

PAPER NAME

Sinta 4 Pfcrt gene polymorphisms in Plasmodium falciparum isolates from malaria patients in malaria

WORD COUNT

2631 Words

CHARACTER COUNT

14245 Characters

PAGE COUNT

7 Pages

FILE SIZE

947.0KB

SUBMISSION DATE

Mar 31, 2023 1:40 PM GMT+7

REPORT DATE

Mar 31, 2023 1:40 PM GMT+7

● **8% Overall Similarity**

The combined total of all matches, including overlapping sources, for each database.

- 8% Internet database
- 0% Publications database
- Crossref database
- Crossref Posted Content database
- 0% Submitted Works database

● **Excluded from Similarity Report**

- Bibliographic material
- Quoted material
- Cited material
- Small Matches (Less than 10 words)
- Manually excluded text blocks

PfCRT GENE POLYMORPHISMS IN PLASMODIUM FALCIPARUM ISOLATES FROM MALARIA PATIENTS IN MALARIA ENDEMIC AREAS USING ACT AS STANDARD THERAPY

¹Jhons Fatriyadi Suwandi, ¹Betta Kurniawan, ¹Hanna Mutiara, ^{2,3,4}Aila Karyus

¹Department of Parasitology, Faculty of Medicine, University of Lampung, Bandar Lampung

²Department of Public Health dan Community Medicine, Faculty of Medicine, University of Lampung, Bandar Lampung

³Program Study of Public Health, Mitra Indonesia University, Bandar Lampung

⁴Health Office of Pesawaran District, Gedong Tataan

Email : jhons.fatriyadi@fk.unila.ac.id

ABSTRACT

The pfcr gene is a biomarker to determine the resistance of *Plasmodium falciparum* to chloroquine and amodiaquine. When chloroquine was switched to ACT, it is very likely that there will be an increase in wild type strains (pfcr K76) due to the absence of exposure to chloroquine. Currently chloroquine has not used for malaria treatment. The aims of this study were to identify polymorphisms in the pfcr gene and phylogenetic analysis of *Plasmodium falciparum* isolates from malaria patients in Pesawaran Regency, Lampung Province. This research is a laboratory research by analyzing blood samples which are stored biological materials (BBT). DNA isolation was carried out using a DNA isolation kit QIAmp DNA Mini kit from Qiagen, followed by amplification using an appropriate primer. The amplification results were followed by sequencing and then analyzed using Mega 10. The results showed that all samples had mutations in codons 76 (K76T) and 72 (C72S) of the pfcr gene. Pesawaran isolates were closely related to other isolates. The conclusion of this study is that polymorphisms were found in codons 72 and 76 of the PfCRT gene, although chloroquine has long been abandoned as an antimalarial. The sequenced pesawaran isolates were also related to other isolates.

Keywords: *pfcr, Plasmodium falciparum, chloroquine, resistans*

1. INTRODUCTION

Falciparum malaria infection is still an important topic to be discussed, with a high incidence in the world^{1,2} and also in Indonesia.³ Lampung Province is one of the provinces with malaria endemic areas. In general, the Annual Parasite Incidence (API) value tends to decrease from 2007 to 2019.^{3,4} Pesawaran Regency is the location of the highest malaria endemic in Lampung Province.⁴

One of the most important aspects in controlling malaria is resistance to antimalarials.⁵ This resistance is closely related to mutations in certain genes such as pfcr, pfmdr, pfk13, pfdhfr and other genes.^{6,7}

Plasmodium falciparum chloroquine resistance transporter (pfcr) gene is a biomarker to determine the resistance of *Plasmodium falciparum* to chloroquine and amodiaquine. Another biomarker to detect resistance to chloroquine, amodiaquine, mefloquine, halofantrine and quinine is the *Plasmodium falciparum* multi-drug resistance 1 (pfmdr1) gene. In previous studies, mutations in these genes have been reported from Lampung isolate samples.⁶⁻⁹ Since resistance to chloroquine was reported, the standard of treatment was changed to use artemisinin-based combination therapy (ACT) in accordance with the 2008 treatment guidelines.¹⁰

