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Research Article

Distribution of *Plasmodium falciparum* Chloroquine Resistance Transporter (*Pfcrt)* and *Plasmodium falciparum* Multidrug Resistance 1*(Pfmdr-1)* Genes in Ilorin North-central Nigeria

***Abdulraheem Jimoh O.[1], Oluwasogo Olalubi[2] and Sunday Awe[3]**

[1]Microbiology unit, Department of Biosciences and Biotechnology, Kwara State University, Malete, Nigeria
[2]Department of Public Health, Kwara State University, Malete, Nigeria
[3]Microbiology unit, Department of Biosciences and Biotechnology, Kwara State University, Malete, Nigeria
****Corresponding Author's email*: yemijimoh@yahoo.com**

ABSTRACT

Malaria remains a major health problem globally, especially in Sub-Saharan Africa. The ease of developing resistance to anti-malaria drugs by *Plasmodium falciparum* is a serious challenge to malaria control and elimination programs. Consequently, continuous monitoring of drug efficacy is necessary to ascertain effective treatment policies. The study investigated the distribution pattern of these genes in *Plasmodium falciparum* isolates in Ilorin metropolis. One hundred and three samples were collected randomly from 5 hospitals in Ilorin metropolis. The samples were amplified at codon 76 and 86 for Pfcrt and Pfmdr-1 respectively using PCR/Restricted Fragment Length Polymorphism (RFLP). Drug susceptible alleles were most prevalent in the study area (K76 and N86) with 31% and 40% respectively, followed by drug resistance alleles (86Y) with 13% and the least prevalence (2%) was the mixed alleles K76T. UITH has the highest number of susceptible alleles followed by CSC 38% and 46% for K76 and N86 respectively. There are significant differences in the distribution of these alleles and the study sites (P55< 0.05). The prevalence of resistance alleles in the area is a welcome development that can be employed for possible re-introduction of CQ for the treatment of malaria in the study area which will serve as an advantage over expensive ACT due to the fact that it is safe cheap and readily affordable.

Keywords: *Plasmodium falciparum;* Chloroquine Resistance Transporter Gene; Multidrug Resistance Gene; Ilorin

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INTRODUCTION

Malaria still remains a significant public health disease in the tropical parts of the world, especially in the African continent. Globally, about 93% of cases in 2018 were from sub- Sahara Africa notwithstanding, 94% of the overall 405,000 malaria death in the same year were from sub- Sahara Africa. (Mangusho *et al*., 2023). Children under 5 years of age accounted for about 78% of all malaria cases and 95% of death (WHO 2023). Eleven countries in sub-Saharan Africa and India carried almost 70% of the global malaria burden and these countries are, Burkina Faso, Cameroon, the Democratic Republic of the Congo, Ghana, India, Mali, Mozambique, Niger, Nigeria, Uganda and the United Republic of Tanzania accounted for 70% of the global estimated case burden and 71% of global estimated deaths (Lukwa *et al*., 2019).

In Africa, malaria in pregnancy is responsible for 400,000 cases of severe maternal anaemia and 200,000 newborn deaths each year. Placental infection, premature birth and low birth weight (a significant factor in infant mortality) are also caused by maternal malaria. In addition, severe maternal anaemia increases the risk of perinatal complications. Malaria, therefore, is seriously hindering the achievement of Millennium Developmental Goal (MDG) 4 & 5 (improve maternal

health), however, this is yet to reach the reduction rate as recommended by MDG because the country is still among the top 13 highest Maternal Mortality Rate in the world (Dadding *et al.,* 2019)

In Nigeria, the disease is responsible for 60% outpatient visits to health facilities, 30% deaths of under-five children, 11% maternal mortality (Lawal *et al*., 2018). The prevalence ranges from 16% in the South and South East Zones to 34% in the North West Zone. The prevalence in rural populations is 2.4% higher than that of urban settings (Severe Malaria Observatory, 2020).

The yearly economic loss due to malaria in Nigeria has been put at 480 billion Naira due to costs of treatment, transportation to sources of treatment, loss of man-hours, absenteeism from schools and other indirect costs. Thus, malaria imposes a heavy cost, not only on the country's income, but also on its rate of economic growth and invariably on its level of economic development (WHO 2022).

Antimalarial agents inhibit growth of *Plasmodium* species by concentrating within acid vesicles and increasing the internal pH of the parasite. They also inhibit haemoglobin utilization and parasite metabolism (Adamu *et al.,* 2021). The major classes of antimalarial drugs are; Quinoline derivatives; i.e Aminoquinoline, Chloroquine, Amodiaquine, Antifolate; Sulfadoxine-Pyrimethamine (SP) and Artemisinin derivatives and combined therapy (ACT). Out of the various antimalarial drugs available, the chloroquine was the agent of choice for many decades because of its safety, efficacy and affordability (WHO, 2019).

However, parasite resistance to this drug was initially observed in Thailand in 1957. Since then, it spread out quickly to Africa in 1970s, and finally surfaced in Nigeria the early 1980's (Mohammed *et al.*, 2017). The dispersion of chloroquine resistance was a paramount factor in the failure of the first malaria control and elimination efforts in the mid of 20th century (Abdulfatah *et al*., 2018). In Nigeria, survey carried out by Federal Ministry of Health in 2002 showed that both Chloroquine and Sulfadoxine were no longer adequate for national first line use. Nigerian authority eventually shifted to the use of ACT for the presumptive treatment of all fevers (Mohammed *et al*., 2017). The report of ACT resistance parasites in Cambodia however, posed a major setback to malaria control and elimination program (WHO, 2023). At present, chloroquine remains effective only in some parts of Central America, where clinical studies have confirmed it as an effective drug. However, recent data on the prevalence of chloroquine-resistant genotypes in these areas present an alarming situation for health officials (Sinha *et al*., 2014).

