Distribution of *Pfmdr* 1 and Kelch 13 Genes Among the Children 5 Years and Below Attending a Secondary Health Facility, South-West, Nigeria.

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Research

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Abstract

Background: Malaria is a major public health concern in some part of the world especially in the tropical Africa where children are more vulnerable. The occurrence of resistant gene in *Plasmodium falciparum* to some antimalarial drugs could increase the malaria morbidity and mortality among the children. The study evaluates the distribution *of P. falciparum* resistant kelch protein gene on chromosome 13 (*PfKelch 13*) and multidrug resistant (*Pfmdr1*) mutant genes among children aged five years and below who attended Mother and Child Hospital, Akure, Nigeria.

Methods: Thin and thick smears were prepared from the blood collected aseptically through venepuncture from five hundred (500) children (age 5years and below). Two hundred (200) malaria positive samples were randomly selected from the 500 samples for PCR analysis to detect *Pfmdr1* and *Kelch 13* mutant genes from the positive samples.

Discussion: The results showed that of the 500 respondents who gave their consent to participate in the study, 288 (57.6%) were males while 212 (42.4%) were females. The distribution of *Pfmdr1* are; mixed group (mutant/wild) 38.5% (77/200), mutant gene 35.5% (71/200), wild gene 20.5% (41/200) and the resistant genes were absent in 5.5% (11/200) of the infected children. The mixed group of *Pfmdr1* gene was higher among infants (51.9%), male (44.3%), children with birth order 4 (60.0%) and children that have blood group B (51.3%), however, there is no significant difference in the distribution of *Pfmdr1* between gender ($\chi 2 = 0.634$, df = 1, $p>0.05$). There was a point mutation in the codon position 557 where the amino acid Alanine was replaced by Serine in the *PfK13.* The research revealed high prevalence of *Pfmdr1* mutant genes and point mutation in the *PfK13* gene of *P. falciparum* among children which may be as a result of treatment of malaria with different antimalarial drugs which the parasite has developed resistance against. It is therefore important to administer other malaria drugs apart from the drugs the parasite has developed resistance against.

1.0 Introduction

Malaria is a major public health concern in some part of the world especially in the tropical Africa where children are more vulnerable. In Nigeria, malaria is still responsible for about 60% and 30% of outpatient visits and hospital admissions respectively (Federal Ministry of Health, 2010). The use of Artemisinin Combination Therapy (ACT) has been the first line drug in the treatment of malaria in Nigeria since approval by WHO in 2001. However, emergence and spreading of clones of *P. falciparum* resistance to the most commonly available antimalarial drugs hinders effective control of the disease. There is evidence of resistance of *P. falciparum* malaria to ACT which is caused by mutations in two genes, the *P. falciparum* multidrug resistance transporter-1 (*Pfmdr1*) and specifically, *Plasmodium falciparum* kelch protein gene on chromosome 13 (*PfKelch 13*) (Muhammad et al., 2017; Ashley et al., 2014). In a multi-centre study done across 10 countries in 2014, there was a report of increasing resistance of *P. falciparum* to the ACTs (Ashley et al., 2014). The study reported that slowly clearing infections (clearance half-life >5 hours) were strongly associated with single point mutations in the “propeller” region of the *P. falciparum* kelch protein

gene on chromosome 13 (*Pfkelch13*). The aims of treatment however, as outlined by the World Health Organization (WHO) are to prevent death, prevent long term deficits, to reduce the duration of morbidity of an acute episode of illness and to clear the parasites entirely from blood so that the malaria infection does not re-occur (WHO, 2014); this however cannot be sustained with the spread of these mutant genes across borders. Consequent upon the danger malaria infection poses to children under the age of 5 years due to problems of resistant strains of *P. falciparum* and dearth of information on the subject matter, it will be apt to study this trend in these group of individuals so as to proffer solutions to the menace of parasite resistance to the available drugs. This present study therefore investigated the presence and distribution of *Pfmdr* 1 and *PfKelch 13* genes among the under-5 children in a secondary health facility, South-West, Nigeria. The aims of treatment however, as outlined by the World Health Organization (WHO) are to prevent death, prevent long term deficits, to reduce the duration of morbidity of an acute episode of illness and to clear the parasites entirely from blood so that the malaria infection does not re-occur (WHO, 2014); this however cannot be sustained with the spread of these mutant genes across borders. Consequent upon the danger malaria infection poses to children under the age of 5 years due to problems of resistant strains of *P. falciparum* and dearth of information on the subject matter, it will be apt to study this trend in these group of individuals so as to proffer solutions to the menace of parasite resistance to the available drugs. This present study therefore investigated the presence and distribution of *Pfmdr* 1 and *PfKelch 13* genes among the under-5 children in a secondary health facility, South-West, Nigeria.