Mutations in the *pfcrt* and *pfmdr1* genes will reduce the parasite's susceptibility to chloroquine, even some studies have stated that these genes are biomarkers to detect *Plasmodium* resistance to chloroquine.^{11–13} However, it was also reported that a mutation in the *pfmdr1* gene codon 86 was associated with an increase in the effectiveness of artemisinin and its derivatives.⁶ Research in Kenya showed that there was an increase in the wild-type strain (*pfcrt* K76) when chloroquine was substituted for artemisinin. But not in the *pfmdr1* gene, where no increase in the wild-type strain was found.¹⁴

Currently, the standard treatment for malaria in Indonesia is using ACT therapy, including in Pesawaran District.¹⁵ In the past 10 years, chloroquine has not been used as antimalarial therapy at all in health care centers. Based on this, it is very possible that the wild type will appear in Pesawaran Regency. The aims of this study were to identify polymorphisms in the *pfcrt* gene and phylogenetic analysis of *Plasmodium falciparum* isolates from malaria patients in Pesawaran Regency, Lampung Province.

2. METHOD

The design of this research is descriptive analytic research. The research was carried out at the Laboratory of Microbiology and Parasitology, Faculty of Medicine, University of Lampung by analyzing blood samples from stored biological materials (BBT). The population in this study were all malaria patients who were treated at the Hanura Health Center in 2016. The sample of this study was stored blood samples, which were taken from malaria patients from a previous study (in 2016) at Hanura Health Center, Pesawaran Regency, Lampung Province based on inclusion criteria and exclusion. The number of samples stored is 16 samples. Inclusion criteria were uncomplicated malaria infection by *Plasmodium falciparum* (single infection with

falciparum malaria) based on microscopic examination; and samples from malaria patients who received ACT therapy. Exclusion criteria were insufficient sample volume for DNA isolation.

DNA isolation was carried out using the QIAamp DNA Mini Kit DNA isolation kit from Qiagen. The isolated DNA was amplified using appropriate forward and reverse primers (Table 1). The PCR conditions were determined by referring to the forward and reverse primer melting temperatures, which were then optimized according to the appropriate temperature. The amplification results were examined by electrophoresis on 2% agarose gel in TBE buffer (Tris Borate 0.045M; ethylene diamine tetra acetic acid 0.001 M pH 8.0) containing 1 µg/ml ethidium bromide.^{7,8,16–19}

The amplified DNA was analyzed by sequencing. The sequencing process for these genes used the appropriate primers (Table 1). The analysis of the sequencing results was carried out in stages, namely sequence editing, reverse complement on the reverse primary sequencing results.

The next stage is to do the alignment with the reverse primer. The last step is multiple alignment to compare the sample sequence with the sequence from the gene bank. If there is a change in the nucleotide base, it indicates a polymorphism in the gene. A change in one nucleotide base, or the loss of a nucleotide base or a nucleotide base substitution, will cause a sequence change, so that amino acids can also change. After the multiple alignment process was completed, a phylogenetic analysis was carried out to determine the relationship with various other isolates. The analysis of the sequen in this study used the Mega 10 computer program and various electroferogram graphic visualization programs.

