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MOLECULAR ANALYSIS OF THE *DHPS* GENE IN *PLASMODIUM FALCIPARUM* USING CLINICAL ISOLATES FROM DALOA TOWN IN CÔTE D'IVOIRE

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ABSTRACT

Plasmodium falciparum, the protozoan responsible for malaria, has repeatedly developed resistance to antimalarial drugs, thereby thwarting efforts to malaria control and elimination. These resistances have contributed significantly to an increase in malaria-related mortality. Among the molecular markers of resistance, point mutations in the dihydropteroate synthase (*dhps*) gene confer resistance to sulfadoxine in *P. falciparum*. The combination of its alleles is strongly correlated with the resistant phenotype. This study aimed to assess the resistance of *Plasmodium falciparum* to sulfadoxine in patients consulting at the Daloa Regional Hospital Centre (CHR), Côte d'Ivoire. The method involved PCR amplification followed by enzymatic digestion (PCR-RFLP) using the *MnII* enzyme. A Total of 380 patients took part in the study, of whom 262 cases were confirmed by thick smear. Of these 262 isolates, 175 samples with high parasitaemia ($\geq 1,000$ trophozoites/ μ L) underwent molecular testing; of these, 148 (84.57%) tested positive by PCR amplification. Enzymatic digestion revealed 36 mutant isolates (24.32%) compared with 112 isolates (75.68%) of wild-type phenotype. These results from a cross-sectional study at Daloa CHR confirm the circulation of sulfadoxine-resistant *P. falciparum* isolates in this area and highlight the need to strengthen surveillance of chemoresistance to antimalarial drugs used in intermittent preventive treatment (IPT), particularly the sulfadoxine-pyrimethamine combination. A future research direction will involve combining an in vivo study of chemosensitivity to sulfadoxine-pyrimethamine with a joint analysis of the *dhfr* and *dhps* genes of *P. falciparum*.

Keywords: Malaria, *Plasmodium Falciparum*, PCR-RFLP, Dihydropteroate Synthase (DHPS), Daloa, Côte d'Ivoire.

1 INTRODUCTION

Erythrocytopathy caused by haematozoa of *Plasmodium* genus, which are transmitted by the bite of an infected female *Anopheles* mosquito, malaria remains one of the world's most serious public health problems, exposing nearly half the world's population to the risk of contracting this parasitic disease [1–3]. *Plasmodium falciparum* and *Plasmodium vivax* are the two most dangerous species, with *P. falciparum* being the most virulent and causing the highest case-fatality rate among the five human-infecting *Plasmodium* species [3]. In 2024, the annual number of reported cases was nearly 282 million, and the reported number of worldwide deaths from malaria was 610,000. Endemic African countries continued to carry the heaviest burden, with 95% of global reported cases and 96% of malaria-related deaths [3]. Indeed, children under five, pregnant women, people living with HIV/AIDS, and travelers from non-endemic countries visiting endemic countries constitute the most vulnerable section of the population [4,5].

Thanks to control strategies based on prevention, rapid diagnosis, curative treatment and vaccination, the global incidence of malaria has fallen from 79.4 cases per 1,000 in 2000 to 64 cases in 2024 [6]. Today, thanks to control strategies based on prevention, rapid diagnosis, curative treatment, and vaccination, the global incidence of malaria has fallen from 79.4 cases per 1,000 in 2000 to 64 cases in 2024 [6].

In Côte d'Ivoire, because of the predominant presence of the most virulent malaria parasite like *P. falciparum* and the main vector like *Anopheles gambiae* (sl), malaria has become the leading cause of hospital admissions and healthcare facilities-related deaths [7,8]. Malaria is hyperendemic in country all regions, with active transmission throughout the year [9,10]. However, the national incidence of the disease has declined from 490.5 cases in 2000 to 267.9 per 1,000 inhabitants in 2024 [6].

