polyclonal infection" OR "mixed infection" OR "MOI") AND ("evidence-based healthcare" OR "evidence-based medicine" OR "evidence-based practice") AND ("Southeast Asia" OR "South East Asia" OR Indonesia OR Malaysia OR Thailand OR Vietnam OR Singapore OR Philippines OR Myanmar OR "Burma" OR "Bangkok Thailand" OR Cambodia OR "Jakarta Indonesia" OR Laos OR "Kuala Lumpur Malaysia" OR "Selangor Malaysia") AND (scholarly journals) AND (publication date: 2014-2024) AND (language: English) AND (document type: article)	1,557

allele distribution between locations, reflecting unique local transmission conditions and selective pressure. Some regions report a predominance of the MAD20 and 3D7 allele families, whereas others show higher frequencies of the K1 or FC27 alleles [20][21]. However, comprehensive syntheses summarizing and comparing genetic diversity and MOI data from Southeast Asia remain limited. The absence of this integrated regional picture hinders a comprehensive understanding of parasite population dynamics, particularly the cross-border gene flow necessary for coordinated control strategies, even though high human population movement in the region can facilitate the spread of certain strains, including drug-resistant strains [22].

A systematic review of existing genetic data can provide a macro view of the geographical distribution of alleles, identify genetic diversity hotspots, and evaluate the correlation between MOI patterns and different transmission intensities in the region. To fill this knowledge gap, this systematic review aimed to collect, evaluate, and synthesize existing evidence on the genetic diversity of the msp-1 and msp-2 genes and the multiplicity of *P. falciparum* infection in Southeast Asia. Specifically, it aims to elucidate how epidemiological factors, such as the geographical distribution of msp-1 and msp-2 alleles, the prevalence of polyclonal infections and MOI values, and determinants that influence the structure of the parasite population in this region. The results of this review are expected to provide a consolidated insight of the *P. falciparum* population structure in Southeast Asia. This information could strengthen regional molecular surveillance programs to monitor the spread of parasite strains of concern and provide an empirical evidence base for the development of effective MSP-based vaccines against relevant antigenic variations in the region.

2. MATERIALS AND METHODS

2.1. Protocol and Registration

This systematic review was conducted following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines [23], and the protocol has been registered in the International Prospective Register of Systematic Reviews (PROSPERO) with registration number CRD42024606943.

2.2. Search Strategy and Study Selection

Before the article search was conducted, the author established a population, exposure, comparator, outcome, setting, time (PECOST) framework to define the key elements, including population (individuals infected with *P. falciparum*), exposure (*P. falciparum* infection), comparator (none), outcome (geographical distribution of genetic diversity, prevalence of polyclonal infection, and determinants of genetic diversity and multiplicity of infection/MOI), setting (all countries in Southeast Asia), and time (1 decade, 2014–2024). A systematic literature search was conducted in the Scopus, PubMed, ProQuest, and Google Scholar databases. The search was limited to articles published in English between 2014 and 2024.

This time frame was chosen because of the relatively high availability of malaria genomic technology, which produced representative data for the region analyzed. In addition, the incidence of malaria in Southeast Asia decreased during this period. A comprehensive search strategy was developed using a combination of Medical Subject Headings (MeSH) terms and relevant free-text keywords, such as “Plasmodium falciparum”, “genetic diversity”, “multiplicity of infection”, “MOI”, “msp-1”, “msp-2”, and “Southeast Asia”. Boolean operators (AND, OR) were used to construct customized search strings for each of the databases [24]. A summary of the databases, keywords, and number of documents obtained is presented in Table 1. The study selection process was conducted in two stages by two independent reviewers (RP and NAH), who examined the titles and abstracts, followed by a full-text review of the potentially relevant studies. Discrepancies were resolved through discussion until a consensus was reached.

2.3. Inclusion and Exclusion Criteria

The studies included in this review met the following inclusion criteria: (1) observational study design, including cross-sectional, case–control, cohort, and/or randomized controlled trials (RCTs); (2) human populations of all age groups infected with *P. falciparum* in Southeast Asia; (3) studies

