Jurnal Epidemiologi Kesehatan Komunitas
8 (2), 2023, 117-130

JEKK

Recent Findings in Malaria Emergence of Piperaquine-Resistant *Plasmodium falciparum* Genes in Malaria Endemic Areas of Indonesia: a Literature Review

Ayu Nurdiantika Sari*, Tri Yunis Miko Wahyono*, Ayleen Alicia Kosasih**, Yoshida Aussiana Samosir***, Rania Rifdah Taufiq****, Inge Sutanto**.

*Department of Epidemiology. Faculty of Public Health. Universitas Indonesia, **Department of Parasitology, Faculty of Medicine, Universitas Indonesia, ***Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, ****Undergraduate Program, Faculty of Medicine Universitas Indonesia

ABSTRACT

Background: In recent years, artemisinin and piperaquine (PPQ) resistance in the Greater Mekong Sub-region alarmed Southeast Asian countries, especially those relying on artemisinin- based combinations as antimalarial drugs. This study aims to review the current status of malaria research and examine the frequencies of piperaquine resistance in *Plasmodium falciparum* isolates originating from malaria-endemic areas in Indonesia.
Methods: We undertook a review to identify empirical data on antimalarial piperaquine-inclusive artemisinin combination therapies (ACT) in Indonesia using studies conducted since 2015. Journal articles were searched using the keywords combination of malaria, piperaquine, *Pfcrt*, *Pfmdr*, *Pfpm2*, and Indonesia. The search was conducted in four databases. Trends in empirical data were summarised in a table and compared with emerging malaria prevalence and conditions in Indonesia.
Results: Our study found that dihydroartemisinin-piperaquine (DHA-PPQ) is still effective in most area. Survey of PPQ resistance in regions using DHA-PPQ as the first-line treatment heavily depends on phenotypic tests of the given drug resistance. Molecular surveys exploring polymorphisms of Pfcrt, Pfmdr1, and Pfpm2 were not found.
Conclusion: This study supports the use of dihydroartemisinin-piperaquine as the first-line antimalarial drug in malaria endemic areas of Indonesia. Further research examining efficacy is required to monitor piperaquine resistance in Indonesia.

Keywords: piperaquine; Pfmdr; Pfcrt; PfPm2; polymorphism.

**Corresponding author*, ayunurdiantika10@gmail.com

Introduction

Malaria is a vector-borne, infectious disease caused by protozoa belonging to the genus *Plasmodium* and transmitted by the Anopheles spp. mosquito. Mild symptoms include high fever, chills, headaches, nausea, vomiting, joint pain, which could lead to various complications, including anaemia, respiratory failure, seizure renal failure, coma, and even death[1]. In 2015, WHO estimated that there were 212 million cases worldwide with a death rate of 429,000[2]. Among those statistics, 90% of the reported cases were from Sub-Saharan Africa and the rest came from Southeast Asia (7%) and the Mediterranean region (2%).

Indonesia is categorised as an endemic area for malarial diseases, with over 100 million individuals at risk of being infected with malaria[3]. Moreover, WHO estimates approximately 1.6 million malaria cases and 1600 deaths all over the archipelago in 2015 alone (World Health Organization 2016). A significant amount of the given cases were caused by *P. falciparum* and *P. vivax* parasites (Elyazar et al. 2012; Elyazar et al. 2011). Dihydroartemisinin-piperaquine (DHP) as one of the artemisinin combination therapy (ACT) have been used to treat malaria falciparum and malaria vivax in Indonesia since 2006, specifically in Papua Province where chloroquine resistance is evident[4]. As a first-line treatment drug for malaria worldwide artemisinin efficacy plays a massive role. In 2008, ACT-resistant Plasmodium falciparum emerged in Greater Mekong Subregion, Southeast Asia and threatened the malaria control and prevention program[5].

In Cambodia, where the resistance first describes the efficacy of DHP for P. falciparum has dropped to less than 80%[4]. P. falciparum propeller domain of the Kelch 13 gene (PF3D7_1343700) on chromosome 13 has been identified to be associated with artemisinin resistance[6]. Amato et al. 2017[7] suggested that piperaquine resistance in Cambodia were modulated by genetic markers, including *P. falciparum multidrug resistance 1 (pfmdr1)* (PF3D7_0523000), *P. falciparum plasmepsin 2 (pfpm2)* (PF3D7_1408000), *P. falciparum exonuclease (pfexo)* (PF3D7_1362500), and *P. falciparum chloroquine resistance transporter (pfcrt)* (PF3D7_0709000), known for specific point mutation. Studies have shown the association between the value of *pfmdr1* copy number variation and piperaquine resistance. Specific point mutations in the *Pfcrt* gene are associated with PPQ resistance at mutation points T93S, H97Y, F145I, I218F, M343L, or G353V, and substitution of C350R in the *Pfcrt* gene resulted in decreased susceptibility to PPQ[8]. Dhingra et al[9]. found that the T93S and I218F-PfCRT mutations have increased in the last 5 years in Southeast Asia.

Artemisinin, which is the standard first-line antimalarial drug in Indonesia, has been used since 2008 due to its high efficacy. However, overuse of this drug has the possibility of leading to resistant strains. First signs of resistance were reported in Cambodia and Thailand in 2017, with suggestions of point mutations of the *Kelch 13*. In addition, the emergence of artemisinin- resistant parasites elevates the risk of mutations from partner drugs, including mefloquine and piperaquine. Studies have found that mutations in the *plasmepsin 2-3 gene* are associated with piperaquine resistance[10].

