Molecular Evaluation of *Plasmodium falciparum* Drug Resistant Genes among Pregnant Women Attending Antenatal Care at Maryam Abacha Women and Children Hospital (MAWCH) Sokoto, Nigeria

Abubakar H[1*], Bala AY[2], Bandiya HM[2], Shuaibu AB[3]
[1]Department of Animal Health and Production Technology, College of Agriculture and Animal Science, Wurno, Sokoto, Nigeria
[2]Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, Nigeria
[3]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria

Submitted:12th Feb., 2022; Accepted:18th Oct., 2022 ; Published online: 31st Oct., 2022
DOI: : https://doi.org/10.54117/jcbr.v2i5.10
*Corresponding author: abubakarhafiz95@gmail.com; Tel.: +2348030510042

Abstract
Malaria continues to cause significant morbidity and mortality across Africa, despite the intensification of control interventions with most of the infections caused by *Plasmodium falciparum*. Previous researches conducted in Sokoto indicated high prevalence of malaria among pregnant women. Other studies reported that resistant *P. falciparum* may have contributed reasonably to the malaria burden in the country and independently recommended molecular surveys for its confirmation in the state. This research was aimed at determining *P. falciparum* drug-resistant genes among pregnant women attending antenatal care (ANC) at Maryam Abacha Women and Children Hospital (MAWCH) Sokoto, Nigeria from February, 2020 to May, 2020. Blood samples of 200 pregnant women who came for ANC in the hospital were collected and questionnaires administered. Thin and thick blood film microscopy and *P. falciparum* monoclonal antibody specific RDT kits were used to detect *P. falciparum* in the samples. *P. falciparum* positive dried blood samples (n = 19) were investigated for the presence of Pfmdr1 and Pfcrt genes using PCR protocol. Results for the investigation of Pfcrt and Pfmdr1 genes revealed 26.30% and 89.50% prevalence respectively, while 4 samples were positive for both genes representing 21.05% prevalence. Prevalence of Pfcrt and Pfmdr1 genes do not associate ($p>0.05$) with age, parity, trimester, marital status, occupation and history of antimalarial rugs used by the pregnant women. The high prevalence of these mutant genes observed in this study could be attributed to pregnant women's indiscriminate use of antimalarial drugs. Pregnant women should attend antenatal care clinics early and on a regular basis, and malaria control programs for pregnant women should be prioritized in Sokoto State and throughout the country.

Key words: *Plasmodium falciparum*, Pfmdr 1, Pfcrt, pregnant women, antenatal care

introduction

Malaria is an infectious disease caused by protozoan parasites from the genus *Plasmodium* that can be transmitted by the bite of the *Anopheles* mosquito with *falciparum* malaria being the deadliest (WHO, 2018; Maina *et al.*, 2017). Malaria Symptoms include cycles of chills, fever, sweats, muscle aches and headache that recur every few days. There can also be vomiting, diarrhea, coughing, and yellowing (jaundice) of the skin and eyes. Persons with severe

falciparum malaria can develop bleeding problems, shock, kidney and liver failure, central nervous system problems, coma, and death (WHO, 2018).

P. falciparum is a protozoan parasite belonging to phylum Apicomplexa, class Aconoidasida, order Haemosporida, family Plasmodiidae, and genus *Plasmodium* (WHO, 2019). The success of *P. falciparum* as a parasite has been attributed partly to the enormous genetic diversity that allows it to adapt to antimalarials and hinder the development of an effective vaccine (Tobias *et al.*, 2019). Malaria continues to cause significant morbidity and mortality across Africa, despite the intensification of control interventions (WHO, 2018).

Research conducted by Buhari *et al.* (2016) in Sokoto indicated prevalence of malaria was higher in pregnant women and need for the promotion of insecticide-treated bed nets (ITNs) and intermittent preventive treatment (IPTp) to protect pregnant women in the area from malaria. Malaria infection during pregnancy can have adverse effects on both mother and fetus, including maternal anemia, fetal loss, premature delivery, intrauterine growth retardation, and delivery of low birth-weight infants (<2500 g or <5.5 pounds), a risk factor for death (CDC, 2018).

Currently, malaria chemotherapy relies mainly on the sustained efficacy of artemisinin-based combination therapy (ACT), which are the recommended drugs for the treatment of adults and children with uncomplicated *P. falciparum* infection (WHO, 2015). The ACT combines artemisinin derivatives and other long-acting antimalarials, with a different mechanism of action, to maximize the chances of eliminating parasites during treatment, but also to limit the possibility of a spontaneous mutation that will confer resistance to both drugs (Tobias *et al*., 2019).

Drug resistance is the reduction in effectiveness of a medication such as an antimicrobial or an antineoplastic in treating a disease or condition (Alfarouk, 2014). The development of drug resistance could be influenced by multiple factors such as polymorphism rate, fitness costs, overall parasite load, strength of drug selection, treatment compliance, transmission intensity, host immunity, and erythrocyte disorders (Himanshu *et al.,* 2018). Naturally acquired immunity plays a major role in the emergence and clearance of artemisinin-resistant parasites (Ataide *et al.,* 2017). Chloroquine-resistant *P. falciparum* parasites arose in the late 1960s and have since spread to malaria endemic area rendering chloroquine ineffective in many patients (Gresty and Karryn, 2014). Chloroquine resistance in *P. falciparum* has been reported to associate with mutations in pfcrt gene. Molecular studies demonstrated that resistance to chloroquine results from a series of mutations in pfcrt located on chromosome 7, of particular importance is the mutation causing a change from lysine (K) to threonine (T) at amino acid 76 (Simon-Oke *et al.,* 2018).

Reports suggest that *P. falciparum* has developed resistance to most Antimalarial Drugs, including chloroquine and its derivatives, sulfadoxine–pyrimethamine, mefloquine, and artemisinin (Ashley *et al.,* 2014; Reteng *et al.,* 2017). It is widely accepted that several polymorphisms play important roles in chloroquine-resistant *P. falciparum*, particularly a threonine substitution at codon 76 in the *P. falciparum* chloroquine-resistant transporter (Pfcrt) and a tyrosine substitution at codon 86 in *P. falciparum* multidrug-resistant protein (Pfmdr1) (Reteng *et al.,* 2017).The management of the disease has become

increasingly difficult and controversial due to emerging multiple drug resistance (Faruku *et al.,* 2016).

