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Prevalence of the 108N and 437G Mutations in *Plasmodium falciparum* Associated with Sulfadoxine–Pyrimethamine Resistance in Clinical Isolates from Moundou (Chad)

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A B S T R A C T

The resistance of *Plasmodium falciparum* to sulfadoxine-pyrimethamine (SP) threatens the efficacy of malaria chemoprevention strategies across sub-Saharan Africa. In Chad, data regarding the prevalence of molecular markers of resistance remain scarce. This study aimed to evaluate the polymorphism of the *Pfdhfr* (codon 108) and *Pfdhps* (codon 437) genes in *P. falciparum* isolates collected in Moundou, southern Chad. A cross-sectional study was conducted among patients infected with *P. falciparum*. Genomic DNA was extracted from dried blood spots (DBS) using the Chelex-100 resin method. Mutations at codons *Pfdhfr* 108 and *Pfdhps* 437 were characterized using the nested PCR-FRLP technique. Molecular analysis revealed a high prevalence of mutant alleles. For the *Pfdhfr* gene, the prevalence of the 108N mutation was 62%, while the S108 wild-type allele persisted in 38% of cases. For the *Pfdhps* gene, the 437G mutant allele was detected in 70.37% of isolates (comprising 59.26% pure mutants and 11.11% mixed infections), compared to 29.63% for the A437 wild-type allele. Haplotype analysis showed that 40.38% of parasites carried the double mutation (108N/437G), which is the cornerstone of SP resistance. Fully wild-type strains (lacking any mutations) accounted for only 3.85% of isolates. Single mutations were more frequently observed in *Pfdhps* (34.62%) than in *Pfdhfr* (21.15%). Antifolate resistance is firmly established in the study area, with a marked reduction in sensitive strains. However, the moderate prevalence of the double mutant and the persistence of wild-type alleles suggest a transitional phase. This indicates potential residual efficacy of SP for chemoprevention, provided that continuous surveillance is maintained.

Introduction

Malaria remains a major global public health concern. It is caused by five species of the genus *Plasmodium*, with *Plasmodium falciparum* being the most virulent species and responsible for the vast majority of cases. According to the WHO, 263 million cases of malaria and 597,000 deaths were reported worldwide, with the bulk of the burden (94%) occurring in Africa (WHO, 2024). In Chad, malaria continues to be the primary reason for medical consultation (30%) and the leading cause of mortality (42.77%) within healthcare facilities (PNLP, 2023). The most vulnerable populations are children under five and pregnant women. In the absence of a widely available vaccine, case management and chemoprevention remain the core strategies in the fight against malaria. The Sulfadoxine-Pyrimethamine (SP) combination is recommended for Intermittent Preventive Treatment in pregnancy (IPTp) and, in combination with amodiaquine (AQ), for Seasonal Malaria Chemoprevention (SMC) in children under five (WHO, 2012, 2022). However, malaria control efforts have been compromised by the emergence of *Plasmodium falciparum* strains resistant to antimalarial drugs.

The resistance of *P. falciparum* to antimalarials is a critical factor influencing the effective control and management of malaria cases globally (Bloland, 2001; Duraisingh *et al.,* 1998b). This resistance primarily stems from the accumulation of mutations in the drug's target genes (Le Bras J., 1999). Mutations in the dihydropteroate synthase (*dhps*) and dihydrofolate reductase (*dhfr*) genes—which encode essential enzymes in the folate biosynthesis pathway—are responsible for SP resistance (Duraisingh *et al.,* 1998; Nyamngee *et al.,* 2025; Peterson *et al.,* 1988; Plowe *et al.,* 1997). The *Pfdhfr* Ser108Asn mutation is recognized as playing a pivotal role in pyrimethamine resistance, while mutations at positions 51Ile, 59Arg, and 164Leu modulate the level of resistance (Peterson *et al.,* 1991; Plowe *et al.,* 1997). Specifically, the *Pfdhfr* triple mutation (51Ile, 59Arg, and 108Asn) has been associated with SP treatment failure, regardless of the *Pfdhps* genotype. Studies have indicated that *in vitro* results often correlate with clinical reports of therapeutic failure within a country (Beshir *et al.,* 2023).

Sulfadoxine resistance in *P. falciparum* is associated with mutations at five codons of the *Pfdhps* gene: 436Ala/Phe, 437Gly, 540Glu, 581Gly, and 613Ser (Mousa *et al.,* 2025; Plowe *et al.,* 1997; Tchuenkam *et al.,* 2024; Triglia *et al.,* 1998). The *dhps* A437G mutation represents an early and predominant alteration conferring sulfadoxine resistance (Zhao *et al.,* 2020). The presence and frequency of these polymorphisms, whether isolated or in various haplotypes, have been linked to high therapeutic failure rates and reduced clinical efficacy of SP in several regions (Djontu *et al.,* 2024; Flegg *et al.,* 2022; Zhou *et al.,* 2024).

