fortune Journals

Research Article

FORTUNE JOURNAL OF HEALTH SCIENCES

ISSN: 2644-2906

Assessing of *Pfdhps* Gene of Sulfadoxine after Five Years of Seasonal Malaria Chemoprevention Implementation at Two Rural Sites in Mali: The Quantitative Method

Hamma Maiga[*,1,2], Robert Morrison[1], Issaka Sagara[3], Jennifer Hume[1], Sara Healy[1], Mahamadoun H. Assadou[3], Abdoulaye Katile[3], Amatigue Zeguime[3], Jillian Neal[1], Irfan Zaidi[1] and Patrick E. Duffy[1]

Abstract

Seasonal malaria chemoprevention (SMC) aims to prevent malaria in children under 5-year of age and this strategy was implemented in Mali since 2012. Mass administration of sulfadoxine-pyrimethamine plus amodiaquine (SPAQ) could potentially generate drug-resistant *Plasmodium falciparum* parasites after several years. In this study, we investigated the mean fraction of resistance gene markers in the drug target of sulfadoxine *Pfdhps* among children and adults after 5 years of SMC implementation at two rural sites in Mali. From June to December 2019 at Doneguebougou and Bancoumana, data were collected from children under 5 years of age who received SMC as well as older individuals did not receive SMC. In Doneguebougou specifically, participants were 5 years and older received an investigational malaria transmission-blocking vaccine (Pfs230D1-EPA/AS01) or a comparator vaccine, and all children received artemether-lumefantrine before vaccination. Genomic DNA was extracted from filter paper blood spots and whole blood, then nested PCR was used to amplify the *Pfdhps* gene. Sanger sequencing of resistance loci fragments and deconvolution of chromatograms were used to quantify *Pfdhps* molecular marker variants and genotypes I431V, S436F/A, A437G, K540E, A581G and A613S/T. A total of 456 children and adults were evaluated in Doneguebougou (n=202) and Bancoumana (n=254). The mean fraction of resistance markers was significantly different between smc-group versus non-smc-group in Doneguebougou, but not in Bancoumana for *Pfdhps* at codons 431V and 613S/T. Highest mean fraction was found with the *Pfdhps* single mutant 436A/F and 437G in smc-group as well as in non-smc-group at both sites. *Pfdhps* 540E was not found in the Doneguebougou smc-group. There was no difference in the mean fraction of *Pfdhps* at all codons by age category (1-4years, 5-8yrs, 9-18yrs and adults), when data from both sites were combined. The mean fraction of *Pfdhps* 540E and 581G were low after five years of SMC implementation, suggesting that sulfadoxine-pyrimethamine remains an effective antimalarial drug in these two sites in Mali. Otherwise, sulfadoxine resistance molecular markers in Bancoumana were as high or higher in the non-smc-group as smc-group.

Keywords: Seasonal malaria chemoprevention, *Pfdhps*, sequencing, deconvolution method, Mali

Introduction

In Mali, malaria is the top cause for clinical consultation, as well as morbidity and mortality in children under 5 years and pregnant women

Affiliation:

[1]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 29 Lincoln Drive, Bethesda, MD, 20892, USA

[2]Institut National de Sante Publique, Bamako, BP: 1771, Mali

[3]Malaria Research and Training Center, Department of Epidemiology of Parasitic Diseases, Faculty of Medicine and Dentistry, Faculty of Pharmacy, University of Sciences, Techniques and Technologies of Bamako, BP: 1805, Mali

***Corresponding author:**
Hamma Maiga, Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 29 Lincoln Drive, Bethesda, MD, 20892, USA.

Citation: Hamma Maiga, Robert Morrison, Issaka Sagara, Jennifer Hume, Sara Healy, Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguime, Jillian Neal, Irfan Zaidi and Patrick E. Duffy. Assessing of *Pfdhps* Gene of Sulfadoxine-Pyrimethamine after Five Years of Seasonal Malaria Chemoprevention Implementation at Two Rural Sites in Mali: The Quantitative Method. Fortune Journal of Health Sciences, 8 (2025): 479-490

Received: April 15, 2025
Accepted: April 22, 2025
Published: June 02, 2025

Maiga H, et al., Fortune J Health Sci 2025
DOI:10.26502/fjhs.298

[1]. In children under 5 years, almost 80-90% are infected during the rainy season. Seasonal malaria chemoprevention (SMC), previously referred to as Intermittent Preventive Treatment in children (IPTc), is a strategy to control malaria in children, defined as intermittent administration of full courses of antimalarial drug treatment during the malaria season in order to maintain therapeutic drug concentrations in the blood throughout the period of greatest risk [2]. SMC currently involves monthly administration of sulfadoxine-pyrimethamine plus amodiaquine (SPAQ) and is a promising strategy to reduce the burden attributable to malaria in children under five. African countries or regions that meet these criteria are mostly in the Sahel sub-region, where both SP and AQ currently retain their efficacy against *Plasmodium falciparum* [2]. In 2012, the World Health Organization (WHO) issued a policy recommendation that children aged 3-59 months living in areas of highly seasonal malaria in the Sahel sub-region should receive SMC. SMC implementation started in 2012 by Medecin Sans Frontiere projects in Mali and Chad; in 2013 SMC implementation started in Niger, Senegal, Togo and Nigeria; and in 2014 in Gambia and Burkina Faso [3-7]. Thirteen countries now have SMC programs, reaching about 20 million children [8].

SMC has been shown to be safe, but as with all new interventions, safety monitoring must be maintained to ensure the drugs remain safe. Thus, it is important to strengthen national safety monitoring systems. In addition, the impact of SMC on child health must be determined by surveillance at clinics and hospitals to assess the reduction in the number of cases of malaria. Although SMC implementation appears to have contributed to an increase in frequency of molecular markers of SP drug resistance after two years implementation in Mali [4, 5, 9]. The overall efficacy of SPAQ may not be compromised at this stage. Indeed, (i) there has been no selection of resistance markers to AQ, (ii) there has been no spread of SP resistance markers in the general parasite population in the same area, and (iii) the rate of dhps 540E at baseline is still at 7%, much lower than the WHO threshold of 50% which is thought to compromise SP efficacy in Intermittent Preventive Treatment in pregnancy (IPTp) [7]. However, progress in malaria control has stalled, with no significant reductions in the number of malaria cases worldwide in the past 4 years, per WHO World Malaria Reports in 2018 and 2019 [8, 10]. Furthermore, the spread of insecticide and mosquito behavioural resistance compromises malaria control measures such as indoor residual spraying (IRS) and insecticide-treated nets (ITN) [11]. This is a potential threat for future control efforts, necessitating new approaches for malaria control and ultimately elimination.

An example of such an approach includes administration of SMC in combination with a malaria vaccine (RTS,S) [12]. A vaccine to interrupt malaria transmission (VIMT), which aims to disrupt parasite transmission to both humans and mosquitoes, would be a valuable additional resource in the fight to eliminate this disease [12, 13]. Transmission-blocking vaccines (TBVs) are among the tools being developed for use during pre-elimination and elimination phases, according to malERA [14], and could be an effective adjunct to vector control. Compared to vector control interventions, TBVs are ecologically safer, cost-effective, and readily enable high coverage of populations. Notably, sexual stages of the malaria parasite are critical for the generation of parasite genetic diversity and regulation of parasite virulence, hence the effects of TBVs on these phenomena also warrant assessment and monitoring [11]. In addition, the preventive use of SPAQ decreases the malaria burden in terms of morbidity and mortality which would also restrict mosquito infection.

