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DISTRIBUTION OF *Plasmodium falciparum* Chloroquine RESISTANCE TRANSPORTER (*Pfcrt)* AND *Plasmodium falciparum* MULTIDRUG RESISTANCE 1*(Pfmdr-1)* GENES IN ILORIN NORTH-CENTRAL NIGERIA

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

[1]Microbiology unit, Department of Biosciences and Biotechnology, Kwara State University, Malete, Nigeria.
[2]Department of Public Health, Kwara State University, Malete, Nigeria.

*Corresponding authors' email: yemijimoh@yahoo.com

ABSTRACT

Globally**,** malaria is a peculiar health challenge particularly in continent of Africa. The ease of developing resistance to anti-malaria drugs by *Plasmodium falciparum* is a serious impediment towards programs aim at control and elimination of malaria. Consequently, drug efficacy surveillance is desirable to institute adequate and effective treatment policies. In this study, distribution of these genes in *Plasmodium falciparum* isolates in Ilorin metropolis were investigated. One hundred and three samples 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). The most prevalent alleles were drug susceptible alleles in the study area (K76 and N86) with 31% and 40% respectively, while drug resistance alleles (86Y) have 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 (P< 0.05). The prevalence of resistance alleles in the area is a welcome development that can be employed for possibility of re-introduction of CQ for the treatment of malaria in the study area which will serve as an advantage over expensive ACT due to fact that it is safe cheap and readily affordable.

Keywords: *Plasmodium falciparum*, Chloroquine resistance transporter gene, Multidrug resistance gene, Ilorin

INTRODUCTION

Malaria, a disease of public health significant in the tropics, particularly in the Africa. Generally, in 2018, about 93% of cases and 94% malaria related death occurred in 2018 were from sub- Sahara Africa notwithstanding, 94% of the overall 405,000 malaria death in Africa. (Mangusho *et al*., 2023). About 78% of all malaria cases and 95% of death occurred in children under 5 years of age. (WHO 2023). India and some countries in sub-Saharan Africa shouldered about 70% of malaria burden in the world 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, cases of severe maternal anaemia and deaths of newborn each year were enormous 400,000 and 200,000 respectively. Placental infection, premature birth and low birth weight are also caused by maternal malaria. In addition, perinatal complications is cause by anaemia in pregnancy, therefore, malaria is seriously impeding the achievement of Millennium Developmental Goal (MDG) 4 & 5 (improve maternal health), although, reduction rate is yet to be attained as recommended by MDG because the country is among the 13 highest Maternal Mortality Rate in the world (Dadding *et al.*,2019)

In Nigeria, 60% of outpatient visits to hospitals, 30% deaths of children under-five years and 11% of maternal mortality were caused by malaria (Lawal *et al*., 2018). The prevalence varies from 16% to 34 % in the South and South East Zone and North West Zone respectively. Rural populations have the higher prevalence 2.4% higher than urban settings (Severe Malaria Observatory,2020).

Malaria in Nigeria causes huge economic loss, about 480 billion Naira annually due to costs of treatment, logistic for treatment, time loss, and inability to be in schools and other indirect costs. Thus, malaria imposes a heavy cost generally on the country's income as well as its rate of economic growth and invariably on its level of economic development (WHO 2022).

Antimalarial agents prevent 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). In spite of different antimalarial drugs available chloroquine was preferred because of its safety, efficacy and affordability (WHO,2019). However, resistance to this drug by the parasite was first observed in Thailand in 1957, and spread to Africa in 1970s, and finally reached Nigeria the early 1980's (Mohammed *et al.*, 2017). The spread 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). Currently, in Central America, chloroquine remains effective according to clinical studies that confirmed it as an effective drug. However, there is concern for health officials about the prevalence of chloroquine-resistant genotypes (Sinha *et al*.,2014).

In Nigeria, In the last decade, this phenomenon has prompted series of study in different parts of the country which revealed varying response to chloroquine from South-eastern states to North-eastern states where most strains of *P. falciparum* found from resistance to chloroquine to fully sensitive to chloroquine (Mohammed *et al*.,2017).