Table 1. Forward dan reverse primers

Reaction	Primers	Sequent
1	Pfcrf F 1	CCGTTAATAATAAATACACGCAG
	Pfcrf R 1	CGGATGTTACAAAACCTATAGTTACC
2	Pfcrf F 2	AGGTTCTTGTCTTGGTAAATTTGC
	Pfcrf R 2	CAAAAACCTATAGTTACCAATTTTG

3. RESULT

The process of amplification and sequencing on 16 blood samples from patients with falciparum malaria in Pesawaran Regency, Lampung Province has been successfully carried out. Multiple alignments of the pfcrf gene along 546 bp of a total of 1275 bp have been carried out to determine polymorphisms and predict amino acid changes. The results of the analysis using the Mega 10 program on this

gene showed that there was a change in the nucleotide base number 225 from the base Thymine (T) to Adenine (A) (Figure 1) in all samples analyzed. Nucleotide base number 225 is one of the nucleotide bases for codon 76. The change in this nucleotide base will cause the amino acid Lysine (K) to change to the amino acid Threonine (T) (Figure 2).

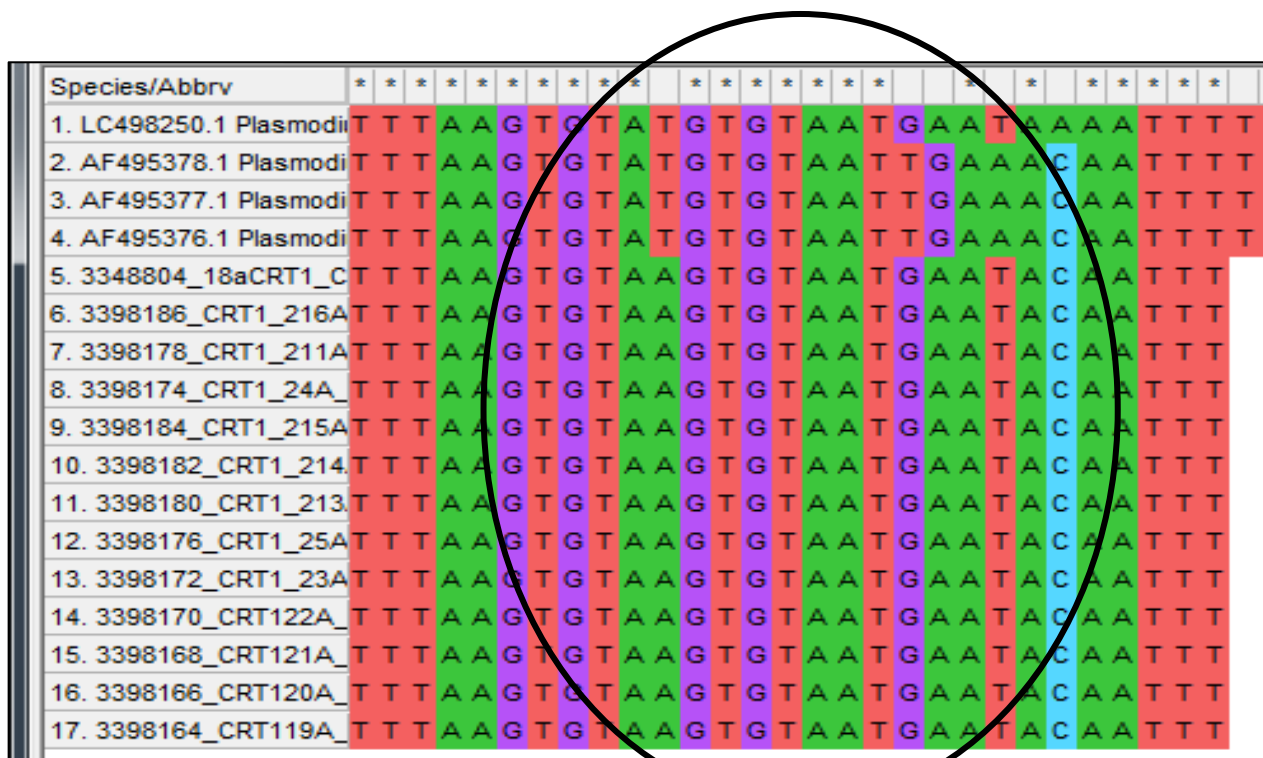


Figure 1. Multiple alignment results of pfcrf gene

In this study, it was also found that there was a change in the nucleotide base number 214 from Thymine (T) to Adenine (A) (Figure 1). The results of the analysis show that the

nucleotide base codes for the amino acid at codon number 72. The change in the nucleotide base causes a change in the amino acid cysteine (C) to the amino acid serine (S) (Figure 2).

