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Prevalence of Malaria and Diversity of *pfmdr1* Gene Polymorphisms among *Plasmodium falciparum* Isolates in Daloa, Western Côte d'Ivoire

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Abstract

Resistance of *Plasmodium falciparum* to antimalarial drugs remains a major public health concern in endemic regions. Polymorphisms in the pfmdr1 gene, particularly N86Y and Y184F, have been implicated in modulating parasite susceptibility to artemisinin-based combination therapies (ACTs). This study aimed to investigate the molecular epidemiology of *P. falciparum* and the distribution of pfmdr1 polymorphisms in Daloa, western Côte d'Ivoire. A cross-sectional study was conducted among individuals with suspected clinical malaria at the Regional Center for Mutuality and Social Works in Schools (CREMOSS) of Daloa. Blood samples were collected for parasitological and molecular analyses. Detection of *P. falciparum* and genotyping of pfmdr1 polymorphisms were performed using standard molecular techniques. Associations between infection status, genetic markers, and sociodemographic variables were also assessed. The overall molecular prevalence of *P. falciparum* was 50.27%, indicating sustained transmission within the study population. No significant association was observed between infection status and sex, whereas age influenced infection risk, with children aged 6 - 15 years being the most affected group. Analysis of the pfmdr1 gene revealed a high prevalence of the mutant alleles 86Y and 184F, with a marked predominance of the double mutant haplotype 86Y-184F (65.00 %). The wild-type haplotype N86-Y184 was infrequent, while the 86Y-Y184 haplotype was nearly absent. No significant asso-

ciation was found between pfmdr1 polymorphisms and sociodemographic variables. The high prevalence of pfmdr1 mutant alleles and the predominance of the 86Y-184F haplotype suggest ongoing selection pressure, likely associated with the use of antimalarial drugs within the study population. The low frequency of wild-type genotypes may reflect a progressive decline in drug-sensitive parasites. These findings highlight the need for continuous molecular surveillance to monitor changes in ACT efficacy and guide malaria control strategies.

Keywords

Malaria, *Plasmodium falciparum*, *pfmdr1* Gene, Codon, Daloa, Côte d'Ivoire

1. Introduction

Malaria remains one of the most concerning parasitic diseases in public health, particularly in tropical and subtropical regions [1]. According to the World Health Organization (WHO), sub-Saharan Africa bears the highest global burden of malaria, accounting for the majority of cases and deaths worldwide. Children under five years of age and pregnant women remain the most vulnerable groups to this disease [2]. In Côte d'Ivoire, malaria continues to be a major cause of outpatient consultations, hospitalizations, and deaths despite ongoing control efforts implemented through national malaria control programs [1].

This situation is exacerbated by the persistence of *Plasmodium falciparum*, the most virulent malaria parasite in humans and the species most prone to developing resistance to antimalarial drugs, thereby compromising malaria control and elimination efforts [3] [4]. Ongoing transmission in several areas of Côte d'Ivoire is driven by environmental, socio-economic, and behavioral factors, including favorable climatic conditions for vector development, insufficient preventive measures, self-medication practices, and limited access to healthcare services [1]. In western Côte d'Ivoire, particularly in the city of Daloa, ecological and demographic conditions contribute to sustained high malaria transmission [5]. High rainfall, increasing urbanization, agricultural activities, and favorable environmental conditions for vector development are key factors sustaining malaria transmission in this area [6].

The World Health Organization currently recommends artemisinin-based combination therapies (ACTs) as first-line treatment for uncomplicated *Plasmodium falciparum* malaria [7]. However, the efficacy of these drugs is increasingly threatened by the emergence and spread of parasite resistance, particularly to artemisinin derivatives [8]. This resistance is associated with genetic mutations affecting several parasite genes, among which the *pfmdr1* gene plays a major role. This gene encodes a membrane transport protein involved in intracellular molecule trafficking and plays a key role in modulating *Plasmodium falciparum* sus-

ceptibility to several antimalarial drugs such as chloroquine, mefloquine, lumefantrine, and certain artemisinin derivatives [9].