Polymorphisms at molecular level of the 2 *P. falciparum* genes are basis for antimalaria resistance [7](Adamu *et al*.,2021). The mutations at codons K76T, A220S, Q271E, N326S, I356T and R371I of *P. falciparum* chloroquine resistance transporter gene and *P. falciparum* multi-drug resistance at codon N86Y have been linked to 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 process which results to reduce CQ sensitivity. The presence of *Pfcrt* and *Pfmdr-1* mutations can change chloroquine sensitivity to high level of resistance (Mohamad *et al*.,2024).

Sensitivity or resistance to other antimalaria drugs has been linked with polymorphisms of the mutations of these genes. For example, mutations at the codons 86,1034 and 1042 of *Pfmdr-1* improve parasites sensitivity to mefloquine, halofantrine and lumefantrine (Mohamed *et al*.,2024). Decreased in *P. falciparum* parasite sensitivity that has emerged in South East Asia presents a threat to future efficacy of the agents. Parasites with mutations conferring reduced partner drug susceptibility have become widespread throughout sub–Saharan Africa, and parasites with reduced in-vivo artemisinin susceptibility were reported for the first time in sub-Saharan Africa in Rwanda and Uganda in the past 2 years. Therefore, the emergence and spread of antimalaria drug resistance should be tracked to prevent further spread of resistance parasites.

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 study looked at the distribution and the prevalence of both *Pfmdr-1* and *Pfcrt* resistance and susceptible alleles in *P. falciparum* isolates in relation to gender, age, accommodation types and study sites in Ilorin metropolis.

MATERIALS AND METHODS

Description of study area

The study was carried out 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**).