Currently, most malaria control programs include vector control, early diagnosis and effective treatment of clinical cases (Kiaco *et al.,* 2015). However, *P. falciparum* esistance to anti-malarial drugs is one of the main challenges for malaria control in endemic countries since resistant parasites are widespread and continue to evolve in response to the selective pressure applied (Sondo *et al.,* 2016; WHO, 2018; Nsanzabana *et al.,* 2018). Chloroquine (CQ) and sulfadoxine–pyrimethamine (SP) had to be discontinued for clinical malaria treatment following increased morbidity and mortality associated with resistance in the past decades. The same could happen in the near future to artemisinin-based combination therapy (ACT), the current first-line therapy, if an alternative would be available (Hanboonkunupakarn *et al.,* 2016; WHO, 2018; Nsanzabana *et al.,* 2018). Resistance to CQ and SP arose in South-East Asia (SEA) and spread to Africa (Slater *et al.,* 2016; Takala *et al.,* 2015). Regular monitoring of these markers across malaria endemic regions will ensure its containment and prevent its emergence or spread to Africa, as was the case with previous antimalarials (Kamau *et al.,* 2015; Tobias *et al.,* 2019).

In Nigeria, data on the susceptibility profile of *P. falciparum* isolates to antimalarial Drugs is inadequate. However, it is imaginable that resistant *P. falciparum* may have contributed reasonably to the malaria burden in the country. It is interesting to note that Abdullahi *et al.* (2009) and Umar *et al.* (2007) reported the first documented evidence of possible *in vivo P. falciparum* resistance to anti-malarial drugs in Sokoto State and independently recommended molecular surveys for its confirmation in the state. Unfortunately, according to Umar *et al.* (2007) such tools were not available at that time. Therefore, this study is aimed at determining the prevalence and molecular survey of drug resistant *falciparum* malaria among Pregnant Women attending Anti-Natal Care at Maryam Abacha Women and Children Hospital (MAWCH) Sokoto, Sokoto State, Nigeria. Determining the prevalence of *P. falciparum* chloroquine resistance transporter (Pfcrt) and *P. falciparum* multi-drug resistant (Pfmdr1) genes will provide awareness to pharmaceutical companies, governments and pregnant women. It will also form basis for further research.

Materials and methods

Study Area

Sokoto town, the capital of Sokoto State is located in the extreme northwest of Nigeria, near the confluence of the Sokoto River and the River Rima. As of 2006 it has a population of 3,702,676 (NPC, 2007). Maryam Abacha Women and Children Hospital is located on Sultan Bello Road in Sokoto. The women and children health-care clinic was built in 1997 and commissioned by her Excellency, Maryam Sani Abacha, to address health issues that women and children face, such as vesicovaginal fistula (VVF). Since its inception, the hospital has successfully performed surgery in such cases (Lema *et al.,* 2019)

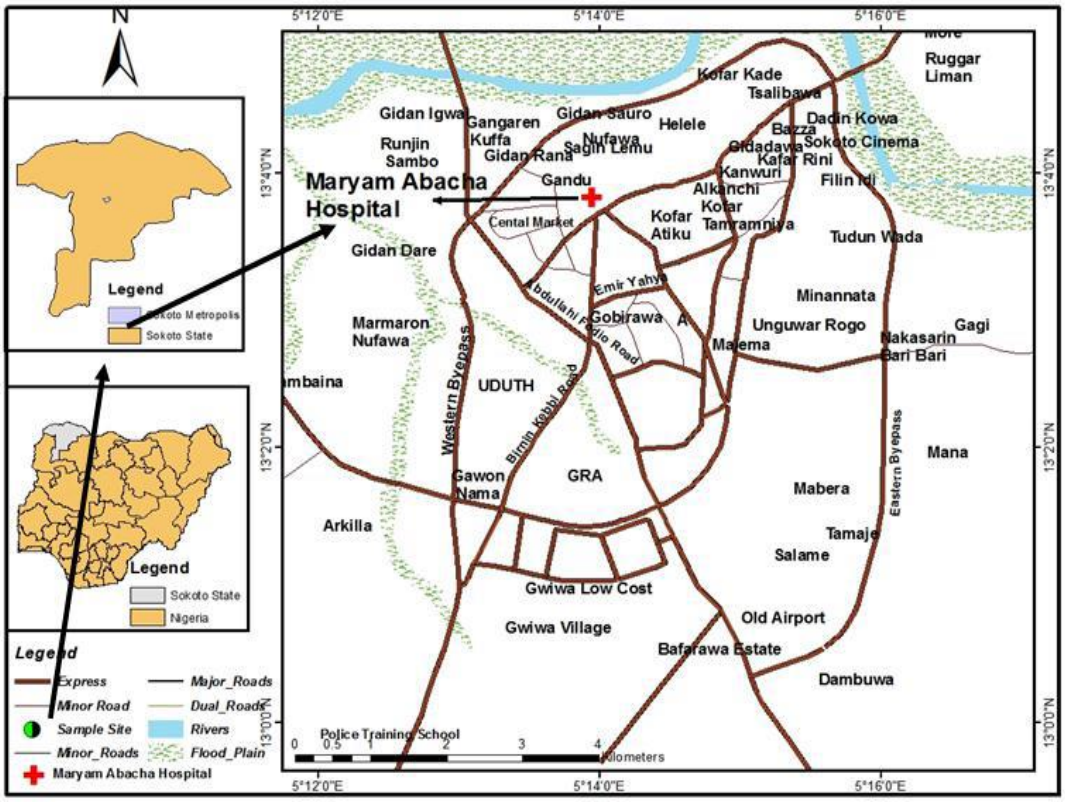

Figure 1: The Study Area – Maryam Abacha Women and Children Hospital, Sokoto.
Source: Department of Geography UDUS (2021).

Hospital Consent

Prior to the commencement of the research work, an approval was obtained from the management of MAWCH, Sokoto State, Nigeria. Blood samples were collected from consenting patients who were clearly informed on the aim and objectives of the research. Introduction letter was obtained from the Head of Biological Sciences department which was addressed to the management of MAWCH seeking them to assist and give access to their facilities. Open ended/closed ended questionnaire was also distributed to participating pregnant women.

