

Full Length Research Paper

Molecular characterization of sulphadoxine-pyrimethamine resistant malaria parasites among pregnant women in Anambra State, Nigeria

Isaac Okezie Godwin¹, Ifeoma Mercy Ekejindu¹, George Uchenna Eleje², Christian Chibuzo Ibeh³, Dorothy Amauche Ezeagwuna⁴, Chigozie Geoffrey Okafor² and Ahaneku Iherue Osuji^{5*}

¹Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Nnewi, Anambra State, Nigeria.

²Department of Obstetrics and Gynaecology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

³Department of Community Medicine, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

⁴Department of Microbiology and Parasitology, Nnamdi Azikiwe University Teaching Hospital, Nnewi Campus, Nnewi, Anambra State, Nigeria.

⁵Department of Microbiology and Immunology, University of Abuja Teaching Hospital, Gwagwalada, Abuja, Nigeria.

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The study determined the prevalence of malaria parasitemia among pregnant women using Sulphadoxine-pyrimethamine (SP) for intermittent preventive treatment (IPT) and assessed *Plasmodium falciparum* dihydropteroate reductase (Pfdhfr) and dihydropteroate synthetase (Pfdhps) resistance genes among the subjects. Three hundred and ninety consenting pregnant women attending antenatal clinics in Nnewi, Anambra State, Nigeria were recruited. Of this, 336 of the women were using SP for IPT, while fifty-four of the women were non-users of SP. Polymerase Chain Reaction was used in the characterization of *Plasmodium* species while the Sanger sequencing method was used in sequencing assay. Of the 336 pregnant women SP users, 73 (21.7%) and, 41 (12.2%) of the neonates had malaria parasitemia while 29 (53.7%) of the 54 non-users of SP and 14 (25.4%) of the babies had malaria parasitemia ($p=0.022$). *Plasmodium falciparum* and *Plasmodium vivax* parasitemia among subjects on IPTp-SP was 38 (11.3%) and 30 (8.9%) respectively. The prevalence of Pfdhfr and Pfdhps resistance genes in pregnant women was 11 (3.3%) and 10 (2.9%) respectively. Malaria parasitemia was significantly higher among non-users of SP. The presence of *P. falciparum* resistance genes among IPTp-SP users could lead to treatment failures. Therefore, novel drugs should be sought to replace SP.

Key words: *Plasmodium*, Sulphadoxine-pyrimethamine, pregnancy, polymerase chain reaction (PCR), Resistant gene, Nigeria.

INTRODUCTION

Malaria is an infectious disease caused by parasites of the genus *Plasmodium* and is transmitted by mosquitoes

(Warrell and Gilles, 2002). Malaria is characterized by chills, fever, and generalized body pain (WHO, 2004).

Plasmodium falciparum, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi* are the species that infect humans (Tangpukdee et al., 2009). The most prevalent species of malaria parasites in Nigeria is *P. falciparum* (greater than 95%) and responsible for the most severe forms of the disease in Nigeria (Umeh et al., 2013). Although *P. ovale* and *P. malariae* parasitemia play a minor role in Nigeria, *P. malariae* is currently isolated from children with mixed infections (Nigeria Malaria Indicator Survey, 2015). Nigeria accounts for the high prevalence of the global malaria burden in sub-Saharan Africa (National Malaria Elimination Programme, 2017; WHO, 2017). This highlights the need to focus on treatment as well as prevention. Nationwide, Malaria prevalence varies widely, ranging from 14% in the South East to 37% in the North West (National Malaria Elimination Programme, 2017; WHO, 2017). In Nigeria, malaria is responsible for the deaths of an estimated 300,000 children per year and contributes to over 4000 maternal deaths annually (WHO, 2017).

Malaria in pregnancy is a serious public health problem that impacts negatively and causes an enormous risk to the mother, fetus, and neonates (Gontie et al., 2020). It is estimated that each year approximately 25 million pregnant women in sub-Saharan Africa live at risk of *P. falciparum* malaria infection (WHO, 2017). Although malaria during pregnancy might be asymptomatic due to a high level of acquired immunity in mothers residing in high transmission areas (Fagbemi et al., 2020), it is still associated with an increased risk of maternal anemia, spontaneous abortion, stillbirth, prematurity, and low birth weight (Kwala et al., 2021).

