Plasmodium falciparum Multidrug Resistant Gene (*Pfmdr-1*) Prevalence among Patients Attending Selected Hospitals in Kaduna North and South Local Government Areas, Kaduna State, Nigeria

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Abstract: Malaria is one of the most common causes of morbidity and mortality, *Plasmodium falciparum* is the most pathogenic species responsible for most malaria cases in Nigeria. Increased prevalence occurs due to evolution of new genotypes resulting in resistance to most common antimalarial drugs used. In malaria endemic regions, drug resistance is a serious public health issue and a major constraint to malaria eradication programmes. This research was aimed at determining the prevalence of molecular marker of *Plasmodium falciparum* multidrug resistant gene (*pfmdr-1*) among patients attending selected public hospitals within Kaduna North and Kaduna South Local Government Areas of Kaduna State Nigeria. A total of 280 blood samples were collected and analysed using standard methods. *Plasmodium falciparum* detection was carried out using rapid diagnostic test and microscopic examination of thick and thin blood films. The *Pfmdr-1* gene was detected using Polymerase Chains Reaction (PCR). Results shows that out of 280 samples screened, 97 (34.6%) were positive for *P. falciparum* and Kaduna South had high prevalence (35.7%) compared to Kaduna North (33.6%). Overall prevalence of 34.3% of *pfmdr-1* gene was recorded, Kaduna North had high prevalence (40.0%) compared to Kaduna South (26.7%). Higher prevalence of *pfmdr-1* was recorded among patients age > 23 years (37.5%). Female had higher prevalence (41.7%) than male (18.2%). There was significant difference ($p>0.05$) in the distribution of *pfmdr-1* based on location, age and gender. This study revealed gradual spread of *pfmdr-1* in the study area, and continuous spread would lead to reduced efficacy of antimalarial drugs. Constant monitoring of *pfmdr-1* would influence and direct drug policies against malaria.

Key word: Malaria, *Plasmodium falciparum, Pfmdr-1* gene, prevalence, drug resistance

INTRODUCTION

Malaria caused by protozoan parasite of the genus *Plasmodium* is transmitted to man through the bites of female Anopheles mosquitoes. *Plasmodium falciparum*, the deadliest species, predominantly occurs in Africa and is known to be responsible for 90% of all world malaria deaths and frequent antimalarial treatment resistance (WHO 2017; Mohammed *et al*., 2019; Huang *et al*., 2021). A significant characteristic of human malaria parasites is their genetic diversity, resulting from allelic polymorphisms, recombination, chromosome rearrangements and antigenic variation (Oyedeji *et al.,* 2013). There are currently five known different *Plasmodium* species that cause humans malaria namely: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*. *Pasmodium falciparum*, which causes more than 90% of all malaria deaths worldwide, is the most critical public health problem (Baird, 2013; Snow, 2015). Malaria symptoms and indicators include the classic paroxysm, a cyclical recurrence of sudden coldness and rigour, followed by fever and sweating, impaired consciousness, significant weakness, lack of appetite, convulsions, low blood pressure, kidney failure, or haemoglobin in the urine. The *P. falciparum* infection can result in either a milder, essentially constant fever or a recurring fever that occurs every 36–48 hours (quartan fever) (Murray *et al*., 2012).

Global malaria cases were predicted to have reached 219 million in 2017, up from 239 million in 2010 and 217 million in 2016 (Angus *et al*., 1999). An estimated 300 million cases of malaria occur worldwide each year, with one million deaths from the disease (Bundy, 2018). Malaria is a major global hazard and a major cause of health and economic disparities in endemic nations, despite numerous and varied attempts to manage the disease (Arbonnier, 2002;

Bundy, 2018). The ten African nations with the highest malaria burden reported a rise in malaria cases in 2017 over 2016 (Bundy, 2018).

Malaria continues to be a major cause of morbidity and mortality worldwide, with significant costs to public health and the majority of the burden occurring in sub-Saharan Africa, despite significant control efforts in many countries (Ashley and Phyo, 2018). Over 95% of cases and 96% of deaths from malaria occur in the WHO Africa Region, mostly as a result of *P. falciparum* infections (WHO, 2022). An estimated 247 million episodes of malaria and 619 000 fatalities from malaria were reported in 2021. Malaria continues to disproportionately affect the WHO African Region; in 2021, with 96% of all malaria cases and deaths worldwide occurred in Africa. 26.6% of malaria cases are in Nigeria; 12.3% are in the Democratic Republic of the Congo; 5.1% are in Uganda; 4.1% are in Mozambique; 3.4% are in Angola; and 3.3% are in Burkina Faso (WHO, 2022).

