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Evaluation of antimalarial prescription pattern and susceptibility of *Plasmodium falciparum* isolates in Kaduna, Nigeria

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ABSTRACT

Nigeria carries the highest burden of malaria in terms of morbidity and mortality. This is compounded by continuous resistance of *Plasmodium falciparum* to antimalarial drugs. This study was designed to evaluate the profile of malaria patients' antimalarial drug prescription and *in vitro* susceptibility of *P. falciparum* isolates to commonly prescribed antimalarial drugs in Kaduna, Nigeria. Three years' records of patients antimalarial drug prescriptions were collated (2013 to 2015) and the *in vitro* antimalarial agent susceptibility was determined for 28 clinical isolates using WHO Mark III microtest. Artemisinin-based combination therapy (ACT) was the most prescribed antimalarial for the period under review (92.3-93.7%). Among the ACTs, Artemether-lumefantrine was most prescribed. Of the 28 *P. falciparum* isolates evaluated, 3 (10.71%) were resistant to chloroquine with a median IC₅₀ of 4.82µM (4.60-8.14µM), while five (17.86%) were resistant to mefloquine with a median IC₅₀ of 25µM (10.3-41µM), 7(25.00%) to artemether with a median IC₅₀ of 2.69µM (2.09-8.77µM), 9 (32.14%) to artesunate-mefloquine combination with a median IC₅₀ of 9.0µM (7.98-35µM) and to artesunate, 11(39.29%) were resistant with a median IC₅₀ of 2.4µM (1.56-5.65µM). This result shows a decline in resistance of *P. falciparum* to chloroquine compared to period prior to artemisinin-combination therapy as well as reduced susceptibility to artesunate and artemether. Further *in vitro* and *in vivo* monitoring will be required to inform antimalarial drug policy change.

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Keywords: Antimalarial, Artemisinin-combination therapy, resistance, susceptibility, microtest.

INTRODUCTION

Malaria is a disease of wide distribution caused by *Plasmodium* (Lucas and Gilles, 2003). The four species infecting man namely *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* are found mainly in tropical and subtropical regions (Shapiro and Goldberg, 2006; Wesolowski, 2015). Malaria remains a disease of major public health importance globally due to the high morbidity

and mortality associated with it (WHO, 2010). Children under-five are most affected and complications such as anaemia resulting from untreated or severe malaria causes death (Ukibe et al., 2010). According to World Health Organization (WHO) recent report, 228 million cases and 405,000 deaths occurred as a result of malaria in 2018 with 93% of these affecting Africa (WHO, 2019).

Plasmodium falciparum has been reported as the most prevalent and causes the

most fatal malaria disease in Africa and it has been estimated to account for up to 99.7% of malaria cases in 2017 (WHO, 2018). Antimalarial drug resistance emerges as one of the greatest challenges facing malaria control today (Olaseinde et al., 2015) and recent reports of *Plasmodium falciparum* resistance to artemisinin and its derivatives in Cambodia threatens the control and elimination efforts (Ariey et al., 2014). Multidrug resistance of *Plasmodium falciparum* to commonly used drugs have been reported to weaken the therapeutic approach to the control of malaria (Ngbolua et al., 2011). Comprehensive and up-to-date understanding of the scope of antimalarial drug resistance is therefore essential for protecting the recent advances which have been attained in malaria control efforts (WHO, 2010).

Nigeria is the highest index country accounting for 25% of malaria cases and 24% of global mortality due to infections related to the most prevalent malaria parasite (WHO, 2018). It is therefore important to continue to monitor drug response of *Plasmodium falciparum* in different parts of Nigeria especially as cases have increased in recent reports. *In vitro* susceptibility testing is an epidemiological tool for assessing baseline sensitivity and for monitoring drug response provides information for early containment of drug resistance and timely revision of treatment policies (WHO, 2001). The use of artemisinin-combination therapy became effective in Nigeria in 2004. However, following reports of resistance to ACTs worldwide and Nigeria bearing the highest burden of malaria disease (WHO, 2019), monitoring for resistance becomes imperative as emergence of resistant strains of *Plasmodium falciparum* to currently used antimalarial drugs especially the artemisinins would further worsen the huge burden of malaria in Nigeria (WHO, 2010).

This study was designed to evaluate *in vitro* effectiveness of currently prescribed antimalarial agents against *Plasmodium falciparum* isolates from patients in Kaduna, Nigeria and to highlight the resistance factors responsible for reducing susceptibility of

Plasmodium falciparum isolates in Kaduna, Nigeria.