Intermittent preventive treatment in pregnancy with sulphadoxine-pyrimethamine (IPTp-SP) is one of the main strategies for protecting pregnant women, fetus, and their newborns against adverse effects of *P. falciparum* infection (Briand et al., 2009). The development of the drug resistance linked to mutations in *P. falciparum* dihydrofolate reductase genes (pfdhfr) and *P. falciparum* dihydropteroate synthetase genes (Pfdhps), is currently threatening the IPTp-SP approach and is one of the greatest challenges of malaria control programs (Fagbemi et al., 2020). Sulphadoxine-pyrimethamine (SP) resistance is linked to the substitution of amino acids in the enzyme dihydropteroate synthetase (dhps) and dihydrofolate reductase (dhfr) in the folate biosynthetic pathway (Brooks et al., 1994; Cowman et al., 1988). Mutations in dhfr confer resistance to pyrimethamine while mutations in dhps confer resistance to sulphadoxine and other sulpha drugs. There are

variations in SP mutations, it may be single, double, or triple: the more the mutations, the stronger the resistance. In sub-Saharan Africa, the dhfr triple mutant (Asn- 108 + Ile- 50 + Arg - 59) and dhps double mutant (Gly- 437 = Glu - 540) have been strongly associated with potential resistance (Duraisingh et al., 1998; Triglia et al., 1997). In a study done in Lagos, Nigeria, the mutant strains of *P. falciparum* were present up to 75.9% of the blood samples of pregnant women (Agomo et al., 2016). In another study done in six districts in the general population of Zambia indicated variations of rates of resistance to SP with mutated dhfr frequency ranging from 71 - 92% and 39- 71% for the double mutant dhps respectively (Akanbi et al., 2009).

Therefore, there is a need for monitoring and surveillance of the efficacy of SP in the treatment of malaria among pregnant women. In the present study, maternal venous blood and neonatal cord blood samples of babies of pregnant women who were users of SP and non-users of SP attending antenatal clinic in Nnewi, a metropolitan city, were assessed for *Plasmodium* species using a molecular approach. The purpose was to evaluate *P. falciparum* resistance markers (dhps and dhfr) to SP in the study area and the Nigerian nation at large and to assess the possibility of looking for a novel drug for the treatment of *falciparum* malaria.

MATERIALS AND METHODS

Study area, design, and population

The study was a cross-sectional study involving pregnant women who were on antenatal visits to maternity and hospitals, primary healthcare centers (PHCCs), and Nnamdi Azikiwe University teaching hospital (NAUTH), Nnewi, Nigeria.

Consent and ethical approval

Ethical approval was obtained from Nnamdi Azikiwe University Teaching Hospital, Nnewi Ethical Committee (NAUTH/CS/66/VOL.11/158/2018, 22/01/2019) and authorization from primary healthcare centers and maternity and hospitals' administration. The written and oral informed consent was obtained from each participant before administering the questionnaires. The anonymity of participants involved in this study as well as the confidentiality of information collected was respected.

Sample size determination

A total of 390 participants were enrolled in the study. This was obtained by calculation using the formula as described by Naing, (2003). The formula is $n = z^2p(1-p)/d^2$, where n= required sample

*Corresponding author. E-mail: dhanekuos@gmail.com. Tel: 08033507775, ORCID ID: 0000-0002-8124-3674).

size, z = confidence level 95% (Standard value of 1.96). p = Prevalence rate of 30% as reported in Imo State, Nigeria by Umeh et al., 2013. d = Desired level of significance 5% (standard value is 0.05). Minimum sample size calculation performed obtained $n = 302$. However to adjust for non-response, $n_2 = n/n-f$, Where n^2 = adjusted sample size; n = unadjusted sample size (302.16); f = non-response rate (10%) = 0.1; $n^2 = 302.16/1- 0.1$; $n^2 = 302.16/0.9 = 335.73$; $n^2 = 336$; Minimum sample size = 336.

A total number of 336 pregnant women taking SP for IPT and 54 pregnant women who were using other anti-malaria drugs for the treatment of malaria were recruited into the study, giving an overall sample size of 390 pregnant women who were recruited and enrolled in the study.

Sampling method / data collection

A stratified sampling method was used in the selection of primary healthcare centers while a convenience sampling method was used in sample collection. The enrolment of the pregnant women into the study was done consecutively until the number of pregnant women required for the study was obtained. Structured questionnaires were administered to the participants after obtaining their informed consent. Data were collected employing structured questionnaires. The study variables included socio-demographic factors, history of use of SP for malaria treatment, and other antimalarial drugs taken during pregnancy, obstetrical and gynecological records.

Sample collection

Blood samples were collected from February to November 2019. Three milliliters of each maternal venous blood and neonatal cord blood sample were aseptically collected from participants and were placed into Ethylene diamine tetraacetic acid (EDTA) containers (Micropiont, United Kingdom). The blood samples were properly labeled in coded numbers and were transported to the laboratory for immediate analysis. For each sample, approximately 20 μ l of blood was spotted on a piece of 3 mm filter paper (Whatman, Maidstone, United Kingdom) and was air-dried. The dried filter paper samples were stored in individual zipper plastic bags with a dryer at -20°C until DNA extraction.