DNA Sequences	Translated Protein Sequences
Species/Abbrev	
1. LC498250.1 Plasmodium falciparum	V F K L I F K E I K D N I F I Y I L S I I Y L S V C V M N K F A K R T L N K I G
2. AF495378.1 Plasmodium falciparum	V F K L I F K E I K D N I F I Y I L S I I Y L S V C V I E T I F A K R T L N K I G
3. AF495377.1 Plasmodium falciparum	V F K L I F K E I K D N I F I Y I L S I I Y L S V C V I E T I F A K R T L N K I G
4. AF495376.1 Plasmodium falciparum	V F K L I F K E I K D N I F I Y I L S I I Y L S V C V I E T I F A K R T L N K I G
5. 3348804_18aCRT1_CRT1R	V F K L I F K E I K D N I F I Y I L S I I Y L S V S V M N T I
6. 3398186_CRT1_216A CRT1R	V F K L I F K E I K D N I F I Y I L S I I Y L S V S V M N T I
7. 3398178_CRT1_211A CRT1R	V F K L I F K E I K D N I F I Y I L S I I Y L S V S V M N T I
8. 3398174_CRT1_24A CRT1R	V F K L I F K E I K D N I F I Y I L S I I Y L S V S V M N T I
9. 3398184_CRT1_215A CRT1R	V F K L I F K E I K D N I F I Y I L S I I Y L S V S V M N T I
10. 3398182_CRT1_214A CRT1R	V F K L I F K E I K D N I F I Y I L S I I Y L S V S V M N T I
11. 3398180_CRT1_213A CRT1R	V F K L I F K E I K D N I F I Y I L S I I Y L S V S V M N T I
12. 3398176_CRT1_25A CRT1R	V F K L I F K E I K D N I F I Y I L S I I Y L S V S V M N T I
13. 3398172_CRT1_23A CRT1R	V F K L I F K E I K D N I F I Y I L S I I Y L S V S V M N T I
14. 3398170_CRT122A CRT1R	V F K L I F K E I K D N I F I Y I L S I I Y L S V S V M N T I
15. 3398168_CRT121A CRT1R	V F K L I F K E I K D N I F I Y I L S I I Y L S V S V M N T I
16. 3398166_CRT120A CRT1R	V F K L I F K E I K D N I F I Y I L S I I Y L S V S V M N T I
17. 3398164_CRT119A CRT1R	V F K L I F K E I K D N I F I Y I L S I I Y L S V S V M N T I

Figure 2. Amino acid changes at Codons 72 and 76 of the pfcr gene

Based on the results of phylogenetic analysis, it was shown that the pesawaran isolates based on the pfcr gene had a slightly different genetic composition from other regional isolates. However, the difference is not

much. The results of the analysis showed that the pfcr gene isolates from Plasmodium falciparum in Pesawaran District, Lampung Province had similarities with the isolates in the gene bank (Figure 3).