Nonetheless, in 2016, SMC implementation expanded nationally in Mali [15]. Of concern, the WHO has not yet standardized methods to monitor the effectiveness of this strategy in a mass campaign. It is essential to establish a standardized monitoring system to assess the effectiveness of SMC while also monitoring for potential development of drug resistance after several years of mass implementation, and SPAQ could potentially generate drug-resistant *Plasmodium falciparum* parasites after several years. One study found that the prevalence of SP resistance increased significantly from 1.6% to 7.1% within after 2 years and 7 rounds of SMC implementation in Mali [9]. However, there was no significant increase of these markers of resistance in the general parasite population (non-SMC population). The widespread deployment of SPAQ for SMC could lead to a selective advantage for more highly resistant parasite variants, such as the “quintuple” haplotype, and thus a progressive loss of efficacy. Therefore, it is essential that any large-scale SMC program should incorporate a resistance monitoring component. We hypothesize that SMC implementation will increase the resistance of molecular makers drug resistance in children population and will extend to the non-SMC population. This finding will contribute to monitoring the SP effectiveness and the spread of drug-resistant parasites. In this study, we investigate the mean fraction (quantitative method) of *Pfdhps* after several years of SMC implementation in two rural sites in Mali.

Materials and Methods

Study sites

This study was performed in Bancoumana and Doneguebougou villages located 60 km southwest and 30 km northwest of Bamako (capital of Mali), respectively. Malaria transmission is highly seasonal and intense in both villages during the rainy season from July to December. *Plasmodium falciparum* represented from 93 to 97% of infecting Plasmodium species, while *P. malariae* represented

Citation: Hamma Maiga, Robert Morrison, Issaka Sagara, Jennifer Hume, Sara Healy, Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguime, Jillian Neal, Irfan Zaidi and Patrick E. Duffy. Assessing of *Pfdhps* Gene of Sulfadoxine-Pyrimethamine after Five Years of Seasonal Malaria Chemoprevention Implementation at Two Rural Sites in Mali: The Quantitative Method. Fortune Journal of Health Sciences. 8 (2025): 479-490.

Maiga H, et al., Fortune J Health Sci 2025
DOI:10.26502/fjhs.298

less than 8%, and *P. ovale* less than 1% of all malaria infections [16]. The prevalence of *P. falciparum* infection in children less than five years of age varies from approximately 30–50% during the dry season to 75% during the rainy season (Doumbia S, 2002. *Determinants of Semi-Immune State in an Area of Seasonal Malaria Transmission in Bancoumana, Mali*. Doctoral Thesis. New Orleans: Tulane University). The major vectors are *An. gambiae* (approximately 95.5%) and *An. arabiensis* (approximately 4.5%). Annual Entomologic Inoculation Rate is > 100 infective bites per person in Doneguebougou and the Koba River near the village heavily contributes to the persistence of anopheline mosquito breeding sites. The mean monthly entomologic inoculation rate in Bancoumana was 2.8 infectious bites per person with marked seasonal variations (*Bagayoko M, 2000. Application des Systèmes d'Information Géographiques à l'Étude Micro-Épidémiologique de la Transmission du Paludisme à Bancoumana (Arrondissement de Sibi, Cercle de Kati)*. Probably, the Niger River near the village heavily contributes to the persistence of anopheline mosquito breeding sites. Both villages are situated in the sudanian area of Mali. The climate is hot, with daily temperatures ranging from 19 to 40°C. The annual rainfall, mean Relative Humidity and Wind Speed wirer 1034.2 mm, 49.5 and 2.2 in both villages, respectively. Precipitation occurs mainly during the rainy season from June to October/November, which corresponds to the period of intense malaria transmission. A population of approximately 9000 and 2500 inhabitants in Bancoumana and Doneguebougou, respectively people (a full census was undertaken, in 200 by the MRTC/DEAP). These two villages contain facilities built by NIAID/NIH for vaccine trials, hosting more than 20 years of collaboration between NIH and the research centers at the University of Sciences, Techniques and Technologies of Bamako, Mali. Doneguebougou and Bancoumana have been chosen as a site for testing malaria vaccines based on examination of malariometric indicators collected over several transmission seasons (including infection rates, disease prevalence and incidence, entomologic inoculation rates (EIRs), and demographic data) and a close relationship between the MRTC and the community. The main economic activity of these populations is agriculture and general income levels are very low. Extended families generally live together in separate compounds within the village. SMC was started in both village in 2015. This study was conducted during the high-transmission season (i.e., July through December 2019).

Study design

The Laboratory of Malaria Immunology and Vaccinology (LMIV) was commissioned in 2009 to conduct basic and applied research relevant to malaria immunology and vaccine development, to pursue novel vaccine concepts, to produce prototype malaria vaccines, and to conduct early-phase clinical trials of promising vaccine candidates in Mali. The LMIV's overarching goal is to develop malaria vaccines that will reduce severe disease and death among African children and pregnant women and will eliminate malaria from low-transmission areas of the world. It has an organizational structure that encompasses both basic discovery and product development within a small, integrated team. Discovery sections within LMIV conduct basic research on malaria pathogenesis and immunology, with an emphasis on studies in humans who are naturally or experimentally infected with malaria parasites. It has completed a Phase 1 study of TBV candidate Pfs230D1M-EPA/AS01 in adults (NIAID Protocol #17-I-N006); results indicate that the vaccine is safe, tolerable, immunogenic, and provides high and durable transmission-blocking activity. These initial results suggest that Pfs230D1M-EPA/AS01 can be employed in a community wide trial to evaluate its efficacy to reduce malaria infections in the community in Doneguebougou. LMIV is now conducting a Phase 2 age de-escalation community trial to ensure the vaccine is safe to administer to children and efficacious in family groups. This Phase 2 study will first determine safety and tolerability of Pfs230D1M-EPA/AS01 in healthy Malian children of decreasing ages: 9-18 years old, followed by 5-8 years old. Children recruited from compounds/families are enrolled in a staggered manner to receive either Pfs230D1M-EPA/AS01 vaccine or comparator, as assigned by block randomization. For this reason, in 2019, children under 5 years old did not receive this vaccine but did receive Artemether-lumefantrine (Coartem^{R}) in Doneguebougou.

Samples collection

Samples for molecular analysis were collected from participants of the Doneguebougou and Bancoumana health centers. In Doneguebougou the participant aged 5 to up received 3 doses of vaccine or comparator from April to Jun and all children aged from 5 to 18 years received one dose of artemether-lumefantrine in July. In Bancoumana children less than 5 years received seasonal malaria chemoprevention and other participants did not vaccine. During a high malaria transmission, children and adults presenting with fever (axillary temperature ≥ 37.5 °C) or history of fever during the previous 24 hours were tested for malaria. The presence of *Plasmodium falciparum* was initially investigated by a rapid diagnostic test (HRP2 SD Bioline®) then confirmed and quantified by microscopy on Giemsa-stained blood smears. The recruitment period extended from July to December 2019. After obtaining informed consent from parents or guardians, 1 ml of venous blood was collected in EDTA anticoagulant tubes among participant up to 5 years among 5 years aged to older and filter paper among children under five years in Doneguebougou. In Bancoumana, filter paper was collected among all participants. Parasitized blood samples were stored frozen at – 80 °C until analysis.