Unfortunately, the effectiveness of antimalarial drugs is constantly under threat from the appearance and spread of resistant strains. Among the available treatments, combinations based on sulfadoxine–pyrimethamine (SP) have played, and still play in certain contexts, an important role in intermittent prophylaxis and treatment, making the monitoring of drug resistance essential. *P. falciparum* resistance to sulfadoxine is mainly associated with point mutations in the gene encoding dihydropteroate synthase (dhps), which reduce the enzyme's affinity for the drug [11,12]. The emergence and spread of these mutations compromise the efficacy of sulfadoxine and may lead to treatment failure, which would increase morbidity and undermine control and elimination strategies. It is therefore necessary to update data on *dhps* gene mutation prevalence to inform clinical and public health decisions. This study aims to characterise, using molecular approaches, the profile of *P. falciparum*'s chemoresistance to sulfadoxine in patients attending the Daloa Regional Hospital Centre (CHR)

2 MATERIALS AND METHODS

2.1 Study site

This study was carried out at Daloa Regional Hospital Centre. Located in south-eastern Daloa municipality, the CHR is the referral hospital for Haut-Sassandra district in central-western Côte d'Ivoire (Figure 1). According to the 2021 General Population and Housing Census report, this hospital serves approximately 421,879 people in the local area [13].

This town is the country's third-largest urban area by surface and is characterised by significant socio-demographic diversity, with a young population and a high proportion of residents living in peri-urban areas [14]. Daloa is situated 141 km from Yamoussoukro, the country's political capital, and covers an area of 5,305 km² with a population density of 79.52 inhabitants per km². With varied socio-economic conditions, the city experiences a humid tropical climate, with annual precipitation totaling 1,335 mm and an average annual temperature of 27.3 °C [15]. Given the bioclimatic conditions, combined with sometimes precarious living conditions and inadequate sanitation management in certain outlying neighborhoods of the city, malaria transmission is holoendemic. It remains active during all seasons, with peaks in incidence during the rainy season. These epidemiological characteristics highlight the unique characteristics of this area of Daloa for the study of malaria drug resistance