Several polymorphisms of the *pfmdr1* gene, notably N86Y, Y184F, and D1246Y, have been reported to be associated with altered therapeutic responses to artemisinin-based combination therapies [10]. Molecular surveillance of *pfmdr1* polymorphisms is therefore an essential tool for monitoring antimalarial drug efficacy and guiding malaria control policies. However, data on the genetic diversity of this gene in *Plasmodium falciparum* isolates circulating in Daloa remain limited. A better understanding of the distribution of *pfmdr1* polymorphisms in this area could contribute to assessing the emerging risk of antimalarial resistance and improving case management strategies.

The present study was conducted to assess malaria prevalence and characterize the diversity of *pfmdr1* gene polymorphisms in *P. falciparum* isolates circulating in Daloa.

2. Methodology

2.1. Study Design and Study Setting

This cross-sectional study was conducted at the Regional Center for Mutuality and Social Welfare in School Settings (CREMOSS) of Daloa, located in the Haut-Sassandra region in west-central Côte d'Ivoire, from April to June 2025. The study site is a referral and primary healthcare facility receiving patients from various districts of the city and surrounding localities, thereby providing access to a heterogeneous population.

2.2. Study Population and Sampling

The study population comprised patients of all age groups who were permanent residents of the Haut-Sassandra region and had been prescribed laboratory testing for malaria diagnosis. All samples meeting the inclusion criteria and available during the study period were consecutively enrolled. The sample size was determined by the number of eligible participants attending the hospital during the recruitment period and by the availability of biological samples for molecular analyses.

2.3. Inclusion and Exclusion Criteria

Participants were included in the study if they were permanent residents of the Haut-Sassandra region, presented with suspected clinical malaria requiring a medical prescription for parasitological examination, and provided informed consent. For minors, assent along with parental or guardian consent was required.

Patients were excluded if they refused to participate, had received antimalarial treatment within the two weeks prior to recruitment, or presented incomplete clinical or biological data.

2.4. Blood Sample Collection and Laboratory Procedures

Following the interview and clinical assessment, 5 mL of venous blood was col-

lected from each participant into EDTA-containing tubes by trained laboratory personnel. Samples were properly labeled and immediately transported to the laboratory under conditions ensuring an appropriate cold chain and full traceability of specimens. Thick blood smears were prepared on clean, grease-free slides, air-dried, and then stained with 10% Giemsa solution. Microscopic examination was performed by an experienced microscopist following World Health Organization (WHO) standardized procedures, using a light microscope with oil immersion (×100 objective). Malaria parasitaemia was quantified by counting asexual parasite forms per 200 leukocytes and expressed as parasites/μL, using a standard leukocyte count of 8,000/μL.

Internal quality control was ensured through systematic re-examination of all positive slides and a random subset of negative slides (10%) to verify consistency and reliability of the results. In cases of uncertainty in slide interpretation, a repeat examination was performed for confirmation. In addition, 2 mL aliquots of whole blood were collected from each participant, independently of microscopy results, and stored in uniquely labeled cryotubes at −20˚C for subsequent molecular analyses.

2.5. Informed Consent and Confidentiality

Participants were informed about the objectives and methodology of the study in French or in a local language. Written informed consent was obtained from all adult participants, while parental consent and child assent were obtained for minors. All procedures were conducted in accordance with the principles of the Declaration of Helsinki. Participant confidentiality was strictly maintained throughout the study.

2.6. DNA Extraction and Molecular Detection of *Plasmodium falciparum*

Genomic DNA was extracted from 200 μL of venous blood using the commercial QIAamp DNA Blood Mini Kit (QIAGEN, Germany), according to the manufacturer's instructions.