Citation: Hamma Maiga, Robert Morrison, Issaka Sagara, Jennifer Hume, Sara Healy, Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguime, Jillian Neal, Irfan Zaidi and Patrick E. Duffy. Assessing of *Pfdhps* Gene of Sulfadoxine-Pyrimethamine after Five Years of Seasonal Malaria Chemoprevention Implementation at Two Rural Sites in Mali: The Quantitative Method. Fortune Journal of Health Sciences. 8 (2025): 479-490.

Maiga H, et al., Fortune J Health Sci 2025
DOI:10.26502/fjhs.298

DNA extraction, PCR and Sequencing

QIAamp DNA blood kit has served to genomic DNA extraction and the measurement of the concentration and purity of DNA was achieved using a NanoDrop2000 UV. The final elute was 50 ul and 100ul for Dried blood spots (DBS) and whole blood DNA extraction, respectively. *Pfdhps* sequences, genes containing mutations I431V, S436A/F/H, A437G, K540E, A581G, and A613S/T (previously associated with parasite responses to sulfonamide) were amplified by nested PCR using previously published primers [17]. Briefly, for the first round of PCR, 0.5 for each primer, Master mix hot-Taq 12.5 µl and 5 µl of DNA in a total volume of 25 µl completed by nuclease-free water was run under the following cycling conditions: 94 °C for 3 min then 30 cycles at 94 °C for 30 s, 55 °C for 30 s and extension at 65 °C for 1 min and final extension at 65 °C for 5 min. For the second round of PCR, a total volume of 50 µl made of 19 µl nuclease-free water, 0.5 for each primer, Master mix hot-Taq 25 µl and 5 µl of DNA was run under the following cycling conditions: 94 °C for 5 min then 30 cycles at 94 °C for 30 s, 60 °C for 30 s and extension at 65 °C for 1 min and final extension at 65 °C for 5 min. PCR products for both genes were purified by Qiagen kit and concentration was measured using a NanoDrop2000 UV. The same primers were sent for cycle-sequencing. Cycle sequencing followed the standard BigDye3.1 dye terminator protocol (AppliedBiosystems) on an MJ-Thermocyler. Sequencing reactions were cleaned on Sephadex G10 columns and analysed on an ABI3130xl Genetic Analyser. Sanger sequencing of resistance gene fragments and deconvolution of chromatograms to quantify molecular marker variants were used to genotype *Pfdhps* [18]. We further filtered the dataset to exclude samples exhibiting either a low fraction of genotype bases because of low sequencing coverage or SNP sites exhibiting a call rate of less than 10% in the remaining samples and/or a minor allele frequency of less than 10%. Samples were excluded from the log sheet if they exhibited calls for fewer than 90% of the SNP loci matching the criteria just described. DNA sequencing analysis of the *Pfdhps* fragment was carried out to detect mutations at codons 431, 436, 437, 540, 581 and 613.

Statistical analysis

This was an exhaustive study. All the confirmed malaria cases detected during the study period in the reference site (Doneguebougou) were collected and the same number were taken in Bancoumana site. At each site we have two groups, the smc-group defined by children under 5 years of age who received seasonal malaria chemoprevention (SMC) and non-smc-group that is defined by the children and adults did not receive smc drugs. Wilcoxon Rank Sum test with a continuity correction was used to compare the mean fraction of *Pfdhps* mutations at codons I431V, S436F/A, A437G, K540E, A581G and A613S/T between smc-group versus non-smc-group in each site. Statistical analysis was performed using R® software version 4 and the significant p-value was inferior to 0.05.

Ethics

Trial protocol (19-I-N086/N°2019/10/CE/FMPOS; 21 March 2019) was approved by ethics committees of the US National Institutes of Health (Bethesda, MD, USA), Faculte de Medecine et d'Odonto-stomatologie, and Mali Ministry of Health (Bamako, Mali). Studies were conducted with the US Food and Drug Administration (FDA) Investigational New Drug Application. This study was registered with ClinicalTrials.gov (NCT03917654). Before the study was started. overall community and local authorities' permission were also obtained in addition to parent or guardian informed consent.

Results

Study characteristics

DNA was extracted from 456 samples, 148 from children under five years of age and 308 from individual aged from 5 to 65 years. The samples were positive for *Plasmodium falciparum* parasites (Figure 1). The total sample in Doneguebougou and Bancoumana were 202 and 254 with (57 and 91) for smc-group and (145 and 163) for non-smc-group, respectively. The mean parasitemia of the participants under 5-year age group (999.41 ul/mm^3) was higher than the mean parasitemia in the 5 to older of age group (385.40ul/mm^3), p= 0.003 (Data not shown).

Mean fraction of sulfadoxine resistance markers in Doneguebougou and Bancoumana

Eight amino acid variants were commonly found encoded in the *Pfdhps* gene at the six codons of interest: I431V, S436F/A, A437G, K540E, A581G, A613S/T in the Figure 2 and Figure 3. In addition, two novel *Pfdhps* mutations were discovered at low prevalence in Bancoumana: D484T (0.35%), D545N (0.27%) in smc-group and D545N (0.17%)

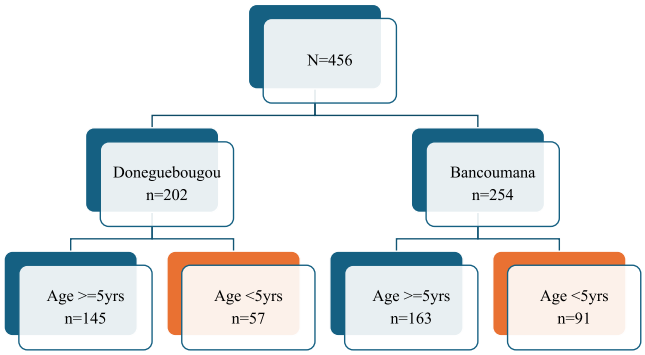

Figure 1: Study profile

Citation: Hamma Maiga, Robert Morrison, Issaka Sagara, Jennifer Hume, Sara Healy, Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguime, Jillian Neal, Irfan Zaidi and Patrick E. Duffy. Assessing of *Pfdhps* Gene of Sulfadoxine-Pyrimethamine after Five Years of Seasonal Malaria Chemoprevention Implementation at Two Rural Sites in Mali: The Quantitative Method. Fortune Journal of Health Sciences. 8 (2025): 479-490.

Maiga H, et al., Fortune J Health Sci 2025
DOI:10.26502/fjhs.298

in non-smc-group (Table not shown). The mean fraction of mutant S436F/A and A437G were presents at high frequency in smc-group and in non-smc-group in Doneguebougou (47.3% vs. 47.0%, p=0.78 and 76.8% vs. 69.0%, p=0.33) and in Bancoumana (42.5% vs. 40.0%, p=0.58 and 74.6% vs. 81.7%, p=0.27), respectively. The *Pfdhps* K540E and A581G mean fraction mutations were lower observed and similar in smc-group versus non-smc-group in Doneguebougou (0.0% vs. 2.7%, p=0.12 and 6.8% vs. 3.5%, p=0.15) and in Bancoumana (0.5% vs. 3.3%, p=0.37 and 1.6% vs. 3.7%, p=0.14), respectively. However, the mean fraction of resistance markers was significantly differed between smc-