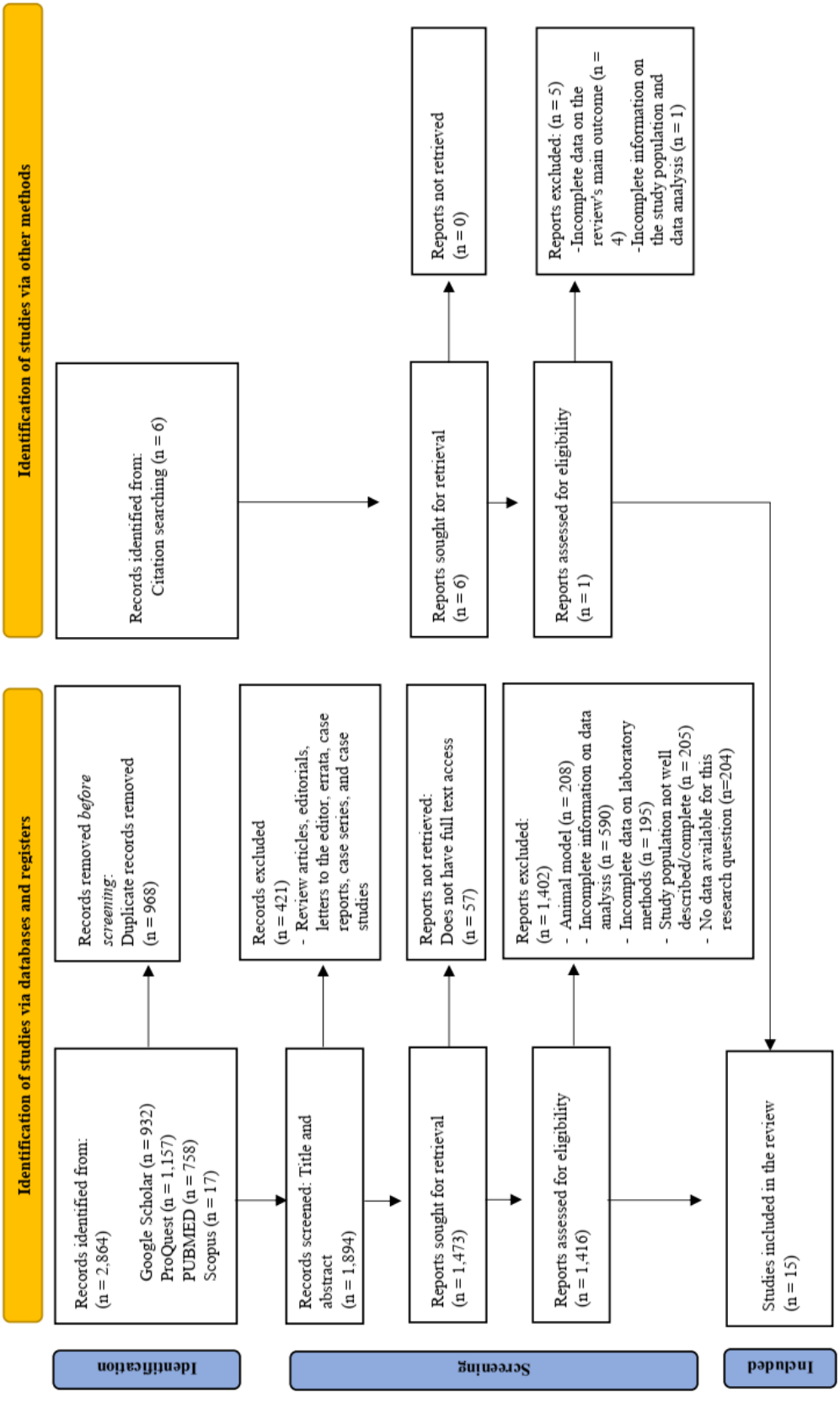

Figure 1. PRISMA 2020 flow diagram for new systematic reviews that included searches of databases, registers, and other sources.

investigating genetic diversity or multiplicity of infection (MOI) using the msp-1 and/or msp-2 genes; (4) studies presenting quantitative data on allele frequency, proportion of polyclonal infections, and mean MOI values; and (5) studies describing the laboratory methods used for genotyping in detail. Review articles, case reports, letters to the editor, editorials, and studies conducted on animals were excluded. Studies were excluded if they did not provide numerical data on allele frequency or MOI values, used genotyping methods other than PCR electrophoresis, or did not include a validated deoxyribonucleic acid (DNA) extraction protocol.

2.4. Data Extraction

Before data extraction, the articles were checked for duplication via Mendeley Reference Manager software. Two reviewers (RP and NAH) independently extracted data from the included articles via a predefined extraction form. This form included variables such as (1) author characteristics, including the first author's name, year of publication, country and location of the study, and sampling period; (2) study characteristics, including study design, sample size, and clinical and demographic characteristics of the population (age, sex, symptomatic/asymptomatic status); (3) transmission characteristics, including the endemicity or intensity of malaria transmission at the study site and the annual parasite index (API); (4) laboratory methodology for confirming malaria cases/diagnoses, specimen types, and DNA extraction methods used; and (5) genetic diversity, including the frequency of msp-1 (K1, MAD20, RO33) and msp-2 (FC27, 3D7/IC1 allele family of merozoite surface protein-2 (3D7/IC)) family alleles and multiplicity of infection (MOI) data, including the prevalence of polyclonal infection, number of genotypes, and/or average MOI value. Any discrepancies in the data extracted by the two reviewers were reverified by referring to the original article until a consensus was reached.

2.5. Critical Appraisal and Study Quality Assessment

Methodological quality assessment was conducted via the Joanna Briggs Institute (JBI) Critical Appraisal Tools appropriate for the design of the studies analyzed. The three instruments used included (1) the JBI critical appraisal checklist for analytical cross-sectional studies with eight evaluation criteria for analytical cross-sectional studies; (2) the JBI critical appraisal checklist for case–control studies with ten evaluation criteria for case–control studies; and (3) the JBI Critical Appraisal Checklist for Studies Reporting Prevalence Data with nine evaluation criteria for prevalence studies. Each study was evaluated independently by two reviewers (DH and IMDMA) using a four-category rating system: "yes" (green), "no" (red), "unclear" (yellow), and "not applicable" (blue).