Determination of parasite density by Giemsa-stained blood smears

Thick blood smears each measuring about 8 μ l was prepared. Each blood smear was labeled with the patient's identification number. The slides were air-dried in a dust-free environment. Staining of the blood smears was done using a 10% Giemsa stain and examined using 100x oil immersion objective lens of a binocular Olympus microscope by two blinded Laboratory Scientists (WHO, 2015). Determination of parasite density was done as described by WHO (2015). Thick blood films of the participants were made using 8 μ l of well-mixed blood. Thin blood films were also prepared. Smears were stained and parasite count was performed as described by Cheesbrough (2006) and WHO (2015) Determination of total WBC was done using Sysmex hematology analyzer as the patient's actual WBC count was used to calculate parasite density as follows:

$$\text{Parasite } /\mu\text{l of Blood} = \frac{\text{No of parasites counted} \times \text{WBCs}/\mu\text{l}}{\text{No of WBCs counted}}$$

The Sysmex KN 21 auto-hematology analyzer used to determine white blood cells (WBC) count is a quantitative automated hematology analyzer for in-vitro diagnostic use in clinical laboratories. The test was performed according to the instructions of the automatic multi-parameter blood counter. Blood films were considered negative if no parasites were detected in oil immersion fields of thick blood films. Thin blood films were also examined for malaria parasites using the 100x objective of the microscope to determine malaria parasite species.

Molecular assay

DNA extraction and purification from dried blood spots (QIAamp DNA Mini kit)

DNA was extracted from a blood spot on filter papers using a QIAamp DNA min kit as described by Chandrasekhar et al. (2016).

Polymerase chain reaction (PCR)

Quick load One Taq one-step PCR master (2X) with catalog number NEB MO486S was purchased from Inqaba Biotech Hartfield South Africa incorporated and used according to the manufacturer's instruction.

The primers for the detection of *P. malariae*:

rMAL 1: 5'-ATA ACA TAG TTG TAC GTT AAG AAT AAC CGC-3'

rMAL 2: 5'-AAA ATT CCC ATG CAT AAA AAA TTA TAC AAA-3'

The primers for the detection of *P. ovale*:

rOVA 1: 5'-ATC TCT TTT GCT ATT TTT TAG TAT TGG AGA-3'

rOVA 2: 5'-ATC TAA GAA TTT CAC CTC TGA CAT CTG-3'

The primers that were used for *P. falciparum* detection:

rFAL 1: 5'-TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT-3'

rFAL 2: 5'-ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC-3'

and for *P. vivax* detection:

rVIV 1: 5-CGC TTC TAG CTT AAT CCA CAT AAC TGA TAC-3'

and

rVIV 2: 5' ACT TCC AAG CCG AAG CAA AGA AAG TCC TTA-3'

The resistance primers are represented in Table 1.

Preparation of agarose gel

One point zero percent (1.0%) Agarose gel was prepared by dissolving 1.0 g in 100 ml Tris EDTA Buffer. The mixture was then heated in a microwave for 5 min to dissolve completely. It was then allowed to cool at 56°C and 6 μ l of Ethidium bromide was added to it. The Agarose gel was poured into the electrophoresis chambers with a gel comb, and allowed to solidify.

Electrophoresis

Five microliters of the amplified PCR products were analyzed on 1.0 Agarose gel containing ethidium bromide in Tris EDTA buffer. Electrophoresis was performed at 90v for 60minutes, after electrophoresis, the PCR products were visualized by Wealth Doc UV transilluminator and photographed. Molecular weights were calculated using the molecular weight standard of the marker.

Table 1. Resistance primers.

Name	Locus/fragment primary PCR amplification	Sequence 5', 3'
P5/ for	dhfr	TTTATGATGGAACAAGTCTGC
P5/rev	dhfr	ATTCATATGTACTATTTATTCTAGT
P8/for	dhps	ATTTTTGTTGAACCTAAACGT GCTGTTCA
P8/rev	dhps	CTTGTCTTTCCCTCATGTAATTCATCT

Source: Agomo et al. (2016).

Polymerase chain reaction product cleaning and purification

The PCR products were cleaned using Exonuclease/Shrimp Alkaline Phosphatase (NEBMO 371) 1 μ /ul in a 0.6 ml microcentrifuge tube. Purification was done with ABI V 3.1 Big dye kit according to the manufacturer's instructions.