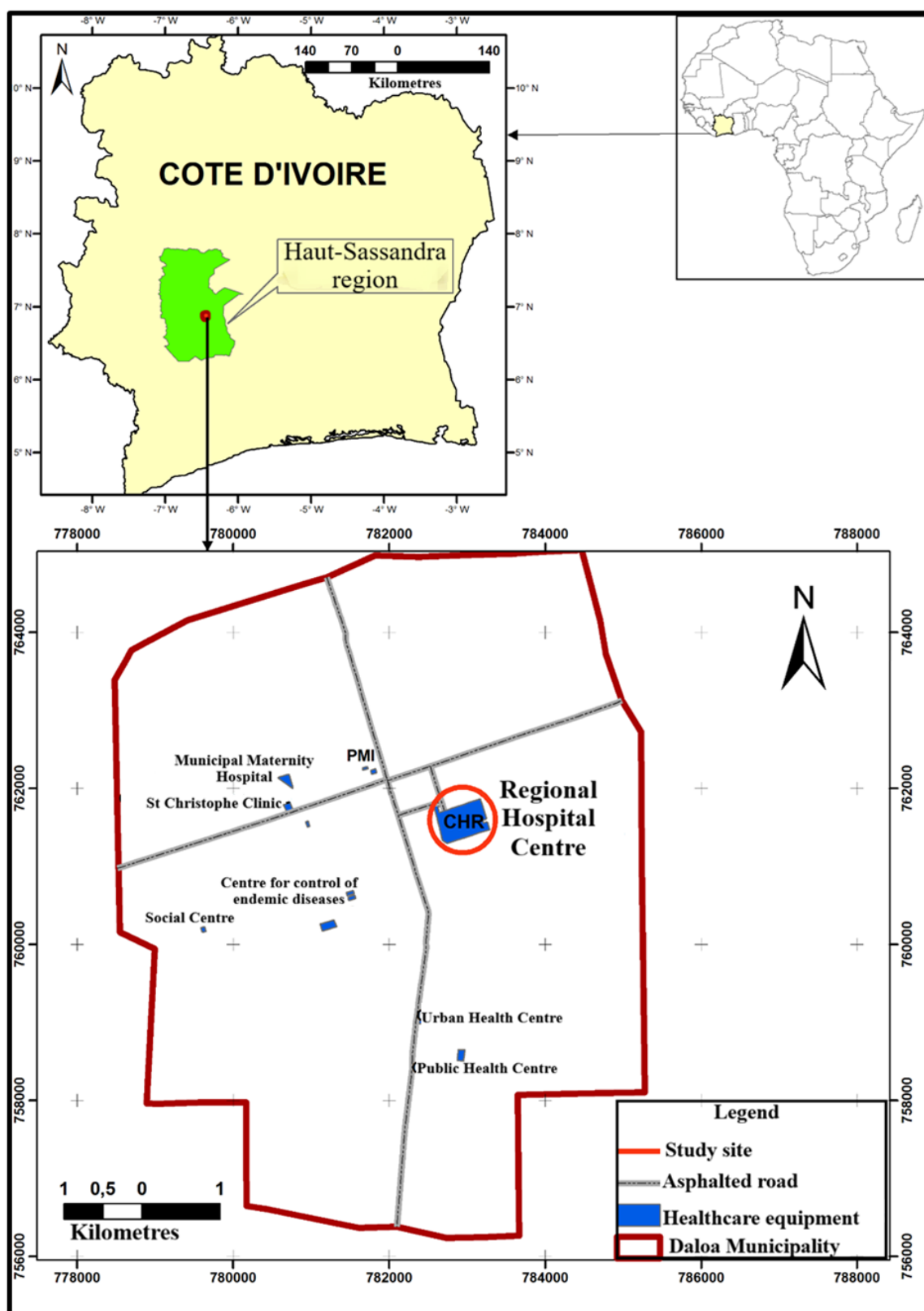


Figure 1: Geospatial representation of the study area, source: BNETD, 2016

2.2 Sampling

This cross-sectional study was conducted from June 29 to October 22, 2015, covering four consecutive months. Patients whose medical history and clinical diagnosis revealed symptoms and clinical signs suggestive of malaria were referred to the CHR's laboratory department. All patients who consented to take part in this study were first interviewed to gather information on socio-economic data, clinical and social assessment, and treatments administered prior to their consultation at CHR. Then, a venous blood sample (3-4 mL) was taken from the crook of the elbow into an

ethylenediaminetetraacetic acid (EDTA)-based anticoagulant tube by a nurse or a clinical laboratory technician for all participants included in the study. In the case of minors, informed consent was obtained from their parents. All blood samples collected were labelled (with the date and patient number) and patient data were anonymised before being stored. Finally, each sample was divided into two parts, one of which was used by the CHR's clinical pathology laboratory to carry out the parasitological test (thick smear) and the complete blood count (CBC) or hemogram. The other part of each sample was kept on ice and then sent to the laboratory of the Research Unit in Genetics and Molecular Epidemiology (URGEM) at Jean Lorougnon Guédé University in Daloa. The various blood samples were aliquoted into cryotubes (4 Nunc tubes per sample) and frozen at -20°C for molecular analysis.

3 DIAGNOSTIC PROCEDURE

3.1 Parasitological diagnosis of malaria at the CHR

The examination for haematzoa was carried out using the thick smear method, which remains one of the gold standard methods for parasitological diagnosis. This method involved using a sterile pipette to deposit 3 to 4 μL of each patient's blood sample on to a sterile slide that had been clearly labelled (with the date and test report number or the patient's name). This blood volume was smeared in a circular pattern (approximately 1 cm) using the tip of another sterile slide, then left to air-dry for 20 to 30 minutes. A simple Giemsa stain, diluted 1:10 in buffered water ($\text{pH} = 7.0\text{--}7.2$), was applied to the smear. The preparation was covered with a sterile coverslip before being examined under an optical microscope using a $\times 100$ objective. This technique enabled the number of asexual forms of *Plasmodium falciparum* (trophozoites) present in the blood to be estimated. Any sample in which the thick smear examination indicated the presence of trophozoites was considered positive.

Parasite density (PD) was calculated using the red blood cell concentration technique, which enables the detection of *Plasmodium* in the blood [16]. Parasite density was recorded as the ratio of the number of parasites to the number of white blood cells in the thick smear. The number of *Plasmodium* parasites was determined relative to 200 white blood cells. Each sample was analysed using a complete blood count (CBC) test on a 'Beckman Coulter AcT Diff' hematology analyser. This test provides information on abnormalities in blood components (hemoglobin level, platelet count, white and red blood cell counts, etc.).

3.2 Genomic DNA extraction

The samples transported to the URGEM laboratory were used to extract genomic DNA. Using the QIAamp® DNA Stool Mini Kit (50), Cat. No. 51504, extraction from whole blood was carried out in accordance with the manufacturer's instructions. The quality of the extracted DNA was assessed by comparing the intensity of the bands on the electropherograms with that of the molecular weight marker supplied by the manufacturer. The extracted DNA was eluted into 20 μL of elution buffer and then stored at -20°C until analysis by polymerase chain reaction (PCR).