Molecular detection of *P. falciparum* was performed by amplification of the 18S ribosomal gene (small subunit ribosomal RNA, SSU rRNA) using nested PCR. Each PCR run systematically included a positive control consisting of reference *P. falciparum* DNA confirmed to carry the target alleles, as well as a negative control (sterile distilled water) to detect any potential contamination. The first amplification was carried out using a pair of primers targeting conserved regions of the 18S gene of *Plasmodium* species (Table 1). The resulting products were then used as templates for a second amplification using *Plasmodium falciparum*-specific primers (Table 1).

PCR reactions were performed in a final volume of 25 μL containing 4 μL of DNA template (1 ng/μL), 1 μL of each primer (10 pmol/μL; Eurogentec, France), 12.5 μL of GoTaq Green Master Mix (Promega, USA), and nuclease-free sterile

water to adjust the final volume.

The thermal cycling program for the first PCR consisted of an initial denaturation at 95˚C for 5 min, followed by 45 cycles of denaturation at 94˚C for 1 min, annealing at 57˚C for 2 min, and extension at 72˚C for 2 min, with a final extension at 72˚C for 5 min. The second amplification was performed under the same reaction conditions using 4 µL of the first PCR product as template. Cycling conditions included an initial denaturation at 95˚C for 5 min, followed by 45 cycles consisting of denaturation at 94˚C for 1 min, annealing at 55˚C for 2 min, and extension at 72˚C for 2 min. A final extension was carried out at 72˚C for 5 min. All amplifications were performed using a conventional thermocycler (Bio-Rad, USA).

Second-round PCR products were analyzed by electrophoresis on a 2% agarose gel containing GelRed and visualized under ultraviolet light. Amplification product sizes were estimated by comparison with a 100 bp molecular weight marker. A band of approximately 1200 bp was interpreted as positive for the *Plasmodium* genus, whereas a 205 bp fragment was specific for *Plasmodium falciparum*.

Table 1. List of primers used for the diagnosis of *Plasmodium falciparum* [11].

nested PCR	Sequences	Expected amplicon size
First-round PCR genus *Plasmodium*	5'-TTTTTATAAGGATAACTACGGAAA-3' 5'-CCTGTTGTTGCCTTAAACTTC-3'	1200 bp
Second-round PCR *P. falciparum*	5'-TTAAACTGGTTTGGGAAAACCAAATA-3' 5-ACACAATGAACTCAATCATGACTACCCGTC-3'	205 bp

Note: bp: base pair.

2.7. Amplification and Genotyping of the *pfmdr1* Gene

Confirmed *Plasmodium falciparum*-positive samples were used for the amplification of *Pfmdr1* gene polymorphisms at codons 86 and 184. The selection of codons 86 and 184 of the *pfmdr1* gene is justified by their central role in modulating susceptibility to major antimalarial drugs used in Africa, including chloroquine, amodiaquine, and artemisinin-based combination therapies. These two loci are highly informative markers in molecular epidemiology, as they exhibit substantial allelic variability and are frequently involved in drug pressure-driven selection processes. In contrast, the D1246Y codon, although biologically relevant, is less prevalent in several West African populations, which limits its discriminative power in studies with moderate sample sizes [12]. Amplification was performed using nested PCR with two sets of specific primers, A1/A3 for the primary PCR and A2/A4 for the secondary PCR (**Table 2**), according to the method described by [13]. Each PCR run systematically included a positive control consisting of reference *P. falciparum* DNA confirmed to carry the target alleles, as well as a negative control (sterile distilled water) to detect any potential contamination. PCR reactions were performed under conditions identical to those previously described.

The thermal cycling program included an initial denaturation at 94˚C for 2 min, followed by 40 cycles of denaturation at 94˚C for 1 min, annealing at 45˚C for 1 min, and extension at 72˚C for 1 min, with a final extension step at 72˚C for 5 min.

Table 2. Primers and amplification conditions for *Pfmdr1* codons 86 and 184.