In Nigeria, this phenomenon has prompted series of study in different parts of the country in the last decade which revealed high resistance to chloroquine especially in South-eastern states, while most strains of *P. falciparum* in North-eastern states were found to be fully sensitive to chloroquine (Mohammed *et al*., 2017). Polymorphisms at molecular level of the 2 *P. falciparum* genes are basis for antimalarial resistance (Adamu *et al*., 2021). The mutations of *P. falciparum* chloroquine resistance transporter gene at codons K76T, A220S, Q271E, N326S, I356T and R371I and *P. falciparum* multi-drug resistance at codon N86Y have been documented to be associated with chloroquine resistance in *P. falciparum.* These 2 genes are found on the food vacuole of the parasite. The susceptible one is believed to transport and accumulate CQ into the parasite food vacuole, mutations on N86Y, S1034C, N1042D, and D1246Y negate this transport which results to reduce CQ sensitivity. The presence of *Pfcrt* and *Pfmdr-1* mutations can alter chloroquine sensitivity to high level of resistance (Mohamad *et al*., 2024).

Sensitivity or resistance to other antimalarial drugs has been linked with polymorphisms of the mutations of these genes. For example, mutations of *Pfmdr-1* at the codons 86, 1034 and 1042 improve parasites sensitivity to mefloquine, halofantrine and lumefantrine (Mohamed *et al*., 2024). The withdrawal from circulation of some drugs could be responsible for the re-emergence of wild types as reported in Malawi following withdrawal of CQ from circulation (Mohammed *et al*., 2017). Also in Tanzania, emergence of wild types following withdrawal of CQ took about five years; from17.1% to 0.7% and seven years from 48% to 89.6% (Malmberg *et al.*, 2013). This present study investigated the distribution and the prevalence of *Pfmdr-1* and *Pfcrt* resistance and susceptible alleles in *P. falciparum* isolates from Ilorin metropolis.

MATERIALS AND METHODS

Description of Study Area

The study was conducted in Ilorin metropolis where selected public healthcare centers were used as study sites. These hospitals are; University of Ilorin Teaching Hospital (**UITH**), General Hospital Ilorin (**GHI**), Sobi Specialist Hospital (**SOBI**), Kwara State Civil Service Clinics (**CSC**) and Comprehensive Health Centre Okelele (**OKELELE**).

Ilorin is the capital of Kwara State with population of about1,000,000 which is about 2.67% increase from 2021(https://www.macrotrends.net). The climate of the study area is typically tropical with alternating wet and dry seasons each of about six months. Malaria is meso-hyperendemic in Ilorin with the peak at the peak of the raining season June/July and with the second peak of transmission around October/November. These two peaks of transmission occasioned by conducive

environmental conditions that favor breeding of mosquito vectors and intense transmission of *Plasmodium falciparum* (Mohammed *et al.,* 2017).

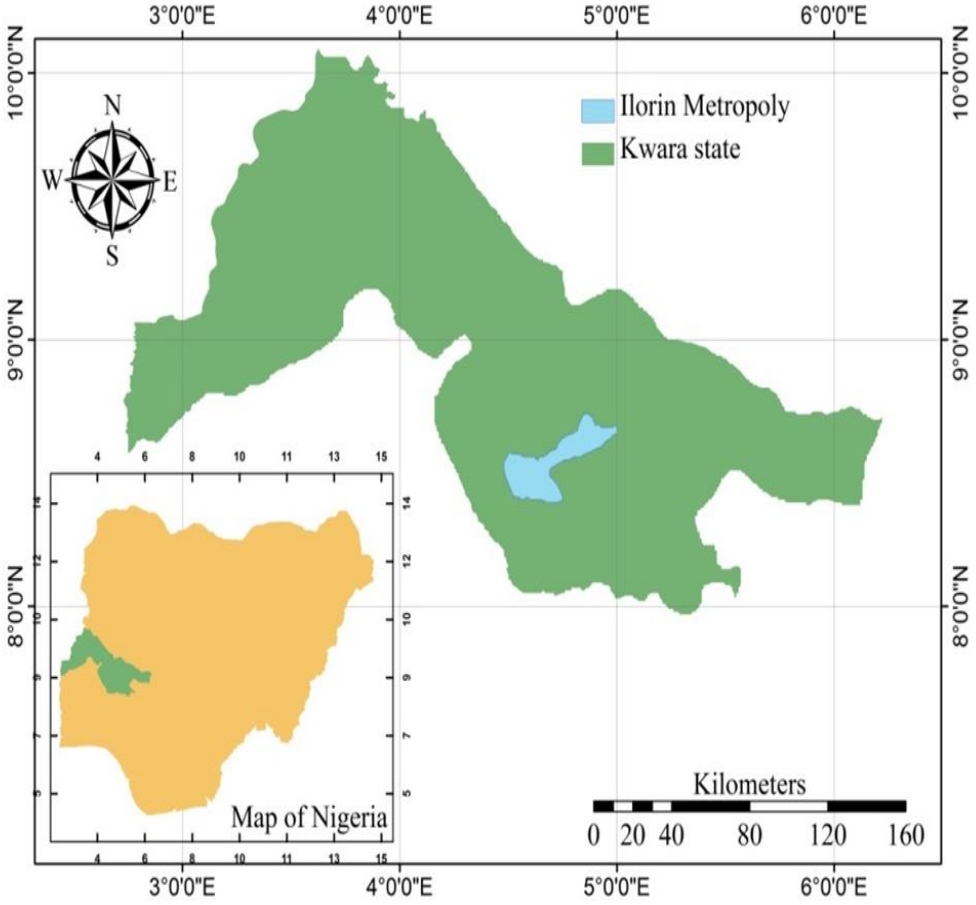

Figure 1. Map of Nigeria showing Kwara State and Ilorin Metropolis

Ethical Clearance

Ethical approval was sought from ethical review committees of both Kwara State Ethical Committee, Ministry of Health, Fate Ilorin (**MOH/KS/EU/777/493**) and The University of Ilorin Teaching Hospital Ilorin, Kwara State Nigeria (**UITH PAN/2022/12/0223)**. The study followed Helsinki ethical code of conduct 2019.

