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Identification of molecular markers of *Plasmodium falciparum* resistance to antifolates by PCR and sequencing in the Republic of Guinea

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Abstract

Introduction: Malaria remains one of the leading causes of medical consultations in the tropics. Objective: The aim of this study was to determine the types of mutations responsible for *Plasmodium falciparum* resistance to antifolates in Guinea. Material and Methods: This is a prospective study lasting eight (8) months, from October 1, 2022, to May 30, 2023.

RESULTS: The diagnosis of *Plasmodium falciparum* was positive in 5.85% of cases (255/4352) by thick blood smear (SBS) and and the thin blood smear. The molecular method (PCR) applied to the 255 SBS-positive samples showed the presence of *Plasmodium falciparum* DNA was positive in 96.47% of the SBS-positive samples (246/255). In individuals over 5 years of age, mutations were higher in the Pfdhfr gene at codons 51Ile, 59Arg and 108Asn with a prevalence of 28.57% for each. On the contrary, in the Pfdhps gene, the highest mutation was in codon 540Glu (5.71%) followed by mutations 436Ala, 437Gly and 613Ser with 2.85% for each. In children under 5 years of age, mutations were more common in the pfdhfr gene codons: 51Ile (42.85%), 59Arg (42.85) and 108Asn (42.85), while in the Pfdhps gene, mutations were common in codon 540Glu (5.71%), followed by codons 436Ala, 437Gly and 613Ser with 2.85% each. Thus, mutations were more common in the Pfdhfr gene than in the Pfdhps gene. After sequencing, only 14.22% (35/246) *Plasmodium falciparum* samples had mutations in both Pfdhfr and Pfdhps.

Conclusion: These results showed generally that mutations in *Plasmodium falciparum* resistance genes, were more common in dhfr gene than dhps gene and the mutation prevalence of these two genes were different i individuals over 5 yeares and those of under 5 years.

Keywords: *Plasmodium falciparum*; Antifolate Resistance; Mutations; Republic of Guinea

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1. Introduction

Malaria remains and continues to be one of the main causes of medical consultations, morbidity, and mortality, which has made it one of the major concerns of the World Health Organization (WHO) for more than half a century. It is a parasitic disease caused by five plasmodial species: *Plasmodium falciparum*, *Plasmodium malariae, Plasmodium vivax, Plasmodium ovale and Plasmodium knowlesi, which is mainly prevalent in Southeast Asia* [1-5]. *Plasmodium falciparum* is the most formidable and dangerous species of the genus *Plasmodium*, responsible for cases of morbidity and mortality in tropical and equatorial countries [2,3,5]. Thus, this species is often considered to be one of the Plasmodium species associated with the most formidable forms of malaria in the world. Moreover, this parasite has developed resistance to most antimalarial drugs, significantly complicating the treatment and control of the disease. Children under 5 years of age represented 76% of the fatalities [7].

The treatment and eradication of this parasitosis, therefore, require an accurate biological diagnosis. Nowadays, in Guinea, as elsewhere in the world, several techniques are used for the diagnosis of malaria. Thus, these different methods generally used in biomedical analysis laboratories for the diagnosis of malaria are rapid diagnostic tests (RDT), thick blood drop (BG) and blood smears. Molecular methods for diagnosing *Plasmodium* are not usually used routinely. In Guinea, the Ministry of Health, through its national malaria control program, and its development aid and public health partners have introduced antifolates such as sulfadoxine and pyrimethamine (SP) in combination with amodiaquine (AQ) into their malaria control policy. These antifolates are administered to children under five years of age and this operation is known as seasonal malaria chemoprevention (SMC). In pregnant women, antifolates are used as intermittent preventive therapy (ITP) [8]. Thus, in recent years, several studies have demonstrated the appearance of strains resistant to antifolates in endemic areas where CPS and ITP have occurred; hence the importance of using molecular diagnostic tests capable of rapidly and accurately detecting these resistant strains of Plasmodium falciparum. Malaria diagnosis remains a concern for all medical biology laboratories. On a daily basis, this diagnosis poses strategic problems: on the one hand, errors or delays in diagnosis can have dramatic consequences. Furthermore, the use of a single diagnostic method is not without consequences. In recent years, the commercialization of new immunochromatographic techniques known as “rapid diagnostic tests” (RDTs) has opened up new perspectives in malaria diagnosis [9].

After decades of dramatic reductions in malaria cases and deaths worldwide, progress towards malaria control and elimination had stalled before the COVID-19 pandemic and malaria cases and deaths increased in 2020 [10,11]. The World Malaria Report 2022 from the World Health Organization (WHO) states that while malaria cases and deaths remained relatively stable in 2021 after pandemic disruptions, the overall fight against the disease has stalled, and efforts are off track to meet 2030 targets [10]. Further erosion of recent progress in malaria control will lead to resurgences, at great cost to health, lives, and economies in the world's poorest countries [12]. Chemoprevention strategies, i.e. the use of antimalarial drugs for prophylactic and preventive purposes, can be effective tools to control and eliminate malaria, but the risks of resistance to antimalarial drugs used for prevention and treatment must be mitigated and managed to regain and maintain momentum. Chemoprevention strategies currently recommended by the World Health Organization (WHO) include intermittent preventive treatment in pregnancy (IPT), intermittent preventive treatment in infants (IPTNI), seasonal malaria chemoprevention (SMC), and mass drug administration (MDA) to reduce disease burden in emergency settings [13]. Since the time these chemoprevention strategies were first conceived, concerns have been raised both about their potential impact on the development and spread of drug resistance that could compromise the treatment effectiveness of the drug classes, and about the impact of drug resistance on the effectiveness of different chemoprevention strategies [13].The increase in Plasmodium falciparum (*P. falciparum*) infections associated with severe and complicated malaria and drug resistance have made malaria control challenging. Significant genetic polymorphism in P. falciparum has been reported in several regions of the world, which affects the efficacy of subunit vaccines. Knowledge of parasite genotypes in a geographical region is therefore important for effective management and control [6]. Having an effective treatment for malaria is key to achieving the goals set by the WHO through the Global Technical Strategy for Malaria 2016– 2030. Artemisinin-based combination treatment associates short-acting artemisinin with a longer-acting partner drug to reduce the emergence of resistance [14].

Malaria, which occurs mainly in tropical and subtropical areas, is the leading cause of consultation in sub-Saharan Africa, including Guinea, where this disease is endemic and remains one of the leading causes of consultations in health facilities. The awareness campaigns, screening, and mass treatment organized by the Ministry of Health and Public Hygiene of Guinea through the national malaria control program supported by development partners including the World Health Organization (WHO), USAID, the United Nations Development Program (UNDP), etc., illustrate its endemicity. The objective of this research is to determine the types of mutations responsible for the resistance of *Plasmodium falciparum* to antifolates (SP) in the Republic of Guinea.