The objectives of this study are: (1) to conduct a review of the literature on malaria and its elimination efforts, (2) to examine current diagnostic methods for malaria, (3) to determine the polymorphism status of the *pfcrt, pfmdr1*, *Pfpm2*, and emerging of piperaquine-resistant genes associated with the P. falciparum in malaria-endemic areas of Indonesia, and (4) to gather research gaps from existing studies. Specifically, this data will inform healthcare experts and stakeholders in designing an effective antimalarial drug in the near future.

Methods

The team conducted a thorough literature search from December 11th, 2020 to January 30th, 2021. Studies were obtained using combined search terms, and relevant titles were selected from the following databases: Pubmed, Scopus, Cochrane, and Google Scholar. Some methodologies for systematic review were adopted for this study. The team used the PICO

framework to assist with database search. Journal articles were searched using the keywords ((malaria) AND (piperaquine marker)) OR (piperaquine)) AND (Pfcrt)) OR (*Pfmdr*)) OR (pfpm2)) AND (Indonesia). The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) was adopted to help identify relevant studies and display some findings. After a thorough selection through these online libraries, it was found that the literature on pfcrt, pfmdr1, and pfpm2 polymorphisms with their relation to piperaquine resistance in Indonesia was mostly published in Pubmed and Scopus.

For this study, the inclusion criteria were: (1) research journals, (2) studies conducted from 2015 until the time of search, (3) studies that examine samples from Indonesia, (4) malaria patients with P. falciparum as its causative agent, and (5) piperaquine trials or observational studies. The exclusion criteria were: (1) review journals, (2) studies conducted before 2015, (3) sample isolates not from Indonesia, (4) causative agent other than P. falciparum, and (5) malaria drugs other than piperaquine, and (6) languages other than English or Bahasa Indonesia. With these search strategy, 577 records were identified during the database search. After screening and eligibility assessment, 12 records were included in the review. The detail of the study identification strategy is shown in Figure 1.

Named authors, as two independent reviewers, extracted and analyzed relevant data. Full articles were evaluated when the abstract did not immediately provide sufficient information to match the paper with designed inclusion and exclusion criteria. Although omitted, reference lists in review articles were examined to identify any missed resources from the search engines. Collected data from initial screening were then narrowed down to 8 studies, which consisted of primary data, which had a variety of tested samples originating from malaria-endemic areas, such as West Papua. Main findings, any discrepancies, limitations and recommendations will be discussed.

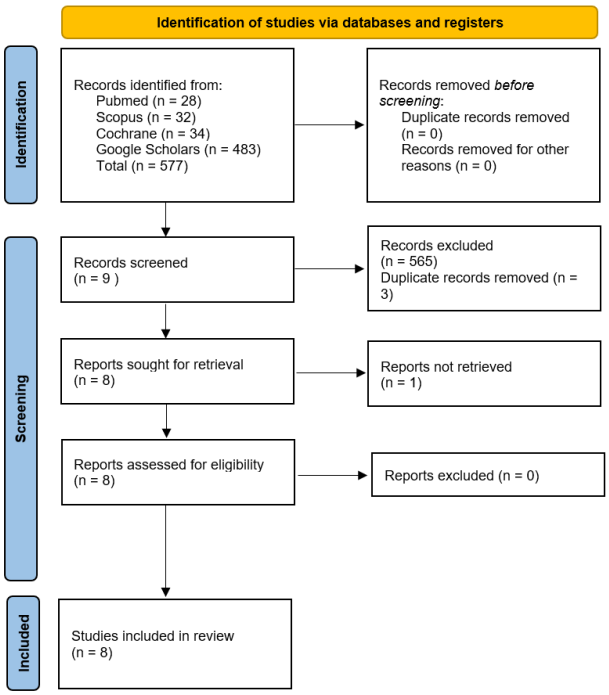

Figure 1. PRISMA Flow Diagram for Identification of Studies

Each study's performance was evaluated by the effectiveness of current ACTs, particularly DHP. Parameters looked out for in these studies were genetic mutation frequencies, copy number variants, and study recommendations, which are known to be significantly associated with piperaquine resistance. Genetic mutation frequency illustrates the proportion of mutant loci in a given population, which is generally evidence of environmental exposure to genotoxic agents [11] . Copy numbers >1.6 was determined as the cut-off value for gene amplification[12].

Result

1. Malaria and Its Elimination

a. Malaria Characteristics

There are many different types of *Plasmodium* parasites, however, only 5 species causes malaria in humans, namely *Plasmodium falciparum* (or *P. falciparum*), *Plasmodium malariae* (or *P. malariae*), *Plasmodium vivax* (or P. vivax), *Plasmodium ovale* (or *P. ovale*), and *Plasmodium knowlesi* (or *P. knowlesi*). The *P. falciparum* parasite is the most pathogenic and deadly, capable of generating severe lesions

of systemic organs (Dickson et al. 2019, Khodzhaeva et al 2019). These parasites are usually located in tropical and subtropical areas, such as Indonesia. *P. falciparum* multiplies rapidly in the bloodstream, leading to severe blood loss (anemia) and clogged smaller blood vessels. If this occurs, severe health conditions may arise, or even fatality if complications occur in the brain.

The mechanisms behind malaria comprise two cycles of human and female *Anopheles* mosquito infection. The mosquito becomes infected by biting an infected person and drawing blood containing the parasite, thereby spreading the disease by biting another person and transferring the sporozoites into the bloodstream[13]. In humans, sporozoites infect liver cells, then after a certain period of maturation, ruptures and releases merozoites. After initial replication in the liver, parasites undergo asexual multiplication in erythrocytes, then differentiating into the sexual stages, yielding gametocytes. This particular stage is responsible for clinical manifestations, and disease progression may be intensified depending on biological characteristics and behavioural traits[14]. Severe malaria is caused by excessive red blood cells rupture (leading to anemia), excessive inflammation, and accumulation of infected red blood cells in small blood vessels (leading to organ damage).

