AFRICAN JOURNAL OF PARASITOLOGY, MYCOLOGY AND ENTOMOLOGY
Official journal of the African Society of Parasitology

Monitoring *Plasmodium falciparum* Drug Resistance Markers in Pregnant Women Attending Antenatal Clinics in Senegal

Marie Pierre Diouf, MSc [1,2,*], Mary Aigbiremo Oboh, PhD [3], Isaac Akhénaton Manga, PhD [2], Safiétou Kande, MD [1], Aminata Colle Lo, PhD [2], Fatimata Bintou Sall, PhD [1], Amadou Seck, MSc [1], Babacar Faye, PhD [2], Corinne Simone Merle, PhD [4], Alfred Amambua-Ngwa, PhD [3,5], Paul Miligan, PhD [5] and Jean-Louis Abdourahim Ndiaye, PhD [1]

[1] Service of Parasitology-Mycology, UFR Santé Thiès, Thies BP A967, Senegal
[2] Service of Parasitology-Mycology, Cheikh Anta Diop University, Dakar BP 5005, Senegal
[3] Disease Control and Elimination,Medical Research council Unit The Gambia at London School of Hygiene and Tropical Medicine, Atlantic Road, Fajara, P.O. Box 273 Banjul, The Gambia
[4] Special Programme for Research & Training in Tropical Diseases (TDR), World Health Organization WHO—TDR, Geneva, Switzerland
[5] Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK
* Corresponding author: mariepierrediouf@outlook.fr or mpfatou.diouf@univ-thies.sn; Tel.: +221-773800832/+221-775885087

Abstract: Background: Malaria control in Senegal relies on antimalarial drugs like sulfadoxine–pyrimethamine (SP) for treatment and chemoprevention. Monitoring drug resistance markers is vital to ensure these interventions' effectiveness. This study assessed the prevalence of SP resistance markers among pregnant women attending antenatal clinics in Senegal in 2019. **Methods:** Cross-sectional surveys were conducted during the high-transmission period of 2019 among pregnant women in low- and high-transmission areas. After obtaining consent, three dry blood spots on Whatman paper were collected. Samples were analyzed by real-time Polymerase Chain Reaction (RT-PCR) to detect the *Plasmodium falciparum* VarATS gene. Positive samples were genotyped by High-Resolution Melting for mutations in the dihydrofolate reductase and dihydropteroate synthase genes. **Results:** Six hundred samples positive for *P. falciparum* were analyzed. The population was predominantly young and resided in high-transmission areas. Key resistance mutations were detected at the following rates: *Pfdhfr*I164L (11.7%), *Pfdhps*S436A (14.8%), *Pfdhps*A437G (19.0%), *Pfdhps*A581G (15.2%) and *Pfdhps*A613S (15.5%). The *Pfdhps*K540E mutation was not detected. The prevalence of these mutations was significantly higher in high-transmission areas. No significant differences in resistance markers were observed based on SP intake or gravidity. **Conclusions:** The low frequencies of SP resistance markers and the absence of the *Pfdhps* K540E mutation suggest that SP remains effective for malaria chemoprevention in Senegal. However, regular surveillance is crucial to monitor and contain any potential resistance of *P. falciparum* to SP and to track the evolution and spread of resistant parasites within malaria control areas. Surveys in antenatal clinics could be a good strategy for monitoring at low cost the emergence of resistance to SP.

Keywords: malaria; sulfadoxine–pyrimethamine; resistance markers; pregnant women; Senegal

How to cite: Diouf, M.P.; Oboh, M.A.; Manga, I.A.; Kande, S.; Lo, A.C.; Sall, F.B.; Seck, A.; Faye, B.; Merle, C.; Ngwa, A.A.; et al. Monitoring *Plasmodium falciparum* Drug Resistance Markers in Pregnant Women Attending Antenatal Clinics in Senegal. *Afr. J. Parasitol. Mycol. Entomol.*, 2024, 2(2): 13; doi:10.35995/ajpme02020013.

1. Background

Despite the global reduction in malaria burden, the disease remains a major public health problem. Globally, there was an increase in malaria cases to an estimated 241 million in 2020 as compared to 227 million recorded in 2019, with most of this increase being observed for countries within the World Health Organisation (WHO) African Region [1]. Senegal is also affected by malaria, and multiple strategies have been implemented to combat the disease, but a significant number of malaria cases are still reported in the general population, rising from 265.624 in 2014 to 354.708 in 2019 [2]. Three regions (Tambacounda, Kolda and Kedougou) in the south-east of Senegal are among the areas where malaria incidence remains the highest. In 2019, for example, these regions recorded 81% of all confirmed malaria cases in the country, 88% of cases in children under 5 years of age and 89% of cases in pregnant women [2]. However, slight but noticeable improvements in control intervention indicators were noted between 2018 and 2019 [2].