The assessment covered key methodological aspects, including the definition of sample inclusion criteria, description of subjects and research setting, validity of exposure measurement, use of objective criteria for condition measurement, identification of confounding factors, strategies for handling confounding factors, validity of outcome measurement, and appropriateness of statistical analysis. Study quality was classified based on the proportion of criteria met into the following categories: high (≥80% of criteria met), moderate (60–79% of criteria met), and low (<60% of criteria met). The classification of evidence levels refers to the 2013 JBI Levels of Evidence with the following designations: Level 3. d. for case–control studies; Level 4. b. for cross-sectional studies; Level 4. b. Prevalence studies. Disagreements between the reviewers were resolved through consensus discussions involving a third reviewer when necessary. The final decision regarding the inclusion or exclusion of studies on the basis of the methodological quality assessment was recorded in the data extraction form, with documentation of the reasons for exclusion.

2.6. Data Analysis

Data extracted from each study were tabulated and synthesized narratively to answer the research questions [25]. The allele frequency, polyclonal infection prevalence, and mean MOI values from each study are presented in tabular form to allow direct comparisons between different geographic regions and transmission conditions. The relationships among malaria transmission intensity, genetic diversity, and MOI were analyzed by

Table 2. Critical appraisal and study quality based on JBI Critical Appraisal Tools.

Author and Year (Study Design)	JBI Critical Appraisal Tools										Level and Quality of Evidence	Special Notes
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10		
Tanabe et al. [27] (Cross-sectional)	+	+	+	+	+	+	+	+			Level 4.b (Quality: High)	The study was very well designed, the methodology was transparent, and the risk of bias was low.
Arwati et al. [4] (Cross-sectional)	+	+	+	+	+	+	+	-			Level 4.b (Quality: Moderate)	The laboratory methodology is adequate but does not report formal statistical tests to support claims of comparisons between groups.
Tanjung et al. [28] (Cross-sectional)	+	+	+	+	?	?	+	+			Level 4.b (Quality: High)	A strong descriptive study with clear methodology appropriate for its purpose. Low risk of bias.
Soe et al. [7] (Cross-sectional)	+	+	+	+	+	+	+	+			Level 4.b (Quality: High)	A well-designed analytical study comparing three locations using appropriate molecular and statistical methods.
Kuesap et al. [29] (Cross-sectional)	+	+	+	+	×	×	+	+			Level 4.b (Quality: Low)	Failed to identify and address potential confounding factors when comparing data from different time periods.
Goh et al. [19] (Cross-sectional)	+	-	+	+	×	×	+	-			Level 4.b (Quality: Low)	Lack of demographic data on subjects, failure to address confounding factors, and absence of formal comparative statistical tests.
Jamil et al. [13] (Cross-sectional)	+	+	+	+	+	×	+	+			Level 4.b (Quality: Moderate)	Did not perform multivariate analysis to control for confounding factors when testing associations with disease severity.
Thái et al. [30] (Cross-sectional)	+	+	+	+	?	?	+	+			Level 4.b (Quality: High)	A very thorough population genetics study using laboratory methods and robust statistical analysis.
Runtuboi et al. [31] (Cross-sectional)	+	+	+	+	×	×	+	-			Level 4.b (Quality: Low)	No identification of confounding factors and no statistical test reports for analytical comparisons.
Mau et al. [32] (Cross-sectional)	+	+	+	+	?	?	+	+			Level 4.b (Quality: High)	A good descriptive study with a methodology appropriate for its purpose.
Zhang et al. [20] (Cross-sectional)	+	+	+	+	+	+	+	+			Level 4.b (Quality: High)	A robust analytical study with advanced laboratory methodology and appropriate statistical analysis.
Long et al. [21] (Cross-sectional)	+	+	+	+	?	?	+	+			Level 4.b (Quality: High)	Strong descriptive study using high-resolution molecular methods with clear objectives.

Table 2. *Cont.*

Author and Year (Study Design)	JBI Critical Appraisal Tools Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Level and Quality of Evidence	Special Notes
Chaorattanakawee et al. [33] (Case–Control)	-	+	+	+	+	+	+	+	+	+	Level 3.d (Quality: High)	A well-designed case–control study. Its main strength is the explicit identification and handling of confounding factors (MOI).
Congpuong et al. [34] (Case–Control)	+	+	+	+	+	X	X	+	+	+	Level 3.d (Quality: Moderate)	A useful case–control study, but it does not identify or statistically adjust for potential confounding factors.
Triajayanti et al. [35] (Prevalence studies)	+	X	X	X	+	+	+	+	+		Level 4.b (Quality: Low)	This study has significant methodological weaknesses for a prevalence study, particularly in terms of sampling, inadequate sample size, and minimal subject description. The risk of bias is high.

Remarks: The assessment is based on standard items from the JBI critical appraisal tools according to the type of research design (**https://jbi.global/critical-appraisal-tools**).