Gene	Codon	Primer	Sequence	Nested amplicon size	Restriction enzyme	Mutation
pfmdr1	86 and 184	A1	5'-TGTTGAAAGATGGGTAAAGAGCAGAAAGAG-3'	560 bp	*ApoI*	N 86Y
		A3	5'-TACTTTCTTATTACATATGACACCACAAACA-3'			
		A2	5'-GTCAAACGTGCATTTTTTATTAATGACCATTTA-3'		*DraI*	184 F
		A4	5'-AAAGATGGTAACCTCAGTATCAAAGAAGAG-3'			

Note: bp: base pair.

Genotyping of the Pfmdr1 N86Y and Y184F polymorphisms was performed using restriction fragment length polymorphism PCR (PCR-RFLP). The secondary PCR products were digested with the restriction enzymes ApoI for codon 86 and DraI for codon 184, in a final volume of 15 µL at 37˚C for 1 h (**Table 3**), according to the method described by [14]. The restriction fragments were then separated by electrophoresis on a 2% agarose gel at 60 V for 1 h. The resulting migration profiles allowed discrimination of wild-type, mutant, and mixed genotypes based on fragment sizes. For the N86Y polymorphism, isolates carrying the wild-type N86 allele showed a single 560 bp band, whereas mutant 86Y isolates exhibited a digested fragment characterized by a 333 bp band. Mixed infections were defined by the simultaneous presence of bands corresponding to both alleles (560 bp and 333 bp). Regarding the Y184F polymorphism, wild-type isolates showed an undigested single band, whereas mutant isolates displayed three fragments of distinct sizes after DraI digestion, consistent with the expected profile. Mixed infections were defined by the simultaneous presence of the undigested wild-type profile and the digested mutant profile after DraI treatment. However, mixed infections were excluded from the final analysis due to the limited resolution of PCR-RFLP for reliably discriminating co-existing allelic profiles, in order to ensure the robustness of allele frequency estimates.

Table 3. Digestion conditions of ApoI and DraI enzymes.

Reaction mixture composition	Volume (µL)
H_2O	9.5
10x Fast digest	1.5
APOI ou *DraI*	1
PCR product	3
Final volume	15

2.8. Statistical Analysis

Data were entered, verified, and cleaned according to standard data management procedures prior to statistical analysis. Participant characteristics were described using descriptive statistics. *pfmdr1* gene genotypes were determined based on the presence or absence of wild-type and mutant alleles at the different loci investigated. Mutation frequencies were estimated by grouping isolates carrying mutant alleles.

Associations between categorical variables and malaria infection were assessed using Pearson's Chi-square test or Fisher's exact test when expected counts were less than 5. Differences were considered statistically significant at a p-value < 0.05, with a 95% confidence interval.

Non-informative genotypes, corresponding to samples showing weak, ambiguous, or non-interpretable banding patterns after PCR-RFLP electrophoresis, were excluded from the final analysis. This exclusion was performed to minimize interpretation bias associated with poor resolution of restriction fragments and to ensure the reliability and robustness of allele frequency estimates and statistical analyses.

3. Results

3.1. Population Characteristics and Prevalence of *P. falciparum*

A total of 179 participants were included in this study, comprising 111 (62%) females and 68 (38%) males, corresponding to a sex ratio (M/F) of 0.61 (Table 4). Molecular analyses revealed an overall prevalence of *Plasmodium falciparum* infection of 50.27% (Table 4). Statistical analyses showed a significant association between age and malaria infection (p = 0.036; Table 4), with a notable difference between children aged 6 - 15 years and adults (>15 years). Moreover, children aged 6 - 15 years had a 2.08-fold higher risk of malaria infection compared to adults.

Table 4. General characteristics.