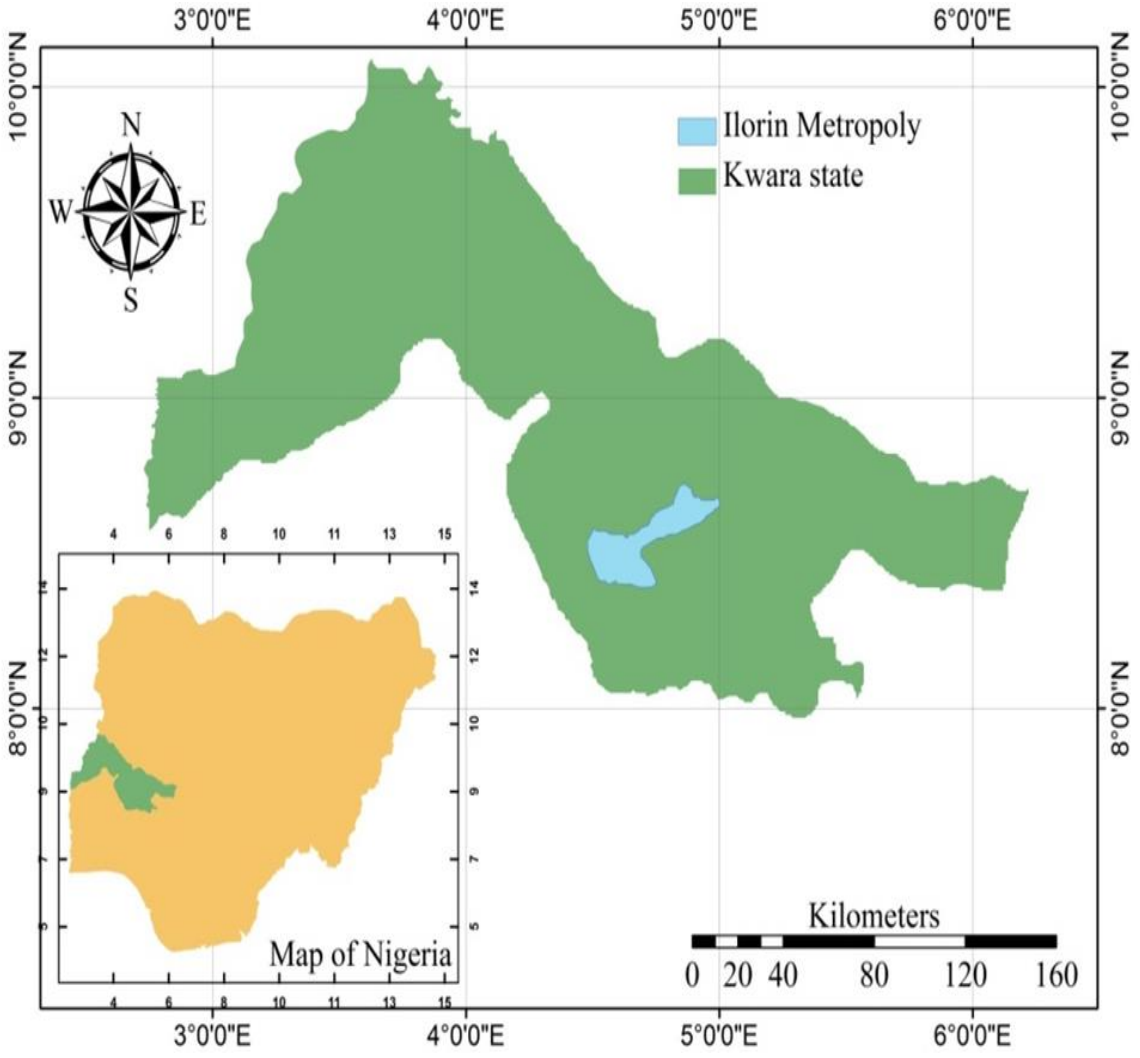

Figure 1: Map of Nigeria showing Kwara State and Ilorin Metropolis
Source: https://www.macrotrends.net

Ilorin is the capital of Kwara State with population of about1,000,000 which is about 2.67% increase from 2021. 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*).

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 antimalaria 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.

Malaria Microscopy and Parasite Density Determination
Thick and thin blood films were made on a grease-free microscope glass slide and stained with 3% Giemsa stain. Malaria parasites was identified using their morphological characteristics in the thin and thick blood films by method described by (WHO, 2022). Parasite identification and estimation of parasite density were 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.

Estimation of Parasite Density
Parasite count per microliter of blood was determined using the formula described by (Oluwasogo *et al*., 2020).

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

TLC means Total Leucocytes Count. (TLC)

Extraction of Parasite DNA
The genomic DNA of parasite was extracted from 103 DBS samples according to the manufacturer's protocol using the Qiagen blood and tissue kit (QIAGEN, Germany). The protocol for DNA purification from blood treated with anticoagulants on filter paper was followed according to manufacturer's instructions. The quality and the yield of the DNA extracted were determined by 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 confirmation of *Plasmodium falciparum* parasites in the samples were done using the forward and reverse primers for parasites 18S ribosomal RNA (rRNA) gene. This involved the first PCR with primers rPLU_5 and rPLU_6 which amplify 18S rRNA gene of human plasmodium parasite. This step is followed by primary PCR product amplification with the specie specific primer pairs: rFAL1 and rFAL2, to identify the species *P. falciparum.* The amplification of Pf-18SrRNA gene was done using the cycling parameters; 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. The PCR products were run on 3% agarose gels pre-stained with ethidium bromide and observed under UV trans illuminator at 312 nm to visualized the band.

Detection of N86Y mutation in *Pfmdr1* gene
Genomic DNA of parasite was extracted from blood samples on filter paper according to the manufacturer's instructions using QIAamp DNA blood kit (Qiagen, Hilden, Germany). 86th codon fragment from the pfmdr1 was amplified successfully from DNA samples. The nested protocol used was as described by (Chebore *et al*.2020). 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 mutation in *Pfcrt* gene (K76T)

Amplification of 76th codon of Pfcrt was carried out by nested PCR followed protocol described by (Chebore *et al*., 2020). 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 PCR conditions for nest 1 included 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 and the 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.