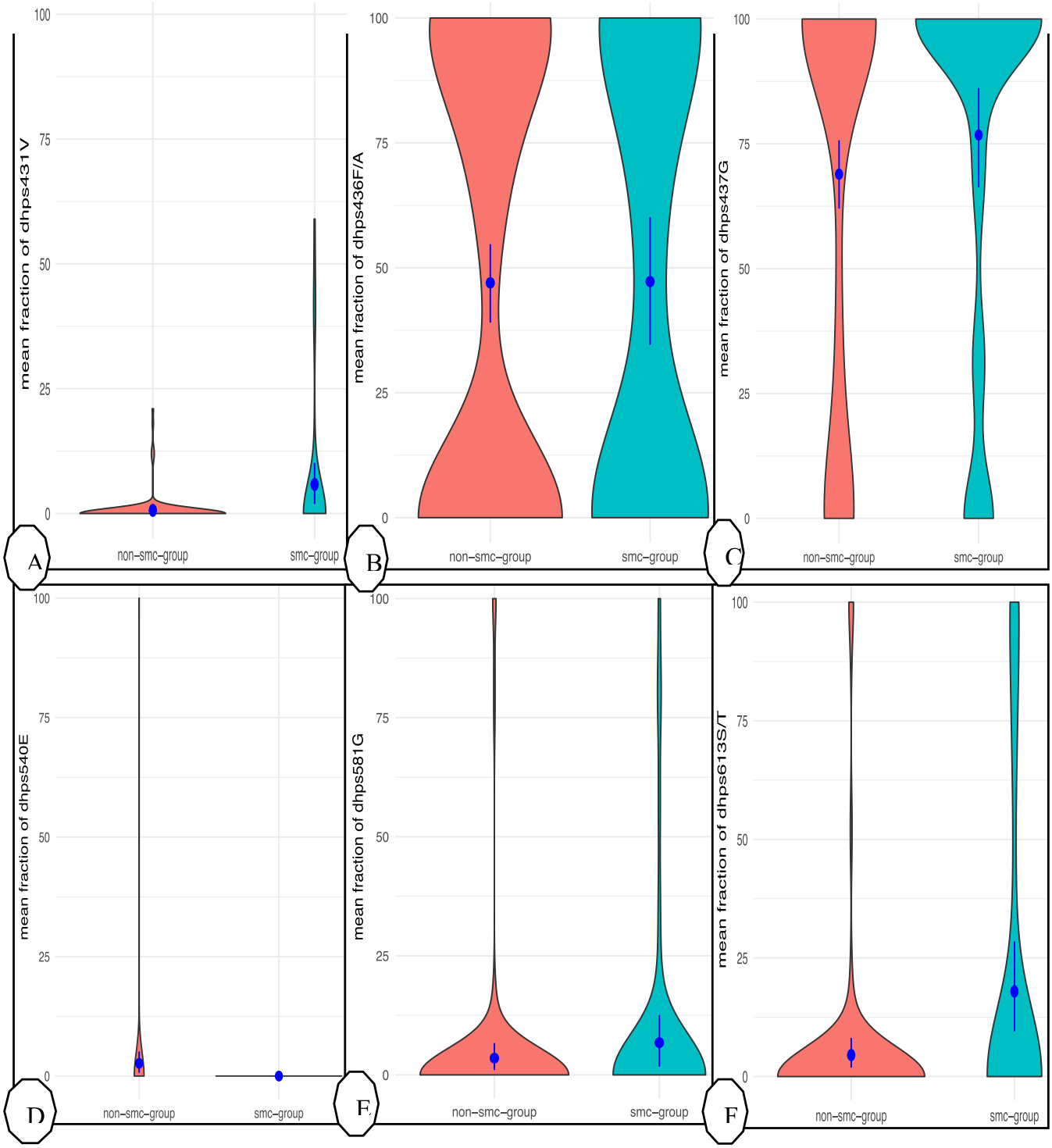

Figure 2: Mean fraction of resistance allele at DHPS codons 431, 436, 437, 540, 581 and 613 in Doneguebougou

Citation: Hamma Maiga, Robert Morrison, Issaka Sagara, Jennifer Hume, Sara Healy, Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguime, Jillian Neal, Irfan Zaidi and Patrick E. Duffy. Assessing of *Pfdhps* Gene of Sulfadoxine-Pyrimethamine after Five Years of Seasonal Malaria Chemoprevention Implementation at Two Rural Sites in Mali: The Quantitative Method. Fortune Journal of Health Sciences. 8 (2025): 479-490.

Maiga H, et al., Fortune J Health Sci 2025
DOI:10.26502/fjhs.298

group versus non-smc-group in Doneguebougou and not in Bancoumana, respectively, for *Pfdhps* 431V (5.8% vs. 0.6%, p=0.01 and 4.3% vs. 4.2%, p=0.84), or for *Pfdhps* 613S/T (18.0% vs. 4.5%, p=0.001 and 7.1% vs. 12.7%, p=0.15). There was no difference in the mean fraction of *Pfdhps* at all codons by categories age (1-4yrs, 5-8yrs, 9-18yrs and adults), p>0.05, respectively, when data were combined from Doneguebougou and Bancoumana in Figure 4. And the high level of mean fraction was observed in *Pfdhps* at codon 437G and low level in *Pfdhps* at codon 540E.