Sequencing and phylogenetic analysis

The Ultra-pure DNA was sequenced with an AB13500XL analyzer with a 50 cm array, using POP7 at Inqaba Biotechnical Industries Ltd (Hatfield, South Africa). The manufacturer's protocols were strictly followed. Sequences data generated were analyzed with Geneious version 9.0.5 and phylogenetic trees were constructed using neighbor-joining.

Statistical analysis

Data were analyzed using SPSS version 25 (Stanford, United States of America). Frequencies of Pfdhps and Pfdhfr were placed in percentages. Pearson's Chi-Square test and Fisher's exact test were employed in the analysis of data. Analysis of variance (ANOVA) was used to determine the mean parasite density and doses of SP intake. A p-value < 0.05 was considered significant.

RESULTS

A total of 390 participants were enrolled in the study. Three hundred and thirty-six women were IPTp-SP users while 54 women were non-users of SP. This study recorded a compliance gap of 13.8%. The pregnant women were enrolled in the study during their antenatal care visits to health facilities. Background characteristics/basic socio-demographic information of the participants were as follows: The participants aged between 18 and 45 years. In consideration of their parity, they were composed of primigravidae 73 (18.7%), secondigravidae 104 (26.7%), and multigravidae 213 (54.6%). Their educational levels: none 3 (0.8%), primary level 140 (35.9%), secondary 165 (42.3) and tertiary 82 (21.0%). Only 117 (30.0%) out of three hundred and ninety pregnant women made their first antenatal care visit during their first trimester, with 234 (60.0%) making their first visit during their second trimester. Thirty-nine 39

(10.0%) made their first antenatal care visit during their third trimester. Most of the participants 387 (99.2%) were married. A total number of 342 (87.7%) of the participants used insecticidal nets. This is shown in Table 2.

Table 3 shows the prevalence and neonatal malaria among participants. Table 3 also shows the analysis of malaria prevalence in pregnant women and babies of SP users and non-SP users. Mean parasite densities of malaria parasites among study participants are shown in Table 3. Also, Table 4 shows the comparative analysis of parasite density and IPT dose. The PCR results of Pfdhfr resistance genes are shown in Figure 1 while Figure 2 shows PCR results of Pfdhps resistance genes. Figure 3 shows two sequence data of *P. vivax*-4 and *P. vivax*-5. The phylogenetic tree of *P. vivax*-4 is shown in Figure 4 while the phylogenetic tree of *P. vivax*-5 is shown in Figure 5.

Resistance genes of *P. falciparum* to Sulphadoxine-pyrimethamine

Sequencing assay

Sequence data generated were analyzed with Geneious version 9.0.5 and phylogenetic trees constructed using neighbor-joining. The sequences were subsequently deposited in the National Center for Biotechnology Information (NCBI) data base with their corresponding accession identities (Figure 3).

Phylogenetic analysis

Figures 4 and 5 shows Phylogenetic tree of *P. Vivax*.

DISCUSSION

The prevalence of maternal malaria among SP- users was 73(21.7%), while 41 (12.9%) of neonates among SP users were infected with the malaria parasite. In non-users of SP, 29(53.7%) were infected with the malaria parasite. The number of neonates among non-users of

Table 2. Socio-demographic profile of study participants.

Sociodemographic profile	Total number recruited (%) N= 390
Age in years	
≤ 19	5 (1.3%)
20 – 29	168 (43.1%)
30 – 39	197 (50.5%)
≥ 40	20 (5.1%)
Parity	
Primigravidae	73 (18.7%)
Secondigravidae	104 (26.7%)
Multigravidae	213(54.6%)
Marital status	
Married	387 (99.2%)
Single	3 (0.8%)
Educational level	
None	3 (0.8%)
Primary	140 (35.9%)
Secondary	165 (42.3%)
Tertiary	82 (21.0%)
Antenatal care first visit	
First trimester	117 (30.0%)
Second trimester	234 (60.0%)
Third trimester	39 (10.0%)
Use of Insecticidal Nets	
Users of Nets	342 (87.7%)
Non-users of Nets	48 (12.3%)

Table 3. Prevalence of maternal and neonatal malaria in the respondents.

Respondents	IPTp-SP Users n= 336		Non-Users n= 54	
	Positive	Negative	Positive	Negative
Pregnant women	73 (21.7%)	263 (78.3%)	29 (53.7%)	25 (46.3%)
Babies (neonate)	41(12.2%)	295(87.8%)	14 (25.9%)	40 (74.1%)
<i>Chi-square (p-value): 65.086(0.00)</i>				

Statistical Significant $p < 0.05$.

Table 4. Mean parasite densities of malaria parasite among study participants.