Consequently, monitoring molecular resistance markers remains a key component of malaria control policies to guide programmatic decision-making. However, in Chad, data on resistance markers in general—and specifically published information on the prevalence of *Pfdhfr* S108N and *Pfdhps* A437G mutations in clinical isolates are limited (Beshir *et al.,* 2023; Souleymane *et al.,* 2018). This lack of data may hinder the National Malaria Control Program's ability to tailor IPTp and chemoprevention policies to local resistance profiles. This study aims to determine the prevalence of S108N and A437G mutations in *P. falciparum* isolates collected from symptomatic patients in Moundou (Chad), thereby providing molecular evidence to inform local malaria control and IPTp policies.

Materials and Methods

Study Site

The study was conducted at the Moundou Regional Hospital (MRH), a referral facility serving a diverse patient population from urban and peri-urban districts. Moundou, the economic capital and administrative center of the Logone-Occidental region, is situated in southern Chad (8°34′00″N, 16°04′59″E) along the banks of the Logone River, approximately 500 km southwest of the capital, N'Djamena. The city holds a strategic position on the road network linking three CEMAC countries: Cameroon, Chad, and the Central African Republic. Moundou is characterized by a Sudanian climate with a rainy season lasting approximately six months, maintaining high malaria endemicity (estimated at 90–100%). The average annual temperature is 27.5°C, with a mean of 76.8 rainy days per year and an average annual rainfall of approximately 1,040 mm (Ngaryamngaye *et al.,* 2024). The city's population was estimated at 200,000 inhabitants in 2020. These climatic and environmental conditions favor malaria transmission, which peaks between July and November.

Under national malaria control policies, sulfadoxine-pyrimethamine (SP) is recommended for intermittent preventive treatment in pregnant women (IPTp) (PNLP, 2023).

Study Design and Population

A descriptive cross-sectional study was carried out from June 2017 to July 2018. The target population included patients of all age groups with suspected malaria, presenting with a temperature ≥ 37.5°C or a history of fever within the previous 24 hours. Data were collected from each participant after obtaining written and oral informed consent.

Whole blood samples were collected via venipuncture. Approximately 1 mL of blood was drawn into EDTA tubes. Standard safety procedures were followed during blood sampling, including the use of sterile equipment and disinfection of the puncture site with 70% alcohol. Following collection, a rapid diagnostic test (RDT) for *Plasmodium falciparum* (HRP2 SD Bioline®) was performed. For positive cases, approximately 100 µL of blood was used to prepare dried blood spots (DBS) on Whatman 3MM filter paper. The DBS samples were air-dried before being individually packaged in envelopes. Samples were then transported to the Public Health and Biotechnology Research Laboratory at the University of Yaoundé I Biotechnology Center. A total of 52 positive samples were selected for the analysis of target gene polymorphisms.

DNA Extraction

Genomic DNA was extracted from the DBS samples using the Chelex-100 boiling method (Bio-Rad Laboratories, SIGMA; Inc. Marnes-la-Coquette, France). The protocol was adapted from methods described by Plowe *et al.,* (1995) and Bereczky *et al.,* (2005), and optimized to minimize the presence of PCR inhibitors (Bereczky *et al.,* 2005; Plowe *et al.,* 1995). A blood spot was first cut from the filter paper, soaked overnight in 0.5% saponin prepared in 1X phosphate-buffered saline (PBS), and subsequently washed with 1 mL of PBS. After washing, the filter paper disc was transferred into 50 µL of 20% Chelex suspended in 100 µL of distilled water and heated at 100°C for 10 minutes. The mixture was vigorously vortexed three times during the heating process. DNA was separated by centrifugation at 13,000 rpm for 2 minutes, and 100 µL of the supernatant was carefully collected, avoiding any Chelex beads. The extracts were stored at -20°C until further use.

Genotyping of Pfdhfr and Pfdhps Genes by PCR-RFLP

The *P. falciparum dhfr* and *dhps* genes were amplified using nested PCR. The primer sequences for the targeted gene fragments are presented in Table I; primers were obtained from Inqaba Biotech (Pretoria, South Africa) and used according to previously described methods adapted from Duraisingh *et al.,* (1998) and Abdullah *et al.,* (2013) (Abdullah *et al.,* 2013; Duraisingh *et al.,* 1998).

Amplification of the *dhps* Gene

The primary reaction (Nested 1) consisted of 3 µL of DNA, 0.25 µM of primers R2 and R/, 12.5 µM of OneTaq® Hot Start (New England Biolabs, MA, USA), and 9 µL of nuclease-free water, for a total reaction volume of 25 µL. The tubes were placed in a thermal cycler (T3 Thermocycler, Biometra, Göttingen, Germany). The amplification program was as follows: initial denaturation at 94°C for 15 min; followed by 45 cycles of denaturation at 94°C for 3 min, annealing at 45°C for 1 min, and extension at 72°C for 1–10 min, with a final extension at 72°C for 3 min (Duraisingh *et al.,* 1998; Plowe *et al.,* 1997). For the secondary amplification (Nested 2), the reaction mixture contained 3 µL of the Nested 1 amplicon, 0.25 µM of primers K1 and K/, 12.5 µM of GoTaq® Hot Start, and 11 µL of nuclease-free water (total volume 25 µL). The secondary amplification was also performed using a Biometra T3 thermal cycler with an initial denaturation at 94°C for 15 min, followed by 35 cycles of 94°C for 3 min, 45°C for 1 min, and a final extension at 72°C for 10 min (Duraisingh *et al.,* 1998; Plowe *et al.,* 1997). Secondary PCR products were resolved by electrophoresis on a 2% agarose gel prepared in TBE buffer containing 0.5 µg/mL ethidium bromide and visualized under UV transillumination. A 100-bp molecular weight marker was loaded on each gel. Electrophoresis was performed at 100 V for 30–45 minutes. The expected size of the amplified fragment was 438 bp.