MATERIALS AND METHODS

Study areas

The study was carried out in two health facilities, Barau Dikko Teaching Hospital (BDTH) and Jowako Specialist Hospital (JSH); both Kaduna North Local Government Area of Kaduna State, Nigeria. The study was carried out from March 2016 to June 2018. Barau Dikko Teaching Hospital is a tertiary health facility while Jowako Hospital is a private health facility both located in the urban centre of Kaduna, Nigeria. Average annual rainfall is 1211 mm and temperature range is 19.3 °C - 46.5 °C. Hospital records of patients were accessed for antimalarial drugs prescriptions made for patients diagnosed to have the disease within the study period.

Ethical considerations

Ethical approval for the study was sought and obtained from the institutions' ethical review committees as well as individual patient's consent.

Statistical analysis

Data obtained from this study was analysed using Microsoft Excel to obtain descriptive statistics and are presented on tables.

Reagents / materials used

Antimalarial drugs: Artesunate-mefloquine 600/750, artesunate, chloroquine, and artemether. RPMI1640 (Sigma Chemical company, Germany), microtitre plates, Giemsa stain, *Plasmodium falciparum* positive patients blood sample, ethanol/methanol, microscope slides with frosted ends, primers (k-13, Pfmdr1, PfATPase6).

Study protocol and sample collection/ in vitro microtest

Blood samples were collected from patients attending the health facility with the assistance of the medical laboratory scientists. A total of 114 patients who consented to be part of the study after informed consent was

obtained were enrolled to participate. Male and female participants were aged 3-60 years. Blood from consenting patients with ancillary temperature above 37.5 °C, mono-infection with *P. falciparum*, and parasitaemia ranging from >1000-<80,000 asexual parasites per µl of blood was used for the study. All patients who had taken any antimalarial two weeks preceding study or with any other symptoms or signs of non-malaria aetiology were excluded from the study. Blood samples which did not have the required parasitaemia were also excluded from the *in vitro* study. Thick and thin smears were prepared to determine species and quantitative count of parasites according to standard protocol (Cheesbrough, 2006).

Stock solutions of the following antimalarial drugs; chloroquine, mefloquine, artesunate and artemether were prepared in appropriate solvents. The solutions were then filtered aseptically in 0.22 millipore membrane filter. The solutions were further diluted to obtain working solutions. Five concentrations of working solutions were prepared in two serial dilutions and these graded concentrations of each test antimalarial agents were used to dose the 96-well flat bottomed microtitre plates aseptically. The *in vitro* micro-test drug susceptibility tests were carried out according to standard techniques described by Trager and Jensen (1976), and adapted Mark III micro-test (WHO, 2001) under strict aseptic conditions.

Examination of the post-culture blood smears / interpretation of results

The thick films obtained following harvesting of post-culture slides were stained using Giemsa (3%) for 30 mins and dried for 48 hours. They were then examined with the oil immersion objectives (100X) and the number of schizonts (matured malaria parasites) were counted in each of the wells and compared with the control for every sample isolate. At the end of the incubation period, schizont maturation in the control well should be ≥ 10% per 200 asexual parasites. Where the value in the control was less than 10%, such results were discarded as unsatisfactory growth.

The counts in each well were expressed as percentages of the control as shown below:

Z=number of schizonts per 200 asexual parasites in control well after incubation

Y=number of schizonts per 200 asexual parasites in wells dosed with drugs after incubation

X=% of schizonts relative to control samples

$$X (\%) = Y/Z \times 100$$

Parasite growth inhibition for each isolate was calculated as:

$$\text{Percentage schizont inhibition} = 100 - X$$

Individual percentage schizont inhibitions were fed directly into Microsoft Excel and using linear regression line analysis, their inhibitory concentrations; IC₅₀ was determined. Drug-resistant parasites were identified as parasites with IC₅₀ values greater than the peak plasma concentration of the antimalarial drugs used.

DNA Extraction and polymerase chain reaction

Extraction of the DNA from dried blood spots (DBS) on filter paper was carried out using the AccuPrep® Genomic DNA Extraction kit and followed Chellex-100 extraction method (Bio-Rad, USA). The DBS was cut into 1.5 ml tubes; 200µl of 1xPBS was added into each tube and placed on a heat block at 50 °C for 1hour. This was allowed to cool at room temperature and then vortex for 10 secs after which 20 µL of Proteinase K was added to each of the tubes containing the sample. 200 µL GB buffer was added and vortex immediately to mix. Mixture was incubated at 60 °C for 10 mins. Absolute ethanol (400 µL) was added to each of the sample, (the binding columns were fixed into the collection tubes) and the lysate transferred into the upper column of the tubes and centrifuged at 8,000 rpm for 1 min. Washing buffer 1 (500 µL) was added to wash and remove ethanol, centrifuged for 1 min and the process repeated using same volume of Washing Buffer 2. The solution from the collection was discarded after each round of wash. It was centrifuged once more at 13,000 rpm for 1 min to completely remove ethanol

and any other droplet remaining on the collection tube.