Digestion of Apo1 and Afl lll with Restriction Enzymes

The product amplified was subjected to restriction digestion with Apo l and Afl lll (The wild type allele and mutant allele) respectively. 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.

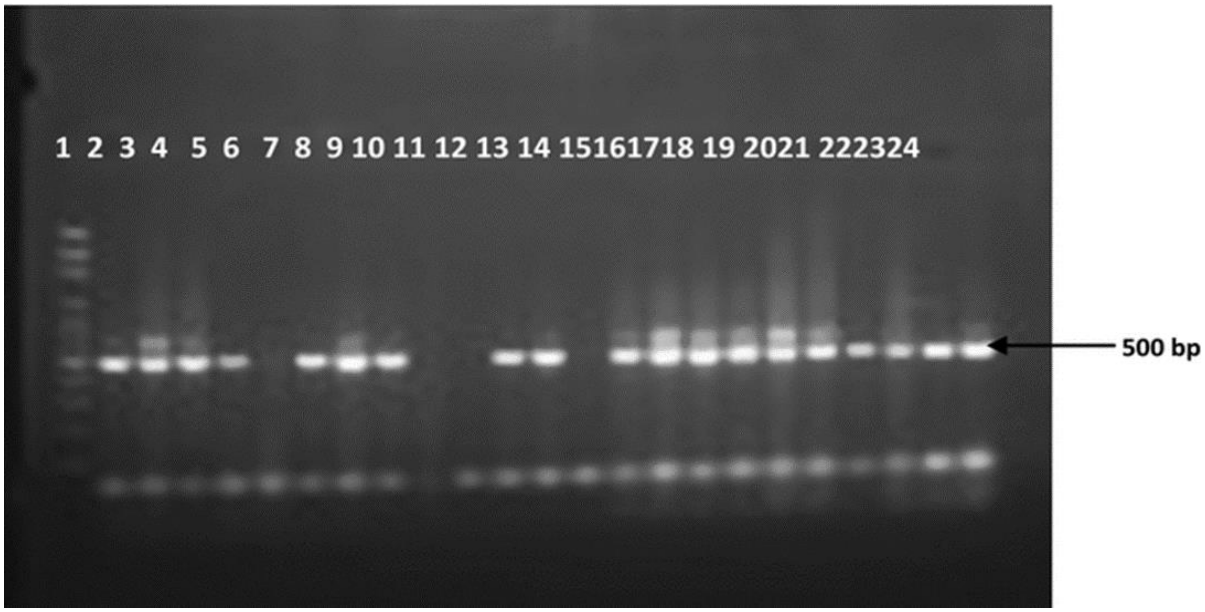

Figure 2: Agarose gel showing confirmation of *Plasmodium falciparum* by18s rRNA gene PCR product. Lane 1 = 100 bp DNA marker; Lane 2 = *P. falciparum* 3D7 control; Lanes 3–24 = Infected blood DNA samples.