Amplification of the *Pfdhfr* Gene

For *dhfr* amplification, the primary reaction (Nested 1) comprised 3 µL of DNA extract, 0.25 µM of each primer

(M1 and M5), 10X ThermoPol® buffer, 200 µM dNTPs, 18.25 µL of nuclease-free water, and 0.25 U of Taq DNA polymerase (total volume 25 µL). The amplification was performed using a T3 Biometra 2000 thermal cycler with an initial denaturation at 94°C for 3 min, followed by 45 cycles of denaturation at 94°C for 3 min, annealing at 45°C for 1 min, and a final extension at 72°C for 10 min (Duraisingh *et al.,* 1998; Plowe *et al.,* 1997). The secondary reaction (Nested 2) included 1 µL of the Nested 1 amplicon, 0.25 µM of primers M3 and F/(F1), 10X ThermoPol® buffer, 200 µM dNTPs, 20.25 µL of nuclease-free water, and 0.25 U of Taq DNA polymerase (total volume 25 µL). The thermal cycling conditions for the 35 cycles were identical to those of the primary reaction (Duraisingh *et al.,* 1998; Plowe *et al.,* 1997). Nested 2 amplicons were separated on a 2% agarose gel in TBE containing 0.5 µg/mL ethidium bromide and visualized under UV light. A 100-bp DNA ladder was used to determine fragment size. Migration was performed at 100 V for 30–45 minutes. The expected size of the amplified fragment was 522 bp.

Digestion of Amplicons

Digestion of the PCR products was performed using restriction enzyme digestion according to a protocol adapted from Abdullah *et al.,* (2013). For the *Pfdhfr* gene (expected amplicon size: 522 bp), restriction was carried out using *BsrI*. Digestion reactions were prepared in a final volume of 20 µL, consisting of 1× restriction buffer, 0.2 µL of BSA, 1 µL of *BsrI*, and 8 µL of the nested PCR product; the remaining volume was supplemented with PCR-grade water.

The digestion mixtures were incubated at 65°C for approximately 16 hours (overnight) in accordance with the manufacturer's instructions. For the *Pfdhps* gene (expected amplicon size: 438 bp), digestion was performed using *AvaII*. The reaction mixtures were identical in composition (20 µL final volume, 1× buffer, 0.2 µL of BSA, 1 µL of *AvaII*, and 8 µL of the second amplification product). Digestion was carried out at 37°C for a minimum of 6–8 hours. The digestion products were resolved on 2% agarose gels prepared in TBE buffer containing 0.5 µg/mL ethidium bromide and visualized under UV transillumination. A 100-bp molecular weight marker (DNA ladder) was loaded on each gel. Electrophoresis was performed at 100 V for 30–45 minutes. The expected fragment sizes for wild-type, mutant, and mixed genotypes are summarized in Table II (Abdullah *et al.,* 2013; Duraisingh *et al.,* 1998).

Statistical Analysis

Data were entered into Microsoft Excel 2010. Statistical analyses were performed using SPSS software (version 25.0). Descriptive statistics were used to calculate the prevalence of *Pfdhfr* 108N and *Pfdhps* 437G mutations, as well as means and standard deviations (SD).

Ethical Considerations

The study protocol was approved by the University Scientific Committee, and authorization for sample collection was granted by the Director of the Moundou Regional Hospital (No. 403/MSP/SG/DGASRLOC/HRM.SA/SP/Sgle/17).

Informed consent (both verbal and written) was obtained from all participants prior to enrollment. For children, informed consent and assent were obtained from parents or legal guardians.

Results and Discussion

Characteristics of the Study Population

A total of 52 participants were enrolled in this study. Participant ages ranged from 5 to 72 years, with a mean age of 26.62 ± 14.66 years. Males predominated, representing 61.05% of the study population compared to 38.95% for females. The mean axillary temperature was 37.86 ± 1.05°C.

Pfdhfr Gene Polymorphism Analysis Results

Figure 1 illustrates the *Pfdhfr* amplicons following nested PCR; characteristic bands at 522 bp were observed for all isolates.

Pfdhfr Genotyping Results (RFLP)

Figure 2 illustrates the DNA restriction fragments. The observed bands correspond to fragments at 522 bp, 190 bp, and 332 bp, characterizing the different *Pfdhfr* genotypes.

Fréquence de polymorphisme du codon 108 du gène *dhfr*

Après digestion avec les enzymes, les résultats montrent 31/50 (62%) des souches mutantes 108N, résistant à la

Pyriméthamine et 19/50 (38%) des souches sauvages S108, sensibles à la pyriméthamine

Results of dhps gene amplification by PCR

The electrophoregram of the amplification products of the dhps gene shows bands at 438 bp for the amplicons.