The binding columns were then transferred to new 1.5 ml tubes for elution by adding 50 μ L EA buffer and left for 1 min at room temperature. It was again centrifuge at 8,000 rpm for 1 min to elute the DNA. The tubes containing primers were spin at 13,000 rpm for 1 min and reconstituted under PCR Hood using nuclease-free water and then mixed. The primers were diluted in nuclease-free water in the ratio (10 μ L:90 μ L) as working solutions.

The premix for the PCR contained dNTPs, Taq polymerase, MgCl₂, PCR buffer. To the premix was added 16 μ L nuclease-free water, 2 μ L primers, 2 μ L DNA of *P.falciparum* (positive control) to make a total volume of 20 μ L. This was mixed gently till it dissolved and spun briefly to allow solution settle down in the tubes. The primers and conditions used for the molecular characterization set on the PCR machines (PTC-100 Programmable Thermal Controller, MJ research Inc.). The primers used are presented in Table 3. The products of the nested PCR were resolved by 1.5% gel electrophoresis, stained with ethidium bromide and visualized with a Bio-Rad Transluminator (Universal Hood II). The results of this were documented and are presented on Plates 1-3.

RESULTS

Retrospective study

Artemisinin-based combination therapy (ACT) was the most prescribed in the two health facilities for the period under review; Barau Dikko Teaching Hospital (92.3%) and Jowako Specialist Hospital (93.7%) (Table 1). Artemether-lumefantrine was most prescribed ACT (71.9% and 59.1%) in the public and private health facility respectively while artesunate-amodiaquine combination was the least ACT prescribed (0% and 0.5%). Table 2 shows pattern of specific ACT prescriptions (%). Other antimalarial drugs prescribed were artesunate (2.7% and 3.0%), sulphadoxine-pyrimethamine (2.4% and 2.6%) and

chloroquine (1.1% and 2.1%) from the public and private facility respectively.

In vitro assay

The degree of schizont inhibition by each of the antimalarial agents resulting in 50% inhibition of *Plasmodium falciparum* isolates are as presented Table 4. Of the 28 *P.falciparum* isolates evaluated, 3 (10.71%) were resistant to chloroquine with a median IC₅₀ of 4.82 μ M (4.60-8.14 μ M) while 25(89.30%) were susceptible with median IC₅₀ of 1.85 μ M (0.49-4.33 μ M) in tandem with the peak plasma concentration of 4.47 μ M. Seven (25.00%) were resistant to artemether with a median IC₅₀ of 2.69 μ M (2.09-8.77 μ M) while twenty-one (75.00%) were sensitive with a median IC₅₀ of 0.62 μ M (0.07-1.71 μ M) based on the peak plasma concentration of artemether at 1.81 μ M. Eleven (39.29%) were resistant to artesunate with a median IC₅₀ of 2.4 μ M (1.56-5.65 μ M) and 17(60.70%) were sensitive with a median IC₅₀ of 0.44 μ M (0.14-1.1 μ M) based on a peak plasma concentration of 1.17 μ M. Five (17.90%) were resistant to mefloquine with a median IC₅₀ of 25 μ M (10.3-41 μ M) while 23(82.10%) were sensitive with a median IC₅₀ of 1.79 μ M (0.09-9.04 μ M) based on the peak plasma concentration of 10.0 μ M. Nine (32.14%) were resistant to artesunate-mefloquine combination with a median IC₅₀ of 9.0 μ M (7.98-35 μ M) while 19(67.90%) were sensitive with a median IC₅₀ of 2.26 μ M (0.43-6.09 μ M) based on a peak plasma concentration of 1.17/7.9 μ M.

Drug resistance characterization

Among the *Plasmodium falciparum* isolates which were resistant *in vitro* to the antimalarial agents tested, five isolates which exhibited *in vitro* resistance to artemisinins, chloroquine and mefloquine were assayed to determine the presence of molecular markers responsible for antimalarial resistance. The molecular markers of resistance assayed were *P. falciparum* multidrug resistant 1 (Pfmdr-1), PfATPase-6 and kelch13 propeller (K-13).