2. Materials and Working Methods

This study was conducted in the Republic of Guinea. The Microbiology laboratories of the Faculty of Sciences and the Faculty of Health Sciences and Technology of Gamal Abdel Nasser University in Conakry, and the biomedical laboratory of the Sino-Guinean Friendship Hospital in Kipé, served as the framework for this study. This is a prospective study lasting eight months, from October 1, 2022, to May 30, 2023. The sampling was simple random, and the sample size (n=4352). All children aged 3 to 59 months were included in this study on the one hand and people aged over 5 years on the other. All these people were residents in the seasonal malaria chemoprevention (SMC) zones in the Republic of Guinea.

3. Variables under study

3.1. Biological variables: Thick blood smear, Blood smear, Mutations

3.1.1. Epidemiological variables: Age, Sex

II-4 Biomaterial Our biomaterial consisted of blood samples, three drops collected on filter paper or confetti (Whitman 3MM), with a total volume of 50 µL each. The number of participants in our study was 4,352, including 4,152 in the seasonal malaria chemoprevention zone of Siguiri Prefecture, which is densely populated due to mining and is one of the first prefectures to benefit from the SMC since 2015. In addition, 200 samples were collected from health centers in other prefectures benefiting from the SMC.

3.1.2. Working Method

II-5-1 Type and Duration of Study: This is a prospective study lasting eight (8) months, from October 5, 2022, to March 30, 2023.

3.1.3. Study Population

Our research involved a total sample of 4,352 individuals, including children aged 3 to 59 months and individuals aged over 5 years, distributed as follows: 4.152 samples collected in the community and 200 samples collected in health facilities.

3.1.4. Sampling

In communities and health facilities, sampling was simple random, and the sample size (n=4352) was obtained using the Schwartz formula (2016) based on the prevalence of malaria in the Republic of Guinea P=15%).

The sample size collected in the study was calculated using the formula.

- n= t2 x p (1-p)/m2
- t= 95% risk of error (1.96)
- P= national malaria prevalence (15%)
- m= margin of error (5%)
- n= 1.962 x 0.15(1-0.15)/0.052 = 196

Our sample size (n=4352) is well above the minimum accepted sample size, making our sample highly representative.

3.2. Selection Criteria

3.2.1. Inclusion Criteria

This study included all children aged 3 to 59 months, as well as individuals aged over 5 years and residing in seasonal malaria chemoprevention (SMC) areas in the Republic of Guinea.

3.2.2. Exclusion Criteria

All individuals who did not meet our inclusion criteria were excluded from our study.

3.3. Study Variables

3.3.1. Biological Variables

The biological variables in our research are: Goutte épaisse, Frottis sanguins, and mutations in Pfdhfr and Pfdhps.

3.3.2. Epidemiological Variables

The epidemiological variables in this research are: Age, Sex

4. Operational Variables

4.1. Sampling

The samples were taken from the third or fourth finger of the left hand on the side, which is less sensitive than the fingertip. The punctured areas were first disinfected using a cotton swab soaked in 70° alcohol, followed by a dry swab to remove all traces of alcohol. After a quick prick with a vaccinostyle, the first drop of blood was wiped off with a dry cotton swab. The following drops of blood were spread thickly in the middle of a slide. This drop of blood was spread with the corner of a clean slide until uniformly thickened.

On a second slide, a second drop of blood was gently placed at one end of the slide. Holding the slide in one hand, the edge of the ground slide was placed just in front of the blood drop with the other hand, then slid until it touched the blood drop. The blood was then spread all along the edge of the ground slide. With a gentle, even movement, the ground slide was pushed to the end of the smear slide. The sample smears were then dried at room temperature, before being stored in storage boxes for Giemsa staining and reading under a microscope. Rapid diagnostic tests were performed on all samples. From each RDT-positive sample, three drops of 50µL of blood each were collected on filter papers or confetti for the search for molecular markers. Each sample collected on confetti filter paper was packed in a plastic bag with silica gel to protect it from moisture.

4.2. DNA Extraction from Blood Samples Collected on Filter Papers Using a DNA Extraction Machine

Blood samples collected on filter papers (confetti) are first scanned on a computer in barcode order and in batches of 96 samples according to the test plates containing 96 reaction wells.

4.2.1. DNA extraction procedure

The filter papers containing the blood sample drops were cut using a scissor punch previously treated with 70% methanol and flamed with a Bunsen burner for each sample. The filter papers containing the blood samples were cut into circles with a diameter of 2 mm. Each sample (small circle) was collected in a well of a reaction microplate containing 96 samples. A volume of 180 µL of DNA extraction buffer solution and 20 µL of protein kinase K enzyme were added to each well corresponding to a sample.

The microplate was then placed in a thermo-mixer for 15 min at 56°C and 900 rpm. The reaction microplate was then transferred to the DNA extraction room. The reagents were placed in the DNA extraction machine. The reagent and consumables boxes were opened successively. The reagents and consumables were placed in the corresponding boxes. The operation was validated by pressing the scan button. The eluent box was opened, along with a new 96-well reaction microplate intended to collect the DNA from the samples after extraction. The wash solution collection reservoir was checked and emptied, if necessary, then scanned.

DNA extraction was performed in the 96-well microplates for 4 hours after the introduction of all the necessary process elements. The DNA extraction products were then collected in a new microplate before being brought to the PCR room.

4.2.2. Study Design

The workflow of this research was designed. The starting point of this work was the collection of blood samples in the CPS areas of Guinea, according to the approval of the Ethics Committee for Science and Research in Guinea. A total of 4352 blood samples were collected in the community and health centers. All participants underwent the rapid diagnostic test (RDT). Subsequently, these RDT-positive samples were analyzed by microscopy (thick drop and blood smear), this allowed us to establish the parasite density and to know the plasmodial species involved. Molecular methods were applied to the RDT-positive samples. Then we collected three drops of 50µL of blood each from the same RDT-positive samples on filter papers or confetti for the search for molecular markers. From the blood drops collected

on filter paper we extracted genomic DNA for each sample using Unclasping Blood mini kit. Then all samples were subjected to a DNA amplification technique, PfSNP-LAMP-LFD (detection of single nucleotide polymorphism of Plasmodium falciparum by Loop-mediated isothermal amplification combined with detection by lateral flow strip) followed by conventional PCR.

4.2.3. Primer sequence and reaction conditions for PCR and nested PCR for Pfdhfr and Pfdhps genes.

For the Pfdhfr gene, the primers used for the detection of SNPs in codons 50, 51, 59, 108, and 164 were: forward primer (5'-TTTATGATGGAACAAGTCTGC-3') and reverse primer (5'-CTAGTATATACATCGCTAACA-3'); the PCR product size was 650 bp. The amplification conditions used were: 95°C for 5min, 94°C for 30s, (54°C for 60s, 65°C for 60s) x 41 cycles; 65°C for 5min, 15°C for 5min. Nested F (5'-CTGGAAAAAATACATCACATTCATATG-3') and Nested R (5'-TGATGGAACAAGTCTGCGACGTT-3') and the amplification product size was 594bp. The amplification conditions: 95°C for min, (93°C for 30s, 68°C for 75s) x 30 cycles, 75°C for 5min.