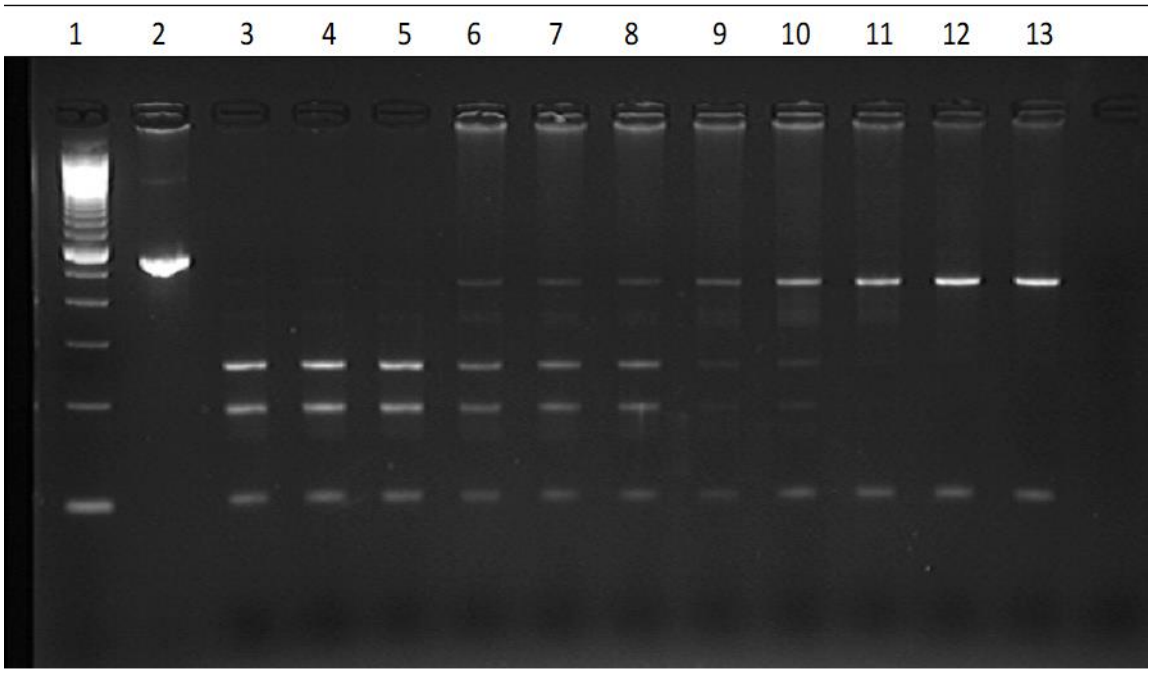

Figure 3: Micrograph of Pfmdr1

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

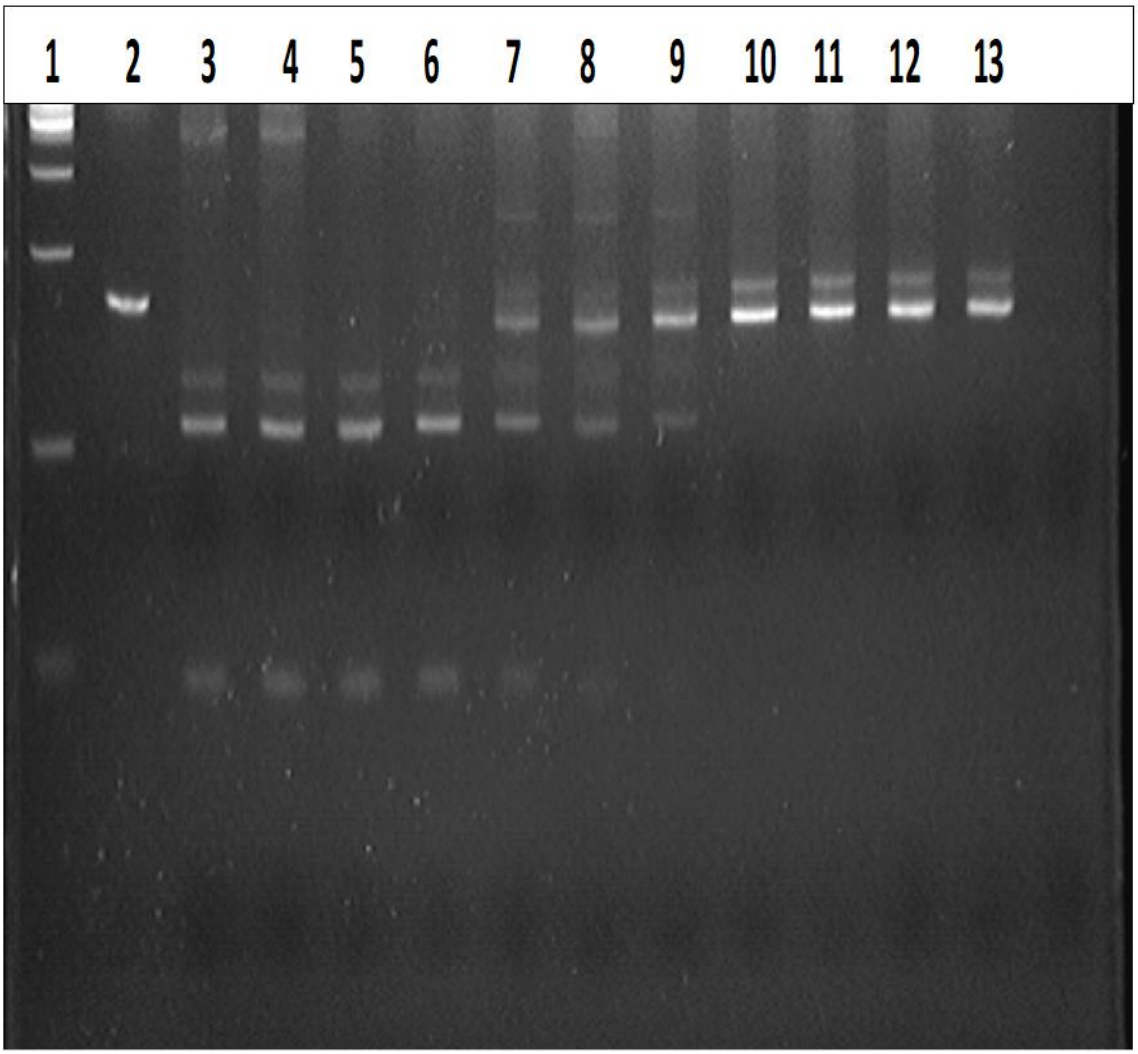

Figure 4: Electro-micrograph of *Pfcrt*
Row 1 Ladder
Row 2 Band sizes (bp) Undigested 164
Row 4, 5, and 6 is 76K – wild type / CQ sensitive 17, 47, 100
Row 7, 8, and 9 is 76K/T – mixture 17, 47, 100, 147
Row 10, 11 and 12 76T – CQ resistant 17, 147
Control DNA
3D7 parasite line – 76K
W2 parasite line – 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 AND DISCUSSION