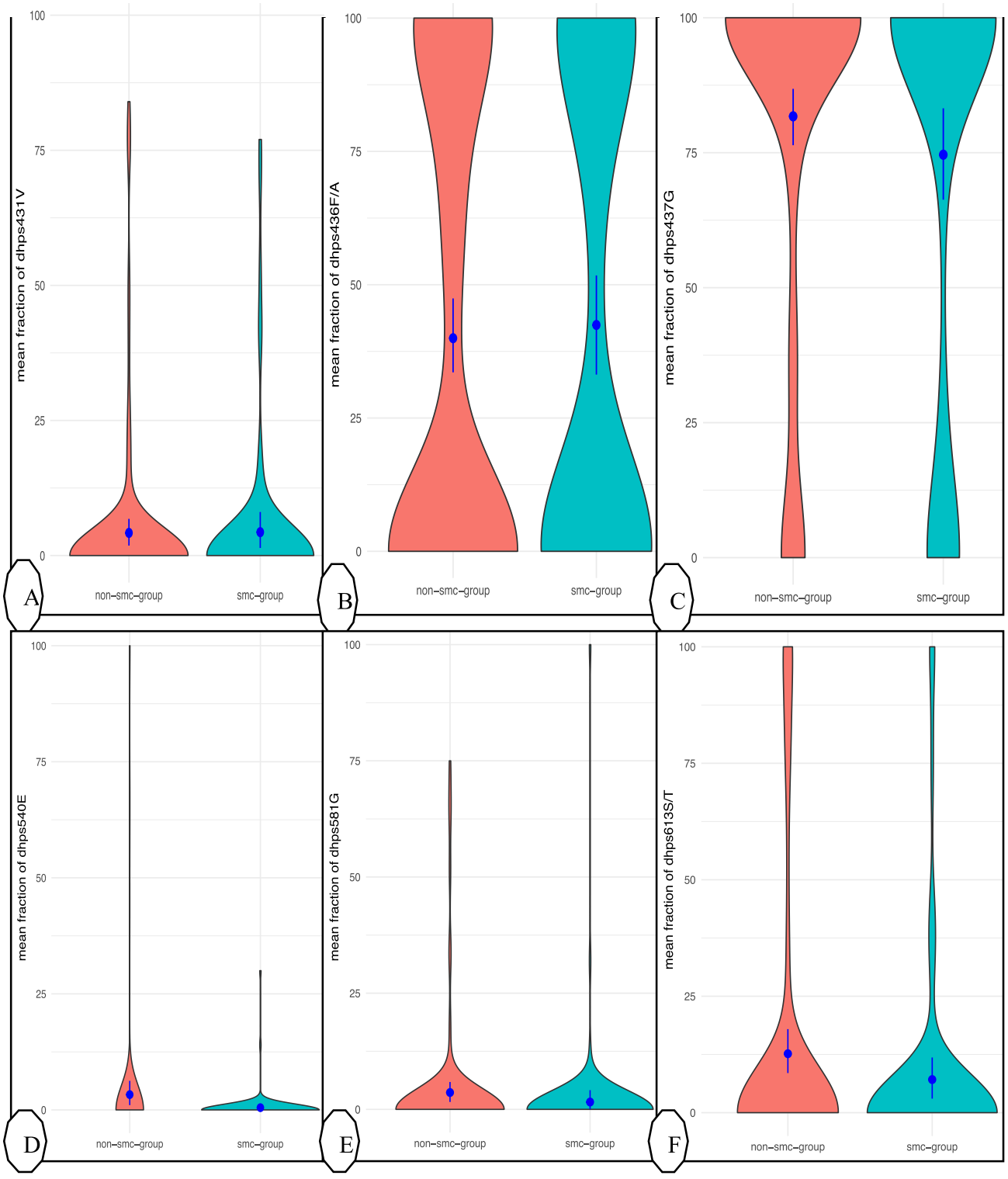

Figure 3: Mean fraction of resistance allele at DHPS codons 431, 436, 437, 540, 581 and 613 in Bancoumana

Citation: Hamma Maiga, Robert Morrison, Issaka Sagara, Jennifer Hume, Sara Healy, Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguime, Jillian Neal, Irfan Zaidi and Patrick E. Duffy. Assessing of *Pfdhps* Gene of Sulfadoxine-Pyrimethamine after Five Years of Seasonal Malaria Chemoprevention Implementation at Two Rural Sites in Mali: The Quantitative Method. Fortune Journal of Health Sciences. 8 (2025): 479-490.

Maiga H, et al., Fortune J Health Sci 2025
DOI:10.26502/fjhs.298

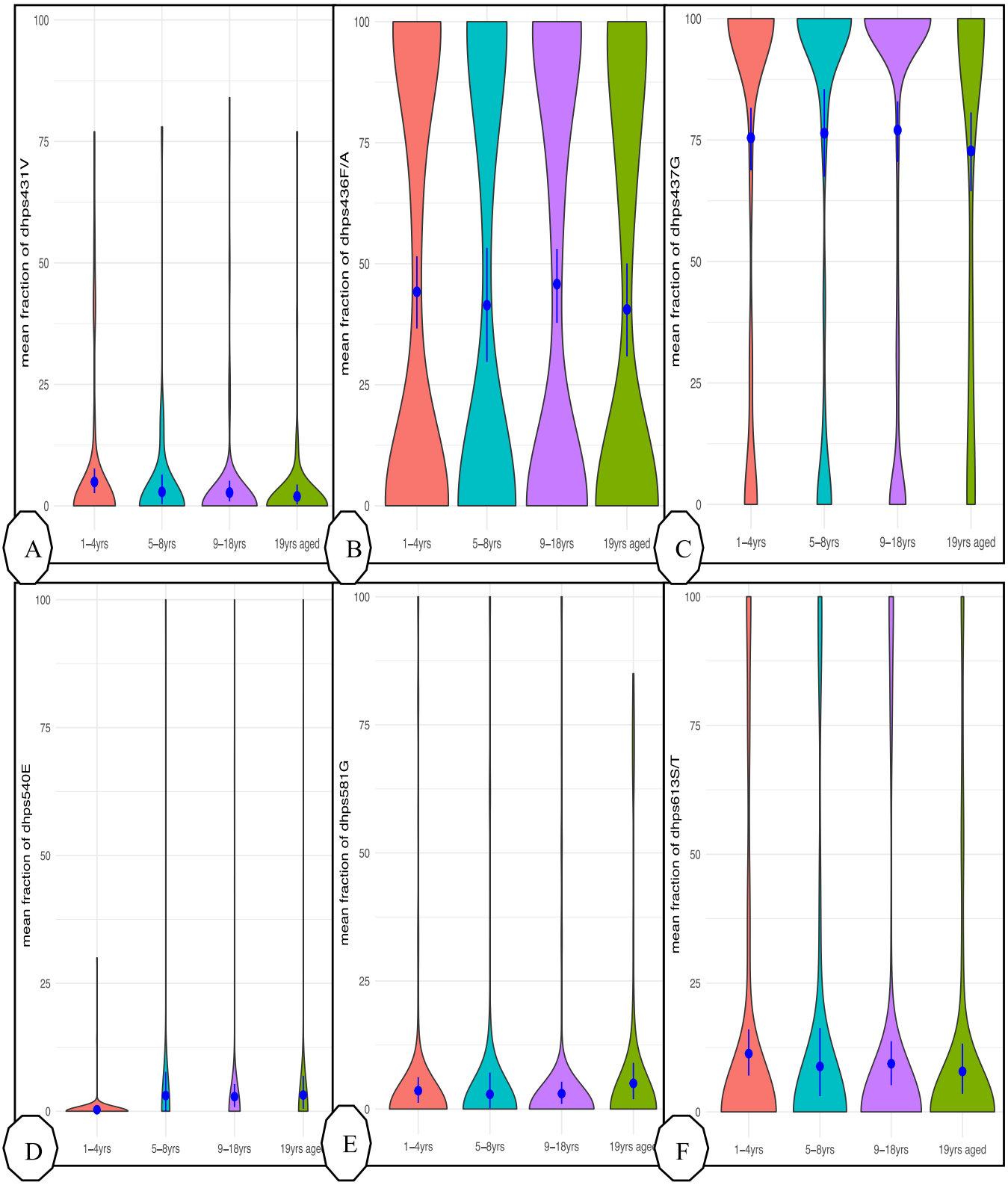

Figure 4: Mean fraction of individual allele of *Pfdhps* 431V, 436F/A, 437G, 540E, 581G, and 613S/T by different age categories in both sites

Citation: Hamma Maiga, Robert Morrison, Issaka Sagara, Jennifer Hume, Sara Healy, Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguime, Jillian Neal, Irfan Zaidi and Patrick E. Duffy. Assessing of *Pfdhps* Gene of Sulfadoxine-Pyrimethamine after Five Years of Seasonal Malaria Chemoprevention Implementation at Two Rural Sites in Mali: The Quantitative Method. Fortune Journal of Health Sciences. 8 (2025): 479-490.

Maiga H, et al., Fortune J Health Sci 2025
DOI:10.26502/fjhs.298

Discussion

This study is the first to report the mean fraction of molecular markers of resistance to sulfadoxine across Mali sites in sub–Saharan Africa as they implement seasonal malaria chemoprevention (SMC). It is also the first study to report the presence of *Pfdhps* in vaccine sites considered as control malaria areas in Mali. In this study, we focused on the *Pfdhps*-540E mutation, which, combined with *Pfdhps*-436F/A or *Pfdhps*-437G, and *Pfdhps*-581G confers resistance to sulfadoxine–pyrimethamine. The aim of this study was to provide an overview of molecular marker drug resistance of SMC to prevent malaria morbidity in children less than 5 years and to measure the impact of this strategy on the participants did not receive sulfadoxine/pyrimethamine plus amodiaquine (SPAQ). In Doneguebougou and Bancoumana, malaria endemic areas, the molecular markers of *Pfdhps* 540E and 581G were low and still absent after five years of SMC implementation, suggesting that sulfadoxine-pyrimethamine remains an effective antimalarial drug in these two sites in Mali. We found that SMC has evidence increased of the mean fraction of resistance alleles of *Pfdhps* at codons 436F/A and 613S/T in SMC participants than participants did not receive SMC. We also found that sulfadoxine resistance molecular markers were as high or higher in the non-smc-group as smc-group in Bancoumana site only. In both sites, the mean fraction of drug resistance was higher in *Pfdhps* at codons 436F/A and 437G. At end the drug resistance molecular marker of *Pfdhps* at all codons was similar by category age (1-4years, 5-8yrs, 9-18yrs and adults) when data were combined from both sites.