Sample Size Determination

Sample size of the study population was calculated using the formula: $n = Z^2 p (1-P)/ e^2$ (Lwanga and Lemeshow, 1991). Where; n = Desired sample size; Z = Confidence interval (95%) = 1.96; p = Previous Prevalence; p = 7% previous prevalence (Ekpereonne *et al.*, 2017);
e = Degree of precision (0.05)
Therefore, substituting Z for 1.96, P for 0.07 and e for 0.05, then the minimum sample size will be: $n= (1.96)^2 \times 0.07 (1 -0.07) / (0.05)^2$
$n= 3.8416 \times 0.07 (0.93)/ 0.0025 = 100$
The minimum sample size was calculated as 100. However, the sample size for the study was adjusted to 200.

Blood Sample Collection

With the use of needle and 5 ml syringe, through the vein, two milliliter of blood samples were collected from pregnant women who came for Antenatal Care in the study area with the help of a trained nurse. After collection, the blood samples were transferred into an ethylene di-amine tetra-acetic acid (EDTA) container to prevent coagulation of the blood sample. The blood samples (in the EDTA container) were

transported to central research laboratory of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto (UDUSok) in a cold chain and store at 4^{o}C until required.

Malaria Parasite Screening

Thick and thin blood films and Rapid Diagnostic Test kits were used to screen blood samples for the presence of malaria parasites.

Thick blood film

With the use of pasture pipette, two drops of blood in the EDTA container was taken and placed on a clean grease-free glass slide, the blood on the slide was allowed to dry after which the film was flooded with Giemsa stain and incubated for 15 min (Agomo *et al.,* 2009) After 15 min, the stain was washed off rapidly with distilled water and allowed to dry. Two drops of immersion oil was added to the film and then viewed under the microscope at x100 objective lens for characteristics features of malaria parasite as adopted by (Simon-oke *et al.,* 2018).

Thin Blood film

Two microlitres (2 µl) of blood was placed on a pre-cleaned, labeled slide, near its frosted end. Another slide was brought at a 30-45° angle up to the drop, allowing the drop to spread along the contact line of the 2 slides. The spreader was quickly pushed toward the unfrosted end of the lower slide, making sure that the smears have a good feathered edge. This was achieved by using the correct amount of blood and spreading technique. The thin smears were allowed to dry. The smears were finally fixed by dipping them in absolute methanol (CDC, 2020) after which the film was flooded with Giemsa stain and incubated for 15 min (Agomo *et al.,* 2009) After 15 min, the stain was washed off rapidly with distilled water and allowed to dry. Two drops of immersion oil was added to the film and then viewed under the microscope at x100 objective lens for characteristics features of malaria parasite as adopted by (Simon-oke *et al.,* 2018).

***Plasmodium falciparum* Rapid Diagnostic Test Kit (RDT Kit)**

All the 200 blood samples were also screened using *P. falciparum* monoclonal antibody specific RDT kit to confirm the presence of the parasite in case if we have false negative via microscopy (*P. falciparum*) The RDT (SD BIOLINE Malaria Ag P.f) Kit was used in accordance with the manufacture's instruction. Positive samples for *P. falciparum* were transported to the central research laboratory of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto (UDUSok) for DNA extraction and molecular screening.

Molecular Screening

Deoxyribonucleic acid (DNA) Extraction

DNA extraction was carried out using Qiagen DNA easy (Qiagen, Krefeld, Germany) extraction kit following manufacturer's instruction (Olasehinde *et al.,* 2014).

Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) was used to determine the resistant genes of *P. falciparum* from the blood of malaria positive pregnant women (Sharma *et al.,* 2015).

PCR amplification of Pfcrt gene

The PCR was carried out in a 0.2 ml nuclease free microfuge tube containing a 25 µl reaction mix with the following components; 12.5 µl of Top Taq master mix (Qiagen, USA), 2 µl of each 10µm of Pfcrt F and Pfcrt R primers, 2 µl of coral load (Qiagen, USA), 1.5 µl of Nuclease-free water (Qiagen, USA) and 5 µl of DNA template. The tubes were transferred to Applied Biosystem (ABS) 9700 thermocycler. The cycling conditions used were: an initial denaturation at 94 °C for 3 min, followed by 45 cycles of final denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, initial extension at 72 °C for 1

min, followed by a final extension at 72 °C for 5 min and post hold at 8°C (Vathsala *et al.,* 2004; Sharma *et al.,* 2015). The PCR amplicons were visualized in 1.5 % agarose gel. The list of primers used for the PCR-amplification is provided in Table 1.

PCR amplification of Pfmdr1 gene

The PCR was carried out in a 0.2 ml nuclease free microfuge tube containing a 25 µl reaction mix with the following components; 12.5 µl of Top Taq master mix (Qiagen,USA), 2 µl of each 10µm Pfmdr v1 F and Pfmdr 1 R, 2.5 µl of coral load (Qiagen,USA), 2 µl of Nuclease free water (Qiagen,USA) and 4µl of DNA template. The tubes were transferred to Applied Biosystem (ABS) 9700 thermocycler. The cycling conditions used were as follows: an initial denaturation at 94 °C for 3 min, followed by 35 cycles of final denaturation at 94 °C for 30 s, annealing at 56 °C for 1 min and extension at 72 °C for 2 minutes, followed by a final extension at 72 °C for 5 min and post hold at 8°C. The PCR amplicons were visualized in 1.5% agarose gel. The lists of primers used for PCR amplification are provided in Table 1.

Table 1: List of primers used in the study

Primer name	Oligonucleotide Sequence (5'-3')	Expected size
Pfcrt- F	G G C T C A C G T T T A G G T G G A	264bp
Pfcrt- R	TGAATTTCCCTTTTTATTTCCAAA	
Pfmdr 1- F	A T G G G T A A A G A G C A G A A A G A	603bp
Pfmdr1 – R	AACGCAAGTAATACATAAAGTCA	

Source: (Vathsala, 2004)

Agarose Gel Electrophoresis

The amplified products were subjected to 1.5% agarose gel electrophovresis pre-stained with ethidium bromide with a 100 bp ladder used as a standard.The electrophoresis was carried out at 80 volts for 1 h using a BioRad agarose gel electrophoresis unit. The gel was visualized using a U-V trans-eliminator in a BioRad XRS gel documentation device.