Inclusion Criteria

The target population consisted of all patients (> 12 months, n = 331) who presented with symptoms of uncomplicated malaria such as fever, headache, general body pains and positive for *P. falciparum* mono-infection and consented were recruited for the study. In the case of children, consent was obtained from their guardian. All subjects were selected on a systematic random sampling method to avoid bias based.

Exclusion Criteria

Patients who are asymptomatic of malaria infection, or those with symptoms of complicated malaria, pregnant women, those that have taken antimalarial drug and those that refused consent

Study Design and Population

A prospective cross-sectional study was designed for this research work and subjects were selected by proportional allocation from these Hospitals.

In this study, patient presented with malaria with symptoms such as fever with axillary temperature ≥37.5^{0}C, headache, muscle pains, chills, and malaise at these selected facilities, examined by the clinician and refer for malaria diagnosis.

Sample Collection

Five milliliters (5ml) of whole blood were collected by venipuncture from each participant into vacutainer bottle coated with Potassium Ethyl diaminetetraacetic acid (EDTA), which was used for thick and thin blood films preparation and also blotted on Whitman filter paper.

Malaria Microscopy and Parasite Density Determination

Blood films (thick and thin) were made on a frosted-end grease-free microscope glass slide and stained using 3% Giemsa stain. The presence of malaria parasites was detected and speciation of the parasites was done using their morphological characteristics in the thin and thick blood films by method described by (Cheesebrough, 2010) and (WHO, 2010). Identification of parasites and parasite density estimation was carried out at the Department of Medical Microbiology& Parasitology Laboratory, University of Ilorin Teaching Hospital. Participant with *P. falciparum* mono infection that has parasite density of ≥ 1000/µl of blood was selected.

Parasite Density Estimation

Parasite count per microliter of blood was calculated using the formula described by (Kolawole *et al*., 2016)

Parasites count per microliter of blood = $\frac{Number\ of\ parasites\ counted\ x\ TLC}{Number\ of\ Leucocytes\ (200)}$

TLC means Total Leucocytes Count. (TLC)

Parasite DNA extraction

Parasite genomic DNA was extracted from 103 DBS samples using the Qiagen blood and tissue kit (QIAGEN, Germany), according to the Manufacturer's protocol. The protocol for purification of total DNA from blood treated with anticoagulants, which has been spotted and dried on filter paper was used according to manufacturer's instructions. The quality and the yield of the extracted DNA were determined using the formula: DNA purity was estimated as OD 260 / OD 280 and the yield in ng/µl. The extraction was conducted at Department of Biochemistry and Nutrition, Malaria Genomic Unit, National Institute for Medical Research (NIMR), Yaba, Lagos, Nigeria.

Confirmation of *Plasmodium falciparum* Infection by PCR

The *Plasmodium falciparum* parasites were confirmed in all samples using the forward and reverse primers for parasites 18S ribosomal RNA (rRNA) gene. The method included the first PCR with primers named rPLU_5 and rPLU_6 that amplify human plasmodium parasite 18S rRNA gene. This step is followed by amplification of the product of primary PCR separately with the specie specific primer pairs: rFAL1 and rFAL2, to identify the species *P. falciparum.* The Pf-18SrRNA gene was amplified using the cycling parameters as follows: 10 minutes initial denaturation at 94ºC followed by 35 cycles of 1minute annealing at 55ºC, 2 minutes extension at 72ºC and a 10 minutes final extension at 72ºC. The primary PCR product was diluted 10 times and 2µl used in nested-PCR to amplify specie specific primers (FALC1 and 2). The cycling parameters for the nested PCR were the same as for primary PCR primers except that the extension at the 72ºC would be carried out for 1 minute. All PCR reactions were carried out in a total volume of 50µl containing 0.2Mm dNTPs, 2Mm $Mgcl_2$, 1µM of each primer, and 1 unit of AmpliTaq polymerase (Perkin Elmer, England). In the nested PCR product was used. Positive and Negative controls were included in each reaction.

Detection of N86Y mutation in *Pfmdr1* gene

Parasite genomic DNA was extracted from blood samples collected on filter paper using a QIAamp DNA blood kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. A fragment from the pfmdr1 encompassing the 86th codon was amplified successfully from DNA samples. The nested protocol used was as described by Shrivastava et al. For first round PCR (Nest 1), 0.5µL of forward and reverse primers 5'AGGTTGAAAAAGAGTTGAAC3' 5'-ATGACACCACAAACATAAAT-3' were used to amplify the region that flanking codon 86. Nested primers 5'-ACAAAAAGAGTACCGCTGAAT-3' and 5'-AAACGCAAGTAATACATAAAGTC-3 were used to amplify PCR products in nest 2 reaction. 5µL Gotaq Green master mix (Promega, Madison, WI, USA) and 3 µL of nuclease-free H2O were mixed on ice. One microliter (1 µL) of gDNA was added to a final volume of 10 µL. Thermocycling conditions were 94 °C for 3min, followed by 35 cycles each of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1min and a 72 °C final extension for 5 min. The PCR products were run on 2% agarose gels pre-stained with ethidium bromide and observed under UV trans illuminator at 312 nm to visualized the band.