IPT dose	Total no. of participants	Mean parasite densities ($\mu\text{l/ml}$)	F (P-value)
0	54	63,553	2.886 (0.022)
1	26	50,110	
2	181	29,254	
3	97	374	
≥4	32	171	

Statistical significant $p < 0.05$.

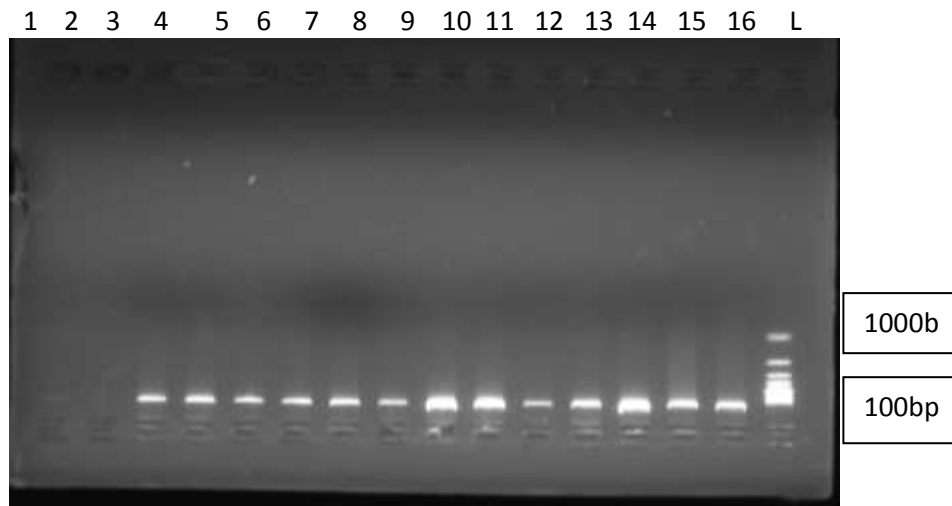


Figure 1. Polymerase chain reaction results for dhfr resistance genes analyzed on a 1.0 % Agarose gel electrophoresis stained with ethidium bromide. L was a 100-1000 bp DNA ladder (molecular marker). Samples 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16 were positive bands for the expressed dhfr resistance genes at 100 bp. Samples 1 and 2 were negative for the dhfr resistance genes.

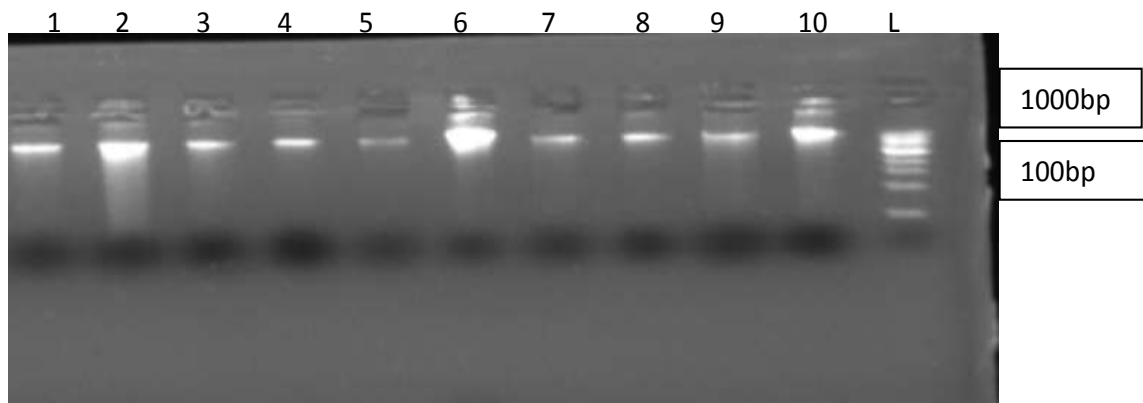


Figure 2. Polymerase chain reaction results for dhps resistance genes analyzed on a 1.0% Agarose gel electrophoresis stained with ethidium bromide. L was a 100bp-1000bp DNA ladder (molecular marker). Samples 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 were positive bands for the expressed dhps resistance genes at 1000 bp.