Twenty per cent (20%) of the *Plasmodium falciparum* isolates were observed to carry the Pfmdr-1 resistance gene

which amplified around 600 bp and PfATPase-6 gene which amplified around 800 bp while 60% carried the K-13 gene which amplified around 850 bp. Plates 1-3 show the results of the gel electrophoresis for the detection of Pfmdr-1, Pfatpase-6 and Kelch 13 genes respectively. The presence of Pfmdr-1 gene has been implicated in quinolone resistance, e.g. chloroquine. It may also be

implicated in artemisinin resistance because of cross-resistance between chloroquine, aminoalcohols (mefloquine) and artemisinins (Basco and Ringwald, 2002). *Plasmodium falciparum* adenosine triphosphate 6 (PfATPase6) gene; a sarco-endoplasmic reticulum Ca²⁺ adenosine triphosphate-ATPase-(SERCA) type protein is the primary target of artemisinins.

Table 1: Average pattern of antimalarial drug prescriptions (%) in two health facilities in Kaduna, Nigeria from 2013-2015.

Drugs prescribed	2013		2014		2015		Average	Average
	BDTH (n=241)	JSH (n=169)	BDTH (n=354)	JSH (n=224)	BDTH (n=380)	JSH (n=217)	BDTH (n=325)	JSH (n=203)
ACT*	91.3	94.7	97.2	92.0	92.6	90.3	93.7	92.3
Chloroquine	0.4	1.8	1.1	3.6	1.8	0.9	1.1	2.1
SP**	2.5	0.6	1.1	1.8	3.7	5.5	2.4	2.6
Artesunate	5.8	3.0	0.6	2.7	1.8	3.2	2.7	3.0

* ACT- Artemisinin-combination therapy

**SP- sulphadoxine/pyrimethamine

Table 2: Pattern of reported Specific ACT prescriptions in two health facilities in Kaduna, Nigeria from 2013-2015 (%).

Specific ACT*	2013		2014		2015		Average	Average
	BDTH (n=220)	JSH (n=160)	BDTH (n=344)	JSH (n=206)	BDTH (n=352)	JSH (n=196)	BDTH (n=305)	JSH (n=187)
I	61.8	58.1	81.3	49.5	72.7	69.9	71.9	59.1
II	6.8	36.9	1.7	48.5	17.0	27.6	8.5	37.7
III	23.6	-	14.8	0.5	7.4	2.0	15.3	0.8
IV	0.5	5.0	-	0.5	-	0.5	0.2	2.0
V	7.3	-	2.0	0.5	0.3	-	3.2	0.2
VI	-	-	-	-	2.6	-	0.9	-
VII	-	-	-	0.5	-	-	-	0.2

*I= Artemether lumefantrine

II= Dihydroartemisinin- piperaquine

III= Artesunate- mefloquine

IV= Artemether- mefloquine

V= Artesunate-sulphadoxine/pyrimethamine

VI= Artemether- sulphadoxine/pyrimethamine

VII=Artesunate-amodiaquine

BDTH- Barau Dikko Teaching Hospital

JSH- Jowako Specialist Hospital

Table 3: Primers used and their sequence.

Gene	Primer	Sequence	Size (bp)
Pfmdr-1	Pfmdr-1(F)	ATGGGTAAAGAGCAGAAAGA	603
	Pfmdr-1(R)	AACGCAAGTAATACATAAAGTCA	
PfATPase6	PfATPase6 1(F)	AATATTGTTATTCAGAATATGATTATAA	896
	PfATPase6 1(R)	TGGATCAATAATACCTAATCCACCTA	
	PfATPase6 2(F)	AGCAAATATTTTCTGTAACGATAATA	798
	PfATPase6 2(R)	TGTTCTAATTTATAATAATCATCTGT	
K-13 propeller	K13-out F	GGAATCTGGTGGTAACAGA	849
	K13-out R	CGGAGTGACCAAATCTGGGA	
	K13-in F	GCCTTGTTGAAAGAAGCAGA	
	K13-in R	GCCAAGCTGCCATTCATTTG	

Table 4: Concentration of antimalarial agents that gave 50% inhibition of *P. falciparum* isolates in two health facilities in Kaduna, Nigeria.