Results of dhps gene digestion

Figure 17 shows the bands of DNA fragments of wild-type, mutated, and heterozygous dhps genes after digestion with the Ava II enzyme. The undigested DNA fragments, wild type at 438 bp, the digested fragments, mutant type at 404 bp, and the mixed fragments (heterozygotes) at 404 and 438 bp.

Frequency of dhps gene polymorphism

In this study population, we detected mutant strains 437G, wild-type A437, and heterozygous A437G with a predominance of mutant strains, namely 31/52 (59.26%), 15/52 (29.63%), and 6/52 (11.11%) respectively. (Figure 5).

Frequency of *Pfdhfr*/*Pfdhps* Haplotype Combinations

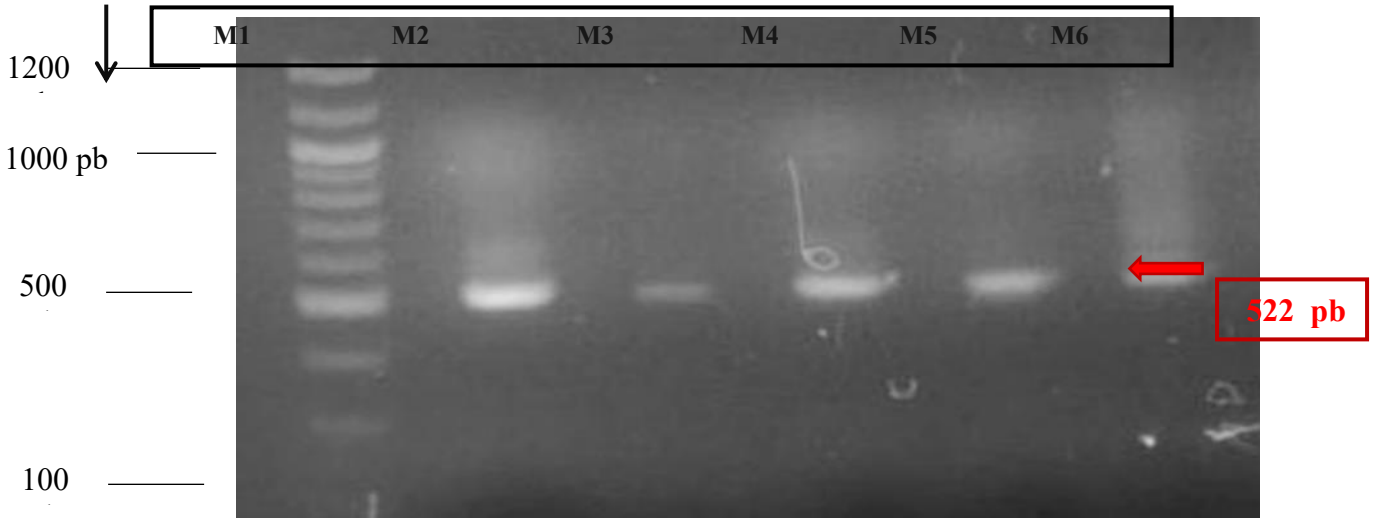
The histogram in Figure 14 illustrates the distribution of the combined genotypes. Among the analyzed samples, 40.38% (21/52) carried the double mutation (108N/437G). Single mutations were observed in 34.62% (18/52) of cases for the *Pfdhps* 437G allele (S108/437G) and 21.15% (11/52) for the *Pfdhfr* 108N allele (108N/A437). Finally, only 3.85% (2/52) of the isolates were fully wild-type, presenting no mutations at these loci.

Molecular surveillance of *Plasmodium falciparum* resistance markers is a critical component for evaluating the efficacy of chemoprevention policies, particularly in high-transmission areas such as southern Chad. This study provides updated data on the prevalence of *Pfdhfr* and *Pfdhps* polymorphisms associated with sulfadoxine-pyrimethamine (SP) resistance in Moundou. Our findings demonstrate a predominance of the *Pfdhps* 437G mutant allele, present in 70.37% of isolates (comprising 59.26% pure mutants and 11.11% mixed infections), while the A437 wild-type genotype persists in 29.63% of cases. This coexistence of wild-type and mutant populations indicates an epidemiological transition phase. These observations confirm that sulfadoxine resistance is firmly established in the Logone Occidental region, although it has not yet reached the level of complete fixation observed in other parts of Central Africa.