We used the relatively novel approach of deconvolution to quantitatively determine the fraction of resistance alleles in individual samples at multiple SNPs in *Plasmodium falciparum* dihydropteroate synthase (*Pfdhps*) [18]. We found that resistance alleles at codons at *Pfdhps* 437G was closed to saturation in parasite samples from Doneguebougou and Bancoumana in smc-group and non-smc-group. By contrast, the mean fraction of parasites carrying the resistance allele at *Pfdhps* at codons 540E and 581G were considerably lower in both sites. Interesting these fractions were similar in parasite samples from participants did or did not use SMC. We also found that smc-group that used SMC harbored a significantly higher fraction of parasites with the resistance alleles of *Pfdhps* at codons 436F/A and 613S/T, suggesting selection for more highly drug-resistant parasites in Doneguebougou. We have not found any difference of all fractions of parasites with the resistance allele in Bancoumana and these data suggesting the extension of resistance from smc-group to non-smc-group. This last step in selection may have clinical relevance, since *in vivo* studies have demonstrated an association between treatment failure among children and adults and the mutant alleles at codons 540E and 581G [19, 20]. SP is currently used for intermittent preventive treatment (IPTp) of women in the last two trimesters of pregnancy in Mali since 2004 [15]. SP as IPTp is known to provide some benefit despite the presence of high level of the *Pfdhps* 540E. However, a study on effectiveness of SP in preventing low birth weight caused by malaria in pregnancy reported that when the estimated prevalence of this quintuple mutation at *Pfdhfr-dhps* reaches 50%, then the effectiveness of SP for IPTp is compromised [21]. And, WHO recommended the replacement of SP as IPTp when the quintuple mutant parasites exceed 50%. Also, WHO recommended the SP-IPTi where parasite resistance to SP is not high – defined as a prevalence of the *Pfdhps* 540E mutation of under 50% [22].

The SMC selection for resistant parasites is not surprising, given that single dose SP treatments increased the prevalence of resistance alleles in children [23-25]. However, our data based on *Pfdhps* 436F/A and 613S/T of Doneguebougou confirmed this effect in children, as was observed in African children receiving SMC [5, 9, 26]. In contrast the mean fraction of *Pfdhps* 540E was not found in Doneguebougou after 5 years of SMC implementation. In both sites we did not find the difference of resistance molecular markers from smc-group compared to non-smc-group that suggests the evidence extension of these markers to older population. This result harbor of the other results from Africa but not with one Malian study implemented in the high seasonal malaria transmission which the molecular marker of SP resistance were high significant compared to the non-smc population [9]. The high level of *Pfdhps* 436F/A and 437G was similar in many smc implemented studies in Africa [4, 5, 9, 17, 27]. The mean molecular markers of resistance to SMC drug (*Pfdhps* 540E and 581G) occurred at low prevalence, consistent with the effectiveness of SMC observed in other studies [9, 26, 28]. However today, there was evidence of selection for resistance to sulfadoxine in parasites sampled from the age group that did not receive SMC but lived in areas where SMC was deployed. Our study was similar with ACCESS-SMC study [28]. Resistance to the sulfadoxine–pyrimethamine should continue to be monitored via standardized methods, across all regions where SMC is used, to provide early warning of loss of effectiveness.

Our data showed that mutations associated with Sulfadoxine resistance were rare in the Sahel region of Mali. The finding of high prevalence of the *Pfdhps* 437G synonym of "quadruple mutant" variant genotype comprising this drug and was consistent with previous reports [19] which may reflect the long history of usage of SP as IPTp. Thus, although our data suggested there is no imminent threat to SMC efficacy in our study, the presence of all the genetic elements for full-blown resistance to the main drug (SP) highlights the need for careful monitoring of resistance markers. Efficacy could deteriorate quickly under strong selective pressure;

an example in a more recent SPAQ effectiveness study in Mali, a four-fold increase in *Pfdhfr-dhps* quintuple mutation was reported within eight months after the last dose of SMC compared to the baseline [9]. Another study from Sahel of Africa have shown the same result [28]. Also, the prevalence of the double *Pfdhps* mutation (437G and 540E) increased one year after a switch to first line SP in Tanzania [29].

Not surprising, we did not find *Pfdhps* 540E in Doneguebougou compared to Bancoumana. In a previous study in Mali, *Pfdhps* 540E was reported in district of Koutiala and Kita, where a four-fold increase in the quintuple mutant genotype was observed after seven rounds of SMC [9, 30]. The *Pfdhps* 540E was reported in Segou after two years of SMC implementation, where they found two individuals harboring parasites with this mutation at baseline [28]. Also, *Pfdhps* 540E was reported in Dangassa with intense seasonal malaria transmission while Nioro-du-Sahel has an unstable and short seasonal malaria transmission where SMC was implemented several years [31]. Other Malian researchers found 540E mutation in Siby, Ouelessebougou and Djioliba after two-year pilot SMC implementation [5], but one study did not find *Pfdhps* 540E. Studies in south Niger were previously reported absence of *Pfdhps* 540E, but this mutation was observed in Gaya [32]. The *Pfdhps* 540E was previously reported in Burkina Faso in Nanoro region [33] and in Boussé health district, Kourweogo province [34], North Burkina Faso (Koupéla) though they did not find any in Koupela, Eastern Burkina Faso and in the district of Lena, southeastern of Burkina Faso [26]. In Nanoro region, SP has been freely available for malaria treatment through clinics run and this high level of drug pressure might have led to the selection of 540E. In Guinea, the *Pfdhps* 540E was previously reported in Laine refugee camp, southeast Guinea [35]. Thus, it is not clear if this important allele may be widespread in Mali despite very low prevalence. Though such increase in the mutant genotypes was still lower than the rates observed in east Africa [36, 37]. We are not observed a similar increase in Doneguebougou and Bancoumana after several rounds of SMC in subsequent malaria seasons.

The two *Pfdhps* mutations 581G and 613S/T, which are thought to enhance resistance to SP in the presence of quintuple mutation in East Africa, were here found in Burkina Faso, Chad and Nigeria for the first time [28] and other sites in Mali [28, 31]. These mutations were more likely to occur in the presence of *Pfdhps* mutations to I431V, S436A and A437G which were high in levels in this study. It was not clear whether the *Pfdhp*s haplotype had any role in modulating parasite susceptibility to SP in the absence of mutant K540E as in the case of Doneguebougou site. Although still a minor variant, the 581G reached less than 7% in Doneguebougou and Bancoumana while 613S reached similar or higher prevalence in West Africa [28]. As the two *Pfdhps* mutations 581G and 613S were thought to increase the resistance to SP in the East African context [38]. Ascertaining the role of each *Pfdhps* mutation using the standard *in vitro* sensitivity test gave highly variable results and poor reproducibility and has not been successful possibly due to *de novo* folate synthesis which necessitate the use of folate free costly media [39]. In the absence of reliable *in vitro* and *in vivo* data, careful monitoring of the prevalence of different *Pfdhps* mutation combinations after large scale SMC intervention could give an insight into the relevance of the different *Pfdhps* mutations. Our data showed no difference in the mean fraction mutations associated with SP resistance between children under 5 years of age and those between 5-8-year, 9–18-year-olds and adults. This was unexpected as older children were more likely to clear mutant parasites compared to children under 5 due to difference in immunity [40]. However, a similar finding was previously reported in Benin seven years after the introduction of SP as first line of treatment [41] and recently in West Africa study [28]. This could change due to a large-scale SMC intervention in the area since 2012. In Mali, children who received SMC had higher prevalence of mutations associated with SP compared to the non-SMC population eight months after SMC and no significant increase was observed in the non-SMC population [9]. And a similar observation was observed in the different studies in Mali and Burkina Faso [5, 26, 34].