For the Pfdhfr gene amplified for the detection of SNPs in codons 50, 51, 59, 108 and 164, the primers used were Forward primer (5'-GATTCTTTTTCAGATGGAGG-3') and Reverse primer (5'-TTCCTCATGTAATTCATCTGA-3') for an amplified product size of 770 bp. The amplification conditions were: 93°C for 5min, (93°C for 20 seconds, 55°C for 30 seconds, 68°C for 75 seconds) x 40 cycles; 68°C for 5min, 15°C for 5min; Nested F primer (5'-AACCTAAACGTGCTGTTCAA-3'), Nested R (5'-AATTGTGTTGATTTGTCCACAA-3'). The amplified sequence size was 711bp with the amplification conditions were 93°C for 5min, (93°C for 30 seconds, 56°C for 30 seconds, 68°C for 75 seconds) x 30 cycles, 72°C for 5min, 15°C for 5 min.

In these techniques we used plasmids like, Puc-18-Pfdhfr-TM/8.2 containing wild type Plasmodium falciparum as negative control and Puc-18-Pfdhps-V1/S containing mutant type Plasmodium falciparum as positive control.

The DNA samples amplified by LAMP technique and by conventional PCR were subjected to electrophoresis on agarose gel and the amplified DNA fragments were visualized under ultraviolet rays after staining the gel with an ethidium bromide solution. Finally, all the positive samples were purified and subjected to sequencing and the chromatograms obtained after sequencing were processed by BioEdit and then by Nucleotide Blast to compare the sequences of the samples to the non-mutated sequence of Plasmodium falciparum to detect the different mutations and their positions. Detection of the Pfdhfr-ts gene by LAMP-LFD

Many attempts have been made to design SNP-LAMP primers to selectively determine the S108N point mutation, but in an AT-rich and highly repetitive polynucleotide sequence, it was not possible to obtain a specific primer to distinguish between wild-type S108 and the mutant-type N108. Therefore, another point mutation conferring resistance to the antifolate pyrimethamine, N51I, was selected for primer design.

Parasites with the highest resistance to pyrimethamine were analyzed by LAMP using specific primers and showed a general N51I mutation. Expression of a mutation in the Pfdhfr gene was detected by RT-LAMP-LFD. The Pfdhfr gene primer sets were designed against the mutated Pfdhfr sequence of chromosome 4 of Plasmodium falciparum 3D7 (GenBank accession number NC_004318, version NC_004318.2).

The LAMP primer set contains four individual primers, one of which is biotin-labeled (FIP). The primer and probe set used for the detection and amplification of the SNP in the Pfdhfr-ts gene at nucleotide position 152 (Asn51), associated with pyrimethamine resistance, by LAMP-LFD. The bold and underlined nucleotide 5' of the forward Pf-SNP-FIP and the reverse Pf-SNP-BIP correspond to the SNP associated with antifolate resistance, an SNP substitution of an adenine (A) to thymine (T) at position 152. The bold nucleotide represents the changes in the oligonucleotides that were modified to optimize the thermodynamic properties of the primer. The primers used for the Pfdhfr-ts gene were (Sequence primers 5'-3') Pf-SNP-F3 (GATGGAACAAGTCTGCGACG), Pf-SNP B3 (GCTTTCCCAGCTTGTTCTTCC), Pf-SNP FIP (F1C-F2 (ATACATTTCCATGGTAATACTCTTTTTCTACACATTTAGAGGTC), and TTTCCCTAGATACGACATATTTTTCAATTTTCCATATTTCGATTCATTC). The probe used was Pf-FITC probe (GTGCAGTTACAACATATGTGAATG).

4.2.4. PfSNP-LAMP-LFD Conditions

The PfSNP-LAMP reaction for mutation detection in Pfdhfr-ts was performed in a total reaction volume of 25 µL containing the following components: 2 µM each for Pf-SNP FIP and Pf-SNP BIP primers, 0.2 µM each for Pf-SNP-F3 and Pf-SNP-B3 primers, 1X reaction buffer provided by Thermopol (20 Mm Tris-HCl, 10 Mm (NH4)2SO4, 10 Mm KCl, 2 Mm MgSO4, 0.1% TritonX-100, pH 8.8), 0.4 M betaine (USB Corporation, Cleveland, OH-USA), 8 mM MgSO4 (Sigma Aldrich, St. Louis, MO, USA), 1.4 mM sorbitol (Sigma Aldrich, St. Louis, MO, USA), and 0.5 mM sorbitol (Sigma Aldrich, St. Louis,

MO, USA). of dNTP (Promega, Madison, WI, USA), 8 U of Bst 2.0 Hot Start DNA Polymerase (New England Biolabs, Ipswich, MA, USA), and sterile distilled water.

A mixture of all reagents and primers was freshly prepared and aliquoted to 23 µL per reaction to perform the LAMP reaction. Then, 2 µL of sample was added to the 23 µL reagent mixture solution to obtain a final volume of 25 µL per reaction. The reaction was incubated at a constant temperature of 65°C for 60 minutes. The negative control (NC) water was performed under the same conditions without the addition of sample or DNA template, and the final volume was adjusted to 25 µL by adding 2 µL of distilled water. Our tests included the wild-type pfdhfr gene as well as non-*Plasmodium falciparum* control genes.. The positive control was performed under the same conditions using 2µl of Pfdhfr mutant gDNA or plasmid DNA containing the Pfdhfr mutant type (pUC18-Pfdhfr-V1/S). The reactions were incubated in a real-time turbidimeter (Turbidimeter Loop AMP Real-time LA-200TERAMECS) and also in the heating or thermal block (SHB200D Stuart) at a constant temperature of 60°C, 61°C, 62°C and 63°C, for 60 minutes, 70 minutes, 80 minutes and 90 minutes.

Table 1 Components of the PfSNP-LAMP reaction

LAMP reaction components	**Sample components (µL)**
SDW	10.9µL
10 µM F3	0.05µL (0.2µM)
10 µM B3	0.05µL (0.2µM)
100 µM FIP	0.5µL (2.0µM)
100 µM BIP	0.5µL (2.0µM)
10X Thermopolis Buffer	2.5 µL (1X)
10 Mm dNTPs	3.5µL (1.4mM)
100 mM $MgSO_4$	2µL (8.0mM)
5 M Betaine	2µL (0.4M)
8 U/µM Bst polymerase	1µL (1U)
DNA template	2µL
Total volume	25.0µL

4.2.5. Mutation Detection in PfSNP-LAMP-Amplified Samples Using the Lateral Flow Strip (LFD)

Hybridization and visualization of PfSNP-LAMP-amplified samples using the lateral flow strip were performed as follows:

A custom-synthesized FITC-labeled probe (Pf-FITC probe) was used. This probe was further used for specific hybridization with the loop region of LAMP-amplified products.

Lateral flow strips (LFD) and HybriDetect assay buffer were purchased from Milenia® GenLine, HybriDetect, GieBen, Germany. The LFD strip is embedded with biotin ligand on the test line and anti-rabbit antibodies on the control line.