Statistical Analysis

Fisher's exact test was done to determine the association between the prevalence of *P. falciparum r*esistant genes (Pfcrt and Pfmdr 1) and occurrence of Pfmdr1 and Pfcrt genes with the socio-demographic characteristics of the pregnant women using InVivoStat version 4.1.0. In all the analysis, confidence level was held at 95% and $P<0.05$ was set as level of significance.

RESULTS

It could be seen in Table 2 that all the participants (100%) were willing to accept new drugs, sleep under bed nets if supplied, and have knowledge on the cause of malaria. A total of 85.50% of the participants use bed nets, and 99.50% had received information on malaria. A total of 92.50% of the participants reported that government has done something to control malaria, 90.80% agreed that the government supplied mosquito treated bed nets and only 17 (9.20%) agreed that government has supplied anti-malaria drugs. It is observed that, the respondents used different drugs (Fansida, chloroquine, artemether and Emal) in treating malaria infection previously.

Two hundred (200) pregnant women were tested for malaria parasites using microscopy and rapid diagnostic test (RDT) kit. The results for microscopy revealed 3 samples that were positive for *P. falciparum r*epresenting 1.50% prevalence. The results of this study with respect to *P. falciparum* monoclonal antibody specific RDT kit revealed 19 samples positive for *P. falciparum r*epresenting 9.50% prevalence (Table 3). The 3 positive samples identified in microscopy were also part of the 19 positive samples identified via RDT kits.

Table 2: Responses from the participating pregnant women in the study area

Variables		Frequency %(N)
Are you willing to accept new drugs	Yes	100 (200)
	No	0 (0)
Are you willing to sleep under Bed net if supplied?	Yes	100 (200)
	No	0 (0)
Do you have knowledge on the cause of malaria?	Yes	100 (200)
	No	0 (0)
Use of Bed net	Yes	85.50 (171)
	No	14.50 (29)
Do you receive information on malaria	Yes	99.50 (199)
	No	0.50 (1)
Has the government done anything to control malaria	Yes	92.50 (185)
	No	7.50 (15)
What does the government done to control malaria	Supply of Mosquitoes treated bed net	90.80 (168)
	Supply of Antimalaria drugs	9.20 (17)
	Others, specify	0 (0)
History of antimalaria drugs used by the respondents in the study area	Fansida	2.0 (4)
	Chloroquine	25.50 (51)
	Fansida and Chloroquine	21.0 (42)
	Fansida and Emal	2.0 (4)
	Fansida, Chloroquine and Artemether	49.50 (99)

Table 3: Prevalence of malaria using RDT kit and Microscopy

Testing Tools	Number examined	Number Positive	Percentage Positive (%)
RDT kit	200	19	9.50
Microscopy	200	3	1.50

Out of the 19 samples tested for Pfmdr1 and Pfcrt genes, 17 samples were positive for Pfmdr1 (89.50%) and 5 samples (26.30%) were positive for Pfcrt gene. While 5 samples (sample 6, 10, 13, 14 and 18) were positive for both genes representing 21.05% prevalence (Table 4).

Table 4: Prevalence of Pfcrt and Pfmdr1 genes

Gene	Number examined	Number Positive	Percentage Positive (%)
Pfmdr1	19	17	89.50
Pfcrt	19	5	26.30

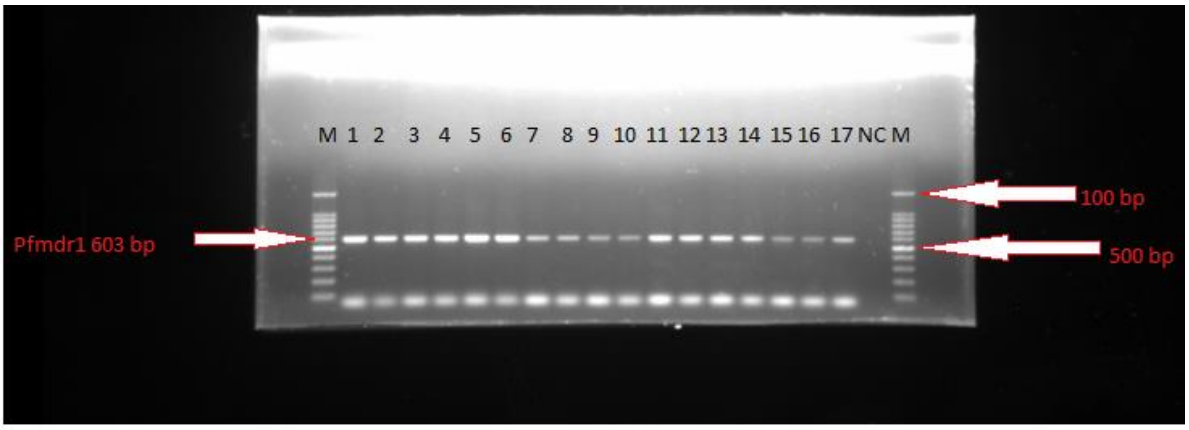

Fig. 2: 1.5% agarose gel electrophoresis analysis for detection of Pfmdr1 gene under UV trans-illuminator in a BioRad XRS gel documentation device

Key: 1= sample 181, 2=186, 3=188, 4=189, 5=191, 6=192, 7=103, 8=105, 9=175, 10=170, 11=167, 12=81, 13=88, 14=106, 15=126, 16=147, 17=144; M=ladder; NC= Negative Control