One crucial strategy for treating malaria patients is the use of effective medications. In the WHO African Region, artemether-lumefantrine (AL), artesunate-amodiaquine (AS-AQ), artesunate-pyronaridine (AS-PY), and dihydroartemisinin-piperaquine (DHA-PPQ) are the first-line therapy for *P. falciparum* (WHO, 2021a).

The complicated biology of the *Plasmodium* parasites, high polymorphism, and rising antimalarial drug resistance, mostly in endemic locations, are some of the factors that have impeded the efficient control of this illness (Lopez *et al*., 2010).

The widespread resistance to antimalarial medications makes it more difficult to eradicate malaria as a significant illness (Xu *et al*., 2019). Because *P. falciparum* develops treatment resistance quickly, antimalarial drug resistance poses a serious obstacle to malaria chemotherapy in Sub-Saharan Africa (Blasco *et al*., 2017). Additionally, there is serious worry about lowering the global malaria burden due to the growing threat of drug resistance to antimalarial medication treatments such artemisinin-based combination therapies (ACTs) (Recht *et al*., 2017; Kunkel *et al*. 2021).

After receiving antimalarial medication for an extended period of time, parasites acquire drug resistance. There is a growing number of locations worldwide where resistance to most available antimalarial medications has been observed and this resistance poses a threat to future advancements in the control of malaria (WHO, 2017). The evolution of parasite strains with molecular processes that undermine or evade the effectiveness of drugs is facilitated by this pharmacological pressure. All of the major advancements in the management of malaria over the years have reportedly been threatened by resistance to artemisinin derivatives and the consequent failure of the ACT, improved coping mechanisms for multidrug resistance, particularly in *P. falciparum* malaria, are proposed. These strategies ought to encompass timely and sufficient handling of clinical cases; recognition and eradication of high transmission foci and asymptomatic reservoirs; sufficient surveillance of resistance; implementation of various antimalarial medication regimens; and customized vector controls. Although, the exact mechanism of action of artemisinin is still unknown, a number of studies have suggested that it involves several cellular targets and the generation of reactive oxygen species (ROS) (Pimpat *et al.*, 2020).

In order to assess antimalarial drug resistance both *in vivo* and *in vitro*, parasite susceptibility testing or molecular techniques such as PCR can be used to identify genetic markers of resistance to lumefantrine, chloroquine, and artemisinin. These markers are *P. falciparum* multidrug resistance protein-1 (Pfmdr-1), *P. falciparum* multidrug Kelch 13 (Pfk13), and *P. falciparum* chloroquine resistance transporter (Pfcrt), *P. falciparum* multidrug resistance transporter (Pfcrt), and *P. falciparum* multidrug Kelch 13 (Pfk13), respectively. Different Rapid Diagnostic

Tests (RDTs) or Antigen Tests can be used to identify different parasite antigens. Some antigens are produced by a specific malaria parasite, such as Histidine Rich Protein-2 (HRP2). Nonetheless, parasite lactate dehydrogenase (PLDH) or aldolase is produced by every malarial species, including *P. vivax, P. ovale*, and *P. knowlesi* (Olasehinde *et al*., 2019). Drug resistance to mefloquine, quinine, halofantrine, and chloroquine is linked to *P. falciparum* multidrug resistance (Pfmdr-1), which is perpetuated by a codon N86Y chromosomal mutation (Olasehinde *et al*., 2019). The P-glycoproteins homolog 1 (Pgh-1) is another name for the pfmdr-1 gene (Hodoameda, 2021). The parasite's reaction to specific antimalarial medications is influenced by the pfmdr-1 protein. The two ways that the pfmdr-1 gene controls the responsiveness of antimalarial drugs are either mutations in the gene or an increase in the copy number of the gene (Wilson *et al*., 1993; Sidhu *et al*., 2006). In an experiment where one of the two copies of drug-resistant FCB strains was knocked out, the resistance reverted and the strain became susceptible to mefloquine, lumefantrine, halofantrine, quinine, and artemisinin (Hodoameda, 2020). This experiment illustrated the role of increased pfmdr-1 copy number in mefloquine, lumefantrine, halofantrine, quinine, and artemisinin resistance.