The assay buffer contains a polyclonal rabbit anti-FITC antibody conjugated to the gold particle. The LFD strip and a microfuge tube should be labeled for each sample to be tested. Then, 120µL of HybriDetect assay buffer were aliquoted into individual microcentrifuge tubes and kept at room temperature. For hybridization of LAMP-amplified samples, 20 pmol of Pf-FITC probe was mixed with 8µL of LAMP product, followed by adding 120µL of assay buffer to the appropriate LAMP reaction tube and allowing hybridization at the single temperature 62°C for 5 min. For detection of LAMP-amplified samples containing the mutation on the lateral flow strip (LFD), we had aliquoted 8µL of the hybridized products (FITC probe and biotin-labeled LAMP amplicons) into the appropriate microcentrifuge tube containing the prepared hybridization assay buffer. Immerse the LFD strip's "sample pad" in the appropriate sample solution for 5 minutes. The sample solution migrates by capillary action through the test line and the control line. Finally, we removed the strip from the sample solution and interpreted the results by observing the bands that appeared on the test line and/or the control line.

4.3. Amplification of Pfdhfr (partial gene) by conventional PCR for DNA sequencing

4.3.1. Components and conditions of the conventional PCR reaction

The presence of SNPs in *P. falciparum* Pfdhfr-ts was detected using a conventional PRC T100 thermocycler, Biorad, USA. The amplification condition was performed in a 25 µL reaction volume consisting of 12.5 µL GoTag Green Master Mix solution (Promega, Medison, USA) which contains Taq DNA polymerase, 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dNTP and 3 pM MgCl2, 0.5 µL forward primer, 0.5 µL reverse primer, 1 µL DNA template and 10.5 µL distilled water, as shown in Table 3.4. The thermal cycle was programmed for 2 min at 95°C for initial denaturation, followed by 35 cycles of 1 min for denaturation at 95°C, 1 min at 51.4°C for primer binding to the template and its amplification, and 1 min at 72°C for extension, followed by 5 min at 72°C for final extension and maintained at 12°C.

The primers and amplification conditions for the Pfdhfr gene are listed below: the sequence GAT GGA ACA AGT CTG CGA- CGT TTT CG served as the Forward primer (5'-3') and the sequence CCC AAG TAA AAC GAT TAG- ATC TTC AAC TTT served as the Reverse primer (5'3'). PCR reactions were carried out in a 25µl reaction volume. The thermal cycle was: -Initial denaturation: 95°C for 2 min whith 35 cycles of Denaturation at 95°C for 1minute, Annealing: 51.4°C for 1minute, Extension: 72°C for 1-minute, Final extension: 72°C for 5 minutes. Holding 12°C for; The expected PCR product size was equal to 460bp.

4.3.2. Detection of PCR Products by Agarose Gel Electrophoresis

A 1.5% agarose gel was prepared by dissolving 1.5 g of agarose powder (Vivantis Bhd, Malaysia) in 1XTAE buffer (40 mM Tris-HCl, 40 mM acetic acid, 25 mM EDTA, pH 8.0). The PCR products were then examined by electrophoresis at 75 volts for 40 minutes in a 1.5% agarose gel (Vivantis, Bhd, Malaysia) in 1X TAE buffer (Tris-acetate + EDTA). The DNA marker used in this study was the Ruler 100 bp plus gene supplied by Thermo Scientific.

The gel electrophoresis was soaked in ethidium bromide for 10 minutes, and then the DNA bands were visualized under ultraviolet (UV) light (Clarechemical.com DR-46B transilluminator; 12VDC, 0.75A, 9W, serial number 13005-1) to detect the size and density of DNA bands. A DNA marker, a positive control, and a negative control were included in each run.

4.4. Pfdhfr Amplification by Gradient PCR for Sequencing

4.4.1. Gradient PCR Primer Design

The Pfdhfr forward and Pfdhfr reverse primers were designed for amplification of the entire Pfdhfr gene using gradient PCR. To amplify the intact dhfr gene, the forward primer 5' upstream of the start codon (ATG) and 3' downstream of the stop codons (TAA) were designed. As the Pfdhfr sequence is an A-T-rich sequence, the forward and reverse primers were manually designed based on the possible primer region. Conventional PCR and PfSNP-LAMP-LFD revealed a single point mutation at position 152 in codon 51 for each sample tested. Therefore, gradient PCR and DNA sequencing were performed using the same samples previously subjected to PfSNP-LAMP-LFD and DNA sequencing to verify whether other point mutations could be found alongside the mutation in codon 51 at nucleotide position 152.

4.4.2. Components and Conditions of the Gradient PCR Reaction

The entire Pfdhfr-ts gene was amplified by gradient PCR using (Thermocycler T100, by Thera Trading No. 186-1096 Biorad, USA). PCR reactions were performed in 25 µL of the mixture containing 12.5 µL of GoTag Green Master Mix solution (Promega, Medison, USA) which contains Taq DNA polymerase, 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dNTP and 3 µM MgCl2), 1.25 µL of forward primer volume, 1.25 µL of reverse primer volume, 1.1 µL of DNA template and 9.0 µL of distilled water, as shown in Table 3.7. All PCR reactions were performed in 25 µL with 35 cycles, initial denaturation at 95°C for 2 minutes and 95°C for 1 minute. The primer-template annealing temperature was 51.2°C at the back and 45°C at the front of the amplifier for 1 minute, extension at 72°C for 2.15 minutes, then a final extension at 72°C for 5 minutes, followed by holding at 12°C for. For this PCR, the expected product size was 1972 bp when using the forward and reverse primer 1 and using the forward and reverse primers 2. The probable size of our amplicon after PCR was 1972 bp when using the forward and reverse primer 1 and using the forward and reverse primer 2, the PCR product size was 869 bp. Detection of Gradient PCR-Amplified DNA Samples by Agarose Gel Electrophoresis

In this study, agarose gel electrophoresis was used to analyze gradient PCR-amplified products. A 1.5% agarose gel was prepared by dissolving agarose powder (1.5 g) weighed using a Precisa 205ACS SWISS (ISO 9001 grade) in 1XTAE (40 mM Tris-HCl, 40 mM acetic acid, 25 mM EDTA, pH 8.0) with heating. The amplified PCR products were electrophoresed at 75 volts for 40 minutes in a 1.5% agarose gel (Vivantis, Bhd, Malaysia) in 1X TAE (Tris-acetate + EDTA) buffer. DNA

bands in the gel were stained with SYBR safe green, a DNA intercalating agent that acts as a fluorescent marker. Amplicon bands were visualized under ultraviolet (UV) light using (Clarechemical.com trans illuminator DR-46B; 12Vdc, 0.75A, 9W, serial number 13005-1) to detect the size and density of DNA bands. One DNA marker, one positive control, and one negative control were combined in each run. Gradient PCR amplified products were purified using the FavorPrep gel extraction and PCR product purification protocol. In addition, to increase the sample DNA concentration for sequencing, DNA from each sample was precipitated using the MRC-Holland (MLPA°) protocol (ethanol precipitation of DNA, description version 01; 24-12-2008).