According to the changing epidemiological profile, targeted control strategies have been recommended for children (Seasonal Malaria Chemoprevention—SMC) and women (Intermittent Preventive treatment in pregnancy—IPTp). In 2012, WHO recommended SMC in areas of highly seasonal malaria transmission for 3- to 59-month-old children [3]. This strategy consists of the monthly administration of sulfadoxine–pyrimethamine plus amodiaquine (SP+AQ) to prevent malaria, which will ultimately decrease morbidity and mortality among children. Additionally, WHO recommended IPTp with sulfadoxine–pyrimethamine (IPTp-SP) in 2004 for pregnant women living in areas of moderate to high malaria transmission [4]. IPTp-SP has been implemented in Senegal since 2006, where pregnant women receive at least two doses of SP starting from the second trimester of pregnancy. This recommendation was revised in 2013, stating that IPTp-SP should be administered as a directly observed treatment to all pregnant women at each scheduled antenatal care (ANC) visit until the time of delivery, provided that the doses are given at least one month apart [4]. Both SMC and IPTp-SP therefore exert drug pressure on the parasite that could amplify historic SP resistance.

The effectiveness and sustainability of SP-dependent chemopreventive strategies is highly dependent on the current level and spread of SP resistance in *Plasmodium falciparum (P. falciparum)* following its continued usage [5]. Considering the limited number of alternative available chemopreventive drugs, the spread of *P. falciparum*-resistant parasites poses a serious threat to these public health interventions, with the risk of a rebound in infections, morbidity and mortality due to malaria in pregnant women and children.

SP acts as an inhibitor of the *P. falciparum* folic acid pathway, and point mutations in two genes, *P. falciparum* dihydrofolate reductase (*Pfdhfr*) and *P. falciparum* dihydropteroate synthetase (*Pfdhps*), confer resistance to SP [6]. Point mutations at *Pfdhfr* codons 50, 51, 59, 108 and 164 act synergistically to increase resistance to pyrimethamine. In particular, S108N is characterized as conferring low-level resistance, the double mutants N51I/S108N and C59R/S108N as moderate levels of resistance, while the triple N51I/C59R/S108N and quadruple N51I/C59R/ S108N/I164L mutants are considered as conferring high-grade resistance to pyrimethamine [7,8]. Similarly, mutations at

Pfdhps codons 436, 437, 540, 581 and 613 decrease the level of parasite sensitivity to sulfadoxine. Mutations at the S436A and A437G codons alone confer low resistance, and when in combination with K540E and/or A581G or A613S/T, the parasite has an increased threshold of resistance to sulfadoxine [9,10]. Previous studies showed that the triple mutation (51-59-108) is very common in Senegal at high frequencies [11]. In Thies, a region of Senegal, this triple mutation rose from 40% in 2013 to 93% in 2011 [12]. It has also been shown that in Senegal, since 2002, the prevalence of this triple mutant increased from 50 to 82.3 % in 2013–2014 [13]. This increase was observed in different regions of Senegal and could reach 100% (personal data). On the other hand, variations have been noted in the *Pfdhps* genes over the years and across different populations [11]. The evaluation of resistance markers is important and can be effectively achieved by targeting certain Single-Nucleotide Polymorphisms (SNPs) of interest. Therefore, this study was carried out in pregnant women in Senegal to assess the proportion of circulating *P. falciparum* parasitic infections resistant to SP. The mutations targeted were the SNPs associated with resistance to pyrimethamine *Pfdhfr* (I164L) and sulfadoxine *Pfdhps* (S436A-A437G-K540E-A581G and A613S/T). This research aims to inform public health interventions and ensure the continued efficacy of malaria control strategies in the face of evolving drug resistance.

2. Study Methods

2.1. Site and Study Design

Data for this cross-sectional survey were collected between September 09, 2019 and January 15, 2020, which was a period of high malaria transmission [14]. Four districts (Tambacounda, Velingara, Kedougou and Saraya) with high endemicity (incidence $\geq$ 25‰) in the south of Senegal and one (Kaffrine) in the center with a low level of transmission (<5‰) were selected. In high-endemicity areas, in addition to IPT for pregnant women, SMC is also used. In the center, however, IPT in pregnant women is the only chemoprevention strategy [2]. Malaria transmission in these five districts is seasonal, occurring during the rainy season (July to November) with peak transmission in October and November [15]. *P. falciparum* is the predominant parasite species, and transmission is mainly due to *Anopheles gambiae* s.l.

2.2. Population of Study and Sampling

The population studied was all pregnant women attending antenatal clinics in the study area. Sampling, recruitment and data collected from these women are described elsewhere [16]. The inclusion criterion in this study was positive RT-PCR for *P. falciparum.* Information on the pregnant women from whom the positive blood samples were taken will be retrieved from the pregnant women study database [16].