Antimalarial drug resistance develops as a result of drug selection of spontaneous *P. falciparum* mutations that give drug tolerance (Adamu *et al*., 2020). Non-synonymous Kelch-13 (k13) mutations have been found in several African countries, despite the fact that ACTs are still effective in Nigeria and the majority of other African countries (Ariey *et al*., 2014; Chilongola *et al*., 2015; Demas *et al*., 2018). Single nucleotide polymorphism (SNP) analysis in treatment resistance-related genes is often carried out to track the occurrence of antimalarial drug resistance in parasite populations. Single nucleotide polymorphisms (SNPs) in the *P. falciparum* multidrug resistance gene (pfmdr-1) influence the parasite's susceptibility to the long-acting partner medication in the ACT (Veiga *et al*., 2016).

Plasmodium falciparum is the most virulent strain of the parasite, according to Cheesbrough (2006), and it causes the bulk of malaria-related illness and mortality, which has a substantial social and economic impact in developing nations. According to Kunkel *et al*. (2021), there is significant concern regarding the reduction of malaria cases worldwide due to the advent of artemisinin-resistant strains of *P. falciparum*. According to studies, the countries with the largest estimated increases—Malaysia, the Democratic Republic of the Congo, and Nigeria—all had more than 500,000 cases (Eluu *et al*., 2019; Bakhubaira, 2013). According to Bulus *et al*. (2011), Nigeria is the nation with the highest number of cases and fatalities worldwide. Nonetheless, there is a dearth of data regarding the prevalence of the *P. falciparum* multidrug resistance gene (pfmdr-1), particularly in endemic regions like Kaduna State. Such data would not only provide a baseline for the surveillance of multidrug-resistant malaria in the State, but also aid in directing and enhancing the country's current drug policy, thereby halting the spread of the pfmdr-1 gene. Therefore, it is necessary to assess the multidrug resistance gene (pfmdr-1) for *Plasmodium falciparum* in patients who visit particular hospitals in Kaduna North and South Local Government Areas of Kaduna, Nigeria.

MATERIALS AND METHODS

Study population: All age groups who were outpatients at the public hospitals in the two Local Government Areas (Chikun and Kaduna North), Kaduna state, Nigeria that were chosen for the study were included in the study population. General Hospital Kawo (GH-K), Teaching Hospital Barau Dikko (TH-BD), 44 Army Reference Hospital (44-ARH), and Yusuf Dantsoho Memorial Hospital (YDMH) are among the hospitals that were chosen at random based

on volume of patients and proximity to the metropolis.

Inclusion and exclusion criteria: Outpatients who exhibited clinical symptoms of malaria and were referred for a test were included in the study; however, outpatients who did not exhibit any clinical symptoms of malaria and were not referred for a test were excluded. The 44 Nigerian Army Reference Hospital Kaduna Ethical Committee and the ethics board of the Kaduna State Ministry of Health gave its approval prior to the commencement of the study.

Determination of sample size: The sample size determination formula, as presented by Araoye (2004), was used to determine the sample size.

$$N = \frac{Z^2 pq}{d^2}$$

Where N = calculated sample size

Z = standard normal deviation at 95% confidence interval (1.96)

p = prevalence (22.4% from Aliyu *et al.,* 2017)

q = 1 – p

d = precision (allowable error) = 5% (0.05)

$$N = \frac{1.96^2 \text{ x } 0.224(1 - 0.224)}{(0.05)^2}$$

N = 267.1

N ≈ 267

However, for convenience in terms of uniformity, a total of 280 samples were collected for the research.

Sample collection: Blood samples were obtained from Yusuf Dantsoho Memorial Hospital (YDMH), 44 Army Reference Hospital (44-ARH), Teaching Hospital Barau Dikko (TH-BD), and General Hospital Kawo (GH-K). A well-structured questionnaire was used to gather pertinent biodata from the respondents. Each patient's venous blood sample was taken in five milliliters (5 ml) and placed into an Ethylene Diamine-Tetra-acetic Acid (EDTA) vacutainer that was labeled with the ID number from the questionnaire. The patients were positioned so that, when the appropriate vein for venepuncture was selected, their arm formed a straight line from their shoulder to their wrist. A tourniquet was placed three to four inches above the collecting location. The puncture site was cleaned by making a smooth circular pass over the collection site, while a 70% alcohol pad was moved outward in a spiral motion from the penetration zone. The syringe's plastic cover was removed, and a sheathed needle or butterfly was placed on it, while the bevel was turned up. To simultaneously puncture the skin and enter the vein, a rapid push was given, while the needle was held parallel to the vein. The tourniquet was relaxed once the required amount of blood was extracted by gradually pushing back on the syringe stopper. A gauze pad was placed over the puncture site, the needle was quickly taken out, and pressure was administered. The patient was told to keep up the pressure for a minimum of two minutes. After the bleeding had stopped, the collection location was covered with a fresh bandage. The blood was extracted and as soon as possible, using a blood transfer device, it was placed into the proper tube to prevent improper coagulation. About 5-8 gentle inversions were performed on the tubes that contained the additive (EDTA). Using a suitable sharp object container, the syringe and needle were disposed of together (https://pathlabs.ufl.edu/client-services/specimen-shipping/blood-collection-process-venipuncture).