4.4.3. DNA Sequencing

All samples confirmed positive, i.e., containing *Plasmodium falciparum* DNA using our various techniques mentioned above, were subjected to sequencing (direct Pfdhfr and Pfdhps genotyping using BigDye Terminator v3.1 cycle sequencing kits and ABI 3730 sequencer from Thermo Fisher Scientific, and the data were analyzed using Geneious v10.1.3 (Biomatters, San Diego, CA, USA).

Finally, all positive samples by PfSNP-LAMP-LFD were confirmed by DNA sequencing. And finally, we compared the results obtained by the PfSNP-LAMP-LFD method and the DNA sequencing of the analyzed samples and then aligned them with the non-mutated sequence to see the mutation points.

5. Results

Amongst the 4352 subjects included in this study, 255 samples were positive for *Plasmodium falciparum* by thick drop. Then all the 255 samples positive for *Plasmodium falciparum* were submitted to PCR. The results showed that that after PCR in 255 samples positive by thick drop, 246 were found to be positive for Plasmodium falciparum DNA (246/255 or 96.47%). Thus, extrapolation of the PCR results to all 4352 subjects included in the study shows that 5.65% of the subjects were found to be positive for Plasmodium falciparum DNA.

Table 2 Distribution of *Plasmodium falciparum* mutant genotypes in children under 5 years of age versus individuals over 5 years

Mutant genotypes *of Plasmodium falciparum* genotypes mutant	**Children under 5years**	**Individuals over 5 years**	**Total**
	Number (%)	Number (%)	Number (%)
dhfr 51Ile	15 (42.85)	10 (28.57)	25(71.42)
dhfr 59Arg			
dhfr 108 Asn			
dhps 436 Ala	1 (2.85)	1 (2.85)	2 (5.71)
dhps 437Gly	1(2.85)	1 (2.85)	2 (5.71)
dhps 540 Glu	2 (5.71)	2 (5.71)	4 (11.42)
dhps 613Ser	1 (2.85)	1 (2.85)	2 (5.71)
Total	20 (57.14)	15 (42.85)	35 (100)

In table 2, we observed that in children under 5 years of age, mutations occurred in the *Plasmodium falciparum* pfdhfr gene: 51Ile (42.85%), 59Arg (42.85%), and 108Asn (42.85%). While in the Pfdhps gene, we successively observed the following mutations: 436Ala (2.85%), 437Gly (2.85%), 540Glu (5.71%), and 613Ser (2.85%). Therefore, mutations occurred in the Pfdhfr gene more than in the Pfdhps gene.

In individuals over 5 years old, mutations are higher in Pfdhfr at codons 51Ile, 59Arg and 108Asn with 28.57% prevalence for each. On the contrary, in Pfdhps the highest mutation was observed in codon 540Glu (5.71%) followed by mutations 436Ala, 437Gly and 613Ser with 2.85% for each.

Table 3 Distribution of *Plasmodium falciparum* mutant genotypes according to age groups

N°	Age groups	Number	Percentage
1	Under 5 years	20	57.14
2	Over 5 years	15	42.85
Total		35	100

In Table 3, we see the presence of *Plasmodium falciparum* mutations in children under 5 years old and in people over 5 years old. This Table 9 shows us that 20 children under 5 years old presented a mutant genotype, i.e. 57.14%, compared to 15 individuals aged over 5 years old, i.e. 42.85%.

Table 4 Prevalence of *Plasmodium falciparum* mutant genotypes relative to the number of *Plasmodium falciparum* positive samples

N°	Age groups (years)	Number	Positive cases	Percentage
1	< 5 yars	246	35	14.22
2	More than 5			

After sequencing, only 35 samples out of 246 *Plasmodium falciparum* samples showed mutations in Pfdhfr and Pfdhps, giving a total prevalence of 14.22%, as summarized in the table opposite.

Table 5 Distribution of Plasmodium falciparum resistance markers to Sulfoxide and Pyrimethamine

Resistance markers	Number	Percentage	IC à 95%
Pyrimethamine: *Pf*dhfr 51I 59R 108N (Haplotype IRN)	25	71.42	[69.60-77.60]
Sulfoxide: *Ph*ips 436Ala 437Gly 540Glu 613Ser (Haplotype AGES)	10	28.57	[26.88-33.59]
Pyrimethamine + Sulfoxide (SP) (Haplotype IRN -GE)	5	14.28	[13.4-15.98]

In table 5 we note that mutations in codons 51I, 59R and 108N appeared in 71.42% of the samples analyzed after sequencing in the Pfdhfr gene (Haplotype IRN: Triple mutation). While in the Pfdhps gene the mutations occurred in codons 436Ala, 437Gly, 540Glu and 613Ser (Haplotype AGES: Quadruple mutation) with a prevalence of 28.57%. Finally, 5 samples presented mutations in both Pfdhfr and Pfdhps genes (Haplotypes IRN–GE: Quintuple mutation), with 14.28%.

Table 6 Distribution of the prevalence of mutations associated with resistance to Pyrimethamine and Sulfoxide according to CPS areas in relation to the number of positive cases after sequencing (n=35)

Zones CPS	Pyrimethamine resistance (Haplotype: IRN) Number (%)	Resistance Sulfadoxine (Haplotype: AGES) Number (%)	Resistance to S+P (Haplotype: (IRN- GE) Numer (%)
Siguiri	4(11.42)	3(8.57)	1 (2.85)
Madiana	2 (5.71)	0	0
Kankan	2 (5.71)	0	0
Kouroussa	1 (2.85)	1 (2.85)	1 (2.85)
Faranah	1 (2.85)	0	0
Dabola	1 (2.85)	0	0
Dinguiraye	3 (8.57)	1 (2.85)	0
Mamou	1 (2.85)	2 (2.85)	1 (2.85)
Tougué	1(2.85)	0	0
Dalaba	1 (2.85)	0	0

Pita	1 (2.85)	0	0
Labé	2 (5.71)	1 (2.85)	1 (2.85)
Koubia	1 (2.85)	0	0
Lélouma	1 (2.85)	0	0
Mali	1 (2.85)	1 (2.85)	0
Gaoual	1 (2,85)	0	0
Koundara	1 (2.85)	1 (2.85)	1 (2.85)

Legend: P: Pyrimethamine; S: Sulfadoxine

All CPS zones, the seventeen prefectures each presented at least one mutation in Pfdhfr, either in pfdhps or both at the same time. The prefectures most affected by the resistance of *Plasmodium falciparum* to pyrimethamine are respectively Siguiri (11.42%), Dinguiraye (8.57%) followed by Kankan, Mandiana and Labé (5.71%) and the others with each (2.85%). The mutations produced in the Pfdhps gene at codons 436Ala, 437Gly, 540Glu and 613Ser mark the resistance of *Plasmodium falciparum* to sulfadoxine were observed in the prefectures of Siguiri (8.57%) followed by the prefectures of Kouroussa, Dinguiraye, Mamou, Labé, Mali and Koundara with each a prevalence of 2.85%. The rest of the prefectures did not present any mutation responsible for the resistance of *Plasmodium falciparum* to sulfadoxine. Mutants responsible for the resistance of *Plasmodium falciparum* to pyrimethamine and sulfadoxine were observed in the prefectures of Siguiri Kouroussa, Mamou, Labé, Koundara with a prevalence of (2.85%) for each. Finally, resistance to pyrimethamine and sulfadoxine was not observed in the other prefectures of the CPS zone.