Malaria caused by *P. vivax* and *P. ovale* infections generally provokes less serious illness. Both species have several biological and morphological similarities, however *P. ovale* can infect individuals who are negative for the Duffy blood group, which is the case for many sub-Saharan Africa residents. Although known to cause less severe malaria-related complications, both species are known to persist in the human liver for many months and remain dormant (hypnozoites), and cause a relapse of symptoms months or even years later[14] .

While other *Plasmodium* species have a two-day life cycle, *P. malariae* is the only species to acquire a three-day cycle. *P. malariae* may cause a long-lasting, chronic infection if untreated, and in rare cases can last a lifetime. *P. knowlesi* infections have been rising in nearly all Southeast Asian countries even though it has previously been known to only cause simian malaria. The pathogen's natural reservoir hosts are the long-tailed and pig-tailed macaque, and banded-leaf monkey [15] . Furthermore, *P. knowlesi* has a 24-hour replication cycle which allows rapid progress to severe infections [14] .

b. Anopheline Vector Distribution and Ecology

Asia contributes to 39% of estimated global malaria clinical cases caused by *P. falciparum*, with endemic areas found in eastern India, western Myanmar and the lowlands of Papua New Guinea (Hay et al. 2009: 295). The influence of *P. vivax* is notably serious, as China, Indonesia, Pakistan, Vietnam, the Philippines, Myanmar and Thailand retrieved relatively high population at risk (PAR) estimates.

According to accumulated surveillance studies, it has been reported that *P. falciparum* and *P. vivax* were the most prevalent in Indonesia, with percentages of 55% and 44%, respectively [3,16] . Almost all malaria cases originated from eastern Indonesia, and have been the study location for a majority of malaria surveys (WHO Indonesia, 2011).

A study by Syafruddin et al. [38] discovered that the total areas at risk for *P. falciparum* (87.4% vs 90.5%) and *P. vivax* (87.6% vs 93.3%) were similar between western and eastern Indonesia. However, the mean prevalence of *P. falciparum* was higher in eastern Indonesia at 8.14% compared to 3.06% in the western region [38]. Nevertheless, Indonesia's high population density contributes to the increased risk of disease manifestation in endemic areas [3] . In addition, this vector-borne disease heavily relies on particular ecological conditions such as warm temperature, high rainfall and humidity, which enhances the survival of malaria parasites and vectors[2].

c. Malaria Elimination

Indonesia lies in the Southeastern tip of Asia, between Malaysia and Papua New Guinea, and comprises 17,504 islands and has an equatorial climate. This climate favours the survival of *Anopheles* spp. mosquitos, allowing malaria to develop rapidly thus constituting to about 9% of total malaria cases in Southeast Asia [17,18] . According to data provided by the

2015 Annual Parasite Incidence (API), malaria can be found in all Indonesian provinces across Indonesia, with several high stratifications in several regions in eastern Indonesia, such as Papua (31.93), West Papua (31.29), Maluku (5.81), North Maluku (2.77), and NTT (7.04). Medium stratifications were located in Kalimantan, Sulawesi and Sumatra. Meanwhile, in Java-Bali were classified as low stratification, although some rural areas in these locations are malaria hotspots [19] . However, changes in diagnostic testing and reporting have occurred over time, jeopardizing real burden representation in Indonesia [20] .

So far, the elimination of malaria has been a global effort to control transmission worldwide. In Indonesia, elimination strategies are carried out with the treatment of combined Artemisinin-based drugs (Ministry of Health of the Republic of Indonesia 2011), as well as insecticide-treated bed nets in endemic areas (Long-Lasting Insecticidal Net/LLIN) (Ministry of Health, Republic of Indonesia 2011). These strategies have been proven to reduce malaria transmission, [21–23] however, does not suffice for elimination [24,25] . Various studies have reported asymptomatic malaria cases may be found within a community, which has a great potential of being misdiagnosed and transmitted because treatment has not been sought after [25,26] . WHO has recommended mass screening and treatment (MST) to correctly identify asymptomatic cases [17] . which has been adopted in Indonesia since 1965 (WHO 2019). This intervention comprises mass microscopic examinations followed by treatment for a targeted community, thus reducing potential infection sources as efforts to reduce transmission. However, studies have shown that rounds of MST have little effect on the community due to scarcity of sensitive point-of-care diagnostic tools and inaccurate representations of how much reduction is needed [10] .

d. History of Antimalarial Drug and Emergence of ACT Resistance

China has initiated research programmes in the early 1970s involving extracted sweet wormwood leaves (*Artemisia annua*), which led to the discovery of artemisinin (or otherwise known as qinghaosu). Artemisinin has been reported to be effective against both asexual and sexual stages of all *Plasmodium* species, but not liver stages [27] . As time progressed, artemisinin was combined with partner drugs requiring a longer half-life. This strategy ensures complete clearance of parasites after rapid reduction by artemisinin's strong potency and prevents resistance development. Since then, artemisinin-based combination therapies (ACT) have been highly effective and had few adverse effects, and are consequently WHO's treatment recommendation for uncomplicated falciparum malaria (WHO 2010). Currently, there are five combinations used worldwide, namely artemether-lumefantrine (AL), artesunate-amodiaquine (ASAQ), dihydroartemisinin-piperaquine (DHA-PPQ), artesunate-mefloquine (AS-MQ) and artesunate-sulfadoxine-pyrimethamine (AS-SP) [28] .

The treatment efficacy of ACTs are regularly monitored and evaluated, however, efforts were not uniform in particular endemic areas, such as Indonesia. DHA-PPQ combination has shown superior efficacy and is considered to be the best ACT option because of the longer serum half-life of partner drugs, which also offers post-treatment prevention and protection (4ABC Study Group 2011) However, major DP efficacy has been reported to decline progressive in Cambodia, with a 98.2% successful rate in 2005, 89.3% in 2010, and 54% in 2013 [29,30] .