The molecular markers prevalence data reported here will provide a crucial information for assessing the impact of a sustained scale-up of SPAQ as a seasonal malaria chemoprevention in sub-Saharan Africa. Vigilance on these markers must be accentuated in view of the increase in these markers in the non-SMC population. It will compromise intermittent preventive treatment (IPTp) in pregnant women in countries where SP is used as IPTp but also increase the risk of respiratory infections in children who will use Cotrimoxazole as treatment and the prevention of opportune HIV infections among the immunosuppressed. One of the limitations in our study was to use the different method to study the molecular marker for better comparison, second, we have not studied the molecular markers of pyrimethamine and amodiaquine. As we know the effectiveness of SPAQ as SMC in sub-Saharan Africa may not be immediately threatened not only because of the low level of quintuple mutant genotype but also due to low prevalence of *Plasmodium falciparum* chloroquine resistance transporter (*Pfcrt)* and *Plasmodium falciparum* multi drug resistance 1 (*Pfmdr1*) mutations associated with Amodiaquine (AQ) resistance. In addition, the effect of SMC drugs on the spread of SP and AQ resistant mutations and in turn on the loss of effectiveness of SPAQ is still unclear. Third, the baseline data is not evaluated in this study.

Citation: Hamma Maiga, Robert Morrison, Issaka Sagara, Jennifer Hume, Sara Healy, Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguime, Jillian Neal, Irfan Zaidi and Patrick E. Duffy. Assessing of *Pfdhps* Gene of Sulfadoxine-Pyrimethamine after Five Years of Seasonal Malaria Chemoprevention Implementation at Two Rural Sites in Mali: The Quantitative Method. Fortune Journal of Health Sciences. 8 (2025): 479-490.

Conclusions

The mean fraction of *Pfdhps* 540E and 581G were low after five years of SMC implementation, suggesting that sulfadoxine-pyrimethamine remains an effective antimalarial drug in these two sites in Mali. Otherwise, sulfadoxine resistance molecular markers of Bancoumana were as high or higher in the non-smc-group as smc-group. These data should be complemented by periodic nationwide molecular surveillance to detect emergence of resistant genotypes.

Conflicts of interest

The authors declare no competing interests.

Acknowledgments

We are very grateful to the study participants, the populations, the members of community health associations, and the staff of the health centres of Doneguebougou and Bancoumana for their cooperation throughout the study. Special thanks to LMIV and MRTC teams for their support to this project. Our specials thanks go also to the members of the Data extraction team of MRTC and Patrick Gorres of LMIV for the critical editing.

Author Contributions

Criteria for authorship read and met: Hamma Maiga, Robert Morrison, Issaka Sagara, Jennifer Hume, Sara Healy, Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguime, Jillian Neal, Irfan Zaidi and Patrick E. Duffy. Agree with the manuscript's results and conclusions: Hamma Maiga, Robert Morrison, Issaka Sagara, Jennifer Hume, Sara Healy, Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguime, Jillian Neal, Irfan Zaidi and Patrick E. Duffy. Designed the experiments/the study: Patrick Duffy. Analyzed the data: Hamma Maiga and Morrisson Robert. Collected data/did experiments for the study: Issaka Sagara, Jennifer Hume, Sara Healy, Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguime, Irfan Zaidi. Enrolled patients: Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguim. Wrote the first draft of the paper: Hamma Maiga. Contributed to the writing of the paper: Patrick Gorres, Hamma Maiga, Robert Morrison, Issaka Sagara, Jennifer Hume, Sara Healy, Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguime, Jillian Neal, Irfan Zaidi and Patrick E. Duffy

Financial support

This work was supported by the Division of Intramural Research of the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH). HM was supported by the African Postdoctoral Training Initiative (APTI) Fellowship program jointly managed by the US NIH, The African Academy of Sciences (AAS) and Bill & Melinda Gates Foundation (BMGF) "Grant Reference Number: APTI-18-01".

References

1. PNLP: INSTAT, INFO-STAT, Institut National de la Recherche en Santé Publique (INRSP) et ICF International. Enquête sur les Indicateurs du Paludisme au Mali (EIPM) (2015).
2. WHO Policy Recommendation: Seasonal Malaria Chemoprevention (SMC) for Plasmodium falciparum malaria control in highly seasonal transmission areas of the Sahel sub-region in Africa. March (2012).
3. Cisse B, Sokhna C, Boulanger D, Milet J, et al: Seasonal intermittent preventive treatment with artesunate and sulfadoxine-pyrimethamine for prevention of malaria in Senegalese children: a randomised, placebo-controlled, double-blind trial. Lancet 367 (2006): 659-667.
4. Konate AT, Yaro JB, Ouedraogo AZ, Diarra A, et al: Morbidity from malaria in children in the year after they had received intermittent preventive treatment of malaria: a randomised trial 6 (2011): e23391.
5. Dicko A, Diallo AI, Tembine I, Dicko Y, et al: Intermittent preventive treatment of malaria provides substantial protection against malaria in children already protected by an insecticide-treated bednet in Mali: a randomised, double-blind, placebo-controlled trial 8 (2011): e1000407.
6. Zongo I, Milligan P, Compaore YD, Some AF, et al. Randomized Noninferiority Trial of Dihydroartemisinin-Piperaquine Compared with Sulfadoxine-Pyrimethamine plus Amodiaquine for Seasonal Malaria Chemoprevention in Burkina Faso. Antimicrob Agents Chemother 59 (2015): 4387-4396.
7. WHO: World Malaria Report 2013. December (2013).
8. World Malaria Report.
9. Maiga H, Lasry E, Diarra M, Sagara I, et al. Seasonal Malaria Chemoprevention with Sulphadoxine-Pyrimethamine and Amodiaquine Selects Pfdhfr-dhps Quintuple Mutant Genotype in Mali 11 (2016): e0162718.
10. WHO: World malaria report 2018. Geneva: World Health Organization (2016).
11. Ogobara K. Doumbo KN, Sara A. Healy, et al. Duffy: Malaria Transmission-Blocking Vaccines. In Present Status and Future Perspectives, Towards Malaria Elimination July 18th (2018).
12. Greenwood B, Targett G: Do we still need a malaria vaccine? Parasite Immunol 31 (2009): 582-586.

Maiga H, et al., Fortune J Health Sci 2025
DOI:10.26502/fjhs.298

13. Birkett AJ: PATH Malaria Vaccine Initiative (MVI): perspectives on the status of malaria vaccine development. Hum Vaccin 6 (2010): 139-145.

14. Aponte JJ, Schellenberg D, Egan A, Breckenridge A, et al. Efficacy and safety of intermittent preventive treatment with sulfadoxine-pyrimethamine for malaria in African infants: a pooled analysis of six randomised, placebo-controlled trials. Lancet 374 (2009): 1533-1542.