2.0 Materials And Methods

2.1 Study area

The study was carried out in the Mother and Child Hospital, Akure (MCHA) from February to July 2019. The hospital (Latitude N7^{0}255'214" and Longitude E5^{0}182'476") was a busy 100-bedded (60 obstetrics and 40 paediatric beds), ultra-modern public facility which provides specialized and effective health care services to the Ondo State capital, ally communities and neighbouring states in the South-Western Nigeria. Akure has two seasons, which includes the rainy (wet) season that ranges from March to October and the dry season that ranges from November to February with an average annual rainfall of 2378mm, temperature range of 25.2^{0}C to 28.1^{0}C and relative humidity of 80% (Simon-Oke et al., 2018).

2.2 Ethical clearance and informed consent

Prior to the commencement of the research, approval was sought from the Research and Ethics Committee of the Mother and Child Hospital and the State Ministry of Health. Informed consent was also obtained from the parents of the participants after the benefits of the research has been fully explained to them.

2.3 Recruitment of study subjects and Sample collection

The study was a cross sectional survey and sampling was done in the hospital on 500 children aged five years and below, recruited from various points of entry into the hospital vis-à-vis emergency room, newborn unit, out-patient department (OPD) and the children's ward.

Five hundred blood samples were collected by venipuncture for malaria parasite test. Thin and thick smears were prepared on sterile slides, which were subsequently stained with Giemsa stain and viewed under the light microscope at X100 magnification. Two to three drops of the positive blood sample were spotted on a 3mm Whatmann filter paper and this was allowed to dry at room temperature (Dry Blood Sample [DBS]). Two hundred positive DBS were then randomly selected for the polymerase chain reaction and other molecular analysis. Demographic data such as age, sex, birth orders, ethnicity, religion, parents' occupation and level of education were collected from the participants and entered into the study questionnaire.

2.4 Malaria parasite Deoxyribonucleic Acid (DNA) extraction from dried blood spots (DBS) using QIAGEN QIAMP DNA extraction mini-kit.

Two pieces of 3 mm disk from the Whatman filter paper dry blood spots were punched out using a sterile hole punch and placed into appropriately labeled 1.5 micro-centrifuge tube, to this, 180 µl of animal tissue lysis buffer was added to ensure the pieces of filter paper were soaked before incubation at 85^{o}C for 10 minutes followed by addition of 20 µl of proteinase K stock solution. The mixture was vortexed and incubated at 56 ^{o}C for 1 hour after which lysis buffer (buffer AL) was added to the sample, thoroughly mixed by vortexing and incubated at 70 ^{o}C for 10 minutes. Absolute ethanol (200 µl) was added and thoroughly mixed. The mixture was then added to a QIAamp Mini kit spin column placed in a 2 ml collection tube and centrifuged at 8000 rpm for 1 minute. The spin column was removed and placed in a clean 2ml well labeled collection tube while the filtrate was discarded with the tube, 500 µL of Wash buffer (Buffer AW1) was then added and the mixture centrifuged at 8000 rpm for 1 minute. The collection tube containing the filtrate was discarded. Buffer AW2 (500 µl) was added to the spin column and then centrifuged at full speed (20,000 x g or 14,000 rpm) for 3 minutes. Again, the filtrate was discarded and the spin column was placed in a 1.5 mL microcentrifuge tube. DNA of the malaria parasite was eluted with 150 µl elution buffer AE, incubated at room temperature for 1 minute and centrifuged at 6,000 x g (8000 rpm) for 1 minute. The extracted DNA was stored in the refrigerator at -20 ^{o}C until it was needed for subsequent molecular studies (Jena Bioscience, 2015). The quality and quantity of extracted DNA yield was 1.87 and 160 ng/µL respectively.