Advancements in medical technology have allowed the frequent use of molecular markers to detect and monitor antimalarial resistance in treatment outcomes. Currently, molecular markers are used to geographically map resistant parasites in endemic areas to suggest better control and elimination strategies, in particular, Indonesia with a high malaria burden [27] . Several studies have been conducted in South East Asia to collect evidence of mutation in the *pfk13*-propeller domains in western Indonesia, in which some genetic polymorphisms have been found. In the early months of 2008, the C580Y mutation had been reported to be associated with reduced drug susceptibility to artemisinin in the Greater Mekong subregion [31] .

As time progressed, research on antimalarial drug resistance has increased. The main findings were point mutations in the genome of *P. falciparum*, which has led to modifications in protein production essential to parasite survival. Study parameters include identification of genetic polymorphisms, mutant alleles, copy number, and genetic mutation frequency. Whilst waiting for the development of effective vaccines and newer licensed drugs, ACT administration is critical to prevent rising failures in malaria treatment. The current antimalarial regimen in Indonesia requires early detection of resistance to correctly identify the spread of resistant parasites.

2. Emergence of Piperaquine-resistant P. falciparum Malaria in Indonesia

Table 1. Ongoing research projects in Indonesia with aims of genetic mutation surveillance in current ACTs used as first-line anti-malarial treatment

No.	Research Title/Aim	Region	Initiation Year
1.	Small Sub. Unit Ribosomal RNA (ssurRNA) Identification in Zoonotic Malaria in Humans and Primates	Jakarta	2015
2.	Genetic Diversity in P. Falciparum, P. vivax and Immune Responses.	Sumba	2015
3.	Genetic Variations of P.falciparum Chlorine Resistance Transporter (*pfrct*)	South Sumatera	2015
4.	Effectivity of Dihydroartemisinin Piperaquine (DHP) in uncomplicated P.falciparum and P. vivax malaria	Central Sulawesi	2016
5.	Development of Malaria Parasite Detection (Culture, Microscopy, PCR)	Jabodetabek	2016
6.	DHA (Dihidroartemisinin) Drug Screening – 6th Trial	Jakarta, West and Central Java	2019

Table 2. Overview of polymorphisms of the *pfcrt*, *pfmdr1*, and *prpm2* genes in the emergence of piperaquine-resistant *P. falciparum* malaria in endemic areas of Indonesia (2004-2016).

Researcher	Origin	Sample	Study Design	Time	Genetic mutation frequency ?	Efficacy Study?	Copy number ?
Rukhsana Ahmed, et al [34]	Sumba and Papua	2279	Cluster-randomised	2013-2016	No	No	No
Sikora SA, et. al [43]	Timika, Papua	10.514	Retrospective study	2004-2013	No	No	No

Ric Price, et al [4]	Timika, Papua	129	Cross-sectional study	2015-2016	No	Yes	Yes
Inke Lubis, et al [44]	North Sumatera	3731	Randomised trial	2015	No	Yes	No
Inke Lubis, et al [41]	North Sumatera	3731	Cross-sectional	2015	Pfmdr1: Variant 86Y (Tyr), 86F (Phe) and 86S (Ser).	No	No
Suwandi et al [42]	Pesawaran District, Lampung	62	Cross-sectional	2012-2013	SNP PfMDR1 N86Y	No	No
Sutanto et al [10]	West Timor	2231	Cluster-randomized trial	2013	No	No	No
Irawati et al [45]	Peasawaran Lampung	52	Cross-sectional	2016	No	No	No

Table 3. Key findings from studies and proposed research gaps

Researcher	**Key findings**	**Research gaps**
Rukhsana Ahmed, et al [46]	Study only surveyed malaria infections in the given population (pregnant women), but did not proceed to examine genetic mutations.	Combination RDTs are sufficient to screen malaria in pregnant cases in rural Indonesia. However, clinical relevance regarding the ability of PCR to detect low-density malaria infections, but undetected by RDTs or microscopy should be evaluated in the future.
Sikora SA, et. al [43]	Intravenous artesunate and DHP reduced hospital stay and recurrence risks.	Other parameters of treatment effectiveness, such as parasite clearance time, were not determined in this study. Additionally, degree of severe manifestations could not be ascertained in this study, and cases with co-morbidities were excluded in this analysis.

Ric Price, et al [4]	In absence of artemisinin resistance, piperaquine has retained high efficacy.	Despite excellent efficacy results, declining efficacy is expected due to the rising mobile population, which affects the distribution and consumption of malaria drug, as well as the potential spread of resistant parasites.
Inke Lubis, et al [44]	Parameter: effectively cleared initial parasitemia. Findings: some parasites persisted in some individuals.	While most treatment successfully cleared initial parasitemia, some *P. falciparum* and *P.malariae* parasites persisted in some individuals. Future deployment of molecular detection should be considered in antimalarial efficacy trials in Indonesia.
Inke Lubis, et al [41]	Findings: some persistent sub-microscopic infection in some patients after drug consumption.	DHA-PPQ is an effective drug of choice for P. falciparum infection in northern Sumatra. However, studies on long term efficacy are still needed in some endemic area in Indonesia.
Suwandi et al [42]	The genetic variation found was the SNP on the N86Y codon of *Pfmdr1* gene, with dominant allele MAD20 and 3D7	DHA-PPQ is quite effective in treating malaria in the Lampung province. The genetic variation is due to transmission rate, transmigration, vector population, environmental condition, human host, and patterns of parasite susceptibility in an area. Studies on said factors are needed to draw a correlation to a genetic mutation.
Sutanto et al [10]	Maximized the use of mass screening and treatment (MST) in areas of low-level dominant Plasmodium species transmission.	Presumptive radical cure of the school children cohorts—to eliminate subpatent malaria infections—did not occur hence further studies on this population group is needed.
Irawati et al [45]	Findings: Falciparum malaria resistance to artemisinin	Treatment failure in this study was very low, indicating P. falciparum resistance to artemisinin is not yet proven. ACT, specifically DHA-PPQ, is still effective as P. falciparum antimalarial treatment. Further monitoring on resistance is needed.