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*. 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 %) as shown in fig 5. There is no significant difference in the prevalence and study sites ($P<0.05$).

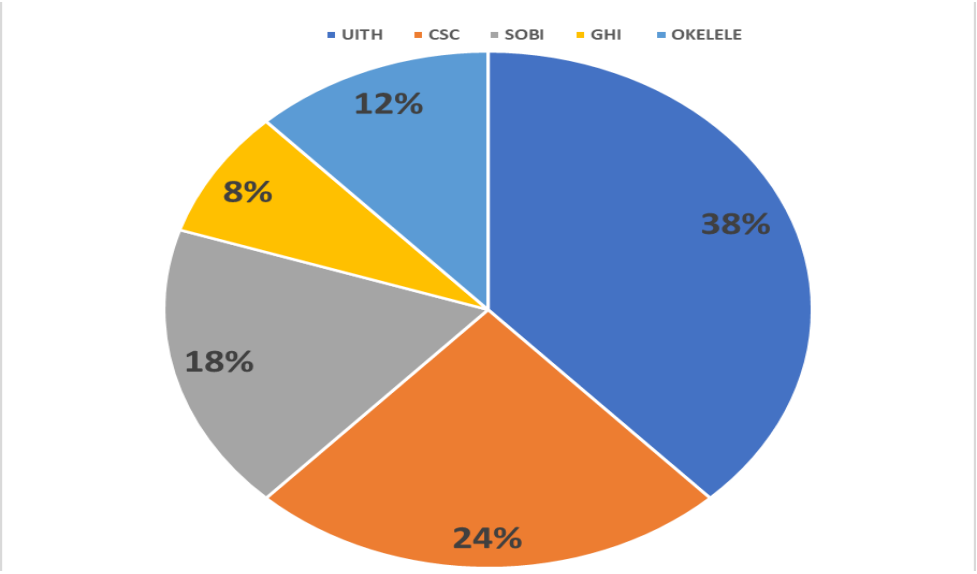

Figure 5: Pie chart showing prevalence of parasitaemia in relation to study sites

The table below shows 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 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**

The table below shows that female has the highest number of resistance alleles 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 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**