2.3. Data Collection

For each individual recruited, a short questionnaire was completed to collect the following information: age, sex, date of interview, place of residence, use of malaria control measures, etc. Three drops of blood were used to make spots on Whatman's filter paper for molecular analysis. Each filter paper was labeled with an identification number for each participant, dried at room temperature and then kept in a sachet with desiccant silica gel.

2.4. Laboratory Methods

2.4.1. Nucleic acid Extraction

Genomic DNA (deoxyribonucleic acid) of all samples was extracted from three punches of a 3 mm dried blood spot using the QIAamp DNA Blood Mini Kit (Qiagen®, Hilden, Germany). Elution was carried out at a 100 µL final volume, and the samples were stored at −20 °C until use.

2.4.2. Molecular Detection of Plasmodium Falciparum

Molecular confirmation of infection was carried out by RT-PCR targeting the *P. falciparum* var gene multi-copy acidic terminal sequence [17]. The primers, probe sequences, and cycling conditions used for this molecular diagnosis have been previously described [18]. The reaction mixture was processed on a CFX 96 real-time system thermocycler (BioRad). For all runs, 3D7 culture isolates served as positive controls, while DEPC-treated water was used as the negative control.

2.4.3. Drug Resistance Genotyping by High-Resolution Melting (HRM)

Primers and probes were reconstituted and diluted to 10× mix. Each HRM assay was constituted from 1 µL of positive gDNA (1 ng/µL) template, 4 µL of PCR-grade water, a 10× primer probe (made previously) and 4 µL of 2.5× Light Scanner master mix (BioFire Defense, LLC, 390 Wakara Way, Salt Lake City, Utah 84108, USA). Cycling was performed on a Light Cycler® 96. For each locus, mutant and wild-type DNA controls from known lab strains of *P. falciparum*, along with PCR negative controls, were run alongside the samples. Post-PCR, the amplicons were melted, and the melt curves were recorded. Normalized melt curves of each sample were compared with those of both the mutant and wild controls in concurrent runs. Allele at drug resistance markers were scored based on their melt curve profile relative to the controls (mutant or wild-type) as previously described [19]. The information obtained from the case report form (CRF) and the results of HRM were recorded in an Excel spreadsheet (Microsoft Excel 2013).

2.5. Statistical Analysis

Statistical analyses were performed using RStudio version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria). Quantitative variables were described based on their measures of central tendency and dispersion, while qualitative variables were presented as absolute and relative frequencies with their 95% confidence interval. Depending on the context, Chi-square or Fisher's exact test were employed for comparisons with a significance level of 0.05.

2.6. Ethical Considerations

This study is part of a project to monitor the effectiveness of IPTp in Senegal. This project was initially discussed with health authorities and community leaders to obtain their approval, followed by approval from the ethics committee (protocol 0404/2019/CER/CAD). Informed consent was obtained from all patients before their enrolment in the study, and anonymity was guaranteed for all participants.

3. Results

3.1. Characteristics of the Study Population

A total of 1050 pregnant women consented to participate in our surveys. After RT-PCR, 600 samples were positive and were all used to analyze resistance markers. These were mainly represented by young women with a mean age of 23.8 $\pm$ 6.3 years with a multigravida status (75.3%; 452/600), with a significant proportion being in the second trimester of pregnancy (41.3%; 248/600). Most participants reside in high-transmission areas (93.3 %; 560/600). Education levels vary, with a majority not having received formal education (67.7%; 406/600). Nearly all participants are married (98.0; 587/600), and the majority (94.3%; 566/600) reported using long-lasting insecticidal nets (LLINs) for malaria prevention. Regarding SP intake, more than half of the participants received at least one dose (54.8%; 329/600). The data also reveal that a significant portion of these women exhibited functional signs, such as fever, affecting 43.3% (260/600) of the population (Table 1).

Table 1: Background information of participants.

Characteristics	N	Absolute Frequence	Relative Frequence (IC 95%)
Age			
• Mean (SD)		23.8 (6.3)	
• $\leq$20 years		247	41.2 (37.5–45.6)
• [21–25 years]	600	143	23.8 (20.6–27.7)
• [26–31 years]		131	21.8 (18.7–25.6)
• >31 years		73	12.2 (9.7–15.2)
• NA		6	1.0 (0.4–2.2)
Area			
• Low transmission	600	40	6.7 (4.8–8.9)
• High transmission		560	93.3 (91.0–95.2)
Gravidity			
• Primigravidae	600	148	24.7 (21.2–28.3)
• Multigravidae		452	75.3 (71.7–78.7)
Gestational age			
• First trimester	600	156	26.0 (22.5–29.7)
• Second trimester		248	41.3 (37.4–45.3)
• Third trimester		196	32.7 (28.9–36.6)
Schooling			
• No		406	67.7 (63.8–71.4)
• Primary school		113	18.8 (15.8–22.2)
• Secondary school	600	30	5.0 (3.4–7.1)
• High school		45	7.5 (5.5–9.9)
• Arabe		5	0.8 (0.3–1.9)
• NA		1	0.2 (0.004–0.9)
Matrimonial status			
• Married		587	98.0 (96.5–99.0)
• Single	600	11	1.8 (0.9–3.3)
• Divorced		1	0.1 (0.004–0.92)
• NA		1	0.1 (0.004–0.9)
Functional signs			
• Fever	600	260	43.3 (39.3–47.4)
• Other signs		340	56.7 (52.6–60.7)