Discussion

1. Emergence of Piperaquine-resistant P. falciparum Malaria in Indonesia

Recent studies have reported declines in efficacy of the Artemisinin combination therapy (ACT) as the first-line of treatment for uncomplicated Plasmodium falciparum malaria in Southeast Asian regions. In particular, treatment failure rates associated with artemisinin and piperaquine resistance have been prominent in Cambodia and Vietnam [34]. Additionally, in-vitro resistance to piperaquine increased rapidly since 2013 and 2015 in the given regions. Genetic mutations in the propeller domain of the kelch gene chromosome 13 (Kelch13), Plasmodium falciparum plasmepsin II (Pfpm2), Pfmdr1, and Pfcrt gene were reported to be associated with piperaquine resistance [34-35]. Duplications in the pfpm2 gene encode a protease involved in hemoglobin degradation, which has elevated susceptibility to DHP combinations in ACTs. Parasitic treatment using piperaquine causes several negative effects such as the reduction of ribosomes, swelling of the food vacuole, undigested hemoglobin vesicles, reduced hemozoin crystals, and the presence of free heme accumulation (Syamsudin, 2006). Although free heme is toxic to parasites, inhibition degradation of hemoglobin will cause parasites to starve, which consequently increases plasmepsin II-III molecules. These products aid amino acid production in parasites when piperaquine inhibits hemoglobin degradation [36].

The main genetic markers associated with piperaquine resistance were found at two copy number variations (CNV), namely, resistance associated with decreased CNV Plasmodium falciparum multidrug resistance 1 (pfmdr1) and resistance associated with increased plasmepsin II-III copy number. A study by Bopp et al. 2018 [36] showed that mutations in Cambodian isolates associated with the genetic marker Pfk13 in relation to PPQ resistance were located in codons C580Y, E270K, R539T, I543T, D584V, and H719N. However, mutations at the Y493H codon were exclusive to piperaquine resistance. In this case, CNV values and point mutations of the reported genetic markers (pfpm II-III and kelch13 gene) play an important role in the emergence of piperaquine resistance [36]. Eastman et al. 2011 [37] reported that Pfcrt mutations were also associated with piperaquine resistance, however, further research is needed.

Previous surveillance studies conducted in the west and east Indonesia identified the wide distribution of mutant alleles of *pfcrt* and *pfmdr1* genes, with a high prevalence of K76T mutation of *pfcrt* but more heterogeneity in the proportion of 86Y, 1034C, 1042D, and 1246Y mutant alleles of *pfmdr1* [29,38–40]. In addition, *The* and *Ala* mutations at codon 474 were observed in some individuals in a surveillance study conducted in west Indonesia (Lubis 2018) [41]. However, results show that DP was highly effective for *P.falciparum*malaria, irrespective of the *pfk13* genotypes, although further studies were suggested to explore phenotypic impacts of *pfk13* mutant alleles and monitoring of drug efficacy against all parasitical species. Resistance to other types of ACTs was not discussed in this study.

ACT has a long history of usage since it was appointed as the standard drug to treat malaria by the Indonesian Ministry of Health in 2004. According to WHO criteria, antimalarial drugs are considered ineffective and no longer recommended if the treatment failure rate is over 10% [17]. As seen in Table 1 and 2, our study included ACT efficacy parameters in endemic areas examined.

Single Nucleotide Polymorphism (SNP) variant 86 Y were commonly found in the *Pfmdr1* gene [41,42]. According to Suwandi et al (2019) [42], reduced effectiveness of AAQ-PQ may be related to polymorphism in the codon N86Y PfMDR1 gene, however, further investigation is required. Polymorphism in the N86Y codon lowers amodiaquine effectiveness in eliminating parasites. Taking into consideration all eight research papers analysed, there was minimal information about single ACT resistance, namely piperaquine. Nonetheless, it was implied in a DHP efficacy study for uncomplicated *P. falciparum* and *P. vivax* malaria in Timika, Papua by Poespoprodjo et al [4] (2018) that in the absence of artemisinin resistance, piperaquine has retained high efficacy.

Overall, study results relating to piperaquine resistance in P. falciparum malaria across

endemic areas in Indonesia in 2004-2016 are compared in Table 2. From the 8 studies analysed, most studies were conducted in Papua, Lampung, North Sumatra, and Timika. The given implication is that more research was carried out in malaria-endemic areas due to higher incidence. Subjects recruited for each study varied greatly, yielding a range of 52-10,514 samples. Moreover, several research designs were utilised, but a majority adopted cross-sectional studies and cluster randomized trials. Research with cross-sectional approaches found that DHP is still effective as the first line of malaria treatment in Indonesia.