The prevalence observed in Moundou is comparable to data reported across the Sahelian and Sudanian basins. Our results align with findings from Cameroon, specifically in the Far North (52.5%) (Tchuenkam *et al.,* 2024), Nkambe (57.6%), and Limbe (60%) (Netongo *et al.,* 2009), as well as among pregnant women in Yaoundé (76.5%) (Chauvin *et al.,* 2015). Similar trends have been documented in Gabon (67.7%) (Ndong Ngomo *et al.,* 2016) and Senegal (54.4% to 67%) (Fall *et al.,* 2013). In contrast, the prevalence in Moundou remains lower than those reported in Brazzaville (89.3%) (Djontu *et al.,* 2024), Mfou, Cameroon (90%) (Tuedom *et al.,* 2021), and rural Fougamou, Gabon, where the mutation has reached 100% fixation (Boukoumba *et al.,* 2021). This spatial heterogeneity may be attributed to differential drug pressure arising from the extensive use of SP in Seasonal Malaria Chemoprevention (SMC) and Intermittent Preventive Treatment in pregnancy (IPTp), or disparities in access to sulfonamides within the informal sector. Notably, the situation in Chad appears less critical than in "super-resistance" zones of Africa and Asia, where the nearly fixed 437G mutation is systematically associated with the *Pfdhps* K540E mutation, resulting in highly resistant mutant haplotypes (Kuesap *et al.,* 2022; White *et al.,* 2025).

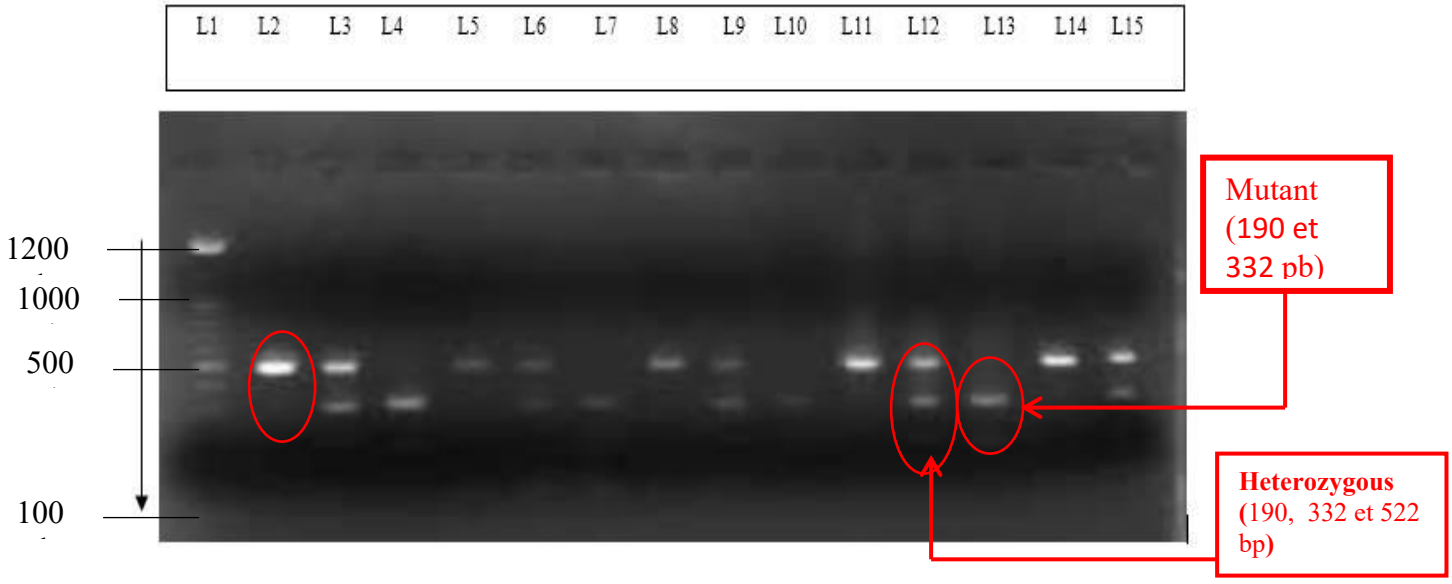
Regarding pyrimethamine resistance, this study reveals a 62% prevalence of the *Pfdhfr* S108N mutation, compared to 38% for the wild-type allele. The 108N mutation is the essential initial mechanism reducing parasite sensitivity; its high frequency indicates that the efficacy of pyrimethamine-containing therapies is under direct threat in Moundou. Its presence is particularly concerning as it is a prerequisite for the acquisition of secondary mutations (N51I and C59R) that confer high-level resistance (Plowe *et al.,* 1995, 1997). However, the 62% frequency remains lower than rates observed in Niger (97.39%) (Issa *et al.,* 2022) or in Cameroon, Congo, and Gabon, where fixation is near-total (100%) (Boukoumba *et al.,* 2021; Djontu *et al.,* 2024; Tchuenkam *et al.,* 2024).

Figure.1 Electrophoretic profile of the Pfdhfr gene (S108N)



Legend: M1: Molecular weight marker (100-bp DNA ladder); M2, M3, M4, M5, and M6: Positive amplicons displaying characteristic bands at 522 bp.

Figure.2 Electrophoretic profile of the *Pfdhfr* gene following *BsrI* digestion.



Legend: L1: Molecular weight marker (100-bp DNA ladder); L2, L5, L8, L11, L14: Wild-type genotypes (single band at 522 bp); L4, L7, L10, L13: Mutant genotypes (two bands at 332 bp and 190 bp); L3, L6, L9, L15: Mixed infections (three bands at 522 bp, 332 bp, and 190 bp).