+ Yes - No X Unclear ? Not/Applicable

comparing the results of studies conducted in hypo-, meso-, and hyperendemic areas. Given the high degree of clinical and methodological heterogeneity between studies, including differences in design, target population, and genotyping techniques, a quantitative meta-analysis was considered inappropriate. Therefore, we used the synthesis without meta-analysis (SWiM) guidelines [26].

3. RESULTS AND DISCUSSIONS

3.1. Study Selection and Inclusion Process

The study selection process was conducted following the PRISMA 2020 flowchart to ensure transparency and reproducibility (Figure 1). An initial search of four electronic databases Google Scholar, ProQuest, PubMed, and Scopus yielded 2,864 articles. After 968 duplicates were removed, 1,894 articles were screened based on their title and abstract. At this stage, 421 articles were excluded because they were not relevant to the research topic of identifying *P. falciparum* species, including review articles, letters to the editor, editorials, case reports, and case studies. Next, 1,473 full reports were evaluated for eligibility criteria. Of these, 57 articles were not fully accessible (not open access) despite attempts to contact the authors and their institutional libraries. The assessment process of the remaining 1,416 reports resulted in the exclusion of an additional 1,402 reports for more specific reasons: (1) use of animal models (n = 208), (2) incomplete analysis data (n = 590), (3) inadequate laboratory methodology data (n = 195), (4) inappropriate or poorly described study population (n = 205), and (5) lack of data relevant to the research question (n = 204). A manual search of additional citations yielded six articles, of which only one was eligible. In total, 15 studies met all the inclusion criteria and were included in the final synthesis.

3.2. Critical Appraisal and Quality of the Included Studies

The distribution of methodological quality in the 15 studies evaluated showed varying patterns (Table 2). Most cross-sectional studies were of high quality (7 of 12 studies, 58.3%), whereas the rest were of moderate (16.67%) or low (25%) quality. Both case–control studies were rated as high or

moderate quality, whereas one prevalence study was classified as low quality. Frequently identified methodological deficiencies were the inability to identify and control for confounding factors found in six studies (37.5%), and seven studies (43.8%) did not report strategies for handling confounding factors. Another weakness was the inadequacy of the statistical analysis for analytical comparisons. This variation in quality underscores the need to consider quality gradation in evidence synthesis. High-quality studies provide more reliable evidence, whereas low-quality studies should be considered supporting evidence with explicit acknowledgment of their limitations.

3.3. Characteristics of the Included Studies

This review synthesized 15 studies from five Southeast Asian countries: Indonesia (n = 6, 40%), Thailand (n = 4, 26.67%), Myanmar (n = 3, 20%), Vietnam (n = 1, 6.66%), and Malaysia (n = 1, 6.66%). The publication time range of the studies was between 2014 and 2023. Most studies used a cross-sectional design (n = 12, 80%). The sample data were collected between 1997 and 2020. Among the 2,130 samples collected, 1,830 were genotyped successfully. The majority of samples were obtained from symptomatic malaria patients, except for one study from Thailand that focused on an asymptomatic majority population, providing important insights into the parasite structure in individuals with different immune responses. The age of the subjects varied from childhood to adulthood. Malaria diagnosis is generally performed via microscopy combined with polymerase chain reaction (PCR). All the samples analyzed were derived from the blood of *P. falciparum-infected* patients and extracted via the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) and/or other methods. The characteristics of each study, including the data collection location, sampling period, and population, are listed in Table 3.

3.4. Characteristics of the Transmission and Geographical Distribution of Alleles

Variations in malaria transmission conditions at the study sites were identified as hypoendemic (n = 2, 13.33%), mesoendemic (n = 5, 33.33%), and hyperendemic (n = 7, 46.67%), which directly shaped the heterogeneous genetic diversity patterns of the *P. falciparum* population in each region (Table 4**)**. Analysis of the msp-1 gene revealed consistent dominance of the MAD20 allele family in most locations, with a frequency of 100% in specific samples from Lampung, Indonesia. The K1 allele family was also commonly found, whereas the RO33 allele consistently had a lower frequency and was undetectable in some areas. In contrast, at the msp-2 gene locus, no single allele was dominant throughout Southeast Asia. The distribution of the 3D7/IC and FC27 alleles varied depending on the location, indicating the presence of strong local selective pressure. The FC27 allele was dominant in Wamena, Papua, Indonesia (96.2%), whereas the 3D7/IC allele was most dominant in Vietnam (97.0%) and Thailand (80.8%). This heterogeneity indicates that local selective pressures, possibly related to population-specific immune responses or other factors, play a strong role in shaping the parasite population structure at the msp-2 locus (Table 5).