2. Past and Future Studies

Resistance reduces anti-malarial drug efficacy, both commercialized and drugs in development. In order to maintain efficacy, antimalarial drugs must be routinely monitored using sensitive molecular methods, as drug resistance has been reported in several renowned anti-malarial drugs across the globe. Efficacy screening and evaluation may be conducted in several ways, such as clinical testing antimalarial agents, calculating sensitivity values of culture parasites *in vitro* or *ex vivo*, identifying genetic polymorphisms related to resistance, and estimating the specificity value of antimalarial drug therapy in subsequent infections [47]. Resistance may be caused by several factors, such as operational, pharmacological, and transmission factors (ie. light intensity, selective pressure by drugs, and host immune response) [48]. Studies that identify and evaluate these factors can help sustain the spread. P. falciparum parasites which cause drug resistance [48]. It should be noted that drug resistance is not entirely related to treatment failure, because treatment failure is caused by many factors, including host immune response to the given drugs. Resistance in particular, tends to be caused by the adaptation of parasites to the designed drug pressure. A study by Klein et al. [49] suggests that the longer the parasites reside in areas exposed to antimalarial drugs, the greater the risk of parasites developing resistance to the drugs [49].

It is likely that the emergence of artemisinin resistance in other malaria-endemic areas, such as Cambodia, were driven by self-treatment by private health facilities, and over-distribution of antimalarial agents. Meanwhile, DHP procurement is highly regulated by the Indonesian Ministry of Health, evident by the current policies of providing malaria treatment at government health facilities and selected private health sites. These selected health centres are also equipped with sufficient laboratory equipment to accurately diagnose malaria by microscopy and RDTs, providing confidence in final diagnoses. Hence, DHP is generally only prescribed to those with confirmed malaria and this degree of control may have contributed to the sustained efficacy of the drugs in the regions mentioned in the studies.

According to Murhandarwati [50], malaria cases in endemic areas in Indonesia showed concentrated occurrences in specific regional areas. A notable challenge was disintegrated coordination between referral systems for malaria surveillance and major constraints for the sustainability of the program [50]. With emerging genetic variance that may jeopardize ACT effectivity, a renewed surveillance approach should be considered, with changes including technical implementation of intervention strategies recommended from literature. Study gaps in molecular mechanisms of genetic variance in ACTs should be closely monitored and funded according to the significance of the research value yielded. Diagnostic methods should integrate a combination of automated molecular and culture methods, to ensure high sensitivity and specificity, without sacrificing highly skilled technicians and medical workers that should be focused on patient care. This approach would more likely fit the latest local disease transmission, intervention, and political system.

In 2019, the Joint Malaria Programme Review has suggested many recommendations after conducting a national survey on the Indonesian malaria control programme. A major portion of these recommendations were highly focused on effective diagnosis capacity, including conducting routine RDT cross-checking with microscopy, ensuring quality

assurance procedures for all ACTs, and monitoring insecticide resistance and supply in vulnerable areas [51] . According to the National Institute of Health Research and Development, current strategies include nationwide availability of diagnosis and treatment, as well as the provision of RDTs and malaria microscopy.

These strategies can be measured by the current research projects that are currently in progress under the supervision of leading public health experts (Table 1). The output of research is the identified genetic variations of pathogenic resistance to current ACTs offered as first-line anti-malarial treatment. All research aims are central to strengthen current diagnosis strategies to accurately illustrate the latest malaria status in Indonesia, which can aid in informing health policies and reduce infection prevalence and malaria morbidity and mortality.

Study Limitations

As this study utilises a literature review method, included studies may consist of incorrect results that have a high risk of misleading reviews. Although efforts have been made to minimise risk, authors may overestimate or underestimate certain studies without objectively examining the content or each study's relevance and value. In this case, there is a possibility of discrepancies with surveillance data from other prominent endemic countries, such as Cambodia, due to scarce amounts of genetic mutation surveillance in Indonesia. Therefore, literature sources were limited, which may affect bias.

Recommendations

Authors have found many polymorphisms for the *pfmdr1* gene from published data from 2020. However, polymorphisms for other genes such as *Pfcrt* and *pfpm2* remain unexplored during the timeframe given in this study. Authors recommend that more research should be conducted in the surveillance of genetic mutation points and frequencies in current antimalarial drugs to inform future policies and efforts in malaria recovery development. A national target has been set, whereby 2030 it is expected that all regions in Indonesia will have reached significant progress in malaria elimination. Current strategies and resources should be maximised with all possible measures to achieve this goal.

Conclusions

Cross-sectional studies included in this review found that DHP is still effective as the first line of malaria treatment in Indonesia. Hence, this study supports the use of dihydroartemisinin-piperaquine as the first-line antimalarial drug in malaria endemic areas of Indonesia. Further research examining efficacy is required to monitor piperaquine resistance in Indonesia.

Acknowledgement

The authors would like to thank Universitas Indonesia for funding this research through PUTI Grant with contract number NKB-5/UN2.RST/HKP.05.00/2020

References

1. Tuteja R. 2007., Malaria - An overview. *FEBS J.* 274(18):4670–9.

2. World Health Organization. 2016. World malaria report 2016.

3. Elyazar IRF, Gething PW, Patil AP, Rogayah H, Kusriastuti R, Wismarini DM, et al. 2011. Plasmodium falciparum malaria endemicity in indonesia in 2010. *PLoS One*.6(6).

4. Poespoprodjo JR, Kenangalem E, Wafom J, Chandrawati F, Puspitasari AM, Ley B, et al. 2018. Therapeutic response to dihydroartemisinin-piperaquine for P. falciparum and P. Vivax nine years after its introduction in southern Papua, Indonesia. *Am J Trop Med Hyg*. 98(3):677–82.

5. Talundzic E, Chenet SM, Goldman IF, Patel DS, Nelson JA, Plucinski MM, et al. 2015. Genetic analysis and species specific amplification of the artemisinin resistance-associated kelch propeller domain in P. falciparum and P. vivax. *PLoS One*. 10(8):1–11.

6. Popovici J, Kao S, Eal L, Bin S, Kim S, Ménard D. 2015. Reduced polymorphism in the kelch propeller domain in plasmodium vivax isolates from Cambodia. Antimi*crob Agents Chemother*. 59(1):730–3.