Figure.3 Frequency distribution of S108N polymorphism of the *dhfr* gene

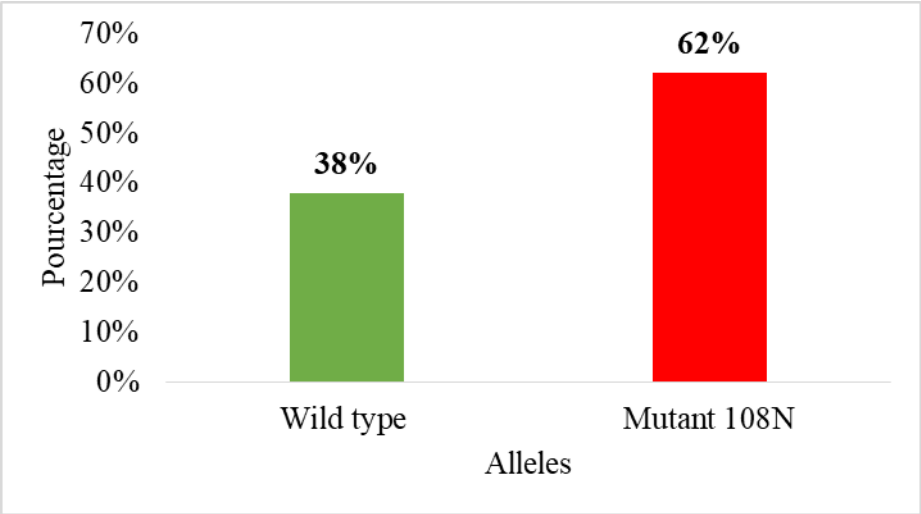
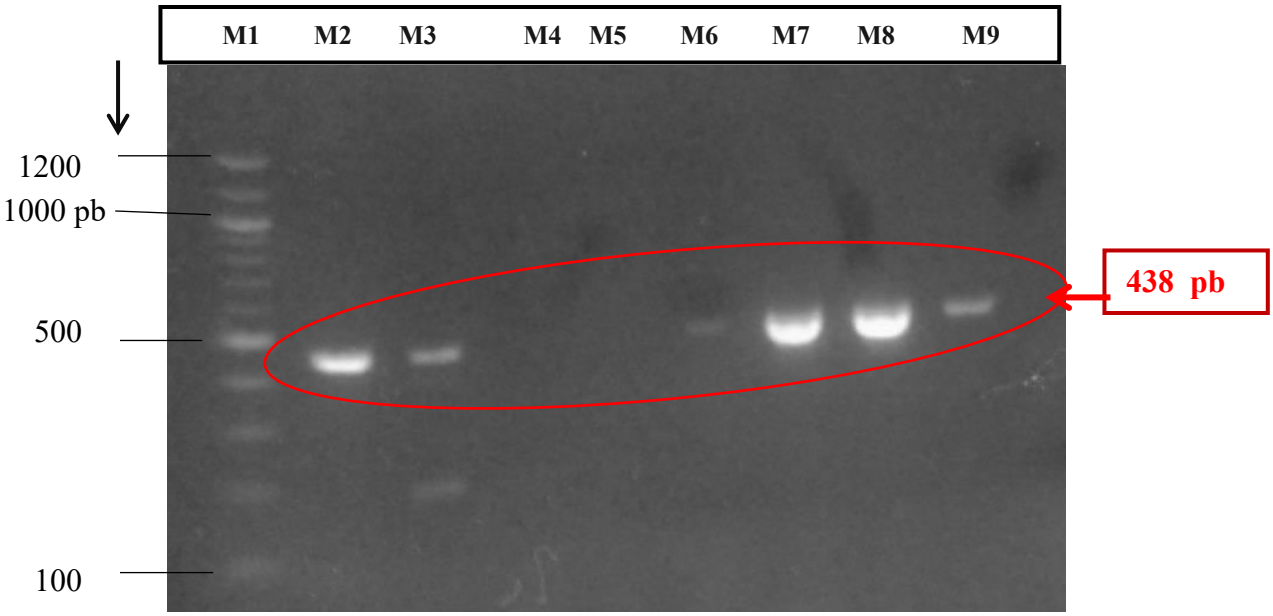
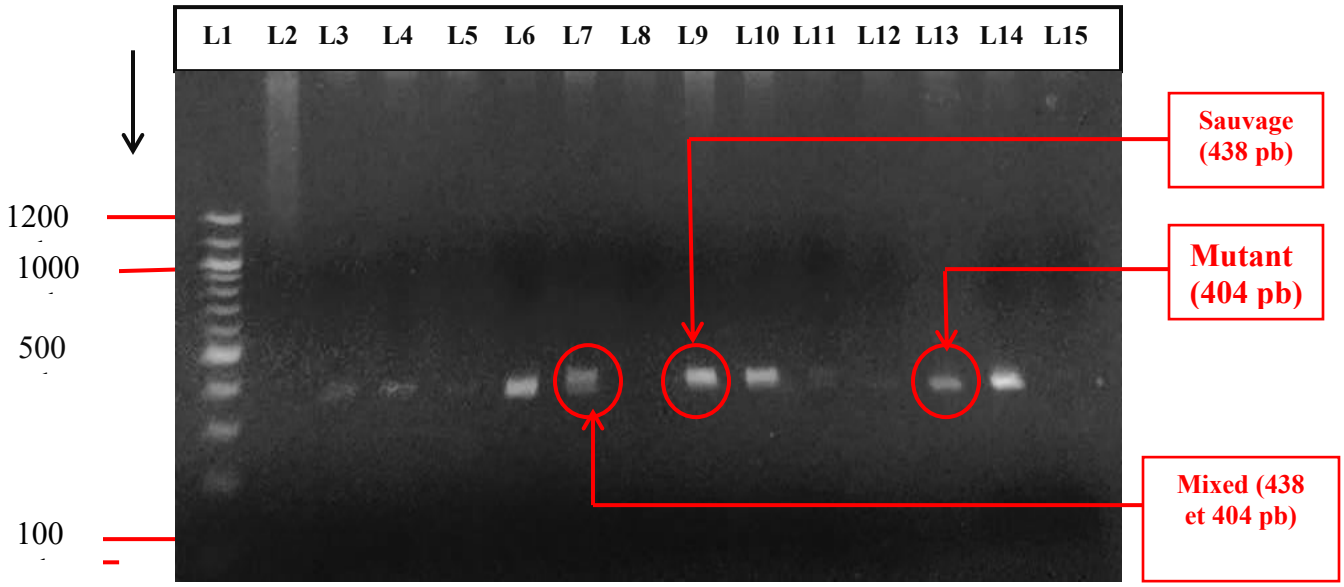