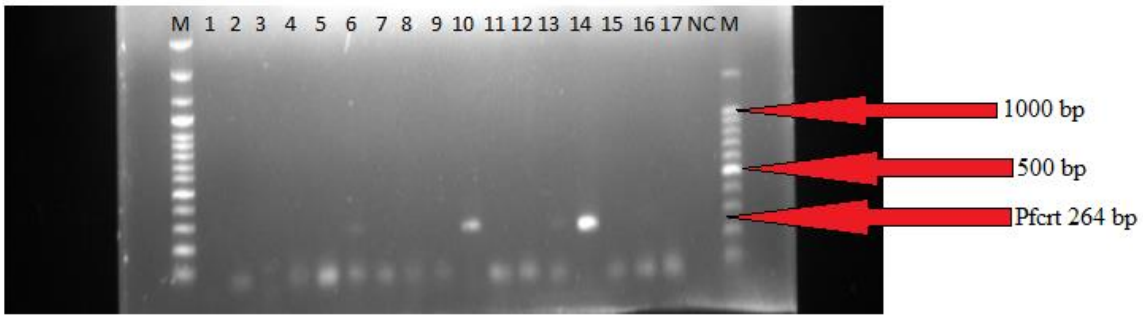

Fig. 3: 1.5% agarose gel electrophoresis analysis for detection of Pfcrt gene under UV trans-illuminator in a BioRad XR documentation device

Key: Positive samples = 6, 10, 13 and 14; Negative Samples= 1,2,3,4,5,7.8,9,11,12,15,16 and 1; M=ladder; NC= Negative Control

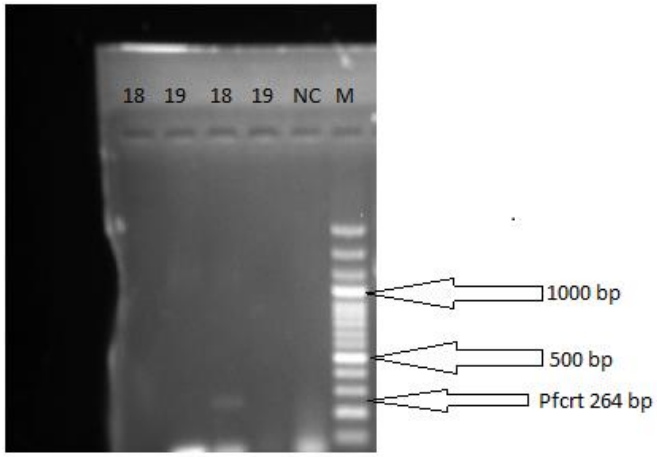

Fig. 4: 1.5% agarose gel electrophoresis analysis for detection of Pfmdr1 and Pfcrt genes under UV trans-illuminator in a BioRad XRS gel documentation device

Key: Positive sample: Pfcrt = 18; Negative samples: Pfmdr1 = 18 and 19, Pfcrt = 19; M=ladder; NC= Negative Control

Relationship between occurrence of Pfmdr1 gene with age of the pregnant women is shown in Table 5 as pregnant women within the age group 17-21, 27-31 and 32-36 years recorded the highest prevalence of Pfmdr1 gene with 100% prevalence. However, those within the age range of 22-26 recorded the least prevalence (80.0% prevalence). There was no significant association (P= 0.6491) between prevalence of Pfmdr1 gene with age of the pregnant women.

Although there was no significant association (P=1.0000) between occurrence of Pfmdr1 gene with Parity of the pregnant women, pregnant women who were Primigravidae recorded higher prevalence of Pfmdr1 compare to those that were Multigravidae with 100% (4/4) and 86.70% (13/15) prevalence respectively (Table 5).

In this study, it was evident (in Table 5) that pregnant women within the first trimester of pregnancy recorded the highest prevalence of Pfmdr1 gene followed by those within second trimester with 100% (2/2) and 93.30% (14/15) prevalence respectively. Those within the third trimester of pregnancy recorded lower prevalence with 50.0% (1/2) prevalence. Even though there was no significant association (P=0.3860) between the occurrence of Pfmdr1 gene with trimester of the pregnant women, prevalence of the Pfmdr1 gene continue to decrease as the pregnant women proceeds to the next trimester of pregnancy.

Although there was no significant association (P=0.5439) between occurrence of the Pfmdr1 gene with occupation of the pregnant women, prevalence of Pfmdr1 gene was recorded higher among participants who were business women, followed by housewives with a prevalence of 100% (1/1) and 92.30% (12/13) prevalence respectively. Pregnant women who were Civil servants recorded lower prevalence of Pfmdr1 gene with 80.0% (4/5) prevalence (Table 5).

Prevalence of Pfmdr1 gene (shown in Table 5) was higher among pregnant women who were widow than married women with 100% (1/1) and 88.90% (16/18) prevalence rates respectively. There was however no significant association (P=1.0000) between occurrence of the gene with marital status of the pregnant women.

Despite the fact that there was no significant association (P=1.0000) between occurrence of Pfmdr1 gene with the type of anti-malaria drugs used by the pregnant women, pregnant women who used Fansida and chloroquine recorded the highest prevalence of Pfmdr1 gene with 100% prevalence each compared to those who used artemether in addition to Fansida and chloroquine with 83.30% (10/12) prevalence (Table 5).

Table 5: Relationship of Pfmdr1 gene with socio-demographic characteristic of the pregnant women

Variables	Number Examined	Number Positive	Percentage positive (%)	p-value
Age				
17-21	2	2	100	0.6491
22-26	10	8	80.0	
27-31	6	6	100	
32-36	1	1	100	
37-41	-	-	-	
Total	19	17	89.50	
Parity				
Primigravidae	4	4	100	1.0000
Multigravidae	15	13	86.70	
Total	19	17	89.50	
Trimester				
First	2	2	100	0.3860
Second	15	14	93.30	
Third	2	1	50.0	
Tota	19	17	89.50	
Occupation				
Unemployed	13	12	92.30	0.5439
Civil servant	5	4	80.0	
Business women	1	1	100	
Total	19	17	89.50	
Marital status				
Married	18	16	88.90	1.0000
Widowed	1	1	100	
Total	19	17	89.50	
Drugs				
Fansida	1	1	100	1.0000
Chloroquine	3	3	100	
Fansida and Chloroquine	3	3	100	
Fansida, Chloroquine and Artemether	12	10	83.30	
Total	19	17	89.50	

Although there was no significant association (p=0.1568) between the occurrence of Pfcrt gene with age of the pregnant women, the prevalence of Pfcrt gene decreases with increase in age of the pregnant women, pregnant women within the age range of 17-21 years recorded the highest prevalence of Pfcrt gene with 100% (2/2) prevalence followed by those within 22-26 years of age with 20.0% (2/10) prevalence. Those within age range of 27-31 years recorded the least prevalence of Pfcrt gene with 16.70% (1/6) prevalence (Table 5).