Table 1: *Cont.*

Characteristics	N	Absolute Frequence	Relative Frequence (IC 95%)
LLIN use			
• Yes	600	566	94.3 (92.2–96.0)
• No		34	5.7 (3.9–7.8)
SP intake			
• No		271	45.2 (41.1–49.2)
• 1		161	26.8 (23.3–30.6)
• 2		108	18 (0.15.0–21.3)
• 3	600	45	7.5 (5.5–9.9)
• 4		13	2.2 (1.1–3.7)
• 5		2	0.3 (0.04–1.2)

NA = not applicable; binomial test for IC95%.

3.2. Plasmodium falciparum Pfdhfr and Pfdhps Mutations in Pregnant Women

Analysis of the frequency of the various SNPs in the study population showed that the I164L mutation had a relative frequency of 11.7% (CI: 9.3%–14.5%). SNPs S436A, A437G, A581G and A613S had relative frequencies of 14.8% (CI: 12.2%–17.9%), 19.0% (CI: 16.1%–22.3%), 15.2% (CI: 12.5%–18.3%) and 15.5% (CI: 12.8%–18.6%), respectively. The K540E mutation was not detected in the study sample (Table 2).

Table 2: Frequencies of SNPs in the study population (N = 600).

SNPs	Absolute Frequence	Relative Frequence (IC 95%)
I164L	70	11.7 (9.3–14.5)
S436A	89	14.8 (12.2–17.9)
A437G	114	19.0 (16.1–22.3)
K540E	0	0
A581G	91	15.2 (12.5–18.3)
A613S	93	15.5 (12.8–18.6)

Binomial test for IC95%.

3.3. Analysis of Resistance Markers According to Population Characteristics

Analysis of resistance markers according to population characteristics showed that gravidity status did not significantly influence mutant prevalence, with rates for primigravidae at 10.8% for I164L, 15.5% for S436A, 23.6% for A437G, 12.2% for A581G and 12.1% for A613S. For multigravidae, the rates were 11.9%, 14.6%, 17.5%, 16.2% and 16.6%. A significant increase in the S436A mutant was observed during the second trimester (8.5%, $p = 0.03$). The prevalence of this S436A mutant was also higher among those not taking SP (18.5%, $p = 0.02$). No significant trends were found with varying SP doses. In high-transmission areas, compared to low-transmission areas, prevalence rates were significantly higher for all mutants: 12.3% for I164L ($p = 0.04$), 15.5% for S436A ($p = 0.04$), 20.2% for A437G ($p = 0.001$), 16.1% for A581G ($p = 0.05$) and 16.6% for A613S ($p < 0.01$) (Table 3).

Table 3: Distribution of *P. falciparum* SP-resistant SNPs according to women's characteristics.

Parameters	I164L		S436A		A437G		A581G		A613T	
	N (%)	*p*-Value	N (%)	*p*-Value	N (%)	*p*-Value	N (%)	*p*-Value	N (%)	*p*-Value
Gravidity										
• Primigravidae	16 (10.8)	0.7	23 (15.5)	0.78	35 (23.6)	0.09	18 (12.2)	0.24	18 (12.1)	0.2
• Multigravidae	54 (11.9)		66 (14.6)		79 (17.5)		73 (16.2)		75 (16.6)	
Gestational age										
• First trimester	18 (11.5)	0.5	24 (15.4)	*0.03* *	25 (16)	0.24	21 (13.5)	0.69	21 (13.5)	0.6
• Second trimester	33 (13.3)		46 (18.5)		55 (22.2)		41 (16.5)		42 (16.9)	
• Third trimester	19 (9.7)		19 (9.7)		34 (17.3)		29 (14.8)		30 (15.3)	
SP intake										
• Yes	35 (10.6)	0.4	39 (11.9)	*0.02* *	61 (18.5)	0.75	53 (16.1)	0.48	45 (13.7)	0.2
• No	35 (12.9)		50 (18.5)		53 (19.6)		38 (14)		48 (17.7)	
SP doses										
• 1	18 (11.2)	0.1	26 (16.1)	0.16	36 (22.4)	0.38	25 (15.5)	0.37	24 (45.3)	0.7
• 2	16 (14.8)		7 (6.5)		19 (17.6)		22 (20.4)		16 (37)	
• 3	1 (2.2)		5 (11.1)		5 (11.1)		6 (13.3)		0 (26.7)	
• 4	0 (0.0)		1 (7.7)		1 (7.7)		0 (0.0)		0 (15.4)	
• 5	0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
Functional signs										
• Fever	49 (18.9)	*<0.01* *	44 (16.9)	0.2	72 (27.7)	<0.01 *	70 (26.9)	<0.01 *	65 (25.0)	<0.01 *
• Other signs	21 (6.2)		45 (13.2)		42 (12.3)		21 (6.2)		28 (8.2)	
Area										
• High transmission	69 (12.3)	*0.04* *	87 (15.5)	*0.04* *	113 (20.2)	*0.001* *	90 (16.1)	0.05 *	93 (16.6)	*<0.01* *
• Low transmission	1 (2.5)		2 (5.0)		1 (2.5)		1 (2.5)		0	