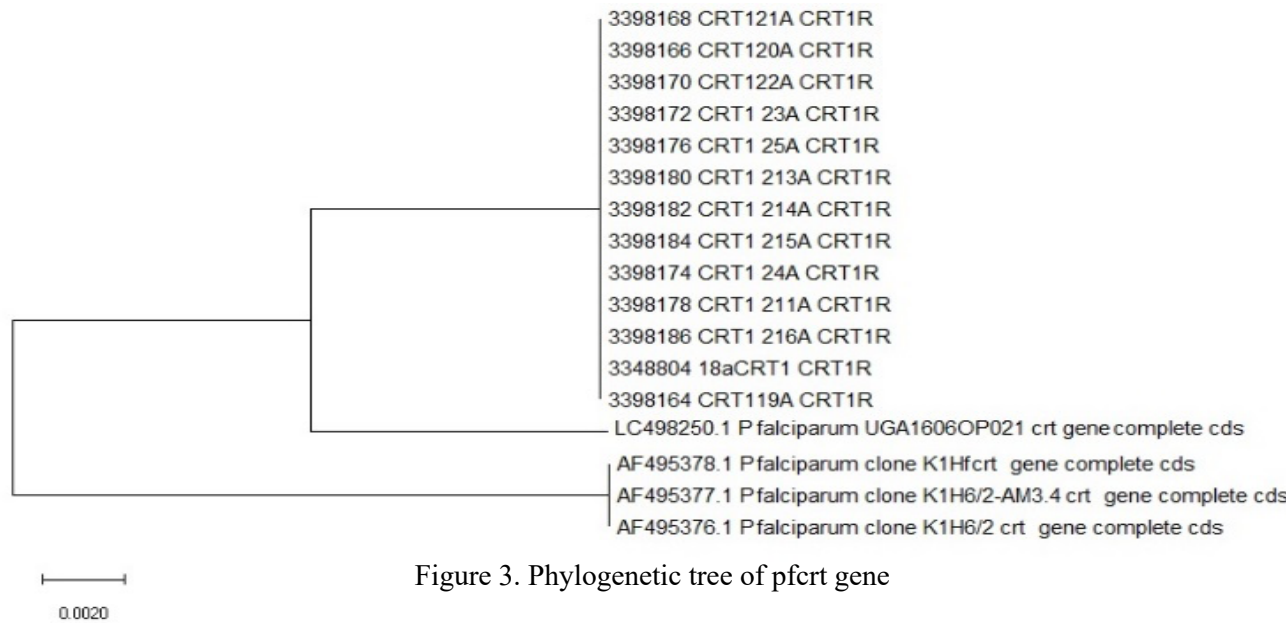


Figure 3. Phylogenetic tree of pfcr gene

4. DISCUSSION

The results of this study showed that no genetic changes were found in the *pfcr*t gene from malaria patients in Pesawaran District even though chloroquine had not been used for more than 10 years. The results of this study are in line with previous studies which stated that the K76T mutation of the *pfcr*t gene remained high after chloroquine was not used.¹⁴ Another study in Kenya and Malawi showed that the K76T mutation rate of the *pfcr*t gene decreased after chloroquine was not used for a long time.^{14,20,21}

The K76T mutation of the *pfcr*t gene was still high, indicating that the susceptibility of *Plasmodium falciparum* isolates from Pesawaran was still low. These results are still the same as the results of previous studies reported in 2005 and 2010 that many mutations of the *pfcr*t gene K76T have been found in *Plasmodium falciparum* isolates from the southern region of Lampung Province.^{7,8} In addition, the results of another study in Malaysia also showed that the high number of K76T mutations indicated a decrease in susceptibility to chloroquine.²²

The emergence of mutant strains is a form of parasite adaptation for survival to the effects of chloroquine. When there are many mutant strains, resistance to chloroquine will increase in the malaria endemic area. However, if chloroquine is not used, the survival of the mutant strain will decrease due to the absence of exposure, while the wild type will begin to develop again. This condition is an ideal thing that should happen if chloroquine is really withdrawn from the community, so that there is no chloroquine exposure at all to *Plasmodium*. The replacement of chloroquine with ACT in malaria therapy will be detrimental to the survival of the mutant parasite, so there is a possibility that the wild type parasite (K76) will reappear.¹⁴

In this study, the opposite happened. Even though chloroquine was not used for more than 10 years, the mutant parasite (76T) was

preserved. This condition can occur due to the presence of chloroquine on the market, especially in endemic areas, both used for individual malaria treatment and for the treatment of autoimmune diseases. This condition certainly cannot be avoided, so that chloroquine exposure to *Plasmodium falciparum* still occurs.