Figure.4 Electrophoregram of the dhps gene (A437G)



Legend: M1: molecular weight marker; M2, M3, M6, M7, M8, and M9: positive samples showing bands at 438 bp; M4 and M5: negative samples with absence of bands

Figure.5 Electrophoregram of the dhps gene after digestion with Ava II



Legend: L1: molecular weight marker; L1, L8, L9, L15, L14: show wild-type fragments at 438 bp; L2, L3, L4, L5, L10, L12, L13, and L14: show mutant-type fragments at 404 bp; and L3, L6, L7, and L11: show mixed-type fragments at 522, 438, and 404 bp

Figure.6 Frequency distribution of A437G polymorphism of the dhps gene

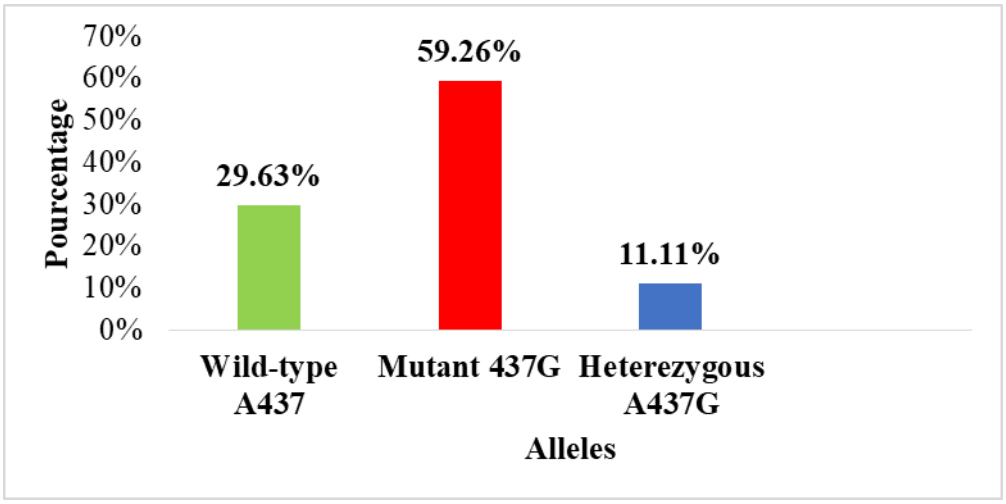
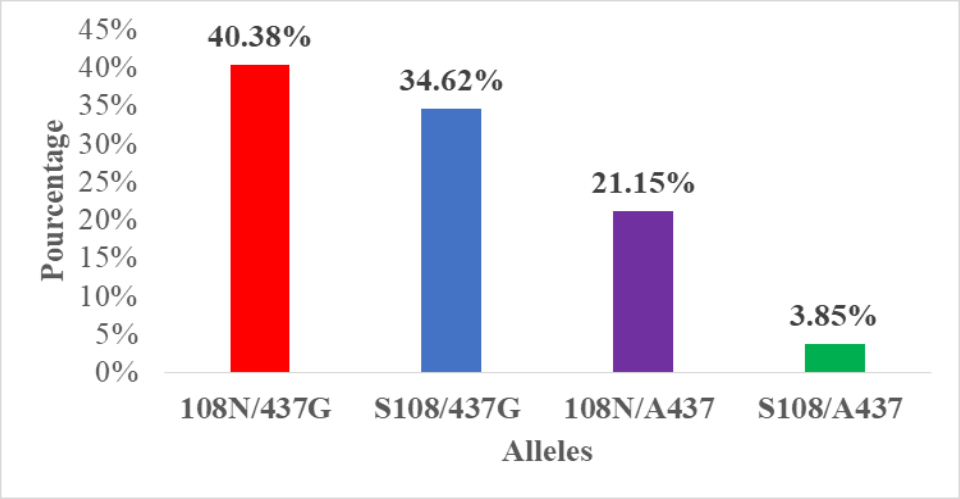


Figure.7 Histogram of frequency distribution of the dhfr/dhps polymorphism combination



Legend: 108N/437G: isolates with double mutation for both genes; S108/437G: isolates with a single mutation for the dhps gene; 108N/A437: isolates with a single mutation for the dhfr gene; S108/A437: isolates with no mutation for both genes.