Detection of the K76T Mutation in *Pfcrt* Gene

Fragment from pfcrt encompassing 76th codon was amplified by nested PCR protocol described by Shrivastava et al. For nest 1, 0.5 µL of both forward and reverse primers 5'-CCGTTAATAATAAATACACGCAG-3' 5'-CGGATGTTACAAAACTATAGTTACC-3' were used and for the nest 2, 5'-AGGTTCTTGTCTTGGTAAATTTGC-3' 5'-CAAAACTATAGTTACCAATTTTG-3' were used. The nest 1 PCR conditions were 94 °C for 3min, followed by 35 cycles each of 94 °C for 30 s, 56 °C for 30 s, 62 °C for 1min and a 62 °C final extension for 5 min. In nest 2 only 30 cycles of PCR were run. The PCR products were run on 2% agarose gels pre-stained with ethidium bromide and observed under UV trans illuminator at 312 nm to visualized the band.

Restriction Digestion with Apo1 and Afl lll

The amplified product was subjected to restriction digestion with Apo l and Afl lll (The wild type allele and mutant allele) respectively by incubating at 37°C for one hour with one unit of each enzyme. The digests were resolved on 3% agarose gel stained with ethidium bromide and visualized for bands.

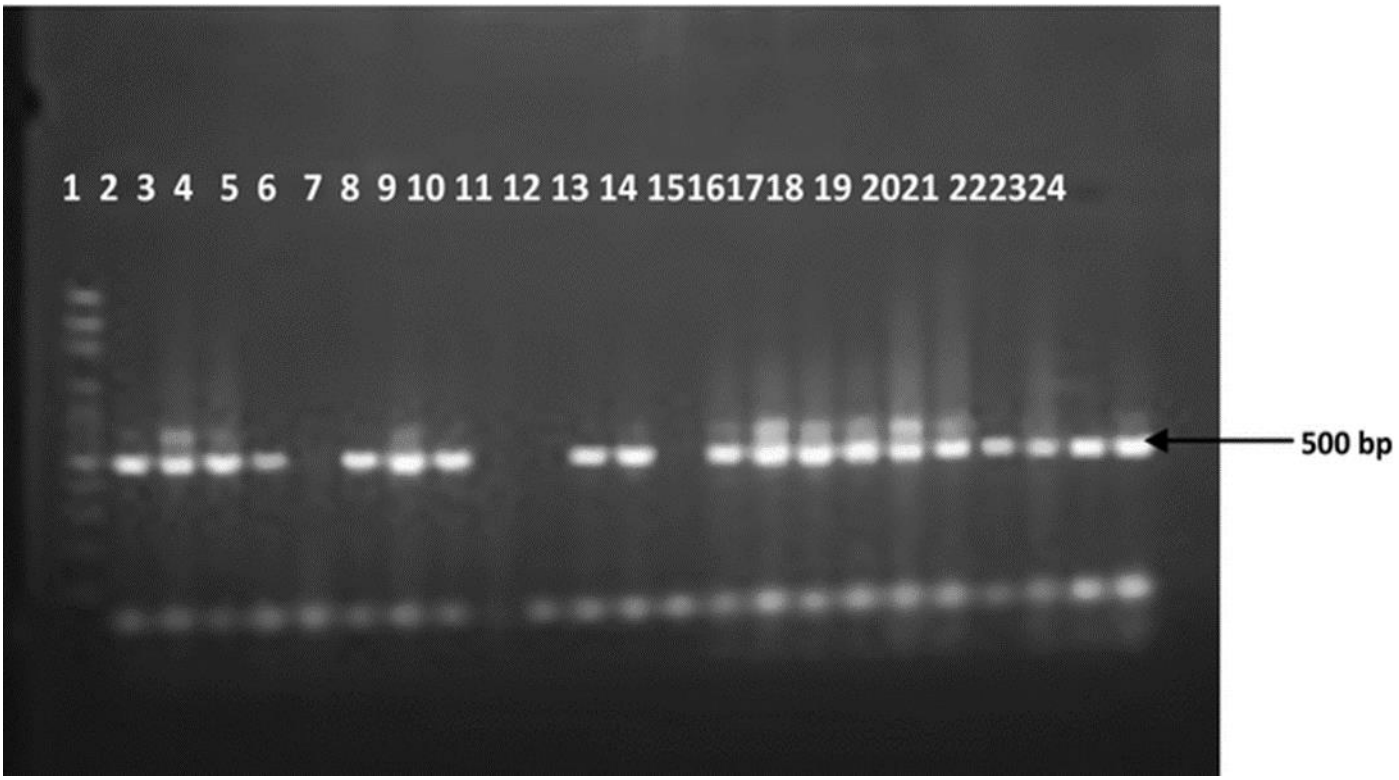

Fig 2: Agarose gel showing PCR confirmation of *Plasmodium falciparum* 18s rRNA gene PCR product. Lane 1 = 100 bp DNA marker; Lane 2 = *P. falciparum* 3D7 control; Lanes 3–24 = Plasmodium infected blood DNA samples.