SP infected with malaria was 14(25.9%). In IPTp-SP users, 263(78.3%) were not infected with malaria while in non-SP users, 25(46.3%) were not infected with malaria. Overall maternal malaria prevalence in SP-users and non-SP users was 102(26.2%) while overall neonatal malaria prevalence in SP-users and non-users of SP was 55(14.1%). The result of the present study is in agreement with a study at Rivers State, Nigeria by Onoja et al. (2019) in which 66(25.3%) of SP users had maternal malaria infection while 17(43.6%) of non-users of SP had maternal malaria infection. Also, in that study; overall

maternal malaria prevalence was 83(27.7%). Neonatal malaria infection was 40(13.3%) and placental infection 66(22.0%). In that study also, overall placental and cord blood malaria infection was 106(35.3%). The present study recorded a low prevalence of malaria among IPTp-SP users; this may be as a result of the majority of the participants adhering to the drug policy guiding the use of SP for malaria treatment in pregnancy. In 2013, malaria prevalence in pregnant women attending antenatal clinics in hospitals in Anambra State, Nigeria was 73.1% (Ukibe et al., 2013). The present study recorded a lower

>Nucleotide alignment Alignment of 2 sequences: 4_Viva-FP_G06_21.ab1, 5_Viva-FP_H06_24.ab1

Score = 346.5, Identities = 88/123 (71%),
Positives = 112/123 (91%), Gaps = 3/123 (2%)

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4_Viva-FP_G06_21.ab1      1 CMARRMMKMG-SWCTTTSWTYGYGCTMMGYKWMGSTGTAGCATTMWTACGTTAGTTCTI
                          C+A+RMMM + +W++TT++T+ + +++++ + ++GT GCATT++TCAC++AGTTCTI
5_Viva-FP_H06_24.ab1      1 CAAARMMMSTGWSYTTCKTCTKCKRWTCTKAAKKGI-GCATTCKTCACRWAGTTCTI

4_Viva-FP_G06_21.ab1      60 GWGCCTTGGYTTWCAKYTCCATCAGGTCCTTTAAGGACTTTCCTTGCTTCGGCTTGGAA
                          G+GCCTTGGYTT+CAKYTCCATCAGGTCCTTTAAGGACTTTCCTTGCTTCGGCTTGGAA
5_Viva-FP_H06_24.ab1      60 GAGCCTTGGYTTYCAKYTCCATCAGGTCCTTTAAGGACTTTCCTTGCTTCGGCTTGGAA

4_Viva-FP_G06_21.ab1      120 TA- 121
                          TA
5_Viva-FP_H06_24.ab1      120 TAA 122
    
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Figure 3. Two sequences data of *P. vivax*; (Accession number: 4 – viva – FPMT57725) and (Accession number: 5 – viva – FPMT57726).