Occurrence of Pfcrt gene with Parity of the pregnant women is shown in Table 6, pregnant women who were primigravidae recorded the highest prevalence of Pfcrt gene with 50.0% (2/4) prevalence compared to pregnant women who were Multigravidae with lower prevalence of Pfcrt

gene having 20.0% (3/15) prevalence. Occurrence of the resistant gene does not associate (P= 0.2722) with parity of the pregnant women.

It could be seen (in Table 6) that there was no significant association (P= 0.1568) between occurrence of Pfcrt gene with Trimester of the pregnant women. Pregnant women within the first and third trimester of pregnancy recorded the highest prevalence of Pfcrt gene with 50.0% (1/2) prevalence compared to those in their second trimester with 20.0% (3/15) prevalence.

Occurrence of Pfcrt genes according to occupation of the pregnant women is shown in table 6. Prevalence of Pfcrt gene was higher among house wives than civil servant with 30.0% (4/13) and 20.0% (1/5) prevalence respectively. There was no significant association (P=1.0000) between occurrence of the Pfcrt gene with occupation of the pregnant women.

Prevalence of Pfcrt gene was recorded only among pregnant women who were married with 27.80% (5/18) prevalence. However, there was no significant association (P=1.0000) between occurrence of the Pfcrt gene with marital status of the pregnant women (Table 6).

In this study, it was observed (in Table 6) that, pregnant women who used Fansida and chloroquine in the treatment of malaria recorded the highest prevalence of Pfcrt gene compared to those who used artemether together with Fansida and chloroquine with 66.70% (2/3) and 25.0% (3/12) prevalence respectively. However, there was no significant association (P=0.5062) between the occurrence of Pfcrt gene with the type of anti-malaria drugs used by the pregnant women.

Table 6: Relationship of Pfcrt gene with socio-demographic characteristic of the pregnant women

Variables	Number Examined	Number Positive	Percentage Positive (%)	p-value
Age				
17-21	2	2	100	0.1568
22-26	10	2	20.0	
27-31	6	1	16.70	
32-36	1	0	0	
Parity				
Primigravidae	4	2	50.0	0.2722
Multigravidae	15	3	20.0	
Trimester				
First	2	1	50.0	0.1568
Second	15	3	20.0	
Third	2	1	50.0	
Occupation				
Unemployed	13	4	30.0	1.0000
Civil servant	5	1	20.0	
Business women	1	0	0	
Marital status				
Married	18	5	27.80	1.0000
Widowed	1	0	0	
Drugs				
Fansida	1	0	0	0.5062
Chloroquine	3	0	0	
Fansida and Chloroquine	3	2	66.70	
Fansida, Chloroquine and Artemether	12	3	35.0	

Total	19	5	26.30

Discussion

Prevalence of Pfmdr1 gene observed in this study (89.50%) was relatively higher than previously reported (45.0%) by Simon-oke *et al.* (2018) in Akure, Ondo State, and 18.90% Pfmdr1 gene prevalence reported in Benin metropolis, Edo State by Okungbowa and Mordi (2013). Similarly, the prevalence of Pfcrt gene observed in this study (26.30%) was higher than earlier reported (24.0%) by Okungbowa and Mordi (2013). High prevalence of these mutant genes in the study area could be as a result of high indiscriminate use of drugs (unprescribed) for treatment of malaria by the pregnant women, due to partial withdrawal of chloroquine in malaria treatment, variation in the environmental conditions or due to presence of the causative agent (*P. falciparum o*f the disease across the country, which may have been due to movement of people from one place to another for recreational activities, employment or education pursuits as reported by Okungbowa and Mordi (2013) and Simon-oke *et al.* (2018).

Lack of association on the occurrence of Pfcrt and Pfmdr1 genes with age of the pregnant women is probably an indication that irrespective of age the pregnant women were at risk of having the resistant genes. This may be attributed to the fact that in all age groups, there is indiscriminate use of chloroquine in the treatment of malaria. However, this study is consistent with previous research conducted in Akure, Ondo State by Simon-oke *et al.* (2018), who reported that there is no association between the occurrence of the two genes (Pfmdr1 and Pfcrt genes) with age of the pregnant women where age group 41-60 years recorded the highest prevalence and attributed it to long time exposure to chloroquine antimalarial drugs.

On the other hand, our findings showed no association in the occurrence of Pfmdr 1 and Pfcrt genes with parity of the pregnant women this indicated that pregnant women were at risk of having the mutant genes (Pfmdr 1 and Pfcrt) regardless of their parity groups. This study is consistent with a previous research conducted among pregnant women in Lagos, Nigeria by Agomo *et al.* (2016) which reported that there is no association between prevalence of Pfmdr1 and Pfcrt genes with gravidity of the pregnant women and attributed it to report that the immune system of semi-immune individuals is able to clear both drug-resistant and sensitive parasites (Bloland, 2001).

In this research, trimester of the pregnant women does not associate with the occurrence of Pfmdr 1 and Pfcrt genes. This is an indication that irrespective of the trimester, the pregnant women were at risk of having the resistant genes. This may be attributed to the fact that there is no level of immunity against having the resistance genes at any level of pregnancy.

It could be seen from this research that there was no association on the occurrence of Pfmdr 1 and Pfcrt genes with occupation of the pregnant women. This is an indication that irrespective of the occupation of the pregnant women, they were at risk of having the resistant genes (Pfmdr 1 and Pfcrt). This may be attributed to the fact that within all the occupation of the pregnant women there was continuous use or partial withdrawal of chloroquine in the treatment of malaria.
Results of this study is consistent with previous research findings among pregnant women in Akure, Ondo State, Nigeria by

Simon-Oke *et al.* (2018) which reported that status of the pregnant women do not associate with the occurrence of Pfcrt genes although, in the finding by Simon-Oke *et al.* (2018) occurrence of Pfmdr1 gene significantly associate with status (staff with 74.10%) and attributed it to the fact that staff are older in age and as such have been exposed to chloroquine treatment.