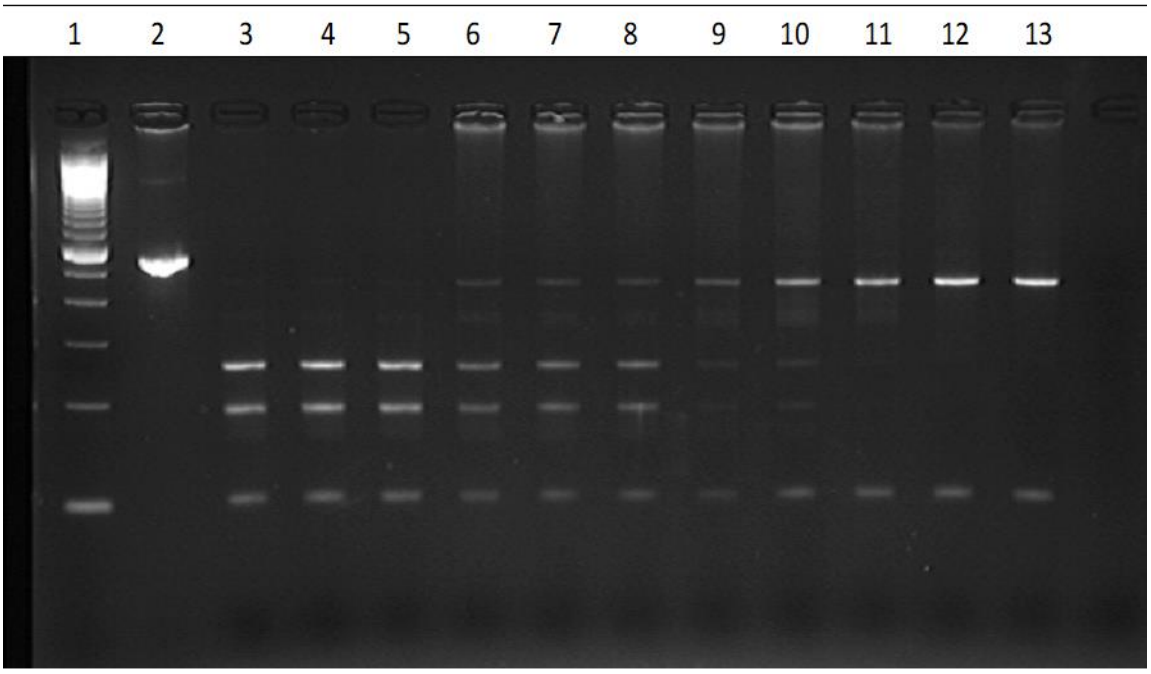

Fig 3: Micrograph of Pfmdr1

Row 1 is LADDER 100 BP
Row 2 is Band sizes (bp)
Undigested

534
Row 4 and 5 is 86N – wild type / CQ sensitive 99, 185, 250
Row 6,7 and 8 is 86N/Y – mixture 99, 185, 250, 435
Row 10,11and 12 is 86Y – CQ résistant 99, 435
Control DNA
3D7 parasite line – 86N
Row 13 is parasite line – 86Y

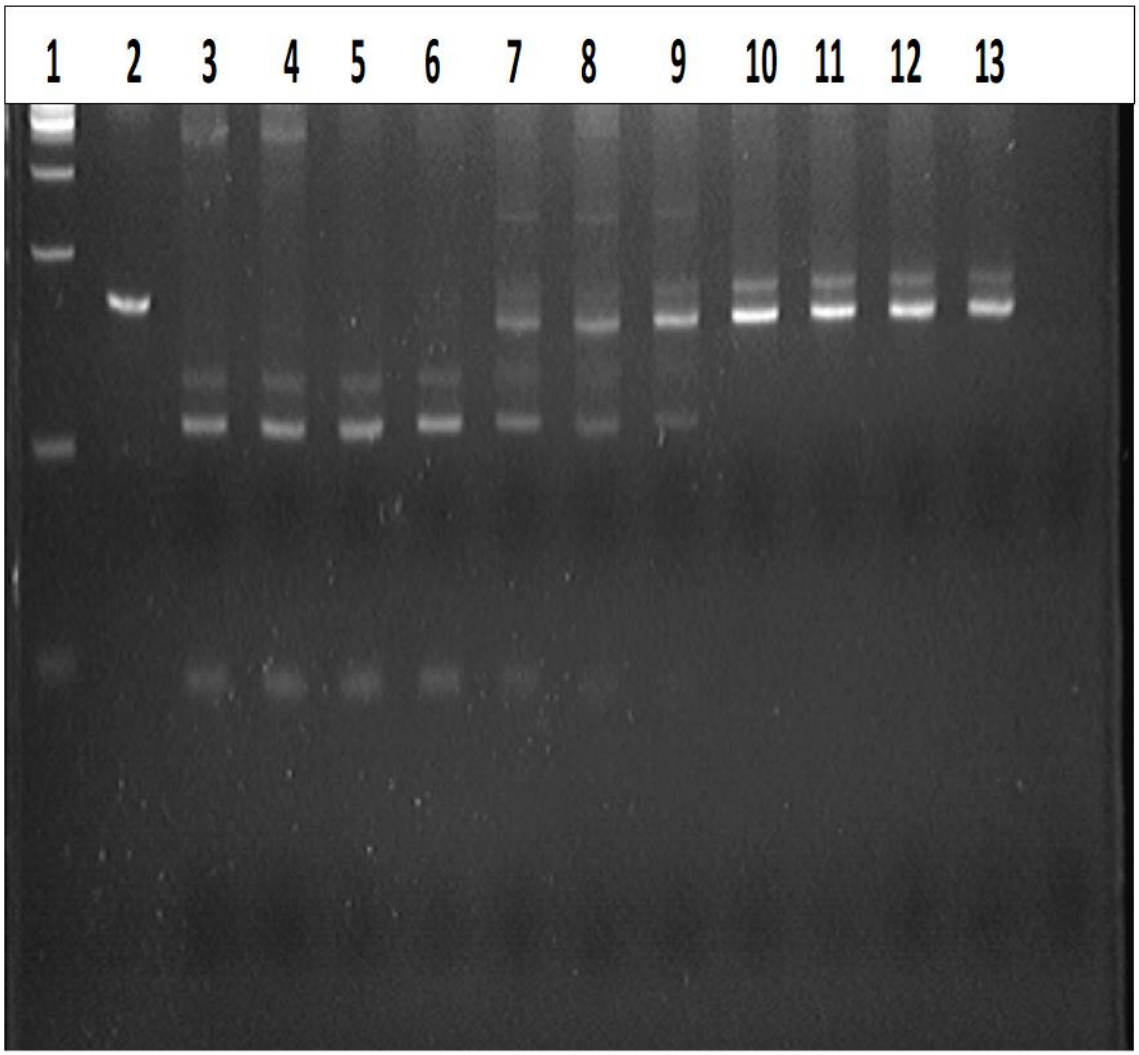

Fig 4: Electro-micrograph of *Pfcrt*

Row 1 Ladder
Row 2 Band sizes (bp)
Undigested 164
Row 4, 5, and 6 is 76K – wild type / CQ sensitive 17, 47, 100
Row 7, 8, and 9 is 76K/T – mixture 17, 47, 100, 147
Row 10, 11 and 12 76T – CQ resistant 17, 147
NB you would not normally see the 17 bp band on an agarose gel
Control DNA
3D7 parasite line – 76K
W2 parasite line – 76T