3.3 Amplification and genotyping of *dhps* gene

Molecular analysis of the samples was based on the nested PCR technique, followed by the restriction fragment length polymorphism (RFLP) technique. The PCR-RFLP method was used to amplify the *dhps* gene of interest in *Plasmodium* DNA and then to digest this DNA with restriction enzymes in order to analyse the length polymorphism of the resulting fragments. The expected size of the nucleotide sequence of interest following PCR amplification of the *Plasmodium falciparum dhps* gene (*pfdhps*) was 711 base pairs (bp), enabling the analysis of several key codons, such as codons 436, 437, 540 and 581, where potential gene mutations occur. Indeed, the *dhps* gene of *P. falciparum* was amplified during the primary PCR using forward primer DHPS1-F: 5'-CCATTCCTCATGTGTATACAACAC-3' and reverse primer DHPS1-R: 5'-CATCTGAAACATCCAATTGTGTGA-3'. Nested PCR was performed using the primer pair DHPS2-F: 5'-TATGATTCTTTTCAGATGGAGGT-3' and DHPS2-R: 5'-CATCTGAAACATCCAATTGTGTGA-3' [17]. The amplification programme and reaction conditions are detailed in Table 1.

PCR was carried out in a BIO-RAD T100 programmable thermal cycler. All products from the two PCR reactions were run on a 2% agarose gel in the presence of a 100 bp molecular weight marker for 30 minutes. Samples in which no products were detectable following visualisation of PCR products under a transilluminator were re-examined at least twice before being declared negative.

Table 1: Protocol and programme for amplifying the *pfdhps* gene

PCR 1			Nested PCR		
❖ Reaction Mix			❖ Reaction Mix		
Reagents	Volume per tube (µL)	Final concentration	Reagents	Volume per tube (µL)	Final concentration
H ₂ O	10,4	✓	H ₂ O	10,4	✓
PCR Buffer	2,5	10X	PCR Buffer	2,5	10X
MgCl ₂	2,5	2,5 mM	MgCl ₂	2,5	2,5 mM
dNTP	2,5	0,4 mM	dNTP	2,5	0,4 mM
DHPS1-F	1	10pm	DHPS2-F	1	10pm
DHPS1-R	1	10 pm	DHPS2-R	1	10 pm
Taq	0,1	5 U/µL	Taq	0,1	5 U/µL
Total mix	20	✓	Total mix	13,4	✓
DNA extracted	5	✓	Amplicon	2	✓
TOTAL	25	✓	TOTAL	25	✓
❖ Amplification programme of PCR 1 and nested PCR					
N°	Steps	Temperature		Time	
1	Initial denaturation	95°C		5 minutes	
2	Denaturation	95°C		40 secondes	
3	Hybridisation	56°C		40 secondes	
4	Elongation	72°C		2 minutes	
	Go back to step 2, 35 times				
5	Final elongation	72°C		15 minutes	
6	End of programme	15°C		∞	

Digestion at codon 436 of *dhps* gene was carried out using restriction enzymes specific to particular sites within the gene. The endonucleases used were *MnII*, *HhaI*, and *HindIII*. Only PCR products that produced bands in nested PCR were used for enzymatic digestion. Briefly, the digestion reaction mixture for each enzyme was prepared in 1.5 mL tube according to the number of samples to be processed per reaction. 15 µL of the reaction mixture was dispensed into each labelled tube, followed by the addition of 10 µL of amplicon to the corresponding tube. The mixture was gently homogenised before being placed in a water bath at 37 °C for a maximum of 2 hours. The products obtained following enzymatic digestion were also run on a 2% agarose gel for 30 to 40 minutes. After visualisation, the gel image was saved for interpretation. The gel photographic image was analysed by comparing the size of the digestion products with the expected sizes and checking for the absence of bands in the negative controls. The classification of a sample as wild-type or mutant was determined based on the difference in the sizes of the fragments obtained following restriction enzyme digestion. The *MnII* restriction enzyme (concentration of 5,000 units/mL) cleaves the DNA fragment into three bands, two of which (127 bp and 234 bp) are identical across the different phenotypes. The mutant strain was identified by the presence of a 254 bp band, whilst the distinguishing phenotype of the wild-type strain exhibits a 348 bp band. Indeed, the wild-type strain indicates the presence of serine (Ser) in the amino acid sequence of the enzyme system, whilst the mutant form of the gene suggests substitution by alanine (Ala). For codon 436, the *HhaI* and *HindIII* enzymes, at a concentration of 20,000 units/mL, were unable to digest the DNA fragment. With these two enzymes, no polymorphism was observed. The fragment sizes were compared with known restriction fragments (band size) obtained in previous studies, using a 1 kb DNA marker as a reference [18].