3.5. Prevalence of MOI

The level of MOI value was clearly correlated with the level of malaria endemicity at the study locations (Table 6). Studies in areas of high transmission, such as the Thailand–Myanmar border and Wamena, Indonesia, reported a high prevalence of polyclonal infection (74.3% and 66.7% for the msp-1 gene and 84.6% and 84.6% for the msp-2 gene, respectively, with an average MOI value above 2.0). These findings indicate that infection with multiple parasite strains is common in these areas. In contrast, low-transmission areas, such as the Thailand-Myanmar and Malaysia borders, presented low rates of polyclonal infection (15.5% and 13.3% for the msp-1 gene, respectively), and the Thailand-Myanmar border with Vietnam (18.4% and 7% for the msp-2 gene, respectively) presented an average MOI value of 1. This pattern confirms that MOI values are effective epidemiological indicators for mapping the gradient of *P. falciparum* parasite transmission intensity in Southeast Asia.

3.6. Genetic Diversity and Multiplicity of P. falciparum Infection in Southeast Asia

The *P. falciparum* population in Southeast Asia

Table 3. General characteristics of the studies and populations included.

Author	Country and Location	Sample Period	Study Design	Sample Size (n, genotyped)	Clinical Category	Age Group (range)	Diagnostic Method	Specimen	Kit/extraction method
Thailand									
Chaorattanakawee et al. [33]	Thailand (Northwestern Thailand, Myanmar border)	N/A	Case–control	480 (471)	Mild, Severe noncerebral, Cerebral	Adults (≥13 years)	Microscopy	Thick and thin blood smears	QIAamp miniblood (QIAGEN, Hilden, Germany)
Congpuong et al. [34]	Thailand (Myanmar border)	2009-2013	Case–control	156 (156)	Symptomatic (uncomplicated)	Adult	Microscopy	Blood	QiaAmp DNA mini (Qiagen, Hilden, Germany)
Kuesap et al. [29]	Thailand (Mae Sot)	1997-2010	Cross-sectional	145 (135)	Symptomatic (uncomplicated)	N/A	Microscopy	Blood	Chelex
Tanabe et al. [27]	Thailand (Kong Mong Tha)	2000-2002	Cross-sectional	60 (59)	Majority asymptomatic (>92%)	All ages (1-92 years)	Microscopy/ PCR	Blood	QIAmp DNA mini kit (Qiagen, Hilden, Germany)
Indonesia									
Arwati et al. [4]	Indonesia (Central Kalimantan)	2017-2020	Cross-sectional	51 (20)	Symptomatic	Adults (15-65 years)	Microscopy/ RDT	Blood	QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany)
Mau et al. [32]	Indonesia (Central Sumba, Nusa Tenggara Timur/NTT)	2015	Cross-sectional	50 (19)	Symptomatic	>1 year	Microscopy	Blood	QIAamp® DNA micro (Qiagen, Hilden, Germany)
Runtuboi et al. [31]	Indonesia (Wamena, Papua)	2018-2019	Cross-sectional	26	Symptomatic	Adults (15-34 years) and Children (12-14 years)	Microscopy	Blood	Genomic DNA mini kit (blood/culture).
Tanjung et al. [28]	Indonesia (Wamena, Papua)	2018	Cross-sectional	26 (24)	Symptomatic	Adults (≥12 years)	Microscopy	Blood	QIAamp® DNA micro (Qiagen, Hilden, Germany)
Triajayanti et al. [35]	Indonesia (Hanura, Lampung)	2016	Descriptive (Prevalence Study)	23 (23)	Symptomatic	N/A	N/A	Blood	QIAamp® DNA Mini Kit (Qiagen)

Table 3. *Cont.*

Author	Country and Location	Sample Period	Study Design	Sample Size (n, genotyped)	Clinical Category	Age Group (range)	Diagnostic Method	Specimen	Kit/extraction method
Indonesia									
Jamil et al. [13]	Indonesia (Aceh)	2013-2015	Cross-sectional	90 (90)	Mild & Severe	Adults (19-63 years)	Microscopy/ PCR	Blood	Chelex-100
Malaysia									
Goh et al. [19]	Malaysia and Thailand	2008-2014	Cross-sectional	173	Outpatients	N/A	Microscopy	Blood	QiAamp® DNA Blood Mini Kit (Hilden, Germany)
Myanmar									
Soe et al. [7]	Myanmar (Shwekyin, Myawaddy, Kyauktaw)	2009-2010	Cross-sectional	267 (184)	Symptomatic (acute)	All ages (6-70 years)	Microscopy/ PCR	Blood	QIAamp® DNA micro (Qiagen, Hilden, Germany)
Thái et al. [30]	Myanmar (Upper Myanmar)	2015	Cross-sectional	69 (69)	Symptomatic	Adults (13-57 years)	Microscopy/ PCR	Blood	QIAamp Blood Kit (Qiagen, Valencia, CA, USA)
Zhang et al. [20]	China-Myanmar border	2006	Cross-sectional	242 (215)	Symptomatic	All ages (<9 - >49 years)	Microscopy/ PCR	Blood	QIAamp® DNA micro (Qiagen, Hilden, Germany)
Vietnam									
Long et al. [21]	Vietnam (Dak Lak, Gia Lai, Dak Nong)	2017-2019	Cross-sectional	222 (166)	Symptomatic (uncomplicated)	Adults (average age = 29.1 years) ± 9.5; 88% female)	Microscopy/ PCR	Blood	QIAamp DNA Mini-Kit (Qiagen, Hilden, Germany)

Remarks: N/A: Not available in the article, PCR: Polymerase Chain Reaction, RDT: Rapid Diagnostic Test

Table 4. Malaria transmission characteristics at the study site.