Note:
First run – Denaturation (95°C for 5mins, then 40 cycles at 94°C for 30secs), Annealing (52°C for 40secs), Extension (72°C for 30secs, then 72°C for 5mins).
Second run – Denaturation (94°C for 5mins, then 40 cycles 94°C for 30secs), Annealing (52°C for 40secs), Extension (72°C for 45secs, then 72°C for 5mins).

RESULTS

A total of three hundred and thirty-one (331) suspected malaria patients were screened and out of these, one hundred and three 103 (31.1%) were positive for malaria parasite. All the parasites encountered were *Plasmodium falciparum*.

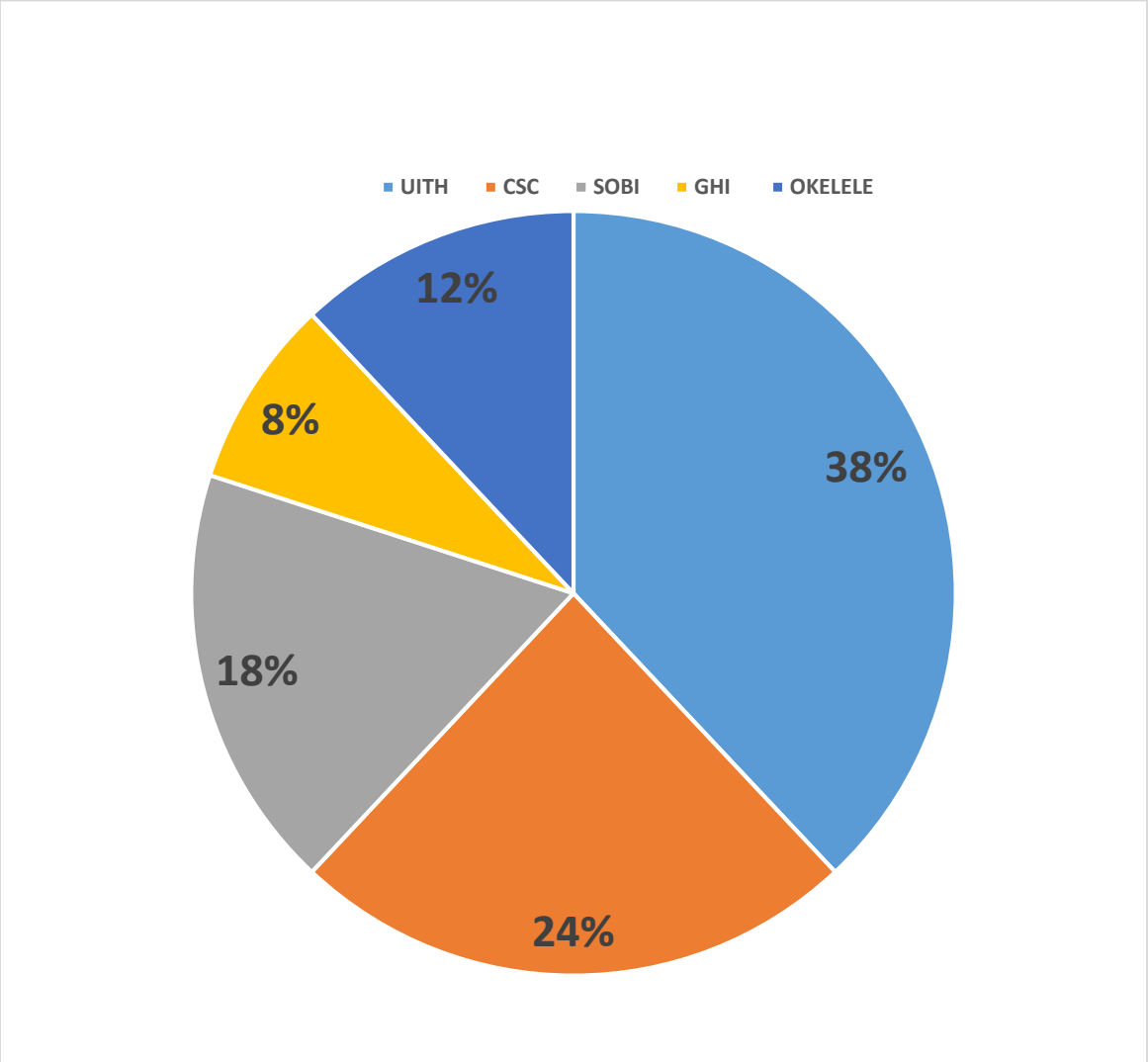

Figure 5. Pie-chart showing distribution of parasitaemia in relation to study sites

The highest prevalence was found in UITH (38 %) which was followed by CSC with (24 %) while the least prevalence was recorded at General Hospital (8 %). There is no significant difference in the prevalence and study sites (P<0.05).

Table 1 depicts that UITH has the highest number of *Pfcrt* (76T) resistance gene 30% followed by CSC, SOBI and OKELELE with 20% each, while GHI has the least prevalence 10%. For *Pfmdr-1* (86Y) UITH has the highest prevalence (46%) followed by CSC (23 %) and GHI has no report *of Pfmdr 1* 86Y allele. There is significant difference in the distribution of (K76, N86 and 86Y) across the study sites (P<0.05).