3.4 Data analysis

Statistical analyses were carried out using the R software, version 4.0.2, and RStudio, version 1.1.456. Descriptive statistics were used to summarise the patients' characteristics. Quantitative data were expressed as mean ± standard

deviation (SD) or as the median with the interquartile range (IQR). Categorical variables were expressed as frequencies and proportions with 95% exact Clopper-Pearson confidence intervals (CI) using the Hmisc package in R. Pearson's chi-square (χ^2) test of independence or Fisher's exact test (for $n < 5$) was used to determine the association between malaria and associated factors. The prevalence of mutations was determined by grouping together isolates carrying the mutations. In all analyses, the significance level was set at 5%.

3.5 Statement of Informed Consent

Written informed consent was obtained from the participants and from the parents or legal guardians of all children who took part before their inclusion in the study. All health problems were managed in accordance with local standards of care.

4 RESULTS

4.1 Patients' sociodemographic and clinical characteristics

Of the 380 patients included in this study, 195 (51.32 %) were women and 185 (48.68 %) were men, giving a female-to-male (F/M) ratio of 0.95. The patients' average age was 8 years, ranging from 0 and 80 years. The mean axillary temperature measured in the patients was approximately 38.17 °C (± 1.13). Based on the complete blood count results, the mean hemoglobin level was 7.8 (± 3.54) g/dl and 72.90% (191/262). Some patients presented anemia cases according to WHO criteria based on hemoglobin levels (Hb). The threshold for anemia is defined as a hemoglobin level of ≥ 10 g/dl for uncomplicated malaria or malaria without anemia. A statistically highly significant association was observed between anemia and the presence of malaria infection in patients ($\chi^2 = 85.11$; $p < 0.0001$). However, a borderline significant association was observed between fever and malaria infection ($p = 0.055$).

4.2 Parasitological diagnosis

The observation of the parasite in the bloodstream revealed an overall prevalence of 68.95% (262/380), comprising 134 male patients and 128 female patients, with a sex ratio (F/M) of 0.95. The bivariate analysis of the relationship between gender and the occurrence of malaria infection revealed no statistically significant association ($p = 0.113$); (Table 2). Following analysis of the samples, the patients were grouped into three categories based on age, defined according to the levels of immunity acquired by the individuals. The age groups most affected by malaria were children aged 0 to 4 and 5 to 14 (217/301), with prevalence rates of 72.52% and 67.86% respectively. Thus, the 0-4 age group was significantly associated with the occurrence of malaria infection (OR = 1.99; 95% CI: 1.19–3.35; $p = 0.008$).

Table 2: Bivariate analysis of selected factors associated with the prevalence of malaria

Factors	Malaria prevalence in the study population (N=380)				
	Total (N)	positive (n(%))	OR	CI 95%	P
Gender					
Female	196	128 (65.31)	1		
Male	184	134 (72.83)	1.42	0.92-2.21	0.113
Age groups (years)					
≥ 15	79	45 (56.96)	1		
5 to 14	28	19 (67.86)	1.60	0.6-3.96	0.312
0 to 4	273	198 (72.52)	1.99	1.19-3.35	0.008

%; percentage of patients infected; OR: odds ratio (Wald method); 95% CI: 95 per cent confidence interval for the odds ratio; N: number of patients examined