Variable	n (%)	PCR-positive for *P. falciparum* (%)	OR	IC 95%	p-value
Sex					
F	111 (62)	56 (50.45)	1.02	[0.56, 1.86]	1
M	68 (38)	34 (50)			
Total	179 (100)	90 (50.27)			
Sex ratio (M/F)	0.61	-			
Age					
0-5	25 (13.97)	15 (60)	2.19	[0.88, 5.42]	0.1113
6-15	68 (37.99)	40 (58.82)	2.08	[1.09, 3.97]	0.036*
> 15	86 (48.04)	35 (40.69)	1		
Total	**179 (100)**	**90 (50.27)**	-	-	-

Note: n: Study population, F: Female, M: Male, IC 95%: 95% confidence interval, OR: Odd ratio, *: significant result.

3.2. Typing of Codons 86 and 184 of the *Pfmdr1* Gene

Genotyping of codon 86 of the pfmdr1 gene was successfully performed on all 90 isolates, whereas that of codon 184 was obtained for 80 isolates due to amplification failure at this codon (Table 5).

Analysis of *Pfmdr1* gene polymorphisms revealed a predominance of mutant alleles in the studied isolates. At codon 86, the mutant allele 86Y was detected in 72.2% of samples compared to 27.8% for the wild-type allele N86. Similarly, at codon 184, the mutant allele 184F accounted for 85.0% of isolates, whereas the wild-type allele Y184 was found in only 15.0% of samples.

Table 5. Frequency of genotypes in the *pfmdr1* gene.

Codon	Allele	Genotype	Number	Frequency (%)	IC 95%
86	N86	Wild-type	25	27.8	[19.6 - 37.8 %]
	86Y	Mutant	65	72.2	[62.2 - 80.4 %]
184	Y184	Wild-type	12	15.0	[8.8 - 24.4 %]
	184F	Mutant	68	85.0	[756 - 91.2 %]

Note: IC 95%: 95% confidence interval.

3.3. Association between *Pfmdr1* Gene Polymorphisms and Sociodemographic Characteristics

According to sex, a higher prevalence of the mutant allele 86Y was observed in males (76.47%) compared to females (69.64%) (Table 6). However, this difference was not statistically significant (OR = 1.42; 95% CI: 0.53 - 3.76; p = 0.484). A similar trend was observed at codon 184, where the frequency of the mutant allele 184F was slightly higher in males (87.5%) than in females (83.33%), with no statistically significant association (OR = 1.40; 95% CI: 0.38 - 5.10; p = 0.61) (Table 6).

Age-based analysis showed that individuals aged 6 - 15 years had the highest frequency of the mutant allele 86Y (82.5%). Compared with subjects older than 15 years, this age group had a 2.46-fold higher risk of carrying the mutant allele, although this association did not reach statistical significance (OR = 2.46; 95% CI: 0.84 - 7.20; p = 0.10). In contrast, children aged 0 - 5 years showed a lower frequency of the 86Y mutant (60%), with no statistically significant difference (OR = 0.78; 95% CI: 0.22 - 2.72; p = 0.70) (Table 6).

For codon 184, the frequency of the mutant allele 184F remained high across all age groups, with prevalences of 73.33% in children aged 0 - 5 years, 88.23% in those aged 6 - 15 years, and 87.09% in individuals older than 15 years. No statistically significant association was observed between age groups and carriage of the 184F mutant allele (p > 0.05) (Table 6).

Overall, mutant alleles of the *Pfmdr1* gene were highly predominant in the study population, with no significant association with sex or age groups. Nevertheless, the high frequency of the 86Y and 184F mutants may reflect persistent antimalarial drug selection pressure in the study area.

Table 6. Analysis of *Pfmdr1* gene polymorphisms (codons 86 and 184) according to sex and age groups.