Another finding is the presence of a mutation at codon 72 (C72S). This is in line with other studies that reported mutations at codons 72 (C72S), 74 (M74I) and 75 (N75E) in addition to codon 76, although in this study, the authors did not find any mutations in codons 74 and 75. The presence of mutations in codon groups 72 to 76 (72-76 SVMNT) will have a very large effect in reducing the sensitivity of *Plasmodium falciparum* to chloroquine. As reported in an in vitro study examining the *pfcr*t gene.¹⁷

The phylogenetic analysis as shown in Figure 1 shows a description of the genetic variation in the *pfcr*t gene from various *Plasmodium* isolates. The sequences from the Pesawaran isolate had a strong homology when compared to the isolates from the gene bank. Some of the comparison isolates found genetic variation in one to three nucleotide bases, when compared to the Pesawaran Lampung isolate.

5. CONCLUSION

Polymorphisms were found in codon 72 and 76 of the *PfCRT* gene, although chloroquine has not been used as an antimalarial for a long time. The sequenced pesawaran isolates were also related to other isolates.

REFERENCES

- [1]. Mayengue PI, Ndounga M, Malonga FV, Bitemo M, Ntoumi F. Genetic polymorphism of merozoite surface protein-1 and merozoite surface protein-2 in *Plasmodium falciparum*

- isolates from Brazzaville, Republic of Congo. *Malar J.* 2011;10(1):276. doi:10.1186/1475-2875-10-276
- [2]. World Health Organization. *World Malaria Report*. World Health Organization; 2020. doi:ISBN 978 92 4 1564403
 - [3]. Ministry of Health Republic of Indonesia. *Indonesia Health Profile 2019*. Ministry of Health Republic of Indonesia; 2020.
 - [4]. Health Office of Lampung Province. *Lampung Province Health Profile 2019*. Health Office of Lampung Province; 2020.
 - [5]. Ministry of Health Republic of Indonesia. *Indonesia Health Profile 2008*. Ministry of Health Republic of Indonesia; 2009.
 - [6]. Suwandi JF. Polymorphism of PfMDR1 and PfATP6 Genes in Plasmodium Isolates from Falciparum Malaria Patients in Pesawaran District. Published online 2014.
 - [7]. Syafruddin D, Asih PBS, Casey GJ, et al. Molecular epidemiology of Plasmodium falciparum resistance to antimalarial drugs in Indonesia. *Am J Trop Med Hyg.* 2005;72(2):174-181. <http://www.ncbi.nlm.nih.gov/pubmed/15741554>
 - [8]. Kamelia M. Study of The Plasmodium falciparum chloroquine resistance transporter (pfert) gene Polymorphism in Patients with Malaria in Bandar Lampung and South Lampung Regency, The Province of Lampung. Published online 2010.
 - [9]. Suwandi JF. *Analysis of Plasmodium Falciparum PfCRT Gene Mutations in Malaria Patients in Lahat District Hospital*. Faculty of Medicine University of Lampung; 2011.
 - [10]. Ministry of Health Republic of Indonesia. *Guidelines for the Management of Malaria Cases in Indonesia*. Ministry of Health Republic of Indonesia; 2008.
 - [11]. Gresty KJ, Gray KA, Bobogare A, et al. Genetic mutations in pfert and pfmdr1 at the time of artemisinin combination therapy introduction in South Pacific islands of Vanuatu and Solomon Islands. *Malar J.* 2014;13(1):1-9. doi:10.1186/1475-2875-13-406
 - [12]. Sondo P, Derra K, Nakanabo SD, et al. Artesunate-amodiaquine and artemether-lumefantrine therapies and selection of pfert and PfmdR1 alleles in Nanoro, Burkina Faso. *PLoS One.* 2016;11(3).doi:10.1371/journal.pone.0151565
 - [13]. Bretscher MT, Dahal P, Griffin J, et al. The duration of chemoprophylaxis against malaria after treatment with artesunate-amodiaquine and artemether-lumefantrine and the effects of pfmdr1 86Y and pfert 76T: A meta-analysis of individual patient data. *BMC Med.* 2020;18(1):1-17. doi:10.1186/s12916-020-1494-3
 - [14]. Tajebe A, Aemero M, Francis K, Magoma G. Identification of chloroquine resistance Pfert-K76T and determination of Pfmdr1-N86Y copy number by SYBR Green I qPCR. *Asian Pac J Trop Biomed.* 2015;5(3):208-220. doi:10.1016/S2221-1691(15)30008-3
 - [15]. Ministry of Health Republic of Indonesia. *Handbook of Malaria Case Management*. Ministry of Health Republic of Indonesia; 2020. <http://www.malaria.id/p/buku-malaria.html>
 - [16]. Sambrook J, Fritsch EF, Maniatis. *Molecular Cloning, A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory Press; 1989.
 - [17]. Humphreys GS, Merinopoulos I, Ahmed J, et al. Amodiaquine and Artemether-Lumefantrine Select Distinct Alleles of the Plasmodium falciparum mdrl Gene in Tanzanian Children Treated for Uncomplicated Malaria □. *Antimicrob Agents Chemother.* 2007;51(3):991-997.