Blood analysis: Rapid Diagnostic Test (HRP-II) described by Tankeshwar (2017) was used for *Plasmodium falciparum* detection.

Rapid diagnostic test (HRP-II) for Plasmodium falciparum detection: *Plasmodium falciparum* antigens were found using a quick diagnostic test. Venous blood samples from patients were used to perform the *P. falciparum* test. With each sample that was obtained, a fresh, unopened test packet containing RDTs unique to *P. falciparum* was employed. After opening each test package, the contents were taken out. The test was administered using the test cassette. Every cassette had the patient ID number

inscribed. Blood samples were transferred from the vacutainer to the hole labeled "A" using a capillary tube, which was then appropriately disposed of. After adding roughly five drops of buffer to the cassette at location "B," it was examined for 15 to 20 minutes, after which the results were noted (Tankeshwar, 2017).

Determination of parasite density (PD): By comparing the number of parasites to a standard number of leukocytes/µl (8000), the parasite/µl of blood could be determined. Two hand tally counters were used to count the leukocytes and parasites. The count was expressed as the number of parasites/200 leukocytes when 200 leukocytes were counted and 10 or more parasites were discovered. Nevertheless, if the number of parasites is nine or less, the counting process is repeated until 500 leukocytes are attained, at which point the count is reported as parasite/500 leukocytes. Following the counting, the quantity of parasites/µl of blood was calculated using a straightforward mathematical procedure (WHO, 2017). Samples with high parasites density were used for molecular analysis.

PD/µl of blood = (Parasite Counted/Number of Leukocytes) x 8000.

Molecular characterization of Plasmodium falciparum multidrug resistant gene (pfmdr-1): Whole blood samples were treated with the AccuPrep Genomic DNA Extraction Kit (Bioneer Inc. Korea) to extract DNA. Twenty microlitre (20 µl) of proteinase K was added to a clean 1.5 ml tube. Following the addition of 200 µl of blood to a tube containing proteinase K, 200 µl of binding buffer was added and the mixture was well homogenized using a vortex mixer. The tube was incubated for ten minutes at 60°C. After mixing the liquid with a pipette, 100 µl of isopropanol was added. The tube was briefly spun down to get the droplets hanging under the cover. Using a 2 ml tube, the lysate was added to the binding column tube's upper reservoir. Following the sealing of the tube, it was centrifuged for one minute at 8,000 rpm. After opening the tube and moving the binding column tube into a fresh 2 ml tube for filtering, 500 µl of washing buffer 1 (W1) was added. The tube was then centrifuged for 1 minute at 8,000 rpm, and the contents of the 2 ml tube were disposed of in a disposal bottle. After carefully adding 500 µl of washing buffer 2 (W2) to the binding column tube, the tube was centrifuged for one minute at 8,000 and 12,000 rpm. The binding column tube was then eluted using a brand-new 1.5 ml tube. This was achieved by filling the binding column tube with 200 µl of elution buffer (EL) and allowing it to sit at room temperature for at least one minute to allow the EL to fully saturate the glass fiber. Next, this was centrifuged at 8,000 rpm for 1 minute in order to elute. Following elution, the DNA was utilized for molecular applications (Ekpa *et al*., 2016).

Amplification of pfmdr-1 gene: For polymerase chain reaction amplification, a 20 *µl* total volume of Accupower Hotstart PCR premix (Bioneer Inc. Korea) was utilized. Using molecular-grade water, the primers were reconstituted and diluted to 10 nM. To a 13 µL premix that also included molecular grade H_20, dNTPs, $MgCl_2$, Taq polymerase, 10× TaqA buffer, and 1 µL of forward and reverse primers, 5 µl of gDNA was added (Table 1). First denaturation for two minutes at 95°C, second denaturation for forty seconds at 94°C, one minute of annealing at 55°C, and one minute of extension at 72°C. The response condition consisted of a final extension at 72°C for 4 minutes after 45 cycles of this, each tube's DNA was extracted and subjected to agarose gel electrophoresis using five microliters (5 µl) of DNA (Kim *et al*., 2013).