Note: Khi 2 and Fisher's exact test; *values marked with an asterisk (*)* indicate statistical significance.

4. Discussion

Malaria control strategies in Senegal have relied heavily on the continued efficacy of antimalarial drugs used in treatment and chemoprevention, which needs to be continuously monitored. The goal of this study was to determine the prevalence of markers of resistance to SP, the antimalarial used for malaria chemoprevention in pregnant women in Senegal. The study's findings indicate a relatively low prevalence of SP resistance markers among pregnant women in Senegal, with notable differences between high- and low-transmission areas. The absence of the K540E mutation suggests that SP remains effective for malaria chemoprevention. However, the presence of other mutations at varying frequencies indicates ongoing selection pressure and potential for future resistance development. The study had a relatively small sample size in low-transmission areas due to the low positivity rate, and it would be beneficial to have a larger sample size in these areas to enhance the study's power and reliability. Some information, such as the possession or use of bed nets, was obtained through interviews, which might have introduced recall bias or inaccuracies. Despite these limitations, this study offers an overview of resistance markers in pregnant women and can guide policymakers in their decisions. It also shows that this type of evaluation done at a lower cost than a drug resistance survey in general population is feasible and can serve as a sentinel strategy to monitor the emergence of resistance to SP in the general population.

Even though the prevalences found in this study are low, compared to previous studies carried out in Senegal, an increase is noted for the A581G, I164L and A613S mutations [13,20,21]. The differences in prevalence observed between this study and previous studies carried out in Senegal can firstly be attributed to methodological factors such as the study design or the localities studied. Depending on the region or sampling method, the prevalence of mutations may vary. However, the most important factor remains the time period during which the studies were carried out. Earlier studies, carried out when SP resistance was less widespread, naturally reported lower frequencies of these mutations. However, with the continued extensive use of SP for IPTp, the selective pressure exerted by the drug promotes the spread of resistant strains, leading to higher mutation frequencies in more recent samples [22]. The A581G mutation is particularly concerning, as it has been associated with the failure of IPTp-SP to improve birth weight outcomes. This is critical because one of the key goals of IPTp-SP is to protect pregnant women from malaria and improve neonatal health by preventing low birth weight. Additionally, the A581G mutation is linked to increased placental inflammation and enhanced parasite growth, even in the presence of SP, which may compromise treatment efficacy [23]. The *Pfdhps* K540E mutation, which is highly associated with sulfadoxine resistance, was not found in the populations sampled. In combination with others variants, K540E confers a higher level of resistance against SP [24]. This mutation is generally less common in West Africa [20,25,26] compared to East Africa, where it could have been imported from southeast Asia. The absence of this mutation in our study population suggests that SP is still effective and can continue to be used for malaria chemoprevention in pregnant women in Senegal. However, recent studies conducted in Senegal have found this mutation, which for a long time was absent in Senegal [13] (Manga, 2024 unpublished). The presence of this mutation could be attributed to the study population, as both studies that identified it were carried out on symptomatic individuals. Even though the prevalences found were low, it is essential to maintain surveillance to monitor and control the potential spread of resistance.

The analysis of resistance markers in relation to the characteristics of the population showed no statistically significant differences based on the number of SP doses taken or gravidity. Several studies support the finding that the prevalence of SP resistance markers does not significantly differ between primigravidae and multigravidae [27,28]. The lack of significant differences in SP resistance markers between primigravidae and multigravidae can be attributed to similar levels of exposure to SP, the overarching influence of local transmission intensity and the genetic characteristics of the malaria parasites rather than individual pregnancy histories. Both primigravidae and multigravidae are likely to receive similar doses of SP during their pregnancies, leading to comparable levels of drug