Table.1 Primer sequences, used to genotype samples in *dhfr* and *dhps*

Genes	Round	Primer sequences	Product size (bp)	Reference
Pfdhps	Nest 1	R2-5' AACCTAAACGTGCTGTTCAA-3' R/(R1)-5- AATTGTGTGATTTGTCCACAA-3		(Duraisingh *et al.,* 1998)
	Nest2	K –5'-TGCTAGTGTTATAGATATAGGATGAGCATC-3' K/(K1) 5'-CTATAACGAGGTATTGCATTTAATGCAAGAA-3'	438	
Pfdhfr	Nest 1	M1-5' TTTATGATGGAACAAGTCTGC-3' M5 -5'AGTATATACATCGCTAACAGA-3'		
	Nest 2	M3-5'TTTATGATGGAACAAGTCTGCGACGTT-3' F/(F1)-5' AAATTCTTGATAAACAACGGAACCTTTTA-3	522	

Table.2 Interpretation of RFLP digestion patterns for *Pfdhfr* and *Pfdhps* genes (Abdullah *et al.,* 2013; Duraisingh *et al.,* 1998b).

Genes	PCR Product Size (bp)	Enzyme	Restriction Site	Digestion Product Size (bp)	Genotype/ Phenotype
Pfdhfr	522	*BsrI*	5'…ACTGGN▼…3' 3'…TGAC▲CN…5'	190 and 332	Mutant
				522	Wild-type
				522, 190, and 332	Mixed
Pfdhps	438	*AvaII*	5'…G▼G(A/T)CC…3' 3'…CC(T/A)G▲G…5'	34 and 404	Mutant
				438	Wild-type
				438, 34, and 404	Mixed

This discrepancy suggests a less intense or more recent selection pressure in Chad, potentially linked to lower pyrimethamine usage in the informal sector compared to Nigerian or Cameroonian markets. Furthermore, a 2018 study in Pala (western Chad) reported a significantly lower frequency (33.3%) for the 108N SNP (Souleymane *et al.,* 2018). This difference could be explained by Moundou's geographical location and status, which favor a massive influx of populations from other regions and neighboring countries, as well as the temporal evolution of resistance over a six-year period.

Combined analysis reveals a complex genetic structure: 96.15% of parasites have acquired at least one resistance marker. The double mutation (108N/437G), the cornerstone of SP resistance, was found in 40.38% of isolates. This rate is significantly lower than those in Niger or Cameroon (>80%), indicating that the parasite reservoir in Moundou has not yet fully shifted toward saturated multi-resistance.

A noteworthy finding is the relative predominance of single mutations in the *Pfdhps* gene (34.62%) compared to the *Pfdhfr* gene (21.15%). This suggests a more pronounced selection pressure for sulfadoxine, likely exacerbated by the frequent use of cotrimoxazole for treating bacterial or opportunistic infections, which facilitates cross-selection (Plowe *et al.,* 1995, 1997). This distribution reflects a population in the midst of genetic transition where resistant haplotypes have not yet stabilized.

Only 3.85% of isolates were entirely free of mutations. The disappearance of sensitive strains is a prevailing trend in sub-Saharan Africa (Amimo *et al.,* 2020), similar to data from Burkina Faso where wild-type strains have become marginal (Bohissou *et al.,* 2024). This confirms that the intensive use of SP (IPTp and SMC) has nearly eradicated sensitive parasites from the Moundou ecosystem. Finally, the presence of mixed infections (11.11%) highlights an intense transmission dynamic that promotes polyclonality and genetic recombination (Lopez & Koepfli, 2021). While this implies a risk of resistance dispersal, the residual presence of wild-type alleles currently justifies the continued use of SP, albeit with an urgent need for reinforced surveillance. The lack of data on *Pfdhps* codons 436, 540, and 581, as well as *Pfdhfr* codons 51, 59, and 164, limits the ability of this preliminary analysis to formally classify the resistance level according to WHO criteria.

In conclusion, this study conducted in Moundou reveals a high circulation of *Plasmodium falciparum* molecular markers associated with sulfadoxine-pyrimethamine (SP) resistance. The elevated frequency of *Pfdhfr* 108N and *Pfdhps* 437G mutations, coupled with a low percentage of wild-type strains, suggests widespread antifolate resistance. However, the moderate prevalence of the *Pfdhfr* 108N / *Pfdhps* 437G double mutant, without complete fixation, indicates a transitional state. This suggests potential residual efficacy of SP for chemoprevention, provided that continuous molecular surveillance is maintained.

Author Contributions

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

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Conflict of Interest The authors declare no competing interests.

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