Author and Year	Transmission characteristics and endemicity	Annual Parasite Index (API)
Thailand		
Chaorattanakawee et al. [33]	High, Hyperendemic	N/A
Congpuong et al. [34]	High, Hyperendemic	N/A
Kuesap et al. [29]	High, Hyperendemic	N/A
Tanabe et al. [27]	Low, hypoendemic	N/A
Indonesia		
Arwati et al. [4]	Low, hypoendemic	0.24
Mau et al. [32]	Moderate, Mesoendemic	N/A
Runtuboi et al. [31]	High, Hyperendemic	N/A
Tanjung et al. [28]	High, Hyperendemic	N/A
Triajayanti et al. [35]	Moderate, Mesoendemic	6.36
Jamil et al. [13]	Moderate, Mesoendemic	0.06–0.08
Malaysia		
Goh et al. [19]	Low, hypoendemic	N/A
Myanmar		
Soe et al. [7]	High, hyperendemic	N/A
Thái et al. [30]	Moderate, Mesoendemic	N/A
Zhang et al. [20]	High, Hyperendemic	N/A
Vietnam		
Long et al. [21]	Moderate, Mesoendemic	N/A

shows high polymorphism in the msp-1 and msp-2 genes, with geographically variable allele distribution patterns. These findings revealed three epidemiological patterns: MAD20 dominance in msp-1, FC27/3D7 variation in msp-2, and an MOI correlation with transmission intensity. These findings answer three key research questions regarding the geographical distribution of genetic diversity, the prevalence of polyclonal infection, and the factors that influence it and provide important implications for malaria control and elimination strategies, including the direction of malaria eradication development and acceleration in Southeast Asia [4][7][13][19]–[21][27]–[35].

Our findings show that the distribution of msp-1 and msp-2 alleles in Southeast Asia is not uniform, reflecting a complex epidemiological landscape in the region. The predominance of the MAD20 allele family in the msp-1 gene in various countries indicates the possibility of a selective advantage, although historical migration patterns and genetic drift may also contribute to this distribution [1][36]-[39]. In contrast, the variation in dominance between the FC27 and 3D7 alleles of the msp-2 gene indicates different selective pressures at each location. These local selective pressures may be the result of the host population's immune response, which has adapted to long-circulating parasite strains, or past antimalarial drug use policies that may have indirectly benefited certain alleles [40]-[42].

This functional link is reinforced by a study by Chaorattanakawee et al. [33] in Thailand, which identified an association between specific sequence variations in the FC27 and 3D7 alleles and malaria severity. These findings confirm that these alleles are not merely neutral markers but potentially have a biological role in parasite–host interactions that determine virulence [43][44]. These findings are consistent with reports from Africa and South America, where genetic diversity is also high, but the frequency and dominance of specific alleles are very different [36]. These differences indicate that although the msp-1 and msp-2 genes are universal

markers, epidemiological history and local selective pressures shape unique genetic patterns in each country [11][45].

The prevalence of polyclonal infections and the MOI values consistently reflected the intensity of malaria transmission, confirming its role as an epidemiological proxy that is indirectly consistent with the global literature. In hyperendemic areas, such as Papua, where individuals are frequently exposed to infectious mosquito bites, the likelihood of infection by several genetically distinct parasite strains is high [28][31]. The high rates of polyclonal infection (>60%) and MOI values (>2.0) in these locations create ideal conditions for genetic recombination during the sexual phase of the parasite within the mosquito vectors. This process allows the exchange of genetic material between different strains, resulting in new variants that potentially have advantages, such as drug resistance or the ability to evade the host immune response. Thus, areas with high MOIs can be considered evolutionary laboratories for parasites that accelerate the adaptation of *P. falciparum*. Conversely, in regions with low transmission rates, as reported by Arwati et al. [4] and Goh et al. [19], the dominance of monoclonal infections is a marker of the success of control programs in reducing the circulation of parasite strains. These findings are reinforced by studies in Africa, where a high MOI (above 2) correlates with hyperendemic transmission areas, whereas a low MOI (~1.0) is often found in low transmission areas [46]. In Southeast Asia, the gradient of MOIs from low to high (e.g., from Malaysia to Myanmar) clearly reflects variations in transmission intensity and can be used as a tool to map the risk of transmission between countries in the future.

Transmission intensity is the main driver of genetic diversity and the MOI. However, other factors, such as human mobility and patient clinical status, also play a role. Studies in border regions, such as Thailand-Myanmar [34] and China-Myanmar [20], have shown high genetic diversity. Border regions facilitate genetic exchange and parasite migration, contributing to increased allelic diversity. This makes border areas critical for cross-border surveillance. Additionally, a comparison between asymptomatic and symptomatic populations by Tanabe et al. [27] in Thailand highlighted the complex interaction between parasite exposure and host immunity. Although the single-nucleotide polymorphism (SNP) diversity levels were similar, the MOI in asymptomatic populations was lower. These findings suggest that the development of clinical immunity in individuals limits the number of parasite clones that successfully survive a single infection, indicating that host immunity acts as a filter that contributes to the complexity of infection at the individual level.