exposure. This uniformity in exposure helps explain why resistance markers are similarly prevalent in both groups [27,28]. While primigravidae generally have less developed immune responses to malaria due to first-time exposure to pregnancy-associated malaria, multigravidae have developed partial immunity from previous pregnancies. However, this difference in immune response does not significantly affect the genetic mutations responsible for SP resistance [27,28]. The intensity of malaria transmission in a given area is a crucial factor. Some studies indicate that areas of high malaria transmission are associated with higher prevalence rates of resistance markers, regardless of the number of pregnancies the woman has had [29]. This observation was made in this study, where higher prevalence was observed in areas of high malaria transmission. This could be explained by the deployment of SMC with SP+AQ in high-transmission areas for over 10 years, in addition to IPTp in Senegal; frequent exposure to antimalarial drugs can accelerate selection pressure on the parasite, leading to the spread of resistant strains. Understanding and addressing these geographical disparities is essential to ensure the effectiveness of malaria control interventions and to design appropriate drug policies.

In this study, we also noticed that for certain codons, the prevalence of mutations was higher in those who had not taken SP. At this stage, we have no data to explain the higher prevalence found for certain mutants in women who were not exposed to SP during their pregnancy. However, several hypotheses have been put forward to explain this trend. Indeed, cross-resistance, drug pressure from multiple treatments and the use of prophylactic drugs in specific populations all contribute to the complex landscape of antimalarial resistance [28]. Other medications, particularly those with similar mechanisms of action as SP like cotrimoxazole (used as prophylaxis in HIV-positive individuals) or those used in combination with SP, can significantly impact the prevalence of SP resistance markers [27]. Also, natural selection and genetic drift can lead to the maintenance of mutations even in the absence of direct drug pressure, as genetic drift can randomly fix or lose alleles in small populations [30]. Further research is needed to elucidate the exact mechanisms and contributory factors behind this observation.

5. Conclusions

This study highlights the importance of ongoing surveillance to monitor SP resistance and inform malaria control strategies in Senegal. Surveys in antenatal clinics could be a good strategy for monitoring at low cost the emergence of resistance to SP. While the absence of a key mutation suggests the continued efficacy of SP, the presence of other resistance markers and regional variations underscore the need for proactive measures. Furthermore, the lack of significant differences in resistance markers based on gravidity or SP dosage supports the continued use of IPTp for malaria prevention in pregnant women.

Author Contributions: M.P.D., S.K., A.S. and I.A.M. carried out the study and participated in the statistical analysis and procedures. M.P.D., M.A.O. and A.A.N. carried out the molecular analysis. F.B.S., M.A.O. and I.A.M. participated in the statistical analysis. J.-L.A.N., C.M., P.M. and M.P.D. wrote the protocol and coordinated and participated in the design of the study and statistical analysis. M.P.D., M.A.O., S.K., I.A.M. and J.-L.A.N. wrote the draft of the manuscript. J.-L.A.N., B.F., A.A.N., A.C.L., S.K., I.A.M., M.A.O. and F.B.S. assisted in the preparation of the article. All authors have read and agreed to the published version of the manuscript.

Funding: Funded by the World Health Organization Tropical Disease Research (Project ID/unit reference B80316, WHO reference 2019/973714-0).

Acknowledgments: The authors wish to thank the dedicated staff of the regional and district health teams and the health post staff nurses and midwives who supported enrollment of the study

participants. We also thank all the participants in this study; WHO/TDR, who provided the funds; the parasitology laboratory of Cheikh Anta Diop University; and MRC Unit The Gambia at LSHTM.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Disclaimer: CSM is currently a staff member of the World Health Organization; the author alone is responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy or views of the WHO.