Gel electrophoresis analysis: In a conical flask, 1.5 grams of agarose powder were mixed in 100 milliliters of buffer TAE (40 milliliters of Tris-acetate and 1 milliliter of EDTA). The mixture was then heated in a microwave to melt. The flask was removed and its contents thoroughly combined. With the complete desolution of the agarose gel, the process was repeated with 5 *µl* of ethidium bromide (EtBr) and poured into the

gel casting tray, fastened with a gel comb. To avoid warping, the gel was left to cool at room temperature on a table top. Tris-acetate EDTA buffer was added after the gel was placed in the gel electrophoresis chamber without the comb. The electrophoresis process involved loading the PCR products and 1000 bp DNA size markers onto an immersed gel, then setting the power supply to oscillate between 65 and 70 volts for 45 minutes. The gel was taken out and loaded into Bio-rad Gel DocTM XR+ at the end of the process of electrophoresis. Photographs of the gel and the predicted amplicon of the gel plate were taken and recorded (Bagriantsev *et al*., 2006).

Data analysis: Data generated from the study were subjected to statistical analysis using Statistical Package for Social Sciences version 23 (SPSSv23). Results were presented using tables, charts, and percentages. Pearson Chi-square was used to test for association between infection, age, gender and location of patients. The mean difference between the groups was calculated using a paired T-test, with $p<0.05$ (5%) considered significant.

RESULTS

A total of 280 samples were screened using Histidine Rich Protein II (HPR-II) *P. falciparum* specific RDT test strips and malaria microscopy to determine the presence of *P. falciparum*. Out of 140 samples screened from Kaduna North, only 47(33.6%) were found to be positive, while 50(35.7%) were positive from Kaduna South samples (Table 2). There was no significant difference ($p<0.05$) in the prevalence of *P. falciparum* between the two Local Government Areas.

A total of 35(36.1%) out of 97 samples that tested positive with *P. falciparum*, were selected randomly for molecular analysis to ascertain the prevalence of *pfmdr-1* in Kaduna North and Kaduna South Local Government Areas (Table 3). The molecular analysis, revealed that 12(34.3%) were positive for *pfmdr-1.* Out of 20 samples examined from Kaduna North, 8(40%) were found *pfmdr-1* positive, while only 4(26.7%) showed positive out of 15 samples screened from Kaduna South (Plates 1, 2, 3 and 4). No significant difference ($p<0.05$) in the distribution of *pfmdr-1* among patients attending the selected hospitals in Kaduna North and Kaduna South LGA was observed. Also, the distribution of *pfmdr-1* is independent of the location of the patients.

Table 1: Primer sequences for *pfmdr-1* gene and PCR reaction conditions

Gene/Primer	Primer sequence
F - MDR1	GCATTTAGTTCAGATGATGAAA
R - MDR1	CCATATGGTCCAACATTTGTATC
PCR Condition	Initial denaturation at 95^0C for 2 mins, denaturation at 94^0C for 40 secs, annealing at 55^0C for 1 min, extension at 72^0C for 1 min for 45 cycles and final extension at 72^0C for 4 mins (Idowu *et al.,* 2018).

Key: F - MDR1 = Forward primer; R - MDR1 = Reverse primer

Table 2: Prevalence of *P. falciparum* in Kaduna North and Kaduna South LGA

Local Government Area	Number examined	Number infected with *P. falciparum*	Percentage infected with *P. falciparum* (%)
Kaduna North	140	47	33.6
Kaduna South	140	50	35.7
Total	280	97	34.6

Table 3: Prevalence of *pfmdr-1* in Kaduna North and Kaduna South LGA

Local Government Area	No examined	No infected with *pfmdr-1*	% infected with *pfmdr-1*
Kaduna North	20	8	40.0
Kaduna South	15	4	26.7
Total	35	12	34.3