Table 2 shows that female has the highest number of both *Pfcrt* (76T) and *Pfmdr-1*(86Y) with 60% and 57% respectively. There is no significant difference between these mutations and gender (P<0.05).

Table 3 shows that age groups 1-5, and **>40years** have the highest prevalence of *Pfcrt* (76T) 33% each followed by age groups **6-20 and** 21-40years each with 17%. The difference is not statistically significant **(P<0.05). For** *Pfmdr -1* (86Y), age group 1-5 and **>40**years recorded the highest prevalence 30% followed by 20% recorded by age groups 6-20 and 21-40years each. The difference is not statistically significant (P<0.05).

Table 4 shows that face to face has the highest distribution of *Pfcrt* (76T) 56% while the prevalence of 25% was reported for *Pfmdr1* (86Y). The difference is not statistically significant (P<0.05)

Table 1: Distribution of *Pfcrt* and *Pfmdr-1* in Relation to Study Sites

SITES	NO POSITIVE	K76 **(%)**	76T **(%)**	K76T **(%)**	N86 **(%)**	86Y **(%)**	N86Y **(%)**
UITH	39	12 **(38)**	3 **(30)**	1**(50)**	15**(37)**	6 **(46)**	2 **(40)**
CSC	25	8 **(25)**	2 **(20)**	1**(50)**	10**(24)**	3 **(23)**	1 **(20)**
SOBI	19	5 **(15)**	2 **(20)**	0 **(0)**	9**(22)**	2 **(15)**	1 **(20)**
GHI	8	3 **(9)**	1 **(10)**	0 **(0)**	4**(10)**	0 **(0)**	0 **(0)**
OKELELE	12	4 **(13)**	2 **(20)**	0 **(0)**	3**(7)**	2**(15)**	1 **(20)**
P value	NA	0.026*	0.519	0.251	0.006*	0.015*	0.269
Total	**103**	**31**	**10**	**2**	**40**	**13**	**5**

.**Table 2: Distribution of *Pfcrt* and *Pfmdr-1* Allele in Relation to Gender**

Gender	No Positive	K76 (%)	76T (%)	K76T (%)	N86 (%)	86Y (%)	N86Y (%)
Male	40	12 **(38)**	4 **(40)**	2**(33)**	18**(41)**	3**(43)**	1**(100)**
Female	63	20**(62)**	6**(60)**	4**(67)**	26**(59)**	4 **(57)**	0
P value	NA	0.937	0.488	0.027*	0.979	0.978	0.424
Total	**103**	**32**	**10**	**6**	**44**	**7**	**1**

Table 3: Distribution of *Pfcrt* and *Pfmdr-1* Allele in Relation to Age

Age	**No Positive**	**K76 (%)**	**76T (%)**	**K76T (%)**	**N86 (%)**	**86Y (%)**	**N86Y (%)**
1-5	33	10**(30%)**	2**(33%)**	1**(25%)**	15**(33)**	3**(30)**	2(50)
6-20	37	13**(39%)**	1**(17%)**	2**(50%)**	18**(39%)**	2**(20)**	1(25)
21-40	22	7**(21%)**	1**(17%)**	1**(25%)**	11**(24%)**	2**(20)**	0 (0)
>40	11	3**(9%)**	2**(33%)**	0	2**(4%)**	3(**30**)	1(25)
P value	NA	0.347	0.630	0.794	0.060	0.648	0.977
Total	**103**	**33**	**6**	**4**	**46**	**10**	**4**

Table 4: Distribution of *Pfcrt* and *Pfmdr-1* Allele in Relation to Accommodation Type

Accommodation Type	**No Positive**	**K76 (%)**	**76T (%)**	**K76T(%)**	**N86 (%)**	**86Y(%)**	**N86Y(%)**
Self-contained	68	18 **(67)**	4 **(44)**	2**(67)**	30**(67)**	**9(75**)	5**(71)**
Face to face	35	9 **(33)**	5 **(56)**	1 **(33)**	15**(33)**	**3(25)**	2**(29)**
P value	NA	0.814	0.282	0.765	0.756	0.763	0.776
Total	**103**	**27**	**9**	**3**	**45**	**12**	**7**

DISCUSSION

Control of malaria depends largely on epidemiological survey of antimalaria resistance genes in the area in order to initiate evidence-based control measures and resistance management. The need for modification and change of treatment policies are desirable when there is widespread of resistance to first line antimalaria drug as experienced with chloroquine in the early 20s which led to its withdrawal. This has resulted to introduction of ACT as drug of choice for the treatment of uncomplicated malaria in Nigeria which has been so effective. However, there have been several reports of treatment failure with the use of ACT which could have resulted in selection and propagation of ACT resistance and re-emergence of CQ susceptible alleles (Mohammed *et al.,*2017). The current study aimed to investigate the distribution of *Pfcrt* K76T and *Pfmdr-1* N86Y codons, in *P. falciparum* isolates collected from five (5) hospitals in Ilorin metropolis.

Generally, this study showed that both *Pfmdr 1* and *Pfcrt* susceptible alleles are prevalent in the study area 40%

and 31% respectively. There is lower distribution of *Pfcrt* **76T** drug resistance alleles across the study sites with highest recorded at UITH (30%), followed by Okelele Comprehensive Health Center, CSC and SOBI each with (20%) prevalence, while GHI has the least prevalence (10%). This is an indication of wild type alleles recovery as a result of CQ withdrawal, the same discovery was observed for *Pfmdr1* N86Y, where there was a decline in the prevalence of the mutant allele **86Y** from 46% to 28% (Mohammed *et al.*, 2017).