Table 7 Distribution of the prevalence of Pf resistance markers to Pyrimethamine and Sulfadoxine according to age groups

Age groups	**Markers**	**Haplotypes**	**Number**	**Percentage**
3 à 59 months	Pyrimethamine	IRN	15	42.85
	Sulfadoxine	AGES	5	14.28
	S + P	IRN-GE	3	8.57
More than 5 years	Pyrimethamine	IRN	10	28.57
	Sulfadoxine	AGES	5	14.28
	S + P	IRN-GE	2	5.71

Legend: P: Pyrimethamine; S: Sulfadoxine

In this table we see that all children from 3 to 59 months presented molecular markers responsible for the resistance of *Plasmodium falciparum* to pyrimethamine (15/35) or 42.85%. However, 0.14% (5/35) of them presented resistance to sulfadoxine and finally 8.57% (3/35) presented resistance to both pyrimethamine and sulfadoxine. In individuals over 5 years, we see that 28.57% (10/35) are resistant to pyrimethamine, while 14.28% (5/25) were resistant to sulfadoxine and only 5.71% (2/35) were resistant to the combination of sulfadoxine and pyrimethamine.

Table 8 Result showing DNA concentration after cloning and transformation of competent *Escherichia coli* cells by plasmids (wild type and pfdhfr gene mutant)

DNA	**Concentration(ng/µL)**	**OD_{260}**	**OD_{260}/OD_{230}**	**OD_{260}/OD_{280}**
pUC18-TM/8.2 (Wild type)	449.365	8.9873	1.97	1.92
pUC18-V1/S (Mutant)	265.196	5.3039	1.81	1.86

Result of DNA amplification of *Plasmodium falciparum* (wild type and mutant type) which served as positive and negative control in our study.

After 100 min of incubation at 63°C in the real-time turbidimeter, the mutant type was successfully amplified; starting amplification time equal to 59.30 minutes, with a crest value equal to 0.100. And in contrast the wild type was not amplified.

As we obtained good results at 63°C, during the amplification of the wild type and mutant type, we studied the same conditions but at four different temperatures to amplify our samples. Our samples were incubated with the wild type (TM4/8.2) in equal volumes (1µl) at 60°C, 61°C, 62°C and 63°C for 100 min, as a result, all wild-type samples were amplified. temperatures. We found that at 60° C the amplification is fastest (47.57 min.) But the yield is lower, the value of crest equal to 0.092. At 61°C and 62 ° C, the amplification time and yield or amount of LAMP amplified product are close to each other, with respectively an amplification time equal to 60.48 min., Equal crest value of 6 min and equal cr value of 0.524 0.127. At 63°C, the amplification is the slowest (86.12min.), But the yield is higher than those at other temperatures. Because we needed a faster condition that also gave more LAMP yield, we concluded that temperatures of 61°C and 62°C were the best conditions for performing SNP-LAMP reactions.

Our samples were incubated with *Plasmodium falciparum* wild-type (TM4/8.2) as a negative control and *Plasmodium falciparum* mutant type (V1/S) as a positive control, respectively, at 60 °C and 62 °C for 100 min. The result showed the amplification of the mutant type (V1/S), and the amplification of the samples at 61°C and at 62°C, the onset points of the amplification times are shorter, the yield of the reactions is higher at any amount of matrix. Consequently, there was no significant difference regarding PfSNP-LAMP results at 61°C and 62°C.

Furthermore, we also analyzed the same samples by incubating at 60°C, 61°C, 62°C and 63°C for 100 min with the wild type (TM4/ 8.2) as a negative control. In summary, only the samples were amplified at the four different temperatures while the wild type was not. Taken together, we confirm that 61°C and 62°C are optimal temperatures for amplifying *Plasmodium falciparum* mutant-positive samples by SNP-LAMP.

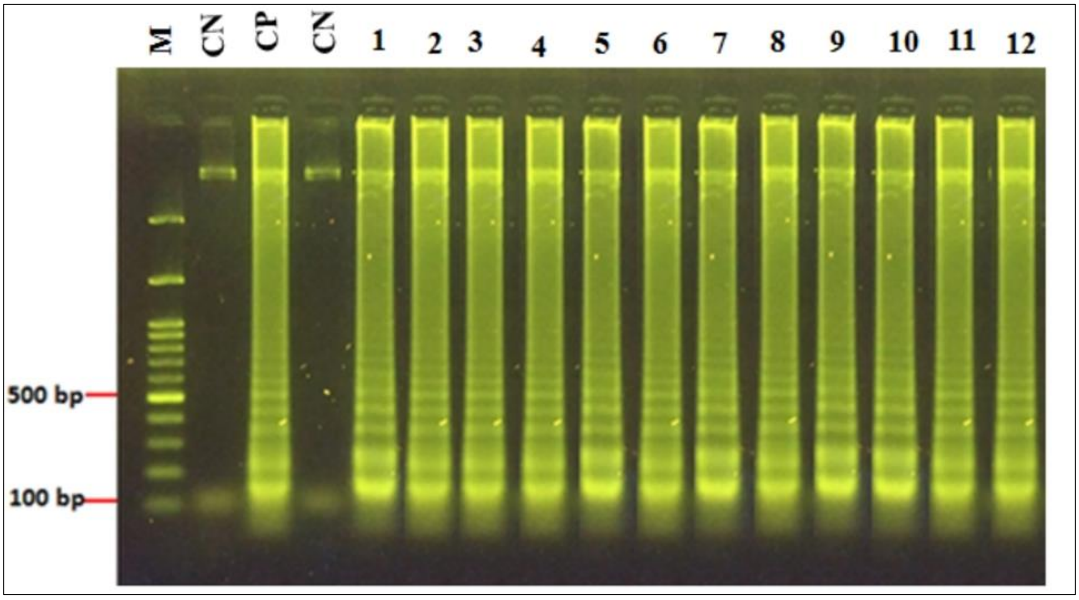

Figure 1 Result of sample amplification by the PfSNP-LAMP method shown in 2% agarose gel after electrophoresis