Overall, the findings of this review provide a comprehensive picture of the structure of *P. falciparum* populations in Southeast Asia. The high level of polymorphism in the msp-1 and msp-2 genes, especially in areas of high transmission, confirms that antigen-based vaccine candidates must induce an immune response that can recognize a variety of allele variants to achieve broad efficacy. Therefore, vaccine development requires a polyvalent approach specifically designed to cover variants that are dominant in Southeast Asia. Furthermore, MOI and allele distribution data can serve as more dynamic and sensitive epidemiological tools for tracking the success of malaria control programs in individual countries [47]-[49]. Changes in the MOI provide real-time indications of decreases or increases in transmission, often faster than traditional metrics such as the annual parasite index (API), which rely on clinical case reporting.

3.7. New Insights for Malaria Eradication in Indonesia

Analysis of the genetic diversity of Plasmodium falciparum in Southeast Asia, including Indonesia, provides several strategic insights for national malaria eradication programs. Parasite genotype data can sharpen malaria risk stratification in Indonesia by going beyond conventional indicators such as the API. Areas with high genetic diversity and high MOIs, such as those identified in several locations in Papua, can be classified as active transmission foci and centers of genetic recombination. These areas require more intensive integrated interventions, including vector control and stricter management of cases. Conversely, areas with low allele diversity and dominated by monoclonal infections, as found in several transmission pockets, indicate a fragmented parasite

Table 5. Frequency distribution of msp-1 and msp-2 family alleles.

Author and Year	Number of Genotypes (*n*)	msp-1 Allele (K1, %)	msp-1 Allele (MAD20, %)	msp-1 Allele (RO33, %)	msp-2 Allele (FC27, %)	msp-2 Allele (3D7/IC, %)
Thailand						
Chaorattanakawee et al. [33]	471	N/A	N/A	N/A	41.2	58.8
Congpuong et al. [34]	156	51.3	41.7	27.6	50.6	80.8
Kuesap et al. [29]	135	94.0	100.0	100.0	58.0	85.0
Tanabe et al. [27]	59	10.0	65.0	25.0	N/A	N/A
Indonesia						
Arwati et al. [4]	20	49.5	27.3	0.0	27.0	33.0
Mau et al. [32]	19	15.8	N/A	N/A	21.1	N/A
Runtuboi et al. [31]	26	N/A	N/A	N/A	96.2	84.6
Tanjung et al. [28]	24	18.4	44.9	36.7	N/A	N/A
Triajayanti et al. [35]	23	N/A	100.0	N/A	N/A	N/A
Jamil et al. [13]	90	37.7	46.6	1.1	41.1	37.7
Malaysia						
Goh et al. [19]	173	19.1	53.2	38.7	N/A	N/A
Myanmar						
Soe et al. [7]	184	24.1	48.9	27.0	35.8	64.6
Thái et al. [30]	69	26.1	53.6	20.3	N/A	N/A
Zhang et al. [20]	215	44.7	53.5	12.6	80.9	75.8
Vietnam						
Long et al. [21]	166	46.0	99.0	0	10.0	97.0

Remarks: N/A: Not available; the % values presented are the result of dividing the sum of n per allele by the number of genotypes.

population and are priority targets for local elimination of malaria. This approach allows for more efficient resource allocation than does relying solely on API data [50]-[52].

The implementation of molecular surveillance in border areas, particularly in Kalimantan and Papua, can serve as an early warning system for the detection of imported parasite strains. Routine monitoring of the msp-1 and msp-2 alleles and sequencing of *P. falciparum* genome can detect the entry of new parasite strains from neighboring countries. Sudden changes in allele frequency can be an early signal of parasite population movements that could carry drug resistance or different virulence traits, enabling faster public health responses. The findings regarding high polymorphism confirm that future malaria vaccine development or adoption strategies must consider locally dominant msp allele variants in Indonesia. Vaccines developed from nonlocal reference strains (e.g., from Africa) are at risk of having suboptimal efficacy in Indonesia because of differences in antigenic composition. Therefore, these genetic diversity data provide a basis for Indonesia to contribute to the design of polyvalent vaccine clinical trials relevant to domestic parasite populations.

3.8. Clinical and Public Health Implications

The findings of this systematic review have several practical implications for public health and clinical practice in Southeast Asia. The high polymorphism of the msp-1 and msp-2 genes and geographical variation in allele distribution indicate that an effective MSP-based vaccine for this region must be polyvalent, targeting several dominant alleles or the most conserved epitopes among circulating strains. Monovalent vaccines developed from a single reference strain have the potential for suboptimal efficacy because they cannot provide broad protection against diverse variants. The varying MOI values between regions can help public health programs prioritize areas for more intensive intervention. In antimalarial drug clinical trials, msp-1 and msp-2 genotype data are the standards for distinguishing between parasite recurrence (treatment failure) and new infection. Understanding local allele diversity and the prevalence of polyclonal infections is the basis for the accurate interpretation of drug efficacy trial results. Therefore, systematic and coordinated molecular surveillance across the region is urgently needed. By monitoring shifts in allele frequencies over time, malaria control programs can detect the spread of specific parasite clones, which may be associated with increased virulence or drug resistance, particularly in border areas with high population mobility.