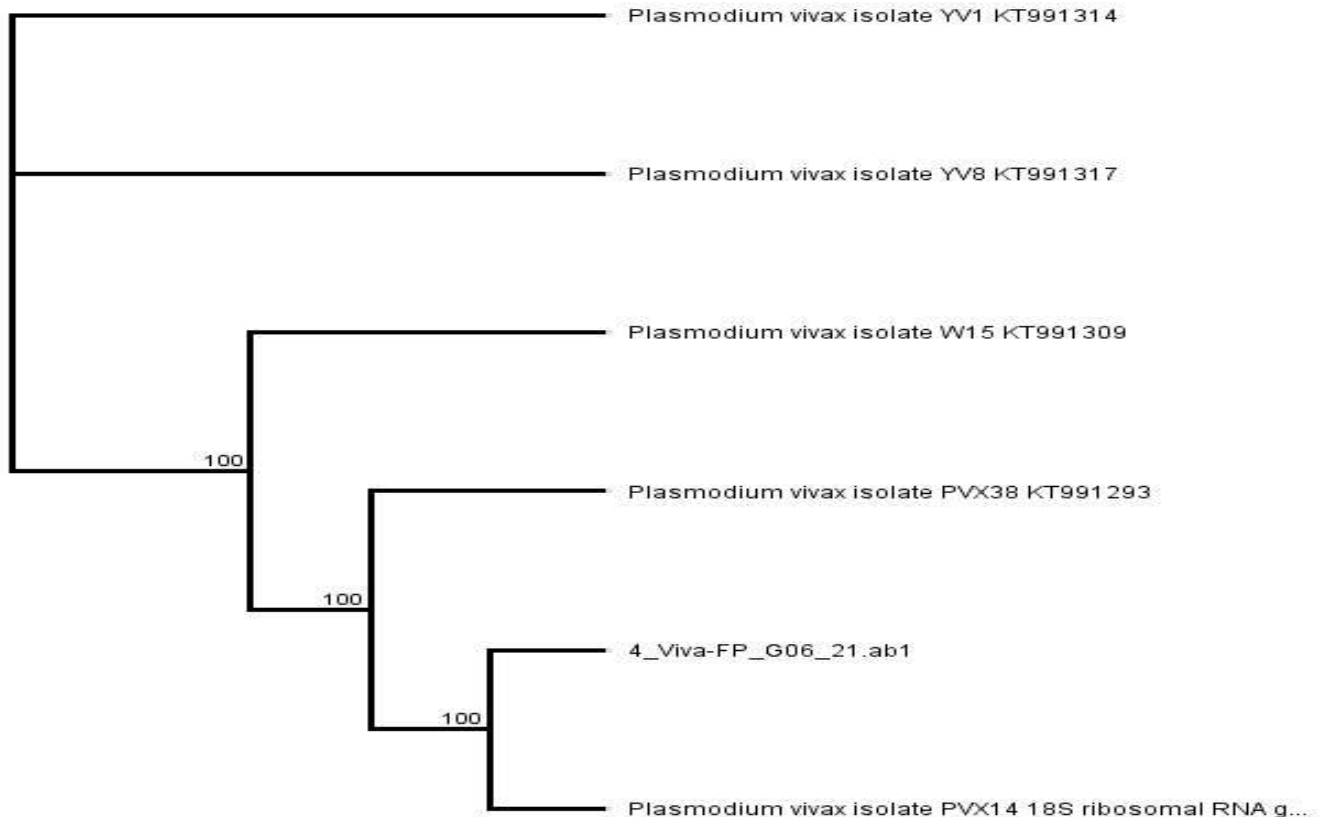


Figure 4. Phylogenetic tree of *P. vivax* (Accession number: 4 – viva - FPMT57725), GenBank, Maryland, USA.

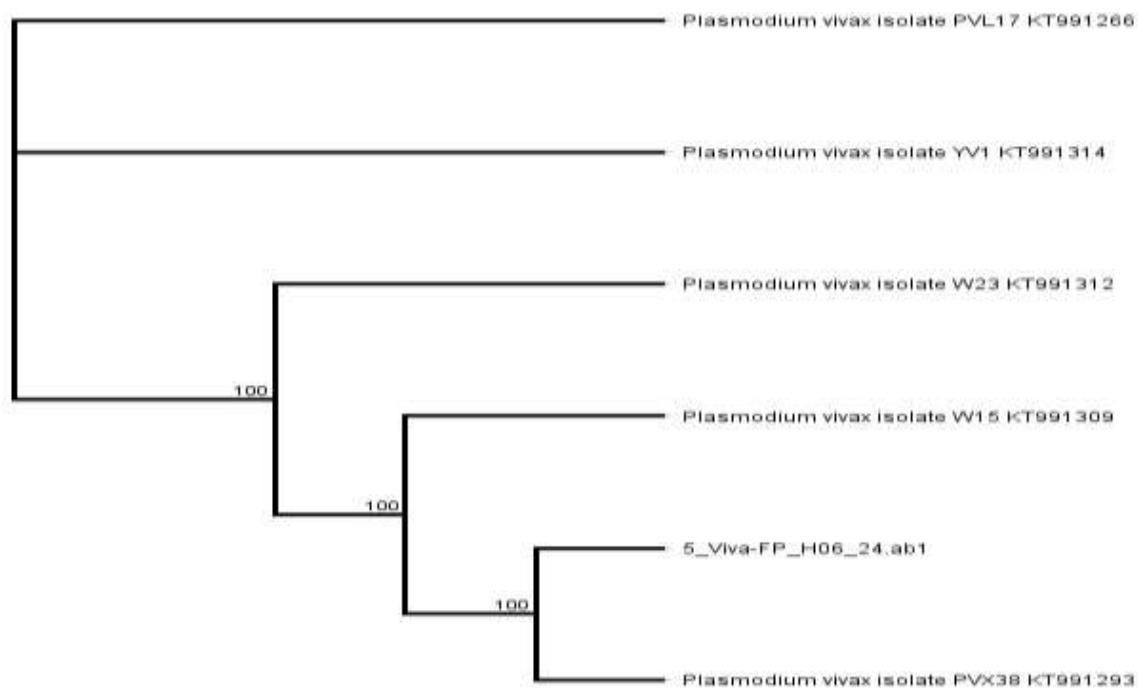


Figure 5. Phylogenetic tree of *P. vivax* (Accession number: 5 - viva - FPMT577726), GenBank, Maryland, USA.