2.5 Genotyping of Pf kelch protein gene on chromosome 13 (kelch13) and Pfmdr 1 mutant genes.

After the molecular screening of the samples for malaria parasites, genotyping analysis of *Pf* kelch protein gene on chromosome 13 (kelch13) and *Pfmdr 1* mutant genes using PCR technique was carried

out only on samples positive for *P. falciparum* using the concentrations of the mixtures as follows: Magnesium chloride; 1.5mM, DeoxyNucleotide TriphosphateS (dNTPs); 0.2mM, forward primer; 0.4mM, reverse primer; 0.4mM, Taq polymerase 0.04 mM, DNA sample 160ng/ µL, PCR water 8.8 µL and Buffer 1.5 µL all in a final volume of 15 µL. The genotyping analysis was done using the appropriate set of primers shown in Tables 1 and 2 after which the PCR products were subjected to electrophoresis analysis (Vathsala et al., 2004).

The primary amplification reaction from the nested PCR method for the *Pfmdr1* involved the use of the primer pair for the forward and the reverse reaction; S 5'-ATGGGTAAAGAGCAGAAAGA-3' and 5'-AACGCAAGTAATACATAAAGTCA -3' respectively for a 250-500 base pair product. The PCR product obtained was used as the template for the secondary PCR using the following primer pairs: S 5'-TGGTAACCTCAGTATCAAAGAA-3' and S 5'- ATAAACCTAAAAAGGAACTGG -3' for a 250-500 base pair product. The PCR reactions were carried out in a final volume of 15 µl, using a DNA Engine Tetrad PTC-225 thermal cycler (MJ Research, USA) with cycling parameters of an initial denaturation at 94°C for 3 minutes (Vathsala et al., 2004) followed by 25 cycles of 92°C for 30 seconds, annealing at 48°C for 45 seconds, extension at 65°C for 1 minutes and a final cycle of extension at 65°C for 5 minutes (**Table 1**). The cycling parameters for the secondary reaction was 94°C for 3mins for initial denaturation, followed by 25 cycles of 92°C for 30secs, annealing at 48°C for 45 seconds, extension at 65°C for 1 minutes and a final cycle at 65°C for 5 minutes all at 25 cycles **(Table 1).**

The primary amplification reaction of the *PfK13* on the other hand involved the use of the primer pair for the forward and the reverse reaction; S 5'-CGGAGTGACCAAATCTGGGA-3' and 5'-GGGAATCTGGTGGTAACAGC-3' respectively for a 849 base pair product. The PCR reactions were also carried out in a final volume of 15 µl. The PCR product obtained from the primary reaction was used as the template for the secondary PCR using the following primer pairs for the forward and reverse reactions: S 5'- GCCAAGCTGCCATTCATTTG-3' and S 5'- GCCTTGTTGAAAGAAGCAGA-3' respectively. The nested PCR was done with initial denaturation at 95°C for 15 minutes (BMRL, 2010) followed by 25 cycles of 95°C for 30 seconds, annealing at 58°C for 2 minutes, extension at 72°C for 2 minutes and a final cycle of extension at 72°C for 2 minutes (Table 2**).** The cycling parameters for the secondary reaction was at 95°C for 15 minutes for initial denaturation, followed by 25 cycles of 95°C for 30secs, annealing at 58°C for 2 minutes, extension at 72°C for 2 minutes and a final cycle at 72°C for 10 minutes all at 25 cycles **(**Table 2**).**

Table 1 Parasite Genotyping for Pfmdr1 (N86Y) -Asparagine to Tyrosine on position 86

Primary

Genes	Primer	Sequence (5_–3_)	PCR Conditions	Size (Base Pair, bp)	References
Pfmdr 1	Forward	ATGGGTAAAGAGCAGAAAGA	Initial Denaturation: 94^{o}C for 3 minutes Denaturation: 92^{o}C for 30 seconds Annealing: 48^{o}C for 45 seconds Extension: 65^{o}C for 1 minute Final extension: 65 ^{o}C for 5 minutes post hold at 4 ^{o}C. All at 25 cycles,	476 (mutant) 256 (wild) 250 (mixed)	Vathsala *et al*., 2004
	Reverse	AACGCAAGTAATACATAAAGTCA			