3.9. Implications for Vector Control

Although the msp-1 and msp-2 genes are not directly related to vector biology, the analysis of parasite genetic diversity offers complementary insights into vector control strategies [53]. High MOI levels generally indicate active and efficient malaria transmission by local mosquito populations. This information can be used to advocate for and focus on vector control efforts, such as the distribution of insecticide-treated bed nets (long-lasting insecticidal nets (LLINs)) or indoor residual spraying (IRS), in areas identified as having high transmission rates on the basis of molecular parasite data. Thus, parasite genotype data can complement entomological data for more precise transmission risk mapping in the future.

3.10. Limitations

This systematic review has several limitations, including methodological heterogeneity, where differences in laboratory techniques, such as the PCR primers used and fragment analysis methods (agarose gel electrophoresis versus capillary electrophoresis), can affect allele size estimates and MOI determination, making direct comparisons between locations was difficult. The data included were from studies conducted over different periods. Parasite population structures are dynamic and can change in response to intervention pressures, such as drug use or vector control. The combination of data from different time periods can obscure temporal trends in genetic diversity. In addition, this review was limited by the availability of data from specific geographic regions in Southeast Asia. Some countries or subnational areas may be underrepresented, making it difficult to generalize the findings to the entire population. Finally, most studies focused on describing genetic diversity and did not directly link it to clinical data (e.g., disease

Table 6. MOI of *Plasmodium falciparum*.

Author and Year	Number of Genotypes (*n*)	Polyclonal Infection (*)				Mean MOI (±SD)	
		Frequency (*n*)	msp-1 (%)	Frequency (*n*)	msp-2 (%)	msp-1	msp-2
Thailand							
Chaorattanakawee et al. [33]	471	N/A	N/A	165	35.0	N/A	1.48
Congpuong et al. [34]	156	116	74.3	132	84.6	2.15	2.93
Kuesap et al. [29]	135	21	15.5	25	18.4	1.03	1.03
Tanabe et al. [27]	59	23	39.0	N/A	N/A	5.15 (±0.19)	N/A
Indonesia							
Arwati et al. [4]	20	N/A	N/A	N/A	N/A	N/A	N/A
Mau et al. [32]	19	N/A	N/A	19	100.0	N/A	N/A
Runtuboi et al. [31]	26	N/A	N/A	22	84.6	N/A	N/A
Tanjung et al. [28]	24	16	66.7	N/A	N/A	2.04	N/A
Triajayanti et al. [35]	23	5	21.7	N/A	N/A	1.22	N/A
Jamil et al. [13]	90	13	14.4	19	21.1	2.27	2.69
Malaysia							
Goh et al. [19]	173	23	13.3	N/A	N/A	1.16	N/A
Myanmar							
Soe et al. [7]	184	97	52.7	N/A	54.4	1.70	2.05
Thái et al. [30]	69	N/A	N/A	N/A	N/A	N/A	N/A
Zhang et al. [20]	215	138	64.2	155	72.1	1.76 (±0.85)	2.21 (±1.29)
Vietnam							
Long et al. [21]	166	77	46.0	12	7.0	2.60 (±1.00)	1.10 (±0.50)

Remarks: *Values refer to the prevalence of polyclonal antibodies for specific genes (msp-1 or msp-2), combined, or varied depending on the study period; N/A: Not available.

severity) or drug resistance phenotypes.

4. CONCLUSIONS

Analysis of the genetic diversity *of P. falciparum* from 1,830 isolates from five Southeast Asian countries confirmed high polymorphism in the msp-1 and msp-2 genes, with heterogeneous geographic distribution patterns. The MAD20 allele dominated the msp-1 locus in most regions, whereas the msp-2 allele showed varying dominance between FC27 and 3D7/IC- s depending on geographic location. The multiplicity of infection correlates directly with malaria transmission intensity, ranging from 1.0 in hypoendemic areas to >2.5 in hyperendemic regions. High polymorphism requires a polyvalent approach in the development of MSP-based vaccines that include locally dominant allele variants, while MOI values are used as epidemiological indicators to evaluate the effectiveness of interventions and prioritize target elimination areas every 6–12 months. Areas with high genetic diversity require intensive control strategies, whereas areas with monoclonal infections are candidates for local elimination. Continuous molecular surveillance is necessary to monitor shifts in parasite population structure, especially in border areas with high mobility. These data provide an empirical basis for malaria control strategies tailored to the complexity of local parasite populations in Southeast Asia.

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Conflicts of Interest

The authors declare no conflict of interest.

SUPPORTING INFORMATION

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DECLARATION OF GENERATIVE AI

Not applicable.

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