- doi:10.1128/AAC.00875-06
- [18]. Moll K, Ljungström I, Perlmann H, Scherf A, Wahlgren M. *Methods in Malaria Research*. 2008th ed. Malaria Research and Reference Reagent Resource Center, University Boulevard, Manassas; 2008.
 - [19]. Ibrahim ML, Khim N, Adam HH, Arie F, Duchemin J-B. Polymorphism of PfATPase in Niger: detection of three new point mutations. *Malar J*. 2009;8:28. doi:10.1186/1475-2875-8-28
 - [20]. Mwai L, Ochong E, Abdirahman A, et al. Chloroquine resistance before and after its withdrawal in Kenya. *Malar J*. 2009;8(1):1-10.doi:10.1186/1475-2875-8-106
 - [21]. Kublin JG, Cortese JF, Njunju EM, et al. Reemergence of chloroquine-sensitive Plasmodium falciparum malaria after cessation of chloroquine use in Malawi. *J Infect Dis*. 2003;187(12):1870-1875. doi:10.1086/375419
 - [22]. Atroosh WM, Al-Mekhlafi HM, Mahdy MAK, Surin J. The detection of pfcrt and pfmdr1 point mutations as molecular markers of chloroquine drug resistance, Pahang, Malaysia. *Malar J*. 2012;11(1):1. doi:10.1186/1475-2875-11-251

● 8% Overall Similarity

Top sources found in the following databases:

- 8% Internet database
- 0% Publications database
- Crossref database
- Crossref Posted Content database
- 0% Submitted Works database

TOP SOURCES

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

1

ejournal.unsri.ac.id

Internet

8%

● Excluded from Similarity Report

- Bibliographic material
- Cited material
- Manually excluded text blocks
- Quoted material
- Small Matches (Less than 10 words)

EXCLUDED TEXT BLOCKS

Majalah Kedokteran SriwijayaTh. 54 Nomor 1, January 2022PfCRT GENE POLYMO...
ejournal.unsri.ac.id

Mutations in the pffcr and pfmdr1 geneswill reduce the parasite's susceptibility to...
ejournal.unsri.ac.id

4. DISCUSSIONThe results of this study showed that nogenetic changes were foun...
ejournal.unsri.ac.id

Majalah Kedokteran SriwijayaTh. 54

Sriwijaya University on 2022-08-09

In this study, it was also found that there was a change in the nucleotide base num...
ejournal.unsri.ac.id