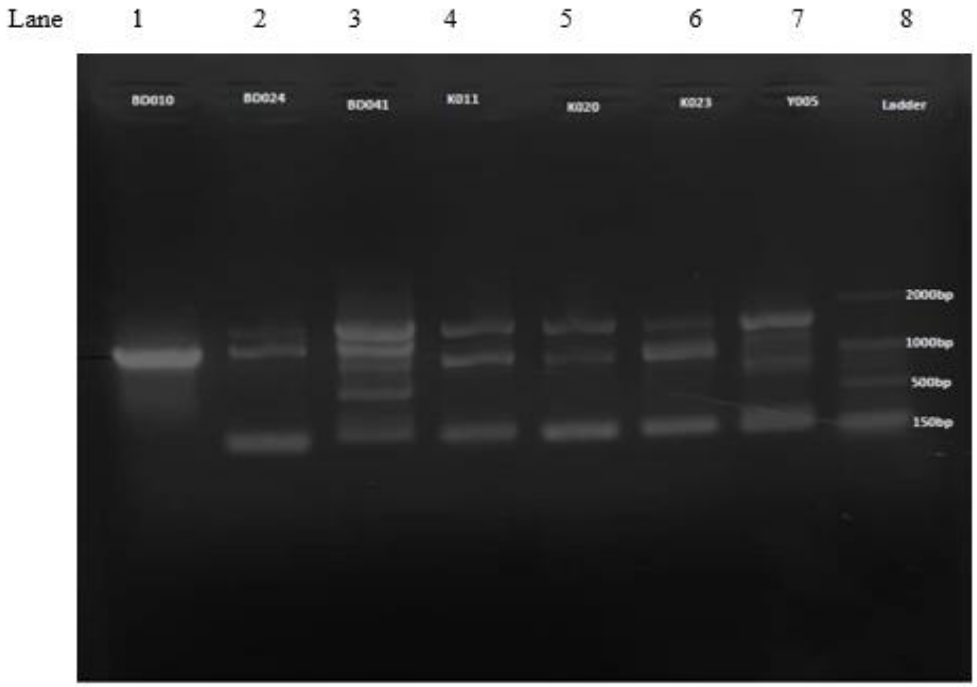

Plate 1: Electropherogram of amplified *pfmdr-1* gene. lanes 1-7, amplified *pfmdr-1* gene (1016 bp), lane 8, molecular ladder (2000 bp)

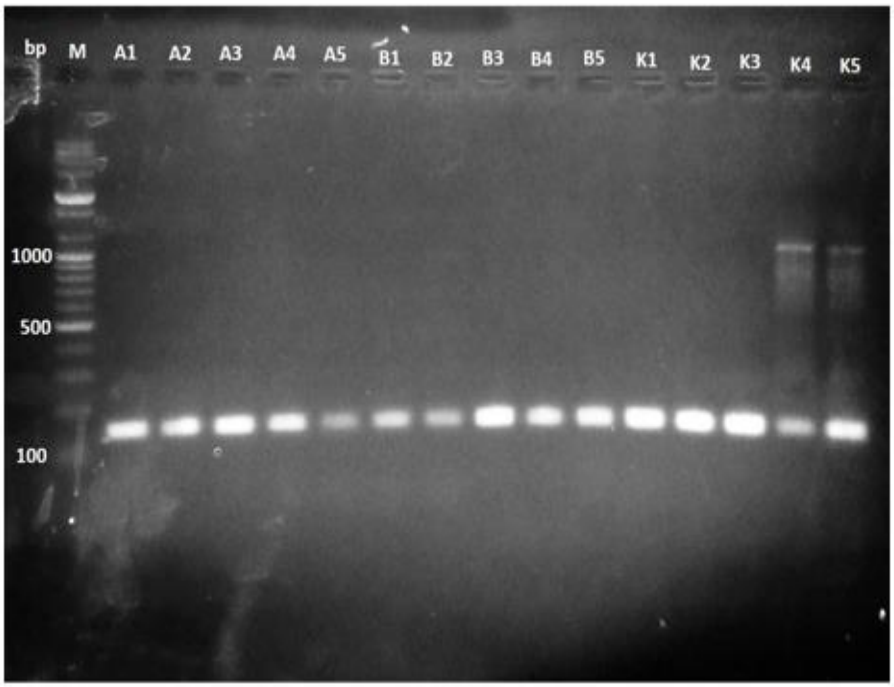

Plate 2: Electropherogram of amplified *pfmdr-1* gene. lane 1, molecular ladder (2000 bp) lanes 15-16, amplified *pfmdr-1* gene (1016 bp)