Polymorphism of the *Pfmdr1* gene										
Variables	Codon 86					Codon 184				
Sex	Mutants (%)	Wild-type (%)	OR	IC 95%	*p-value*	Mutants	Wild-type (%)	OR	IC 95%	*p-value*
M	26 (76.47)	8 (23.53)	1.42	[0.53 - 3.76]	0.484	28 (87.5)	4 (12.5)	1.4	[0.38 - 5.10]	0.61
F	39 (69.64)	17 (30.36)				40 (83.33)	8 (16.67)			
Total	65 (72.22)	25 (27.78)				68 (85)	12 (15)			
Age group										
>15	23 (65.71)	12 (34.29)	1			27 (87.09)	4 (12.91)	1		
0 - 5	9 (60)	6 (40)	0.78	[0.22 - 2.72]	0.7	11 (73.33)	4 (26.67)	0.41	[0.09 - 1.93]	0.25
6 - 15	33 (82.5)	7 (17.5)	2.46	[0.84 - 7.2]	0.1	30 (88.23)	4 (11.77)	1.11	[0.25 - 4.88]	0.88
Total	65 (72.22)	25 (27.78)				68 (85)	12 (15)			

Note: OR: Odd ratio, M: Male, F: Female, IC 95%: 95% confidence interval.

3.4. Prevalence of *pfmdr1* Gene Haplotypes

Haplotype analysis of the *pfmdr1* gene revealed a predominance of the double mutant haplotype 86Y-184F, accounting for 65.00% of successfully genotyped isolates (Table 7). The single mutant haplotype N86-184F represented 20.00%, while the double wild-type haplotype N86-Y184 was observed in only 13.75% of isolates (Table 7). The low prevalence of the 86Y-Y184 haplotype suggests a preferential co-selection of the 86Y and 184F mutant alleles within the local parasite population.

Table 7. Haplotype analysis of *pfmdr1* gene mutations.

Haplotype (*pfmdr1* 86/184)	Statut	Number (n)	Frequency (%)
M-M (86Y-184F)	Double Mutant	52	65.00 %
WT-M (N86-184F)	Single Mutant	16	20.00 %
M-WT (86Y-Y184)	Single Mutant	1	1.25 %
WT-WT (N86-Y184)	Double wild-type	11	13.75 %
Total		80	100 %

Note: WT: wild-type, M: Mutant.

4. Discussion

The present study allowed the characterization of the molecular epidemiology of *Plasmodium falciparum* as well as the distribution of *pfmdr1* gene polymorphisms among patients attending the Regional Centre for Mutuality and Social Welfare in the School Environment (CREMOSS) in Daloa. Overall, a high molecular prevalence of *P. falciparum* (50.27%) was observed, reflecting active and sustained parasite circulation within the population. This level of prevalence confirms that malaria remains a major public health problem in the study area despite implemented

control strategies [2]. Similar levels have been reported in several regions of sub-Saharan Africa where transmission remains stable and intense [15] [16].

At the sociodemographic level, no significant association was found between sex and malaria infection, suggesting a generally homogeneous exposure of both sexes to the parasite. These findings are consistent with those reported in other endemic settings in sub-Saharan Africa, particularly in Burkina Faso and The Gambia [17] [18]. In contrast, age appeared to be a factor influencing infection distribution, with children aged 6 - 15 years showing relatively higher vulnerability. This may be explained by incomplete immunity and increased exposure to mosquito bites. School-aged children therefore represent a particularly exposed group and play an important role in community transmission as potential asymptomatic reservoirs [19] [20].

Analysis of *pfmdr1* gene polymorphisms revealed a strong predominance of the mutant alleles 86Y and 184F, indicating substantial selection pressure within local parasite populations. This trend is widely reported in sub-Saharan Africa, where these mutations are frequently associated with drug pressure, particularly artemisinin-based combination therapies (ACTs) [12]. These polymorphisms are known to modulate *P. falciparum* sensitivity to amino-4-quinolines as well as to certain ACT partner drugs, suggesting progressive parasite adaptation to used treatments [21] [22]. The high prevalence of the 86Y mutant allele observed in this study despite chloroquine withdrawal in several regions suggests residual selection pressure, possibly maintained by the use of related molecules, particularly amodiaquine [23] [24]. Furthermore, several studies have shown that the N86Y mutation differentially influences responses to major ACTs, particularly artemether–lumefantrine and artesunate–amodiaquine, confirming its role in modulating parasite sensitivity [24].