There was no association between the occurrence of Pfmdr 1 and Pfcrt genes with marital status of the pregnant women this indicated that pregnant women were at risk of having the resistant genes regardless of their marital status. This may attributed to the fact that the resistant genes were widely spread across Nigeria as earlier reported (Simon-oke *et al.,* 2018).

Occurrence of Pfmdr 1 and Pfcrt genes do not associate with history of antimalarial drugs used by the pregnant women which is an indication of widespread of the resistant genes among the pregnant women regardless of the type of antimalarial drugs they used. This may be attributed to the fact that the parasite has developed resistance against the anti-malarial drugs. Continues use or partial withdrawal of the drugs increases the chances of occurrence of these resistance genes. However, Jelagat *et al.* (2014) reported significant associations between polymorphism in Pf*mdr1*-86Y, Pf*mdr1*-184F, or Pf*crt*-76T and quinine susceptibility unlike previous researches conducted and attributed the difference in the observations to the genetic backgrounds of the parasites.

In this study, it was observed that four (4) samples have both the two mutant genes (Pfcrt and Pfmdr1 genes). They therefore have tendency of conferring resistance to chloroquine in addition to mefloquine, halofantrine, and lumefantrine. While the remaining 13 samples (1-5, 7-9, 11, 12, 15, 16 and 17) with only Pfmdr1 gene have tendency of conferring resistance to mefloquine, halofantrine, and lumefantrine as earlier reported (Price *et al.,* 2004, Rohrbach *et al.,* 2006).

CONCLUSION

The study has revealed high prevalence of *P. falciparum* multidrug-resistant gene (Pfmdr1) and *Plasmodium falciparum* chloroquine resistant transporter gene (Pfcrt) among the participating pregnant women where Pfmdr1 gene recorded higher prevalence over Pfcrt gene. The research had provided the first documented evidence of PCR based detection of the mutant genes (Pfmdr1 Pfcrt) in Sokoto State, Nigeria. The study has provides a distribution of the two resistant genes (Pfmdr1 and Pfcrt) within the participants sub-groups (women who are pregnant) even though, occurrence of the resistant genes do not significantly associate with age, parity, trimester, occupation and history of antimalarial drugs of the pregnant women.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abdullahi K, Usman IA, Tukur A, Daneji AI (2009). Malaria in Sokoto, North Western Nigeria. *African Journal of Biotechnology,* **8** (24), 101-105.

Agomo CO, Oyibo WA, Anorlu R, Agomo PU (2009). Prevalence of Malaria in Pregnant Women in Lagos, South-West Nigeria. *Korean Journal Parasitol*ogy. **47**(2): 179–183.

Agomo CO, Oyibo WA, Sutherland C, Hallet, R, Oguike M (2016) Assessment of markers of antimalarial drug resistance in *Plasmodium falciparum* isolates from

pregnant women in Lagos, Nigeria. *PLoS ONE*,**11**(1). https://doi.org/10.1371/journal.pone.0146908

Ataide R, Ashley EA, Powell R, Chan JA, Malloy MJ, O'Flaherty K, (2017). Host immunity to *Plasmodium falciparum* and the assessment of emerging artemisinin resistance in a multinational cohort. Proc Natl Acad Sci U S A. 2017. **114**(13):3515-3520.

Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, (2014). Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *New England Journal of Medicine*. **371**:411–423.

Alfarouk KO, Stock CM, Taylor S, Walsh M, Muddathir AK, Verduzco D. … Rauch C (2015). Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp". *Cancer Cell International*, **15**: 71. doi:10.1186/s12935-015-0221-1. Bloland, P. B. (2001). Drug resistance in malaria. WHO/CDS/CSR/DRS/2001.4. World Health Organization. Geneva, 2001. http://www.who.int/csr/resources/publications/drugresist/malaria.pdf. (pp 9-15).

Buhari HA, Erhabor O, Momodu I (2016). *Plasmodium falciparum* malaria among pregnant women attending Ante-Natal Clinic in Sokoto, north western, Nigeria. *Sokoto Journal of Medical Laboratory Science*. **1**(1), 187–193.

CDC (2018). Intermittent preventive treatment of malaria for pregnant women (IPTp). Centre for Disease Control and Prevention 2018 malaria report. https://www.cdc.gov/malaria/malaria_worldwide/reduction/iptp.html.

CDC (2020). DPDx – Laboratory Identification of Parasites of Public Health Concern-Blood Specimens - Specimen Processing. Centre for Diseases Control and Prevention. https://www.cdc.gov/dpdx/diagnosticprocedures/blood/specimenproc.html.

C-GIDD (2008). Canback Global Income Distribution Database Location of Sokoto State, Nigeria 1-2pp.

Faruku N, Magaji UF, Bunza NM, Bello M (2016). *Parity and malaria parasitaemia among pregnant women attending ANC,* UDUTH *Sokoto.International Journal of Medical and Health Research.* **2**(9), 61-64.

Gresty O, Karryn J (2014). Genetic mutations in Pfcrt and Pfmdr1 at the time of artemisinin combination therapy introduction in South Pacific Islands of Vanuatu and Solomon Islands. *Malaria Journal.* **13** (1), 406.

Hanboonkunupakarn B, White NJ (2016). The threat of artemisinin resistant malaria in Southeast Asia. *Travel Med Infect Dis*. **14**(6), 548-550. https://doi.org/10.1016/j.tmaid.2016.11.016.

Himansh G, Eusebio M, Helder B, Crizolgo S, Marian W, Eva C,… Alfredo M. (2015).Drug-resistant polymorphisms and copy numbers in *Plasmodium falciparum*, Mozambique. *EID Journal,* **24**(1), 22-29.

Jelagat C, Luicer A, Ingasia A, Omondi A, Dennis W, Juma B, Joan M (2014). Polymorphisms in Pf*mdr1*, Pfcrt, and Pfnhe1 genes associated with reduced *In Vitro* activities of quinine in *Plasmodium falciparum* isolates from western Kenya. *Antimicrobial Agents and Chemotherapy Jorunal.* **58**(7) 3737-3743; DOI**:** 10.1128/AAC.02472-14.