4.3 Molecular diagnostics

Of the samples collected, 175 with a parasitaemia level of 1,000 trophozoites per μ L of blood were selected for genomic DNA extraction and subsequently tested using the PCR technique. Samples showing specific electrophoretic bands following the second round of nested PCR amplification were classified as positive. The expected fragment size was 711 bp (Figure 2). Malaria prevalence following amplification of 175 isolates was 84.57 % (148/175). Of the 148 positive isolates, 75 (50.68 %) were from male patients, compared with 73 (49.32 %) from female patients. With regard to enzymatic digestion, the restriction profiles obtained are shown in Figure 3. The three (03) restriction enzymes used

for the *pfdhps* gene at codon 436 cleaved the 711 bp fragment obtained after the second amplification differently, depending on the specific mutations present at this codon in the *Plasmodium* genome. Of these, only the *MnII* enzyme revealed a polymorphism characteristic of digestion. Out of the 148 samples, we observed 112 (75.68 %) wild-type (native) profiles, and 36 (24.32%) mutant profiles. The other two enzymes showed no digestion activity on the amplified DNA fragment. The distribution of the two genotypes by age showed that the 0-4-year-old age group, which had the largest number of samples (132), accounted for 30 mutant profiles, representing 83.33 % of all mutants (30/36), and 102 wild-type profiles, meaning 91.07 % of all wild-type profiles (102/112).

As for the other two age groups, 4 (11.11 %) mutant profiles and 3 (2.68 %) wild-type profiles were recorded in patients aged 5 to 14 years, compared with 2 (5.56 %) mutant profiles and 7 (6.25 %) wild-type profiles in patients aged 15 years and over. No statistical association was observed between age and the presence of mutant parasites ($p = 0.1196$; Fisher's exact test) (Table 3). With regard to gender, 15 (41.67 %) mutant profiles were observed in women, compared with 21 (58.33 %) in men. As for wild-type profiles, there were 58 (51.79 %) in women compared with 54 (48.21 %) in men.

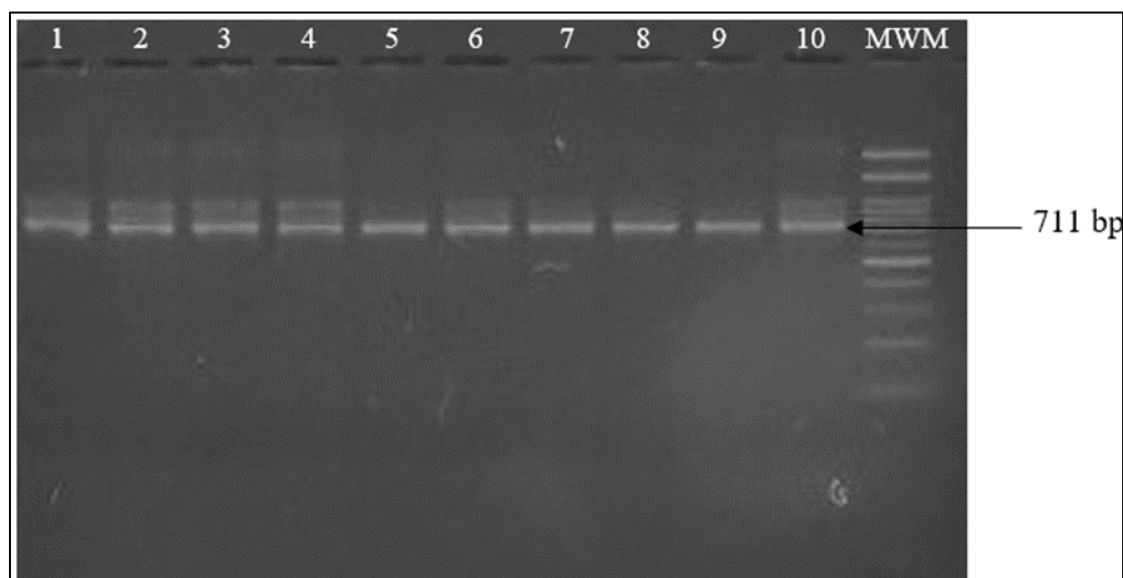


Figure 2: Detection of the products of the second amplification (nested PCR)

1 to 10: sample numbers; MWM: Molecular weight marker; 711 bp: expected fragment size.