prevalence of malaria when compared with the study by Ukibe et al. (2013) on malaria prevalence in women attending antenatal clinics in Anambra State. The result of this study showed that the majority of malaria-infected mothers that took SP admitted having taken the dosage once, twice, thrice while others four times and above. The mean parasite densities of non-users of SP range between 73,000 to 82,400 $\mu\text{l/ml}$. Pregnant women who used SP for malaria treatment for once, twice, thrice and four times and above had mean malaria parasite densities in ranges of 2,800 to 56,700 $\mu\text{l/ml}$, 1,230 to 6,344 $\mu\text{l/ml}$, 137 to 618 $\mu\text{l/ml}$, and 137 to 200 $\mu\text{l/ml}$ respectively. Non-users of SP had the highest mean malaria parasite density 63,553 $\mu\text{l/ml}$, followed by those who took SP once, twice, thrice, and four times and above respectively. Mean malaria parasite densities of participants who took SP for malaria treatment for once, twice, thrice, and four times and above were 50110, 29,254, 374 and 171 $\mu\text{l/ml}$ respectively. There was a significant difference in parasite densities of SP users and non-SP users ($p=0.022$). Therefore mean parasite density was reduced in SP users than in non-users of SP ($p<0.001$). Pregnant women who took SP had reduced malaria parasitemia than those who did not take SP ($p<0.001$). Babies of SP users had reduced malaria parasitemia when compared with babies of non-SP users ($p=0.002$). This finding also agrees with the result of a study carried out by Chukwurah et al. (2016) which showed that pregnant women that took SP once had

higher malaria parasitemia than those that took SP twice and thrice respectively. However, low compliance to SP directives increases the risk of maternal, placental, and neonatal malaria in endemic regions (Mdetele and Kidima, 2017).

Molecular genotyping of *Plasmodium* revealed two species: *P. falciparum* and *P. vivax* were prevalent in the study population. *P. ovale* and *P. malariae* were not seen among the study participants. The number of pregnant women who were positive for molecular genotyping in IPTp-SP users was 68(20.2%). *P. vivax* infection which was discovered in this study should be a matter of public health concern because *P. vivax* infection is not common malaria parasite species seen in the study area. This may be probably a result of misdiagnosis which is common in most laboratories in sub-Saharan Africa. Misdiagnosis of *P. vivax* is common especially in African countries (Ghai et al., 2016; Yegorov et al., 2016). However, the result of this study is in agreement with the result of a study carried out by Howes et al. (2013) in which they reported that there has been an incidence of *P. vivax* infection among the Duffy negative population in sub-Saharan Africa. There has been also evidence of mortality associated with acute *P. vivax* malaria (Baird, 2013). The number of pregnant women that had *P. falciparum* parasitemia was 38(11.3%) of pregnant women on IPTp-SP while 30(8.9%) of pregnant women on IPTp-SP had *P. vivax* parasitemia. The number of pregnant women on IPTp-SP that had co-infection of *P.*

falciparum and *P. vivax* was 27(8.0%). *P. vivax* infection is not absent in sub-Saharan Africa. In this study, two resistance genes were assessed in the malaria parasite genes. The two resistance genes were dihydrofolate reductase (dhfr) and dihydropteroate synthetase (dhps). The result revealed that 13(3.9%) of the participants expressed the genes for dhfr while for dhps resistance genes, 10(2.9%) of the participants had expression for them. The number of participants that had expression for both dhfr and dhps resistance genes was 8(2.3%) among the study participants. The result of the present study when compared with the results of other researchers showed lower frequencies of these resistance genes (Agomo et al., 2016; Akanbi et al., 2009). These researchers observed higher frequencies of these resistance genes and they also studied mutations in these genes. In the present study mutations of these resistance genes were also studied. In the present study, the frequencies for dhfr and dhps resistance gene mutations were 64.86 and 91.9% respectively. Agomo et al 2016 observed mutated resistance genes up to 75.9% while another researcher Akanbi et al 2009 observed mutated resistance genes of up 71- 92% frequency for dhfr and 39 -71% frequency for double mutant dhps respectively among the participants.

CONCLUSION AND RECOMMENDATIONS

In conclusion, the molecular analysis indicated the presence of *P. falciparum* dhfr and dhps resistance genes in the study area which are associated with SP treatment failure, although, the SP drug has been recommended by the World Health Organization (WHO, 2001) as a first line antimalarial medicine and adopted in Nigeria for IPTp in 2005 (Federal Ministry of Health, 2005). Similarly, the present study, which indicated the presence of *P. falciparum* dhfr and dhps resistance genes of which mutations of these resistance genes are associated with SP resistance should be of public health concern. Our results suggest that the presence of these resistance genes can lead to treatment failures among IPTp-SP users. Therefore, novel drugs should be sought to replace SP in near future. The result of the present study might be used as a basis for surveillance as molecular markers for anti-malarial resistance in Nigeria and continuous surveillance using these molecular markers can provide a valuable aid to developing and updating national anti-malarial policy guidelines.

Limitations of the study

Distant primary healthcare centers, poor road network especially in rural places. The financial burden particularly in molecular studies imposed great constraints. Collection

of cord blood samples during deliveries and clotted blood samples. These limitations did not in any way, however, bias the results of the present study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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