Kamau E, Campino S, Amenga-Etego L, Drury E, Ishengoma D, Johnson K (2015). K13-propeller polymorphisms in

Plasmodium falciparum parasites from sub-Saharan *African Journal of Infectious Disease*, **211**(8):1352-1355 doi: 10.1093/infdis/jiu608.

Kiaco K, Teixeira J, Machado M, Rosário V, Lopes D, Guthmann J (2015). Evaluation of artemether–lumefantrine efficacy in the treatment of uncomplicated malaria and its association with pfmdr1, pfatpase6 and K13-propeller polymorphisms in Luanda, Angola. *Malaria Journal*. **14**: 143.

Lema S Y, Suleiman J, Rabiatu MS, Aisha U & Yakubu MS. (2019). Prevalence of *Trichomonas vaginalis* among pregnant women attending antenatal care (ANC), Maryam Abacha women and children hospital, Sokoto, Nigeria. *European Journal of Pharmaceutical and Medical Research.* **6**(3), 52-56.

Lwanga SK, Lemeshow S, WHO (1991). Sample size determination in health studies: a practical manual / Lwanga, S. K. and Lemeshow, S. World Health Organization. https://apps.who.int/iris/handle/10665/40062.

Maina AT, Auta IK, Nock IH (2017). Prevalence of *Plasmodium falciparum* in participants at selected hospitals, Kaduna Metropolis, Nigeria. *Nigerian Journal of Parasitology*, **38**(2).

Nsanzabana C, Djalle D, Guérin PJ, Ménard D, González IJ (2018). Tools for surveillance of anti-malarial drug resistance: an assessment of the current landscape. *Malaria Journal*, **17** (pp 75).

NPC (2007). Report of Nigeria's National Population Commission on the 2006 Census. Population and Development Review, **33**(1), 1-210.

Okungbowa MAO, Mordi RM (2013). Prevalence and distribution of malaria, Pfcrt and Pfmdr1 genes in Benin metropolis, Edo State, Nigeria. *Nigerian Journal of Parasitology*, **34**(2), 47-54.

Olasehinde GI, Ojurongbe OO, Fagade EO, Ruchi S, EgwariL O, Ajayi AA, Adeyeba OA. Detection of Molecular Markers of Antimalarial Drug Resistance in *Plasmodium* Falciparum from South-Western Nigeria. *Covenant Journal of Physical and Life Sciences (CJPL)*. **1**: 2.

Price RN, Uhlemann AC, Brockman A (2004). Mefloquine resistance in *Plasmodium falciparum* and increased pfmdr1 gene copy number, *The Lancet*, **364**(9432), 438–447.

Reteng P, Visia V, Inka S, Ilham HD, Jane AK, George EJ (2017). Genetic polymorphisms in *Plasmodium falciparum* chloroquine resistance genes, Pfcrt and Pfmdr1, in North Sulawesi, Indonesia**.** BMC Research Notes, **10** (147). https://doi.org/10.1186/s13104-017-2468-1

Rohrbach P, Sanchez CP, Hayton K (2006). Genetic linkage of pfmdr1 with food vacuolar solute import in *Plasmodiumfalciparum*, *The EMBO Journal*, **25**(13), 3000–3011.

Sharma D, Manila L, Prashant K, Mallick T, Adak AS, NeenaValecha D (2015). Polymorphism in drug resistance genes dihydrofolate reductase and dihydropteroate synthase in *Plasmodium falciparum* in some states of India. *Parasite vector*, **8**:471

Simon-Oke IA, Obimakinde ET, Afolabi OJ (2018). Prevalence and distribution of malaria, Pfcrt and Pfmdr 1 genes in patients attending FUT Health Centre, Akure,

Nigeria. *Beni-Suef University Journal of Basic and Applied Sciences*, **7** (pp 98–103).

Slater HC, Griffin JT, Ghani AC, Okell LC (2016). Assessing the potential impact of artemisinin and partner drug resistance in sub-Saharan Africa. *Malaria Journal.* doi: 10.1186/s12936-015-1075-7.

Sondo P, Derra K, Diallo NS, Tarnagda Z, Kazienga A, Zampa O (2016). Artesunate-Amodiaquine and Artemether-Lumefantrine therapies and selection of *PFCRT* and *PFMDR1* Alleles in Nanoro, Burkina Faso. *PLoS ONE***11**(3). https://doi.org/10.1371/journal.pone.0151565.

Takala-Harrison S, Jacob CG, Arze C, Cummings MP, Silva JC, Dondorp AM, Bethell D (2014). Independent emergence of artemisinin resistance mutations among *Plasmodium falciparum* in Southeast Asia. *Journal of Infectious Disease,***211**(5),670-679. doi:10.1093/infdis/jiu491.

Tobias OA, Amed O, Vincent PK, Titanji AD, Alfred A (2019). Genetic diversity and drug resistance surveillance of *Plasmodium falciparum* for malaria elimination. *Malaria Journal.* **18**(p 217).

Umar RA, Jiya NM, Hassan SW, Abdullahi K, Ahmed JM, Nata`ala U (2007). Apparent drug failure following artesunate treatment of *Plasmodium falciparum* malaria in Sokoto, Nigeria: Three Case Reports. *Trends in Medical Research*, **2**(pp 113-116).

Vathsala PG, Pramanik A, Dhanasekaran S, Devi CU, Pillai CR, Sub-barao SK (2004). Widespread occurrence of the *Plasmodium falciparum* chloroquine resistance transporter (Pfcrt) gene haplotype SVMNT in *P. falciparum* malaria in India. *American Journal of Tropical Medicine and Hygeine*. **70**(pp 256-259).

UDUS (2021). Department of Geography, Geographic Information System Laboratory, Usmanu Danfodiyo University Sokoto, Nigeria.

WHO (2015). Global Malaria Program. The role of mass drug administration, mass screening and treatment, and focal screening and treatment for malaria: recommendations. Geneva: World Health Organization.

WHO (2018). World malaria report 2018. WHO. Retrieved 2 December 2018. https://www.who.int/malaria/publications/world-malaria-report-2018/en/.

WHO (2019). World Malaria Report 2019. Switzerland: World Health Organization. pp. xii–xiii, 4–10. ISBN 978-92-4-156572-1.