7. Amato R, Lim P, Miotto O, Amaratunga C, Dek D, Pearson RD, et al. 2017. Genetic markers associated with dihydroartemisinin–piperaquine failure in Plasmodium falciparum malaria in Cambodia: a genotype–phenotype association study. *Lancet Infect Dis.* 17(2):164–73.

8. Pelleau S, Moss EL, Dhingra SK, Volney B, Casteras J, Gabryszewski SJ, et al. 2015. Adaptive evolution of malaria parasites in French Guiana: Reversal of chloroquine resistance by acquisition of a mutation in pfcrt. *Proc Natl Acad Sci U S A*. 112(37):11672–7.

9. Satish K Dhingra, Jennifer L Small-Saunders, Didier Ménard DAF. 2019. Plasmodium falciparum resistance to piperaquine driven by PfCRT Satish. *Physiol Behav*. 176(1):139–48.

10. Sutanto I, Kosasih A, Elyazar IRF, Simanjuntak DR, Larasati TA, Dahlan MS, et al. 2018. Negligible impact of mass screening and treatment on mesoendemic malaria transmission at west timor in eastern Indonesia: A cluster-randomized trial. *Clin Infect Dis*. 67(9):1364–72.

11. Green MHL, O'Neill JP, Cole J. 1995. Suggestions concerning the relationship between mutant frequency and mutation rate at the hprt locus in human peripheral T-lymphocytes. *Mutat Res Mutagen Relat Subj*. 334(3):323–39.

12. Duru V, Khim N, Leang R, Kim S, Domergue A, Kloeung N, et al. 2015. Plasmodium falciparum dihydroartemisinin- piperaquine failures in Cambodia are associated with mutant K13 parasites presenting high survival rates in novel piperaquine in vitro assays : retrospective and prospective investigations. *BMC Med* [Internet]. 1–11.

13. Arrow KJ, Panosian C GH. 2004. Saving Lives, Buying Time. Saving Lives, Buying Time.

14. Prevention C for DC and Malaria. 2021;1–2. Available from: https://www.cdc.gov/malaria/about/biology/index.html

15. Amir A, Cheong FW, De Silva JR, Lau YL. 2018. Diagnostic tools in childhood malaria. Parasites and Vectors. 11(1):1–12.

16. Sulistyaningsih E, Loeki EA, Loscher T B-RN. 2008. Plasmodium knowlesi: The fifth human malaria parasite. *Clin Infect Dis* [Internet]. 46(2):172–3.

17. World Health Organization, Indonesia M of H. 2011. National malaria control programme review: Republic of Indonesia. *Handb Falciparum Malar*ia. 200–200.

18. Dale P, Sipe N, Anto S, Hutajulu B, Ndoen E, Papayungan M, et al. 2005. Malaria in Indonesia: A summary of recent research into its environmental relationships. *Southeast Asian J Trop Med Public Health.* 36(1):1–13.

19. Kementrian Kesehatan Republik Indonesia. Info Datin. 2016. p. 1–8.

20. Kemenkes RI. 2013. Profile Kesehatan Indonesia. Ministry of Health Indonesia. 107–108 p.

21. Aregawi M, Malm KL, Wahjib M, Kofi O, Allotey NK, Yaw PN, et al. 2017. Effect of anti-malarial interventions on trends of malaria cases, hospital admissions and deaths, 2005-2015, Ghana. *Malar J*. 16(1).

22. Hung LQ, De Vries PJ, Giao PT, Nam N V., Binh TQ, Chong MT, et al. 2002. Control of malaria: A successful experience from Viet Nam. *Bull World Health Organ.* 80(8):660–6.

23. Heng S, Durnez L, Mao S, Siv S, Tho S, Mean V, et al. 2017. Passive case detection of malaria in Ratanakiri Province (Cambodia) to detect villages at higher risk for malaria. *Malar J*. 16(1):1–12.

24. Moonen B, Cohen JM, Snow RW, Slutsker L, Drakeley C, Smith DL, et al. 2010. Operational strategies to achieve and maintain malaria elimination. *Lancet*. 376(9752):1592–603.

25. Cotter C, Sturrock HJW, Hsiang MS, Liu J, Phillips AA, Hwang J, et al. 2013. The changing epidemiology of malaria elimination: New strategies for new challenges. *Lancet* [Internet]. 382(9895):900–11.

26. Waltmann A, Darcy AW, Harris I, Koepfli C, Lodo J, Vahi V, et al. 2015. High Rates of Asymptomatic, Sub-microscopic Plasmodium vivax Infection and Disappearing Plasmodium falciparum Malaria in an Area of Low Transmission in Solomon Islands. *PLoS Negl Trop Dis*. 9(5):1–18.

27. White NJ. 2008. Qinghaosu (artemisinin): The price of success. Science (80). 320(5874):330–4.

28. Fairhurst RM, Nayyar GML, Breman JG, Hallett R, Vennerstrom JL, Duong S, et al. 2012. Artemisinin-resistant malaria: Research challenges, opportunities, and public health implications. *Am J Trop Med Hyg*. 87(2):231–41.

29. Song J, Socheat D, Tan B, Seila S, Xu Y, Ou F, et al. 2011. Randomized trials of artemisinin-piperaquine, dihydroartemisinin-piperaquine phosphate and artemether-lumefantrine for the treatment of multi-drug resistant falciparum malaria in Cambodia-Thailand border area. *Malar J* [Internet]. 10(1):231.

30. Leang R, Barrette A, Bouth DM, Menard D, Abdur R, Duong S, et al. 2013. Efficacy of dihydroartemisinin-piperaquine for treatment of uncomplicated plasmodium falciparum and plasmodium vivax in Cambodia, 2008 to 2010. *Antimicrob Agents Chemother*. 57(2):818–26.