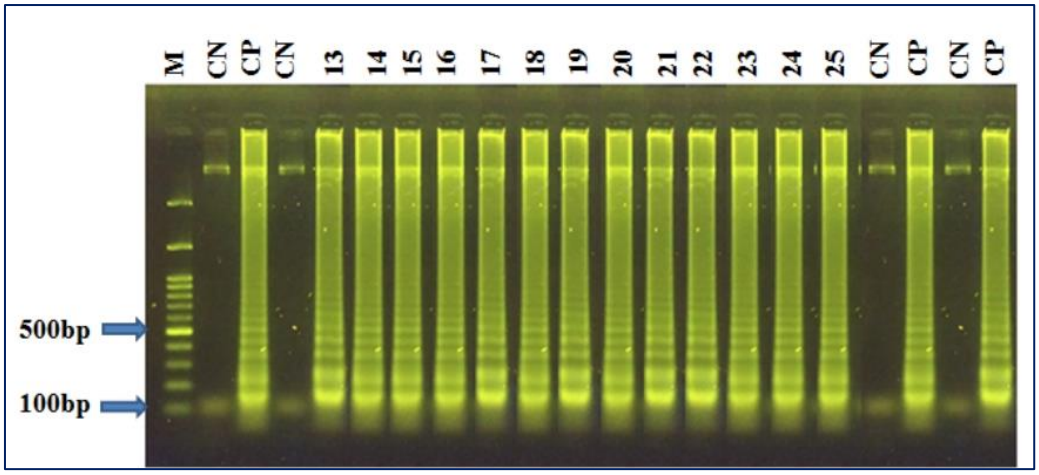

Figure 2 The result of sample amplification by the PfSNP-LAMP method was shown in 2% agarose gel after electrophoresis

6. Discussion

6.1. Technique of PCR and Sequencing.

The primary objective of this study was to detect mutant *Plasmodium falciparum* by the PCR technique as well as by the PfSNP-LAMP-LFD technique using samples from CPS areas, all while exploring the robustness of the method under these conditions. Our results demonstrated the detection of mutations by PCR sequencing and the PfSNP-LAMP-LFD technique was able to accurately identify SNPs (AAT → ATT, N51I) associated with resistance to pyrimethamine test.

Amongst of 4352 individuals were included in our study including children less than 5 years (2176) and individuals aged more than 5 years (2176), either 50 percent of some and other. Our results are comparable to those of Beshir et al., (2023) who in 2016 tested 29,274 children younger than 5 years and individuals aged 10 to 30 years and in 2018, 28,546 children aged less than 5 years and less than 3 individuals sahelian d'Africa [15].

We screened 4352 individuals by rapid malaria diagnostic test (RDR) and obtained 222 cases positive for *Plasmodium falciparum* (5.10%) against 4130 negative cases (94.89%). Our results are lower than those of Tarama, et al., (2023) who found a prevalence of malaria by TDR of 29.1% (338/1134) versus 33.7% (382/1134) for microscopy if this was disseminated in 2023 size of samples tested partly and partly or better Guinea has been using the CPS since 2015 and is a means of reducing the prevalence of malaria in Guinea [16].

After performing thick drop and thin smear we had found 255 positive individuals (5.85%) versus 4097 negative individuals (94.14%). Our results are lower than those of Mbaigane GD et al., (2021) who found in their study among children aged 0 to 5 years a prevalence of 305/320 either 95.31% against 4.68% of negative children [17]. Our results are also lower than those of Tarama et al., (2023) who found microscopy detected 33.7% (382/1134) of positive cases in 2023 [16].

After PCR, out of 4353 samples, only 246 were found to be positive for *Plasmodium falciparum* DNA (246/4352) either 5.65.00%, as opposed to 94.34% (4106/4352) of non- falciferous Prumcipa Our results are lower than those of Beshir et al., (2023) who found an overall prevalence of malaria by PCR among children aged less than 5 years and individuals aged 10 to 30 years respectively 17.5% in 2016 versus 7.6 in 2018 [15].

In the study 132 children under 5 years of age were positive carriers of *Plasmodium falciparum* with a prevalence of 48% versus 138 individuals aged over 5 years also carriers of *Plasmodium falciparum* with a prevalence of 52% [15]. Our results are higher than those of Beshir et al., (2023) who, in their study published in 2023 found country-specific prevalence rates among children under 5 years in 2016 (2.2.9416% for the Gambia) la (2.194162 la (20.9%), Mali 704/2189 (32.2%), Burkina Faso 161/2281 (7.1%), Niger 725/2146 (33.8%), Nigeria 601/1786 (33.7%) and Chad 101/108/2195 individuals in Gambia 85/2012 (4.2%), Guinea 305/1893 (16.1%), Mali 585/2176 (26.9%), Burkina Faso 65/2275 (2.9%), Niger570/206217 (24.6%), and Nigeria TChad181/2194 (8.2%) [15]. The same authors in their 2022 publication showed that in 2018 among children under 5 years of age different prevalences of *Plasmodium*

falciparum were found in seven African countries including The Gambia 53/1383 (2.383%) and Guinea (3.812%) 154/2156 (7.1%) and Burkina Faso73/2264 (3.2%) Niger143/1783 (8.0%) Nigeria 224/2110 (10.6%) and Chad 26/2141 (1.2%)and among Gambian 26/2141 (1.2%)and 291.2956 individuals aged 10 to 3 years Guinea134/2191(6.1%) and Mali 322/2106 (15.3%) and Burkina Faso 106/2168 (4.9%) and Niger 232/2586 (9.0%) and Nigeria 391/1770 (22.1%) and Tchad 128/1547 (8,3%)

In our study we observed that in children under 5 years of age, mutations occurred in the pfdhfr gene of *Plasmodium falciparum*: 51Ile (42.85%), 59Arg (42.85) and 108Andh nosffs in gndius (42.85). planes observed successively the following mutations: 436Ala (2.85%), 437Gly (2.85) ,540Glu (5.71) and 613Ser (2.85). Therefore, mutations occurred in the Pfdhfr rather than in the Pfdhps gene. Our results are comparable to those of Beshir et al., (2022) who found in their previous study the following 5 mutations in Guinea (86.67%),59Arg (83.63%),108Asn (91.44%) in pfdhfr gene while 436Ala (44.12%), 437Gly (79.17%) and (26.13%) Slu (26.13%) mutations were found as mutations in pfdhfr [15]. At the same age bracket the following mutations 51I (98.50%), 59Arg (94.50%), 108Asn (99.29%) were observed in pfdhfr and in pfdphs mutations as 4362.7%Aly% (G 540Glu (3.77%) and 613Ser (5.59%) were also observed in individuals older than 5 years, mutations are most prevalent in Pfdhfr at the level of codons 51Ile, 59Arg and 108Asn with for each 28.57% prevalence. On the contrary in Pfdhps the highest mutation rate was observed in codon 540Glu (5.71%) followed by 436Ala, 437Gly and 613Ser mutations with for each 2.85%. Our results are comparable to those of Beshir, et al., who reported in 2022 the mutation prevalences in Plasmodium falciparum obtained from seven African countries for the same age class as follows 51I (88.52g%),59Ar (90.26%) in pfdhfr gene while in pfdhps gene mutations were found as 436Ala (43.59%),437Gly(71.73%),540Glu(1.83%) and 613Ser(4.69%) mutations compared to 2016 presented as follows:51I (96.28%), 59Arg (97.07%),108Asn (99.25%) in pfdhfr gene while in pfdhps they found as mutations 436Ala (35.01%),437.7Gly (8207%)Gly (8207%) and Gly 613Ser (4.51%) [15].