References

1. World Malaria Report 2021. Available online: https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021 (accessed on 1 June 2024).
2. PNLP: Bulletin-Epidemiologique-ANNUEL-2019-du-Paludisme-au-SENEGAL-VFinale.pdf.
3. WHO Policy Recommendation: Seasonal Malaria Chemoprevention (SMC) for Plasmodium Falciparum Malaria Control in Highly Seasonal Transmission Areas of the Sahel Sub-Region in Africa. 2012. Available online: https://www.mmv.org/sites/default/files/ (accessed on 1 June 2024).
4. WHO Policy Brief for the Implementation of Intermittent Preventive Treatement of Malaria in Pregnancy Using Sulfadoxine-Pyrimethamine (IPTp-SP). April 2013. Available online: https://iris.who.int/bitstream/handle/10665/338350/WHO-HTM-GMP-2014.4-eng.pdf?sequence=2 (accessed on 1 June 2024).
5. Venkatesan, M.; Alifrangis, M.; Roper, C.; Plowe, C.V. Monitoring antifolate resistance in intermittent preventive ther0061py for malaria. *Trends Parasitol.* **2013**, *29*, 497–504. [CrossRef] [PubMed]
6. Hayton, K.; Su, X.-z. Genetic and biochemical aspects of drug resistance in malaria parasites. *Curr. Drug. Targets Infect. Disord.* **2004**, *4*, 1–10. [CrossRef] [PubMed]
7. Cortese, J.F.; Plowe, C.V. Antifolate resistance due to new and known Plasmodium falciparum dihydrofolate reductase mutations expressed in yeast. *Mol. Biochem. Parasitol.* **1998**, *94*, 205–214. [CrossRef] [PubMed]
8. Plowe, C.V.; Kublin, J.G.; Doumbo, O.K.P. falciparum dihydrofolate reductase and dihydropteroate synthase mutations: epidemiology and role in clinical resistance to antifolates. *Drug Resist. Updat.* **1998**, *1*, 389–396. [CrossRef] [PubMed]
9. Gregson, A.; Plowe, C.V. Mechanisms of Resistance of Malaria Parasites to Antifolates. *Pharmacol. Rev.* **2005**, *57*, 117–145. [CrossRef]
10. Triglia, T.; Wang, P.; Sims, P.F.; Hyde, J.E.; Cowman, A.F. Allelic exchange at the endogenous genomic locus in Plasmodium falciparum proves the role of dihydropteroate synthase in sulfadoxine-resistant malaria. *EMBO J.* **1998**, *17*, 3807–3815. [CrossRef]
11. Ndiaye, Y.D.; Wong, W.; Thwing, J.; Schaffner, S.F.; Brenneman, K.V.; Tine, A.; Diallo, M.A.; Deme, A.B.; Sy, M.; Bei, A.K.; et al. Two decades of molecular surveillance in Senegal reveal rapid changes in known drug resistance mutations over time. *Malar. J.* **2024**, *23*, 1–14. [CrossRef]
12. Ndiaye, D.; Dieye, B.; Ndiaye, Y.D.; Van Tyne, D.; Daniels, R.; Bei, A.K.; Mbaye, A.; Valim, C.; Lukens, A.; Mboup, S.; et al. Polymorphism in dhfr/dhps genes, parasite density and ex vivo response to pyrimethamine in Plasmodium falciparum malaria parasites in Thies, Senegal. *Int. J. Parasitol. Drugs Drug Resist.* **2013**, *3*, 135–142. [CrossRef]
13. Boussaroque, A.; Fall, B.; Madamet, M.; Wade, K.A.; Fall, M.; Nakoulima, A.; Fall, K.B.; Dionne, P.; Benoit, N.; Diatta, B.; et al. Prevalence of anti-malarial resistance genes in Dakar, Senegal from 2013 to 2014. *Malar. J.* **2016**, *15*, 1–8. [CrossRef]
14. Programme national de lutte contre le paludisme. Plan stratégique national de lutte contre le paludisme au Sénégal 2021-2025. Available online: https://senegal-cocreation.com/wp-content/uploads/2021/02/PSN_PNLP_Senegal_Version-finale_-Fevrier-2021.pdf (accessed on 5 December 2023).