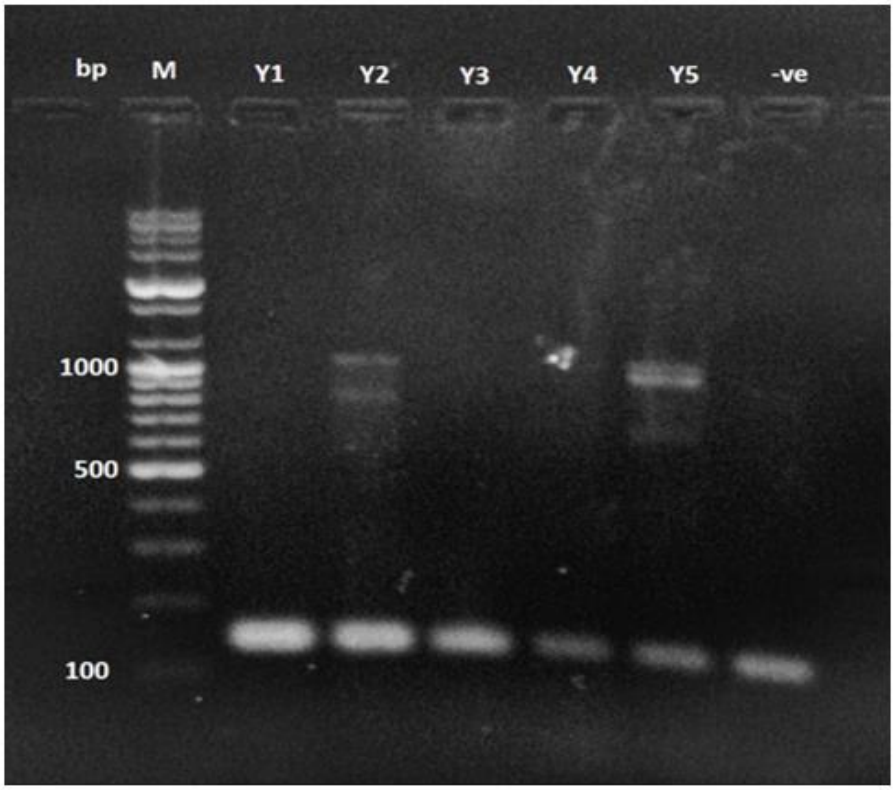

Plate 3: Electropherogram of amplified *pfmdr-1* gene. lane 1, molecular ladder (2000 bp), lanes 3 and 6, amplified *pfmdr-1* gene (1016 bp), lane 7, negative control

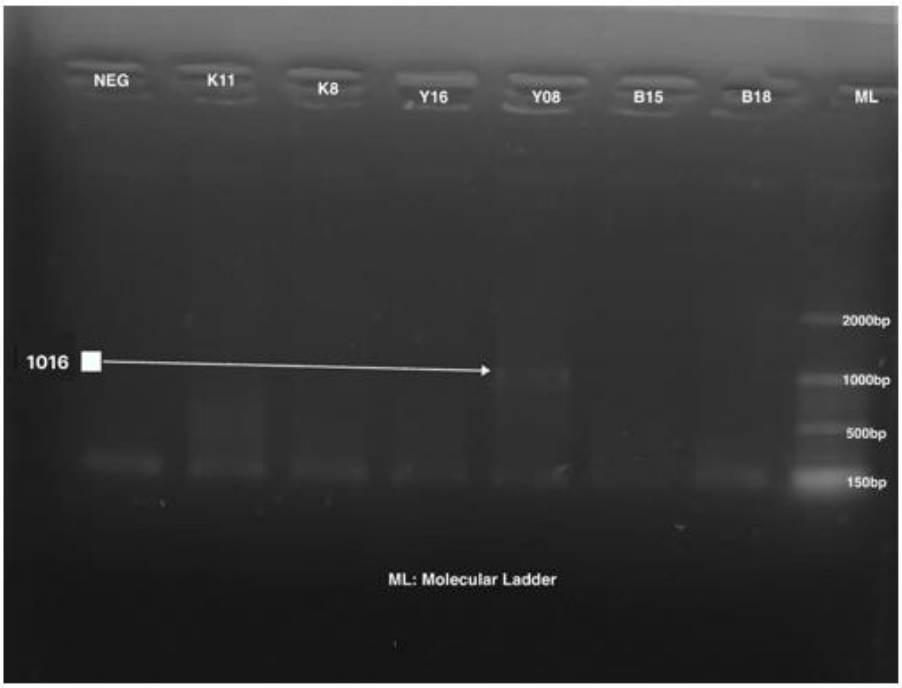

Plate 4: Electropherogram of amplified *pfmdr-1* gene. lane 1, negative control, lane 5, amplified *pfmdr-1* gene (1016 bp), lane 8, molecular ladder (2000 bp)

DISCUSSION

Despite numerous efforts and interventional programs implemented to control malaria, *Plasmodium falciparum*—the parasite that causes malaria—remains a major public health concern in Nigeria. Transmission of malaria occurs year-round, despite the fact that it is preventable and treatable (Kebede *et al*., 2014; Dikwa *et al*., 2020).