Secondary Reaction

Genes	Primer	Sequence (5_–3_)	PCR Conditions	Size (Base Pair, bp)	References
Pfmdr 1	Forward	TGGTAACCTCAGTATCAAAGAA	Initial Denaturation: 94^{o}C for 3 minutes Denaturation: 92^{o}C for 30 seconds Annealing: 48^{o}C for 45 seconds Extension: 65^{o}C for 1 minute Final extension: 65 ^{o}C for 5 minutes post hold at 4 ^{o}C. All at 25 cycles,	476 (mutant) 256 (wild) 250 (mixed)	Vathsala *et al*., 2004
	Reverse	ATAAACCTAAAAAGGAACTGG			

Table 2: PCR Amplification of *Plasmodium falciparum K-13* Propeller Gene (*Pfk13*) Primer Sequences:

Primary

Genes	Primer	Sequence (5_–3_)	PCR Conditions	Size (Base Pair, bp)	References
K13	Forward	CGGAGTGACCAAATCTGGGA	Initial Denaturation: 95^{o}C for 15:00mins, Denaturation: 95 ^{o}C for 30s, Annealing: 58 ^{o}C for 2minutes, Extension 72 ^{o}C for 2 mins, Final extension 72 ^{o}C for 10 minutes	849	BMRL, 2010
	Reverse	GGGAATCTGGTGGTAACAGC			

Secondary

Genes	Primer	Sequence (5_–3_)	PCR Conditions	Size (Base Pair, bp)	References
K13	Forward	GCCAAGCTGCCATTCATTTG	Initial Denaturation: 95^{o}C for 15:00mins, Denaturation: 95 ^{o}C for 30s, Annealing: 58 ^{o}C for 2minutes, Extension 72 ^{o}C for 2 mins, Final extension 72 ^{o}C for 10 minutes	849	BMRL, 2010
	Reverse	GCCTTGTTGAAAGAAGCAGA			

2.6 Data Analysis

Carl Pearson Chi-Square was used to determine the significance of resistant genes between gender. Analyses were done using the Statistical Package for the Social Sciences (SPSS) version 20.0 statistical software for Windows (IBM, Armonk, N.Y., United States).

3.0 Results

3.1 Distribution of Pfmdr **1 and** Kelch 13 **genes among the age groups, gender, birth orders and blood groups.**

The molecular study indicated that the *Pfmdr1* presented itself in various forms among the samples examined. The genetic constitutions were classified as mutant, wild, and a mixture of mutant and wild. The mixed group (mutant/wild) was the highest being expressed in 77 (38.5%) children infected with malaria, followed by the mutant gene (35.5%, n = 71), wild gene (20.5%, n = 41) and the resistant genes were absent in 11 infected children (5.5%) (Figure 1). The wild type is the normal chloroquine sensitive genes, while the mutant and the mutant/wild ones are the *Pfmdr1* resistant strains.

The results further showed that 11 (37.9%) of the neonates had the *Pfmdr1* mutant genes, 12 neonates (41.4%) had both mutant and wild genes and 6 neonates (20.6%) had the wild type (Table 3). Among the infants, 30 (37.0%) had the mutant genes, 42 infants (51.9%) had both mutant and wild genes while 9 (11.1%) had the wild type. In the age groups >12 months to 5 years, 32 children (40.5%) had the mutant genes, 40 children (50.6%) had both the mutant and the wild genes and 7 (8.9%) had the wild genes. Forty-four (38.3%) of the males had the mutant genes, 51 (44.3%) had both the mutant and wild genes and 20 males (17.4%) had the wild genes. Similarly, 29 females (39.2%) had the *mutant* genes, 30 females (40.5%) had both mutant and wild genes and 15 females (20.3%) had only the wild genes. However, Chi-square analysis showed that there is no significant difference in the distribution of *Pfmdr1* between gender ($\chi 2= 0.634$, df = 1, $p>0.05$). The distribution of malaria parasite wild mutant genes (*Pfmdr1*) was present in all birth orders except birth order greater than 5. However, the mutant/wild genes were highest (60%) in birth order 4 and lowest (33.3%) in birth order greater than 5. The wild type was highest (66.7%) in birth order greater 5 and lowest (10%) in birth order 4 (Table 3).