15. WHO: Global Technical Strategy for Malaria 2016–2030. Geneva: World Health Organization (2016).

16. Doumbo OKO, Traore SF, Sangare O, Coulibaly A, et al. Les aspects parasitologiques de l'épidémiologie du paludisme dans le Sahara malien. Médecine d'Afrique Noire (1991).

17. Lo AC, Faye B, Ba el H, Cisse B, et al. Prevalence of molecular markers of drug resistance in an area of seasonal malaria chemoprevention in children in Senegal. Malar J 12 (2013): 137.

18. Maiga H, Morrison RD, et Duffy PE. Sanger sequencing and deconvolution of polyclonal infections: a quantitative approach to monitor drug-resistant Plasmodium falciparum. Ebiomedicine 103 (2024).

19. Gesase S, Gosling RD, Hashim R, Ord R, et al: High resistance of Plasmodium falciparum to sulphadoxine/ pyrimethamine in northern Tanzania and the emergence of dhps resistance mutation at Codon 581 4 (2009): e4569.

20. Plowe CV, Djimde A, Wellems TE, Diop S. Community pyrimethamine-sulfadoxine use and prevalence of resistant Plasmodium falciparum genotypes in Mali: a model for deterring resistance. Am J Trop Med Hyg 55 (1996): 467-471.

21. Muanda FT, Chaabane S, Boukhris T, Santos F, et al. Antimalarial drugs for preventing malaria during pregnancy and the risk of low birth weight: a systematic review and meta-analysis of randomized and quasi-randomized trials. BMC Med 13 (2015): 193.

22. WHO: Intermittent preventive treatment in infants (IPTi) (2017).

23. Wang P, Lee CS, Bayoumi R, Djimde A, et al. Resistance to antifolates in Plasmodium falciparum monitored by sequence analysis of dihydropteroate synthetase and dihydrofolate reductase alleles in a large number of field samples of diverse origins. Mol Biochem Parasitol 89 (1997): 161-177.

24. Marks F, von Kalckreuth V, Kobbe R, Adjei S, et al. Parasitological rebound effect and emergence of pyrimethamine resistance in Plasmodium falciparum after single-dose sulfadoxine-pyrimethamine. J Infect Dis 192 (2005): 1962-1965.

25. Dicko A, Sagara I, Sissoko MS, Guindo O, et al. Impact of intermittent preventive treatment with sulphadoxine-pyrimethamine targeting the transmission season on the incidence of clinical malaria in children in Mali. Malar J 7 (2008): 123.

26. Some AF, Zongo I, Compaore YD, Sakande S, et al. Selection of drug resistance-mediating Plasmodium falciparum genetic polymorphisms by seasonal malaria chemoprevention in Burkina Faso. Antimicrob Agents Chemother 58 (2014): 3660-3665.

27. Kweku M, Liu D, Adjuik M, Binka F, et al Seasonal intermittent preventive treatment for the prevention of anaemia and malaria in Ghanaian children: a randomized, placebo controlled trial 3 (2008): e4000.

28. Coldiron MG, RF; ACCESS-SMC Partnership: Effectiveness of seasonal malaria chemoprevention at scale in west and central Africa: an observational study. The Lancet 396 (2020): 1829–1840.

29. Malisa AL, Pearce RJ, Abdulla S, Mshinda H, et al. Drug coverage in treatment of malaria and the consequences for resistance evolution--evidence from the use of sulphadoxine/pyrimethamine 9 (2010): 190.

30. Diawara F, Steinhardt LC, Mahamar A, Traore T, et al. Measuring the impact of seasonal malaria chemoprevention as part of routine malaria control in Kita, Mali. Malar J 16 (2017): 325.

31. Diakite SAS, Traore K, Sanogo I, Clark TG, et al. A comprehensive analysis of drug resistance molecular markers and Plasmodium falciparum genetic diversity in two malaria endemic sites in Mali 18 (2019): 361.

32. Grais RF, Laminou IM, Woi-Messe L, Makarimi R, et al. Molecular markers of resistance to amodiaquine plus sulfadoxine-pyrimethamine in an area with seasonal malaria chemoprevention in south central Niger. Malar J 17 (2018): 98.

33. Ruizendaal E, Tahita MC, Traore-Coulibaly M, Tinto H, et al. Presence of quintuple dhfr N51, C59, S108 - dhps A437, K540 mutations in Plasmodium falciparum isolates from pregnant women and the general population in Nanoro, Burkina Faso. Mol Biochem Parasitol 217 (2017): 13-15.

34. Konate AT, Yaro JB, Ouedraogo AZ, Diarra A, et al. Intermittent preventive treatment of malaria provides substantial protection against malaria in children already protected by an insecticide-treated bednet in Burkina Faso: a randomised, double-blind, placebo-controlled trial 8 (2011): e1000408.

Citation: Hamma Maiga, Robert Morrison, Issaka Sagara, Jennifer Hume, Sara Healy, Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguime, Jillian Neal, Irfan Zaidi and Patrick E. Duffy. Assessing of *Pfdhps* Gene of Sulfadoxine-Pyrimethamine after Five Years of Seasonal Malaria Chemoprevention Implementation at Two Rural Sites in Mali: The Quantitative Method. Fortune Journal of Health Sciences. 8 (2025): 479-490.

35. Bonnet M, Roper C, Felix M, Coulibaly L, et al. Efficacy of antimalarial treatment in Guinea: in vivo study of two artemisinin combination therapies in Dabola and molecular markers of resistance to sulphadoxine-pyrimethamine in N'Zerekore. Malar J 6 (2007): 54.

36. Shah M, Omosun Y, Lal A, Odero C, et al. Assessment of molecular markers for anti-malarial drug resistance after the introduction and scale-up of malaria control interventions in western Kenya 14 (2015): 75.

37. Harrington WE, Mutabingwa TK, Muehlenbachs A, Sorensen B, et al. Competitive facilitation of drug-resistant Plasmodium falciparum malaria parasites in pregnant women who receive preventive treatment. Proc Natl Acad Sci USA 106 (2009): 9027-9032.

38. Naidoo I, Roper C. Mapping 'partially resistant', 'fully resistant', and 'super resistant' malaria. Trends Parasitol 29 (2013): 505-515.

39. Johnson JD, Dennull RA, Gerena L, Lopez-Sanchez M, et al. Assessment and continued validation of the malaria SYBR green I-based fluorescence assay for use in malaria drug screening. Antimicrob Agents Chemother 51 (2007): 1926-1933.

40. Djimde AA, Doumbo OK, Traore O, Guindo AB, et al. Clearance of drug-resistant parasites as a model for protective immunity in Plasmodium falciparum malaria. Am J Trop Med Hyg 69 (2003): 558-563.

41. Ogouyemi-Hounto A, Ndam NT, Kinde Gazard D, d'Almeida S, et al. Prevalence of the molecular marker of Plasmodium falciparum resistance to chloroquine and sulphadoxine/pyrimethamine in Benin seven years after the change of malaria treatment policy 12 (2013): 147.

Citation: Hamma Maiga, Robert Morrison, Issaka Sagara, Jennifer Hume, Sara Healy, Mahamadoun H. Assadou, Abdoulaye Katile, Amatigue Zeguime, Jillian Neal, Irfan Zaidi and Patrick E. Duffy. Assessing of *Pfdhps* Gene of Sulfadoxine-Pyrimethamine after Five Years of Seasonal Malaria Chemoprevention Implementation at Two Rural Sites in Mali: The Quantitative Method. Fortune Journal of Health Sciences. 8 (2025): 479-490.