The Pfmdr-1 gene, which gives *Plasmodium falciparum* multidrug resistance, and its presence and distribution were evaluated in this work. The results of the study showed that *P. falciparum* is still common in the studied area. This could be because treated bed nets are not always used, the parasite is more common during certain seasons, or chemo-preventive methods are not used correctly. However, a higher percentage of participants (34.6%) from the study area claimed to sleep under treated, long-lasting insecticidal nets. Similarly, the prevalence reported by Ruqayyah *et al*. (2017) was greater than the findings of this investigation at 49%. Despite the fact that this study's malaria incidence is lower than that of Ruqayyah *et al*. (2017), 34.6% nevertheless indicates a noteworthy rate of malaria infection in the research area. Aliyu *et al*. (2017) reported a slightly different finding of a 22.4% incidence of malaria. Given that malaria accounts for more than 26.6% of cases worldwide, it is a major health concern in Sub-Saharan African nations, particularly Nigeria (WHO, 2022).

High levels of pfmdr-1 were found in Kaduna North and South Local Government Areas. This could be attributed to the movement of people with the parasite and resistance genes for work, play, education, or natural disasters, which could have contributed to the national spread of pfmdr-1 (Okungbowa and Mordi, 2013; Simon-Oke *et al*., 2018). Although, the primary reasons for the high prevalence of pfmdr-1 in Kaduna North are unknown, it is possible that environmental variables, selection pressure on certain ACT partner drugs, or inefficient antimalarial drug administration are implicated (Kayode *et al*., 2021). Benjamin *et al*. (2021) found that three hospitals within three Senatorial Zones in Kaduna State had a 36% incidence of pfmdr-1. A number of study sites in Nigeria have detected the pfmdr-1 mutant gene (Simon-Oke *et al*., 2018; Olasehinde *et al*., 2019; Adamu *et al*., 2020). This is noteworthy because the pfmdr-1 gene has been linked to decreased susceptibility to antimalarial chemotherapies like lumefantrine, halofantrine, mefloquine, dihydroartemisinin, and artesunate. This is because, the gene can introduce a mutation in the gene and/or increase its copy number (Nguetse *et al*., 2017; Hodoameda, 2021).

The findings in this study is of great concern because patient between the ages of 0 and 5 was discovered to have pfmdr-1 infection. This suggests that pfmdr-1 is extensively distributed across different age groups in the study area, which may put children younger than 5 years old at risk of developing a mutant pfmdr-1 infection caused by *P. falciparum*, which could lead to an inability to treat malaria with different antimalarial medications.

However, with 41.7% of the female samples testing positive, the pfmdr-1 gene was more common in female patients than in male patients. This could be because there were more female participants in this study than male participants. This is because women are usually the ones responsible for taking care of people in the house and may also be pregnant; as a result, it is possible that women may finally end up getting tested for malaria by coincidence (Okiring *et al*., 2022). Simon-Oke *et al*. (2018) found that more males than women were included in their analysis, men had a higher prevalence of pfmdr-1. Nevertheless, there is not enough information to draw the conclusion that gender affects the higher frequency of pfmdr-1 (Ruqayyah *et al*., 2017).

CONCLUSION

The prevalence of *pfmdr-1* gene recorded in the study area was 34.3% with Kaduna North Local Government Area having higher value (40.0%) compared to Kaduna

South Local Government Area (26.7%). Field isolates from malaria-endemic areas need to have their parasite susceptibility to artemisinin and its derivatives constantly monitored, in order to prevent the spread of the malaria parasite. There is need for constant monitoring of *pfmdr-1* to influence and direct drug policies and administration against malaria. This is because continuous spread could lead to reduced efficacy of antimalarial drugs.

Acknowledgement

The authors greatly acknowledge Muhammed Muazu, all staffs of the Department of Biological Sciences of Nigerian Defence Academy, Nigeria and Precision Biomedicals Limited, Kaduna State, Nigeria.

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