The result also revealed that mutant gene was highest (60%) among AB blood group and lowest in blood group B. Similarly, the wild type resistant gene was highest (30%) in blood group AB and lowest (15.4%) in both blood group A and B. In contrast, the mixed type (mutant/wild) was highest (51.3%) in blood group B and lowest in blood AB (10%).

Table 3
The Distribution of malaria Parasite resistant genes (*Pfmdr1*) among various groups

Variables	Mutant gene (%)	Mutant /wild (%)	Wild (%)	df	P-value
Age Groups:					
Neonates	11(37.9)	12 (41.4)	6 (20.7)		
Infants	30 (37.0)	42 (51.9)	9 (11.1)	0.005	
>12months-5yrs	32 (40.5)	40 (50.6)	7 (8.9)		
Gender:					
Male	44 (38.3)	51 (44.3)	20 (17.4)	1	0.634
Female	29 (39.2)	30 (40.5)	15 (20.3)		
Birth Orders:					
1	37 (44.6)	33 (39.8)	13 (15.6)		
2	21 (35.0)	24 (40.0)	15 (25.0)	4	0.003
3	15 (45.5)	13 (39.4)	5 (15.2)		
4	3 (30.0)	6 (60.0)	1 (10.0)		
≥5	0 (0.0)	1 (33.3)	2 (66.7)		
Blood Groups:					
A	9 (34.6)	13 (50.0)	4 (15.4)		
B	13 (33.3)	20 (51.3)	6 (15.4)	3	0.029
AB	6 (60.0)	1(10.0)	3 (30.0)		
O	51 (44.7)	43 (37.7)	20 (17.5)		

NB: Total Positive by DNA PCR out of the randomly selected 200 positive samples =189

The results as presented in Table 4 revealed that there was a point mutation in the codon position 557 where the amino acid Alanine was replaced by Serine in the *PfK13* detected in some of the blood samples of the participants in the current study. Table 4 also showed the codon positions in the first column, the second column showed the reference amino acid for the wild type (sensitive), the third column showed the amino acids for the mutant genes and the last column showed the observed amino acids for the test samples. The codon positions 580, 612, 476, 569, 449, 557, 458, 617 and 112 have the amino acids Cysteine (C), Glutamic Acid (E), Methionine (M), Alanine (A), Glycine (G), Alanine (A), Asparagine (N), Alanine (A) and Glycine (G) respectively for the reference amino acids.

Plate 1 showed the screen shot of the *PfK13* amino acid sequence alignment and when observed closely, there were point mutations in some of the amino sequence in some of the codon positions, for example, the amino acid Proline had replaced Alanine in the DNA sequence of the *PfK1*3_6 species in the last yellow column, there were also two point mutations in the 6th and 9th column of the same *PfK13* species.

Table 4
Sequence Comparison with global reported SNPS in *pfk13*

CODON POSITION	REFERENCE AMINO ACID (wild type)	MUTANT TYPE AMINO ACID	OBSERVED AMINO ACID
580	C	Y	C
612	E	D	E
476	M	I	M
569	A	S	A
449	G	A	G
557	A	S	S
458	N	Y	N
617	A	T	A
*112	G	E	G
* Non-propeller mutation			

Number of Mutant alleles Detected = 0

SNPs: single nucleotide polymorphisms

Amino Acids: Cysteine (C), Glutamic Acid (E), Methionine (M), Alanine (A), Glycine (G), Asparagine (N), Tyrosine (Y), Aspartic Acid (D), Isoleucine (I), Serine (S), Threonine (T).

3.2 DNA Electrophoresis of *Pfmdr1 (N86Y)* and *PfK13* genes

Two hundred blood samples were randomly selected from the 261 positive samples for molecular tests. The image gel in Plate 2 showed the electrophoregram of *Pfmdr*1 (N86Y) gene Apo1 digest products resolved on 2% Agarose gel. Restriction enzyme was used to identify the mutated gene and to express the percentages of the manifestation of various genotypes such as mutant, wild and mixed types. Lanes 1 and 10 are the 100 base pairs (bp) DNA Ladder. The loaded wells showed bands that separated from the well to a base pair of 476 bp for the mutant type, 250 bp for the wild type and 226 bp for the mixed type. The 226 bp being the lightest migrated fastest ahead of 250bp and the 476bp mutant genes. Lanes 2 to

5, 7 and 9 were the undigested mutant (86Y) strains. Lanes 6 and 8 were digested (wild type or the sensitive genes).