31. Ocan M, Akena D, Nsobya S, Kamya MR, Senono R, Kinengyere AA, et al. 2019. K13-propeller gene polymorphisms in Plasmodium falciparum parasite population in malaria affected countries: A systematic review of prevalence and risk factors. *Malar J* [Internet]. 18(1):1–17.

32. Mbanefo A, Kumar N. 2020 Evaluation of malaria diagnostic methods as a key for successful control and elimination programs. *Trop Med Infect Dis*. 5(2).

33. Wilson ML. 2012. Malaria rapid diagnostic tests. *Clin Infect Dis*. 54(11):1637–41.

34. Leroy D, Macintyre F, Adoke Y, Ouoba S, Barry A, Mombo-Ngoma G, et al. 2019. African isolates show a high proportion of multiple copies of the Plasmodium falciparum plasmepsin-2 gene, a piperaquine resistance marker. *Malar J* [Internet]. 18(1):1–11.

35. Gupta H, Galatas B, Chidimatembue A, Huijben S, Cisteró P, Matambisso G, et al. 2020. Effect of mass dihydroartemisinin–piperaquine administration in southern Mozambique on the carriage of molecular markers of antimalarial resistance. *PLoS One*. 15(10 October):1–14.

36. Bopp S, Magistrado P, Wong W, Schaffner SF, Mukherjee A, Lim P, et al. 2018. Plasmepsin II-III copy number accounts for bimodal piperaquine resistance among Cambodian Plasmodium falciparum. *Nat Commun*. 9(1).

37. Eastman RT, Dharia N V., Winzeler EA, Fidock DA. 2011. Piperaquine resistance is associated with a copy number variation on chromosome 5 in drug-pressured Plasmodium falciparum parasites. *Antimicrob Agents Chemother*. 55(8):3908–16.

38. Syafruddin D, Asih PBS, Siregar JE, Tjitra

E. 2003. Molecular basis of antimalarial drug resistance in Indonesia. *Adv Exp Med Biol*. 531:103–15.

39. Huaman MC, Yoshinaga K, Suryanatha A, Suarsana N, Kanbara H. 2004. Short report: Polymorphisms in the chloroquine resistance transporter gene in Plasmodium falciparum isolates from Lombok, Indonesia. *Am J Trop Med Hyg*. 71(1):40–2.

40. Asih PBS, Dewi RM, Tuti S, Sadikin M, Sumarto W, Sinaga B, et al. 2009. Efficacy of artemisinin-based combination therapy for treatment of persons with uncomplicated Plasmodium falciparum malaria in West Sumba District, East Nusa Tenggara Province, Indonesia, and genotypic profiles of the parasite. *Am J Trop Med Hyg*. 80(6):914–8.

41. Lubis IND, Wijaya H, Lubis M, Lubis CP, Beshir KB. 2020. Plasmodium falciparum Isolates Carrying pfk13 Polymorphisms Harbor the SVMNT Allele of pfcrt in Northwestern Indonesia. 1–11.

42. Suwandi JF, Asmara W, Kusnanto H, Syafruddin D, Supargiyono S. 2019. Efficacy of artemisinin base combination therapy and genetic diversity of plasmodium falciparum from uncomplicated malaria falciparum patient in district of pesawaran, Province of Lampung, Indonesia. *Iran J Parasitol*. 14(1):143–50.

43. Sikora SA, Poespoprodjo JR, Kenangalem E, Lampah DA, Sugiarto P, Laksono IS, et al. 2019. Intravenous artesunate plus oral dihydroartemisinin-piperaquine or intravenous quinine plus oral quinine for optimum treatment of severe malaria: Lesson learnt from a field hospital in Timika, Papua, Indonesia. *Malar J* [Internet]. 18(1):1–12.

44. Lubis IND, Wijaya H, Lubis M, Lubis CP, Beshir KB, Staedke SG, et al. 2020. Recurrence of Plasmodium malariae and P. falciparum following treatment of uncomplicated malaria in North Sumatera with dihydroartemisinin-piperaquine or artemether-lumefantrine. In: *Open Forum Infectious Diseases*. Oxford University Press US. p. ofaa116.

45. Irawati N, Kurniawan B, Suwandi JF, Hasmiwati H, Tjong DH, Kenedi M. 2016. Determination of the falciparum malaria resistance to artemisinin-based combination therapies in Pesawaran, Lampung, Indonesia. *Asian J Epidemiol*. 10(1):19–25.

46. Ahmed R, Poespoprodjo JR, Syafruddin D, Khairallah C, Pace C, Lukito T, et al. 2019. Efficacy and safety of intermittent preventive treatment and intermittent screening and treatment versus single screening and treatment with dihydroartemisinin–piperaquine for the control of malaria in pregnancy in Indonesia: a cluster-randomised, open-la. *Lancet Infect Dis*. 19(9):973–87.

47. Meshnick S. 2012. Perspective: Artemisinin-resistant malaria and the wolf. *Am J Trop Med Hyg*. 87(5):783–4.

48. Simamora D, Fitri LE. 2007. Antimalarial Drug Resistance : Mechanism and the Role of Drug. Kedokt Brawijaya. 23(2):82–92.

49. Klein EY. 2013. Antimalarial drug resistance: a review of the biology and spread. *Int J Antimicrob Agents*. 41(4):311–7.

50. Murhandarwati EEH, Fuad A, Sulistyawati, Wijayanti MA, Bia MB, Widartono BS, et al. 2015. Change of strategy is required for malaria elimination: A case study in Purworejo District, Central Java Province, Indonesia. *Malar J*. 14(1):1–14.

51. WHO. 2019. Joint Malaria Programme Review reveals Indonesia is on track for malaria elimination. *World Health Organization*.