It is obvious that, there is no base line data regarding the prevalence of 76T allele when CQ was replaced with ACT as first line treatment drug for uncomplicated malaria in the area. However, the study conducted in Ethiopia showed fixation of 76T in parasite isolate in an area where CQ had been withdrawn for the treatment of malaria overtime (Hassan *et al*.,2022). In addition, in high malaria transmission areas, competition between drug sensitive and drug-resistant parasites may slow the spread of drug-resistant parasites (Hassan *et al*.,2022)

The **76T** prevalence recorded in this study (10%) is lower than that recorded by (Oluwasogo *et al*., 2020) who reported 68% of mutant allele (**76T**) among rural communities in Kwara State and 28.3% recorded in Northwest Nigeria (Adamu *et al*., 2021), while (Dokunmu *et al.,* 2019) reported 75.9% in southwestern Nigeria, but higher than 4.0% and 8.2% reported in River state, Nigeria by (Alade *et al*.,2019) and in Kenya (Chebore *et al.*, 2020). Variation in distribution pattern of (**76T**) mutation could be attributed to differences in the disease epidemiology, transmission intensity, host immunity mutation compensatory, infection clonality, and treatment policy in different countries (Muhamad *et al*.,2024).

The distribution of susceptible allele of *Pfmdr1* **N86** was highest in UITH (37%), followed by CSC (24%) and the least prevalence of 7% reported at GHI. The studied population revealed presence of wild (Susceptible) alleles for both *Pfcrt* and *Pfmdr1* genes (32%) and (41%) respectively which exceed the number of mutant (Resistance) alleles for the two genes (10%) and (13%). These findings may be due to withdrawal of chloroquine (CQ) for the treatment of malaria or drug pressure that is mounting on artemether-lumefantrine (AL) (Mohammed *et al*.,2017). Although there is paucity of information concerning distribution of these resistance markers in the study area, however, there are evidences that removal of CQ in circulation for treatment of *P. falciparum* malaria for considerable period of time usually ten years and above or the pressure from artemether-lumefantrine eventually result to replacement of *Pfmdr1* resistance genes by susceptible parasites populations (Oluwasogo *et al*.,2020).

This plays a significant role for the selection of *Pfcrt* K76 susceptible parasite following substitution of CQ with AL nationwide (Mohammed *et al* 2017). The re-emergence of CQ susceptible alleles in the study area is synonymous to the finding of (Balogun *et al*.,2016) who reported 76T prevalence of 5.4% among the Almajir in Northeastern Nigeria and (Chebore *et al.*,2020), who also reported similar prevalence in Kenya. The increasing rates of *Pfmdr 1* N86 susceptible allele could also be responsible for decreasing sensitivity to lumefantrine and artemisinin (Chebore *et al.*,2020),

Mixed infection mutations K76T and N86Y were 2% and 5% respectively in this study. This is lower than 3.6% of K76T reported in Northwestern Nigeria by (Mohammed *et al.*, 2017). The mixed mutation, *Pfcrt* K76T is usually used as biomaker of CQ resistance and directly linked to both *in vitro* and clinical resistance.

The distribution in terms of gender showed that females have the highest prevalence of mutant alleles 76T (60%), while males have (40%). The mixed mutant infection K76T was more common in female (67%) which indicates that females are more likely to develop drug resistance than male, because the presence of K76T mutation is a pre requisite for the parasites to develop multidrug resistance property against CQ. It was also found that N86Y were found exclusively in male (100%), it suggests that CQ may be used to treat the disease in male.

Distribution in terms of age showed that susceptible alleles K76 and N86 were seen amongst all age groups and most prevalent in age groups 1-5 and 6-20 years. Withdrawal of CQ in circulation for malaria treatment could be possible cause or as a result of mounting pressure on AL. The presence of resistance alleles among age groups depicting possibility of mounting drug pressure on the age groups that eventually resulted to loss of susceptibility to AL and emergence of susceptible parasites population to CQ.

In terms of accommodation, self-contained has the highest prevalence of the mutant alleles (44% and 75%) for **76T and 86Y** respectively, while face to face has (56% and 25%) for **76T and 86Y** respectively. While the mixed mutations (**K76T and N86Y**) were highest in self-contained type of accommodation (67% and 71%). It then means that people living in self-contained type of accommodation are likely to develop multidrug resistance property against the parasites (Mohammed *et al*., 2017).

Moreover, studies have shown that removal of CQ for malaria treatment or the pressure on AL will consequently give rise to replacement of resistance genes by parasites population that are susceptible to CQ (Mang'era *et al.,* 2012). This is synonymous with the observations in Kenya, Tanzania and Malawi where the

withdrawal from circulation of CQ led to spontaneous spread of a CQ susceptible *Pfcrt* K76 population from less than 15% to 100% within 13 years (Mang'era *et al.,* 2012). This Phenomenon is common in Africa due to abundant of immune individual and higher rate of transmission compare to Southeast Asia where CQ resistance alleles predominate in virtually all the areas (Mang'era *et al.,* 2012).

However, the use of ACT in future may likely lose its efficacy because of high level of susceptible K76 alleles encountered especially in recrudescent samples after AL use (Mohammed *et al*., 2017)

CONCLUSION

This study confirmed a high prevalence of *falciparum* malaria in Ilorin metropolis, North-central Nigeria as reported in various previous studies in the area. It was also established further that susceptible alleles of both *Pfcrt* and *Pfmdr 1* are more prevalent in the area of study. Therefore, it is pertinent to ensure rational use of antimalaria agents for the treatment of *P. falciparum* infection to prevent increase in resistant alleles and the spread of resistance parasite, (*P. falciparum*).

This study is looking at the possibility of re-introducing CQ in combination therapy with other short acting drugs as additional anti-malaria option, since CQ-resistant alleles are relatively low in the study area. This will reduce financial burden of high cost of ACT and the shift to CQ makes treatment accessible to the populace because of its cheapness, safe and readily available. Moreover, sustained regular and expanding molecular surveillance covering entire Kwara State is recommended to allow for the early detection of resistance, informing treatment policy and facilitating prompt containment efforts should cases of resistance are identified.

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