The image in Plate 3 showed the electrophoregram of *PfKelch13* propeller gene nest 2 PCR amplicons resolved on 1.0% Agarose gel. Lane 1 showed the 1.3kB DNA ladder while lanes 2-7 showed the undigested *PfKelc*h 13 amplicons (mutant genes).

4.0 Discussion

In the current study, the prevalence of the *Pfmdr1* mutant gene among the neonates was 37.9%, 37.0% among the infants and 40.5% among the children 12 months to 5 years. These values are comparable to the reports of Muhammad et al. (2017) from some states in the northern parts of Nigeria, the authors reported prevalence of *Pfmdr1* mutant gene to be 32.6% in Jigawa State, 31.1% in Kebbi State and 33.0% in Katsina State. The prevalence of *Pfmdr1* mutant gene is however, higher than the prevalence of 18.9% reported in Kaduna and 11.3% in Kano (Muhammad et al., 2017). The authors also reported that prevalent across the various age groups had no clear demarcation except in age 1 to 5 years where highest prevalence of 31.1% was reported. Factors such as farmland activities, especially irrigation farming and late night farming have been suggested to have contributed to increased transmission of malaria and the higher prevalence of resistance alleles obtained in Jigawa and Katsina States but the current study center is in the metropolitan city with relatively round the year malaria transmission but with peaks in the wet season. The prevalence of the mutant genes in the current study is higher than the prevalence of 15.0% reported by Bui Quang *et al*. (2019) in the Vietnam. Higher prevalence of mutations in *Pfmdr1* confers resistance to chloroquine, artemether, mefloquine, artemisinin, ACT and non-ACT, lumefantrine, primaquine, tafenoquine, piperaquine, and mefloquine (Dokunmu et al., 2019) and this was also previously reported in Nigeria by Olasehinde et al. (2014) and Oladipupo et al. (2015).

The *Pfmdr1* prevalence of 38.3% among the males and 39.2% among the females in the current study is higher than reports from the northern parts of Nigeria with prevalence of 32.1% (male) and 25.7% (female) and the resistant genes are common among the males in the north but there is no significant difference between the male and female in the current study.

The nested PCR reaction showed that there was a point mutation in the codon position 557 where the amino acid Alanine was replaced by Serine in the *PfK13* detected in some of the blood samples of the participants in the current study. The sequencing also revealed that there were point mutations in some of the amino sequence in some of the codon positions, the amino acid Proline had replaced Alanine in the DNA sequence of the *PfK1*3_6 species and the electrophoresis showed the undigested *PfKelc*h 13 amplicons bands. However, restriction enzymes were not applied and hence the phenotypes and the percentages of the mutated genes could not be determined. This result is similar to findings in some African countries where *K13* non synonymous polymorphisms have been reported at low frequencies in isolates in some African countries such as Cameroon, Central African Republic, Democratic Republic of the Congo, Gabon, Gambia, Kenya, Madagascar, Malawi, Mali, Rwanda, Togo, Uganda, Zambia and

Equatorial Guinea (Isozumi et al., 2015; WHO, 2015; Gupta et al., 2018). On the other hand, *K13* mutant falciparum isolates were highly prevalent in Binh Phuoc and Dak Nong provinces of Vietnam and in these 2 provinces, only the C580Y mutant was detected. In Ninh Thuan province, however, the majority of isolates had wild-type *K13* and only 1 isolate with both *K13* (C580Y) was reported (Bui Quang *et al., 2019*). The current study also differ from the report from Mozambique where all the three hundred and fifty-one *P. falciparum* isolates all carried *K13* wild-type alleles (3D7-like) (Gupta et al., 2018).

5.0 Conclusions

The study has been able to reveal that both *PfK13* and *Pfmdr 1* mutant genes were identifiable in children aged 5 years and below in Akure, Ondo State, South-west, Nigeria. The genes were identified in both genders, in all age groups, blood groups, and birth orders and these were responsible for development of resistance to so many of the drugs currently in use for the treatment of malaria; more research into the discovery of newer and more efficacious antimalarial drugs aside the ACTs currently in use for the treatment of malaria is therefore advocated.