The table below shows the distribution of pfcrt and pfmdr 1 alleles in relation to age group. The 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 >40years 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 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 Shows distribution of pfcrt and pfmdr 1 alleles in relation to accommodation type with face to face has the highest prevalence of pfcrt resistance allele (76T) with 56 %, while self- contained has the highest prevalence of pfmdr 1 resistance allele (86Y) and that of mixed infection (K76T) 67 %. The difference is not statistically significant (P<0.05)**.**

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

Malaria is the most causes of mortality and morbidity in tropical parts of the world especially in Africa among children and pregnant women. The prevalence observed in this study is 31% which is higher than 26.7% reported by (Oluwasogo *et al*., 2020). This is quite high and individual should be encouraged to observe various preventive measures such as the use of insecticide treated net (ITN), clearing of bushes around human's dwellings and screening of windows and the doors with net to prevent mosquitoes access to the house. 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 in the early 20s with chloroquine 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 led to 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 common in the study area 40% and 31% respectively while the distribution of *Pfcrt* 76T and *Pfmdr1* 86Ydrug resistance alleles across the study sites are low 10% and 13% respectively. In term of susceptible alleles, the highest prevalence was recorded at UITH, followed by CSC and the least prevalence was recorded at GHI. The difference is statistically significant (P< 0.05) table 4.1. This is an indication of wild type alleles recovery as a result of CQ withdrawal, the same discovery was observed for *Pfmdr1* N86Y, where a decrease in the prevalence of the mutant allele 86Y from 46% to 28% was reported (Mohammed *et al.*, 2017). Moreover, the prevalence of resistance alleles Pfcrt 76T is (10%) which is lower than that reported by (Oluwasogo *et al*., 2020) who documented 68% 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% reported in River state, Nigeria by (Alade *et al*.,2019) and 8.2% in Kenya (Chebore *et al.*, 2020). Variation in distribution pattern of (76T) mutation could be attributed to differences in epidemiological factors and treatment policy adopted by different countries (Muhamad *et al*.,2024).

It is obvious that, there was 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 stagnation 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 down the spread of drug-resistant parasites (Hassan *et al*.,2022).

The studied population revealed presence of wild (Susceptible) alleles for both *Pfcrt* and *Pfmdr1* genes which exceed the number of mutant (Resistance) alleles for the two genes table 4.1 above. These findings may be due to withdrawal of chloroquine (CQ) for the treatment of malaria or drug pressure that continue to mount 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 of both pfcrt 76T and pfmdr 1 86Y table 4.2 above 60% and 57% respectively. This is contrary to the findings of (Mohammed *et al.*, 2017) who reported high frequency of mutant alleles among the male. There is no significant difference between this allele's distribution and gender P<0.05. The mixed mutant infection K76T was more common in female 67% which indicates that females are susceptible to developing 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 shows that age groups 1-5year and >40year have the highest prevalence of both 76T and 86Y mutant alleles whereas, susceptible alleles K76 and N86 were seen amongst all age groups and most prevalent in age groups 1-5 and 6-20 years. This is near in agreement with the reports of (Mohammed *et al.*, 2017) who reported the highest

prevalence in the age group 6-15years .These age groups are more susceptible to malaria due to their immunity as well as outdoor activities that expose them to mosquito bites. The possible cause of this may be linked to CQ withdrawal from circulation for malaria treatment as well as mounting pressure on AL (Mang'era *et al.*, 2012).

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 are highest among the people living in self-contained type of accommodation (67% and 71%). It then signifies 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 the 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 immuned 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

In this study, high prevalence of *falciparum* malaria in Ilorin metropolis, North-central Nigeria was confirmed 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. Consequently, it is pertinent to ensure rational use of antimalaria drugs for the treatment of *P. falciparum* infection to prevent increase in resistant alleles and the spread of resistance parasite, (*P. falciparum*).

RECOMMENDATIONS

This study is suggesting 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 it is cheap, 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, facilitating prompt containment efforts should cases of resistance are identified and informing treatment policy.

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