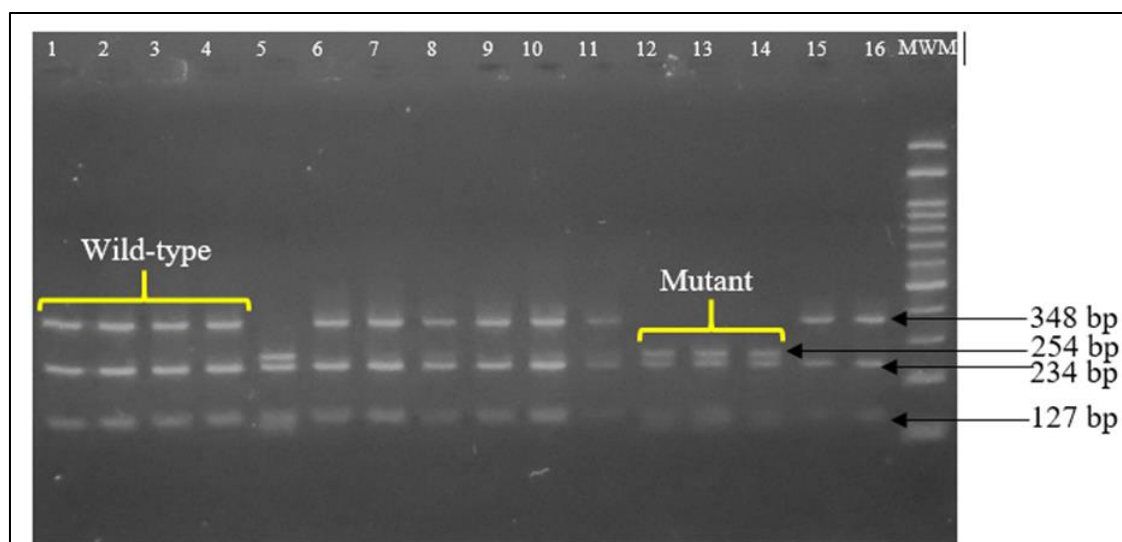


Figure 3: Visualisation of the enzymatic digestion products using the *MnII* enzyme, showing wild-type and mutant profiles

Samples 1 to 4; 6 to 11; 15 and 16 = wild-type profiles; samples 2, 12, 13 and 14 = mutant profiles

The statistical results confirmed that there was no association between sex and the presence of mutant parasites ($p = 0.291$). Table 3 shows that, out of a total of 148 patients with malaria, 30 subjects carrying parasites with a mutant profile and 98 subjects carrying parasites with a wild-type profile had anemia. Anemia therefore does not appear to be associated with carrying mutant parasites ($\chi^2 = 0.40$; $p = 0.525$).

Table 3: Distribution of patients' genotypes by age, sex and anemia

	dhps gene genotypes at codon 436			Statistical test	
Description	Mutant	Wild-type	Total (N)	χ^2	P
Age					Fisher
≥15	2	7	9	0.863	0.1196
5 to 14	4	3	7		
0 to 4	30	102	132		
Sex					Pearson
Female	15	58	73	1.116	0.2908
Male	21	54	75		
Anaemia					Pearson
Yes	30	98	128	0.4047	0.5347
No	6	14	20		

χ^2 : chi-squared; Fisher: Fisher's exact test; Pearson: Pearson's chi-squared test.

The parasite density distribution across the two profiles (Table 4) shows an association between median parasitemia (10,600 Tz/ μ L of blood) and mutations ($p = 0.0102$). The mean hemoglobin level was 5.9 g/dL in subjects with mutant profiles and 6.4 g/dL in patients with wild-type profiles. However, this parameter showed no association with mutations ($p = 0.504$). Similarly, the mean temperature was 38.5 °C in subjects with mutant parasites and 39 °C in those with wild-type parasites; this clinical parameter was also not associated with the presence of mutant parasites ($p = 0.345$).

Table 4: Comparison of clinical and biological parameters by genotype

	dhps gene genotypes at codon 436		Statistical test	
Clinical and biological parameters	Mutant	Wild-type	χ^2	P
Median PD (Tz/ μ L)	10600	1200	6.5981	0.01021
Median T°C	38.5	39	0.8906	0.3453
Median THb (g/dL)	5.9	6.4	0.4456	0.5044
Total	36	112		

THb: Hemoglobin level, PD: Parasite density, T°C: Temperature in degrees Celsius, χ^2 : chi-squared