Declarations

Ethics approval and consent to participate: Ethical clearance and informed consent of the participants were obtained as discussed in the materials and methods.

Consent for publication: The authors gave their consent to the BMC Malaria Journal to publish this manuscript if accepted for publication.

Availability of data and materials: All data generated or analyzed during this study are included in this published article

Competing interests: The authors declare that they have no competing interest

Funding: Not applicable

Authors' contributions: ORO, AOJ and OMO involved in the study design. ORO and AOJ involved in the field and the laboratory work. Data analysis was undertaken by the authors ORO and AOJ. ORO and AOJ were the major contributors in writing the manuscript. The authors read and approved the final manuscript.

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Figures

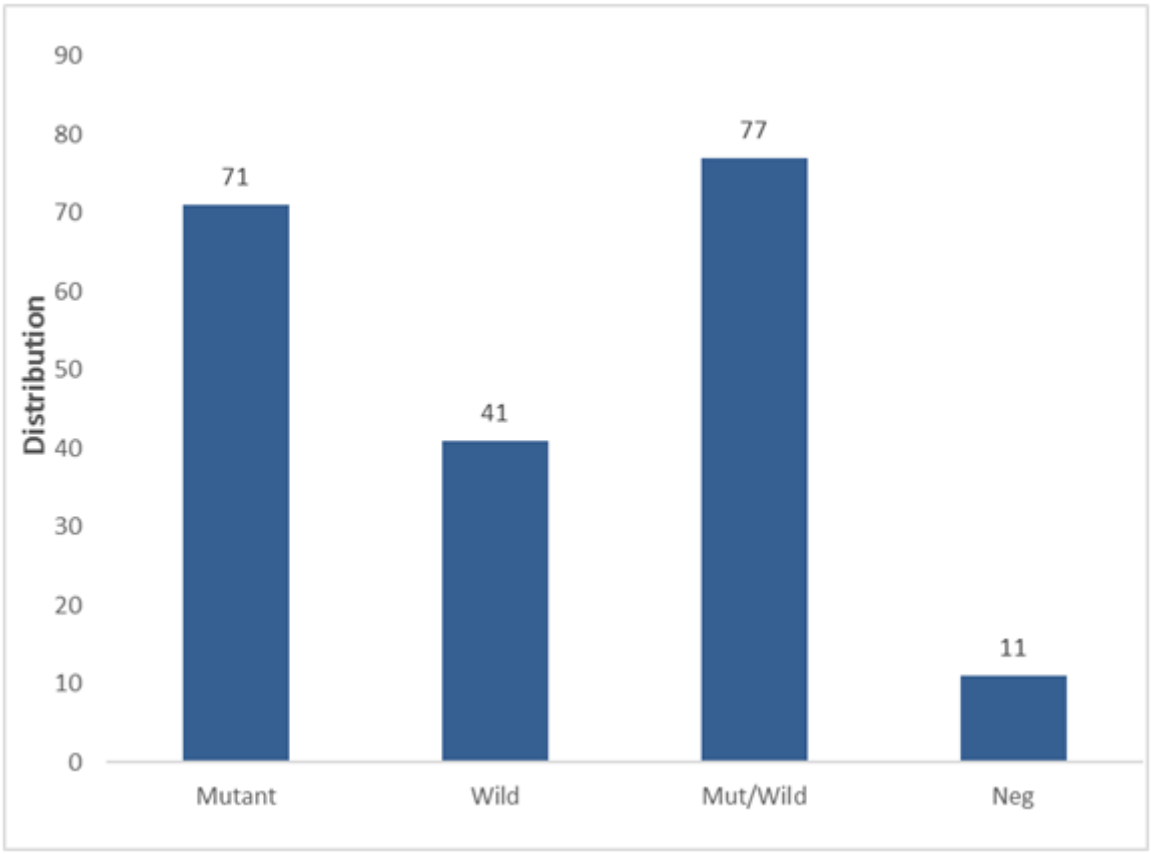

Figure 1

Phenotypic presentation of Plasmodium falciparum (Considering 200 samples after sequencing for Pfmdr1)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Plate1.png
- Plate2.png
- Plate3.png