Similarly, the high frequency of the 184F allele indicates a marked expansion of this polymorphism in the studied population. Although its functional role is not fully understood, it is increasingly recognized to contribute to antimalarial response modulation, particularly through interactions with other *pfmdr1* mutations [25]. The high coexistence of the 86Y and 184F mutations may therefore reflect progressive adaptation of local *P. falciparum* populations under continuous drug pressure, as suggested by [26], who highlighted the combined influence of therapeutic pressure and parasite fitness in the evolution of *pfmdr1* haplotypes.

Haplotype analysis showed a clear predominance of the double mutant haplotype 86Y-184F (65.00%), followed by the single mutant haplotype N86-184F (20.00%), while the wild-type haplotype N86-Y184 was poorly represented (13.75%). The near absence of the wild-type haplotype 86Y-Y184 suggests preferential co-selection of the 86Y and 184F mutations, indicating a potential selective advantage of this combination in the local therapeutic context. Similar findings were reported by [12] in Burkina Faso, where some wild-type haplotypes were absent while mutant haplotypes persisted under drug pressure. This situation is concerning, as haplo-

types combining these mutations have been associated with altered parasite responses to ACTs, particularly artesunate-amodiaquine and artemether-lumefantrine [10] [27]. Thus, the high circulation of these polymorphisms may represent an early signal of parasite adaptation requiring continuous surveillance. Moreover, the low frequency of the wild-type alleles N86 and Y184 suggests a progressive reduction of drug-sensitive genotypes, likely driven by positive selection under local treatment pressure [26].

Finally, the absence of significant associations between polymorphisms and sociodemographic variables suggests that drug pressure is the main driver of the observed genetic structuring. This is consistent with the findings of [12], who showed that *pfmdr1* polymorphism dynamics are primarily determined by antimalarial drug exposure rather than demographic characteristics of the studied populations. However, the lack of phenotypic drug susceptibility testing and additional markers such as *pfcrt* and *kelch13* represents an important limitation, restricting functional interpretation of the observed mutations. Despite these limitations, this study provides important data on the genetic structure of *P. falciparum* and the dynamics of *pfmdr1* polymorphisms in the study area. The high prevalence of 86Y and 184F mutations, together with the dominance of the 86Y-184F haplotype, underscores the need to strengthen molecular malaria surveillance in order to anticipate potential changes in antimalarial drug sensitivity and adapt control strategies accordingly.

5. Conclusions

This study highlights active circulation of *Plasmodium falciparum* within the study population, accompanied by a high prevalence of the *pfmdr1* 86Y and 184F mutations. The predominance of these mutant alleles, together with the rarity of wild-type genotypes and the dominance of the 86Y-184F haplotype, suggests a genetic structuring of the parasite that is strongly influenced by local drug pressure. The absence of a significant association with sociodemographic factors reinforces the hypothesis that selection is mainly driven by exposure to antimalarial drugs rather than demographic determinants.

These findings indicate a progressive adaptation of parasite populations to current therapies, particularly ACTs, and highlight the potential role of *pfmdr1* haplotypes in modulating treatment response. In this context, the persistence and co-selection of the observed mutations may represent an early signal of evolving parasite susceptibility, warranting continuous surveillance. However, the lack of phenotypic data and additional resistance markers limits the functional interpretation of the observed associations. Additional studies incorporating in vitro analyses, as well as the investigation of other resistance genes in a larger sample size, are needed to further elucidate the clinical implications of these polymorphisms. Finally, these results reinforce the importance of regular molecular monitoring of *P. falciparum* in order to anticipate changes in antimalarial drug sensitivity and guide malaria control strategies in endemic areas.

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All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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