5 DISCUSSION

Molecular analysis of the *pf dhps* gene at codon 436, carried out on 148 clinical isolates of *Plasmodium falciparum* collected at Daloa Regional Hospital Centre, revealed a prevalence of 24.32 % (36/148) for mutant profiles, compared with 75.68 % (112/148) for wild-type profiles. The finding that a quarter of isolates were mutant at codon 436 in our study therefore warrants particular attention, as it may reflect significant selective pressure resulting from the excessive use of sulfadoxine in the study area. Similarly, the percentage of isolates with the mutant profile could be explained by the predominant proportion of children aged 0 to 4 years, for whom this drug is prescribed for chemoprophylaxis. Sulfadoxine-pyrimethamine (SP), although no longer used as first-line treatment for uncomplicated malaria in many countries, remains widely used in Côte d'Ivoire as part of intermittent preventive treatment for pregnant women (IPTp-SP) and, in certain contexts, for infants (IPT) [19,20]. The rate of mutations at position 436 observed in this study was significantly lower than the 42.9 % reported nationally in children under five by Djaman et al. in 2010 [21]. Furthermore, a study conducted in São Tomé and Príncipe reported a prevalence of codon 436 mutants as high as 58 % [22]. Moreover, the variation in the prevalence of mutants observed across the different study sites could be due to unequal exposure to sulfadoxine.

One of the study's main strengths is that it is one of the first to document, using a molecular epidemiological approach, the profile of resistance to antifolates in this locality. These findings also make a valuable contribution towards addressing the lack of local data. Most previous studies on drug resistance in the country have focused on chloroquine, a compound that for a long time was the first-line treatment for malaria in Côte d'Ivoire [23,24].

Unfortunately, these results must be viewed within certain methodological limitations. Firstly, the analysis focused on a single codon (436) of the *pf dhps* gene, yet clinical resistance to sulfadoxine-pyrimethamine most often results from a combination of haplotypes involving several codons (437, 581, 613, 540) of the *pf dhps* gene. Furthermore, the combination of mutations 436 and 437 in the *pf dhps* gene constitutes the highly resistant haplotype in West and Central Africa [25,26]. A more comprehensive characterisation of these additional codons would enable a better assessment of the actual level of resistance in the locality. Secondly, the PCR-RFLP technique used only allows for a binary classification of the observed profiles (wild-type or mutant). However, more sophisticated analyses enable the detection of polyclonal infections, which, incidentally, are common in areas of high transmission, and, above all, the detection of rare or emerging mutations in the region. This limitation could lead to an underestimation of the actual prevalence of mutants.

With regard to socio-demographic factors, no significant association was observed between sex and the carriage of mutants, suggesting that the biological mechanism of resistance selection cannot be attributed to a susceptibility linked to the host's sex. This result supports the hypothesis that the distribution of mutant genotypes depends more on prior exposure to antifolates and local transmission dynamics than on individual patient characteristics [27,28].

Our results showed that the parasite load in the blood has a statistically significant effect on the presence of the mutation at codon 436 ($p = 0.0102$). This statistically significant association could be explained by a selective advantage conferred by the mutation in the context of drug pressure. Indeed, in patients who have previously been exposed to suboptimal treatment, mutant parasites, that is, those less sensitive to the activity of sulfadoxine, may multiply more efficiently and result in a higher parasite density at the time of diagnosis [29,30]. By contrast, neither hemoglobin levels ($p = 0.504$) nor body temperature ($p = 0.345$) were significantly associated with genotypic status (mutant or wild-type). These findings are consistent with the mechanism of resistance to antifolates, which affects therapeutic efficacy rather than the pathogenicity of the parasite. Furthermore, the mutation at codon 436 alone is insufficient to account for the severity of the infection, which was assessed on the basis of anemia and fever.

The confirmed presence of mutants at codon 436 in this area remains a public health concern. This study highlights the importance of comprehensive and regular molecular monitoring of resistance markers to sulfadoxine-pyrimethamine in the area, as sulfadoxine-pyrimethamine is currently recommended for intermittent preventive treatment in pregnant women and children.

6 CONCLUSION

Clinical and molecular surveillance of malaria in a locality such as Daloa is of paramount importance and represents a significant asset in controlling the endemic disease at national level. Our analysis shows that the detection of mutations in *pf dhps* gene by PCR-RFLP, specifically at codon 436 in the isolates, indicates that the Ser436Ala mutation is a candidate for sulfadoxine resistance in Daloa area. Whilst this study does not address gene amplification or other factors indirectly linked to resistance to antifolates, it provides essential baseline data on the prevalence of a genetic marker of resistance; a foundation that must now be expanded upon to better guide public health policies in Côte d'Ivoire.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

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