Our results are also comparable to those obtained by Tarama et al., (2023), who also found in 2023 mutations in *Plasmodium falciparum* in children, detailed as follows: Mutations were high in Pfdon9.589sR (Co6% 51I (66.6%) and 108N (14.7%) For the mutation in pfdhps gene, the highest prevalence was observed in codon 437K, (89.3%) followed by codons 436A (62.2%) and 41.2% [16].

Our study shows that 20 children younger than 5 years presented a mutant genotype or 57.14%, versus 15 individuals older than 5 years or 42.85%. After PCR and sequencing, only 35 out of 246 *Plasmodium falciparum* DNA-positive samples showed mutations in Pfdhfr and Pfdhps, an overall prevalence of 14.22%. Our result is comparable to the findings of Floriano et al., (2020) who found in 2020 and through surveys conducted across Africa, different prevalences of *Plasmodium falciparum* resistance to sulfadanza Lathamine, as follows: So6 (55.4%), Mozambique (45.7%), Kenya (29.7%), Malawi (8.7%), observed prevalence between 2000 and 2010. Followed by Sudan (76%), Kenya (65.7%), Tanzania (17.4% prevalence [18]. In addition to Equatorial Guinea (28.9%), DRC (85.3%), Nigeria (14.4%), Mali (7.0%), Malawi (5.5%), Kenya (4.7%), and Tanzania (2%), the prevalence observed between 2000 and 2020 will not increase. of Malawi, Kenya and Tanzania between 2000 and 2020 and lower than those observed in the other countries mentioned above [18].

After genotyping we note that mutations in codons 51I ,59R and 108N occurred in 71.42% of the samples showing at least one mutation after sequencing in the PfRdh-Iplor-8 gene PfRdhfr-8: 5 IRN:Triple mutation). While in the Pfdhps gene mutations occurred at codons 436Ala, 437Gly, 540Glu and 613Ser (AGES haplotypes: Quadruple mutation) with a prevalence of 28.57%. Finally, 5 samples showed mutations in both Pfdhfr:51I -59R-108N and Pfdhps: 437Gly-540Glu genes (IRN-GE haplotypes: Quintuple mutation). with a prevalence of14, 28%. Our results are higher than those found by Beshir KB et al., (2023), who found in their study the IRN haplotype in pfdhfr (corresponding to 51Ile-59Arg-108Asn) in their analyzes of this result could be explained by the very high size of their sample from seven countries [15].

On the contrary they did not find the AGES and IRN-GE haplotypes in their study but rather the ISGEAA haplotype (431Ile-436Ser-437Gly-540Glu-581Ala-613Ala) in pfdh2 which was also rare [15]. Our results are also comparable to those of Kayiba et al., (2023) who observed moderate resistance in pfdhps gene, A437G (99.5%), K540E (47.7%) among which there was the quintuple N Participant N ParfhI mutant-GE C59R- S108N and Pfdhps A437G-K540E mutations that we also obtained in our study [19]. Our results are also comparable to those of Traoré which on a total of 28325 collected samples were analyzed [20]. These authors found in Chad national prevalences of mutant parasites to be 97.1%, 10.3% and 3.9% respectively for the dhfr triple mutant, dhps-540 and the quintuple mutant [20].

In addition, our results are consistent with those of Issa et al (2022), who found in 2022 in Niger a high prevalence of mutations in the Pfdhfr gene at the level of N51I (84.97%), C59R (92.62%), and S10.28N [21]. In their study the key mutation in the Pfdhps gene was not observed in the K540E codon which in contrast we observed in our study. The N51I/C59R/S108N triple mutant haplotype (IRN) was detected in 97% of their sample. They observed single-mutant

(ICS and NCN) and double-mutant (IRS, NRN, and ICN) haplotypes with 97% and 95% accuracies, respectively, which we did not observe in our study.

Double mutant haplotypes of Pfdhps (581 and 613) and Pfmdr (86 and 184) were found in 3% of cases which we did not observe in our study [21]. In another study, Sugaram et al., (2020), reported that for the pfdhfr-pfdhps genes, a septuple mutation was observed at the Thai–Myanmar border (50% =73/146) and between Thailand (65%) and Cambodia (25%) [22]. At the Thai–Malaysian border, the prevalence of pfdhfr-pfdhps haplotypes increased from fourfold (90% = 9/10) to fivefold (65% = 24/37) during 2008–2016. In the absence of pfdhfr-pfdhps haplotypes, between 2008 and 2013, the pfdhps A/S436F mutation was only observed at the Thai-Myanmar border (9 %= 10/107), whereas it was not identified there [22].

In our study or tes CPS areas, seventeen prefectures each presented at least one mutation in Pfdhfr, either in pfdhps or both simultaneously. The prefectures most affected by Plasmodium falciparum resistance to pyrimethamine are Siguiri (11.42%), Dinguiraye (8.57%) respectively followed by Kankan, Mandiana and Labé (5.71%) and the rest with low levels (2.85% in each of the Pfdhs mutations). Six of the 436Ala, 437Gly, 540Glu and 613Ser codons marking Plasmodium falciparum resistance to sulfadoxine were observed in the prefectures of Siguiri (8.57%) followed by the prefectures of Manguira, Dinguira and Kouroussa, Kouroussa with each having a prevalence of 2.85%. The rest of the prefectures did not show any mutation responsible for Plasmodium falciparum resistance to sulfadoxine. The mutants responsible for the resistance of Plasmodium falciparum to pyrimethamine and sulfadoxine were observed in the prefectures of Siguiri Kouroussa, Mamou, Labé, Koundara with a prevalence of (2.85%) for each. Finally, pyrimethamine and sulfadoxine resistance were not observed in the other prefectures of the CPS area.

Definitively all children aged 3 to 59 months showed molecular markers responsible for Plasmodium falciparum resistance to pyrimethamine (15/35) either 42.85%. About 14% (5/35) of them showed resistance to sulfadoxine and finally 8.57% (3/35) showed simultaneous resistance to pyrimethamine and sulfadoxine. Among individuals older than 5 years, we found that 28.57% (10/35) were resistant to pyrimethamine, whereas 14.28% (5/25) were resistant to sulfadoxine and only 5.71% (2/35 were resistant) to sulfadoxine pyrimethamine.

7. Conclusion

The search for pyrimethamine resistance genes in the 4352 samples made it possible to detect mutations in the Pfdhfr and Pfdhps genes using the PfSNP-LAMP technique. Indeed, among the 246 samples positive for Plasmodium falciparum DNA, 25 individuals were detected as carriers of pyrimethamine-resistant Plasmodium falciparum strains, corresponding to a mutation rate of 10.16% (25/246). Extrapolation to all the analyzed samples studied shows a mutation prevalence of 0.57% (25/4352) compared to 99.42% (4327/4352) negative individuals with a prevalence of 99.42%. Children under five years of age were the most affected by the mutation (60%) compared to individuals aged over 5 years with a prevalence of 40%. These results agree well with those obtained after PCR and sequencing of the same samples.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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