15. Programme national de lutte contre le paludisme. Plan stratégique national de lutte contre le paludisme au Sénégal 2016-2020. Available online: https://malariaportal.org/sites/default/files/2023-11/SNG-501.1_%20Plan%20Strategique%20National%20PNLP%202016-2020.pdf (accessed on 10 July 2024).
16. Diouf, M.P.; Kande, S.; Oboh, M.A.; Manga, I.A.; Tairou, F.; Seck, A.; Diallo, A.; Lo, A.C.; Sow, D.; Sylla, K.; et al. Prevalence of Malaria Infection in Pregnant Women Attending Antenatal Clinics in Southern Senegal. *Am. J. Trop. Med. Hyg.* **2024**, *110*, 214–219. [CrossRef]
17. Hofmann, N.; Mwingira, F.; Shekalaghe, S.; Robinson, L.J.; Mueller, I.; Felger, I. Ultra-Sensitive Detection of Plasmodium falciparum by Amplification of Multi-Copy Subtelomeric Targets. *PLoS Med.* **2015**, *12*, e1001788. [CrossRef] [PubMed]
18. Umunnakwe, F.A.; Idowu, E.T.; Ajibaye, O.; Etoketim, B.; Akindele, S.; Shokunbi, A.O.; Otubanjo, O.A.; Awandare, G.A.; Amambua-Ngwa, A.; Oyebola, K.M. High cases of submicroscopic Plasmodium falciparum infections in a suburban population of Lagos, Nigeria. *Malar. J.* **2019**, *18*, 1–8. [CrossRef] [PubMed]
19. Daniels, R.; Ndiaye, D.; Wall, M.; McKinney, J.; Séne, P.D.; Sabeti, P.C.; Volkman, S.K.; Mboup, S.; Wirth, D.F. Rapid, Field-Deployable Method for Genotyping and Discovery of Single-Nucleotide Polymorphisms Associated with Drug Resistance in Plasmodium falciparum. *Antimicrob. Agents Chemother.* **2012**, *56*, 2976–2986. [CrossRef] [PubMed]
20. Ndiaye, M.; Tine, R.; Faye, B.; Ndiaye, J.L.; Lo, A.C.; Sylla, K.; Abiola, A.; Dieng, Y.; Ndiaye, D.; Hallett, R.; et al. Selection of Antimalarial Drug Resistance after Intermittent Preventive Treatment of Infants and Children (IPTi/c) in Senegal. *Am. J. Trop. Med. Hyg.* **2013**, *88*, 1124–1129. [CrossRef] [PubMed]
21. Ndiaye, Y.D.; Diédhiou, C.K.; Bei, A.K.; Dieye, B.; Mbaye, A.; Mze, N.P.; Daniels, R.F.; Ndiaye, I.M.; Déme, A.B.; Gaye, A.; et al. High resolution melting: a useful field-deployable method to measure dhfr and dhps drug resistance in both highly and lowly endemic Plasmodium populations. *Malar. J.* **2017**, *16*, 1–10. [CrossRef]
22. SP Resistance Data Access Group. Infectious Diseases Data Observatory. Available online: https://www.iddo.org/wwarn/working-together/study-groups/sp-resistance-data-access-group (accessed on 28 June 2024).
23. Gutman, J.; Kalilani, L.; Taylor, S.; Zhou, Z.; Wiegand, R.E.; Thwai, K.L.; Mwandama, D.; Khairallah, C.; Madanitsa, M.; Chaluluka, E.; et al. The A581G Mutation in the Gene Encoding Plasmodium falciparum Dihydropteroate Synthetase Reduces the Effectiveness of Sulfadoxine-Pyrimethamine Preventive Therapy in Malawian Pregnant Women. *J. Infect. Dis.* **2015**, *211*, 1997–2005. [CrossRef]
24. Kublin, J.G.; Dzinjalamala, F.K.; Kamwendo, D.D.; Malkin, E.M.; Cortese, J.F.; Martino, L.M.; Mukadam, R.A.G.; Rogerson, S.J.; Lescano, A.G.; Molyneux, M.E.; et al. Molecular Markers for Failure of Sulfadoxine-Pyrimethamine and Chlorproguanil-Dapsone Treatment of *Plasmodium falciparum* Malaria. *J. Infect. Dis.* **2002**, *185*, 380–388. [CrossRef]
25. Lo, A.C.; Faye, B.; Ba, E.-H.; Cisse, B.; Tine, R.; Abiola, A.; Ndiaye, M.; LA Ndiaye, J.; Ndiaye, D.; Sokhna, C.; et al. Prevalence of molecular markers of drug resistance in an area of seasonal malaria chemoprevention in children in Senegal. *Malar. J.* **2013**, *12*, 137–137. [CrossRef]
26. Niba, P.T.N.; Nji, A.M.; Chedjou, J.P.K.; Hansson, H.; Hocke, E.F.; Ali, I.M.; Achonduh-Atijegbe, O.; Evehe, M.-S.B.; Jørgensen, M.H.M.; Fomboh, C.T.; et al. Evolution of Plasmodium falciparum antimalarial drug resistance markers post-adoption of artemisinin-based combination therapies in Yaounde, Cameroon. *Int. J. Infect. Dis.* **2023**, *132*, 108–117. [CrossRef]
27. Mama, A.; Ahiabor, C.; Tornyigah, B.; Frempong, N.A.; Kusi, K.A.; Adu, B.; Courtin, D.; Houzé, S.; Deloron, P.; Ofori, M.F.; et al. Intermittent preventive treatment in pregnancy with sulfadoxine–pyrimethamine and parasite resistance: cross-sectional surveys from antenatal care visit and delivery in rural Ghana. *Malar. J.* **2022**, *21*, 1–9. [CrossRef]
28. Plowe, C.V. Malaria chemoprevention and drug resistance: a review of the literature and policy implications. *Malar. J.* **2022**, *21*, 1–25. [CrossRef] [PubMed]
29. Ruizendaal, E.; Tahita, M.C.; Geskus, R.B.; Versteeg, I.; Scott, S.; D'alessandro, U.; Lompo, P.; Derra, K.; Traore-Coulibaly, M.; de Jong, M.D.; et al. Increase in the prevalence of mutations associated with sulfadoxine–pyrimethamine resistance in Plasmodium falciparum isolates collected from early to late pregnancy in Nanoro, Burkina Faso. *Malar. J.* **2017**, *16*, 1–15. [CrossRef] [PubMed]
30. Star, B.; Spencer, H.G. Effects of Genetic Drift and Gene Flow on the Selective Maintenance of Genetic Variation. *Genetics* **2013**, *194*, 235–244. [CrossRef] [PubMed]