S.No	Chloroquine PPL(4.47µM)	Artemether PPL(1.81µM)	Artesunate PPL(1.17µM)	Mefloquine PPL(10µM)	Art/mef* PPL(1.17/7.9µM)
1	0.69	0.62	0.41	5.21	0.55
2	4.82	0.07	1.86	1.07	0.66
3	3.23	0.28	0.52	2.31	0.43
4	2.73	2.69	4.06	2.74	2.26
5	2.81	0.27	0.14	0.75	1.29
6	3.15	2.76	2.53	1.77	2.20
7	1.82	1.71	3.73	1.56	2.87
8	3.05	3.07	2.49	5.09	3.31
9	1.75	0.85	1.56	1.53	1.21
10	3.56	0.43	1.96	0.56	1.77
11	4.60	2.65	2.28	0.54	1.85
12	3.26	0.75	2.40	2.09	2.79
13	0.67	0.16	0.87	0.09	2.87
14	3.56	2.11	1.97	1.79	2.26
15	1.64	0.33	0.36	0.42	2.53
16	1.55	0.8	0.79	4.07	8.29
17	0.96	0.53	0.91	0.25	0.92
18	1.85	1.56	1.10	35.8	9.70
19	4.33	0.71	0.44	6.85	13.1
20	0.69	0.34	0.37	5.57	8.60

21	8.14	8.77	5.65	36.4	35.0
22	2.10	0.42	0.44	41.0	7.98
23	1.88	0.52	0.18	4.42	8.02
24	0.68	0.7	0.40	0.61	8.99
25	2.72	2.09	1.06	18.90	5.47
26	1.33	1.03	0.94	10.25	5.25
27	0.49	0.76	0.67	7.86	6.09
28	1.35	1.36	0.39	9.04	9.12
%Resistance	3(10.71)	7(25.00)	11(39.29)	5(17.86)	9(32.14)

*PPL- Peak plasma level

Art/mef- artesunate- mefloquine

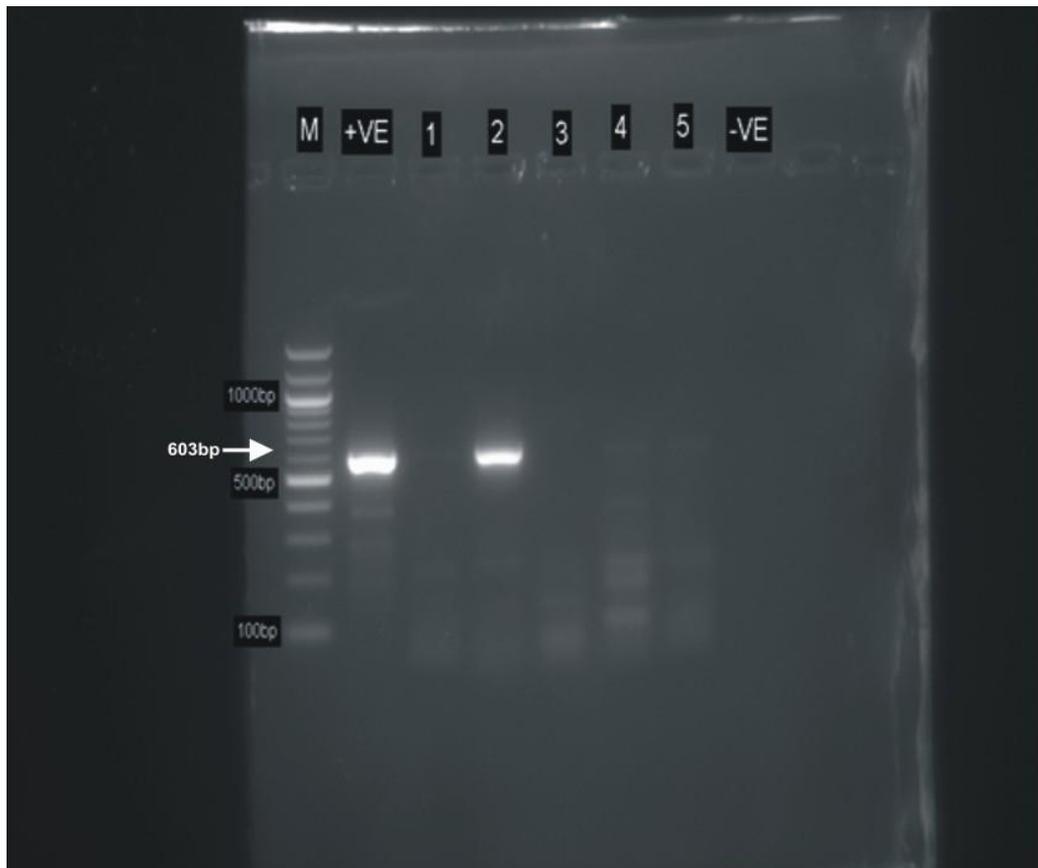


Plate 1: Amplification of Pfmdr-1 gene @ 603bp from DNA of *P. falciparum* isolates from patients in two hospitals in Kaduna, Nigeria.

*M=Marker

+ve= positive control (standard *P. falciparum* isolate)

-ve=negative control (PCR mix and water)

Nos.1-5= resistant *P. falciparum* sample isolates.

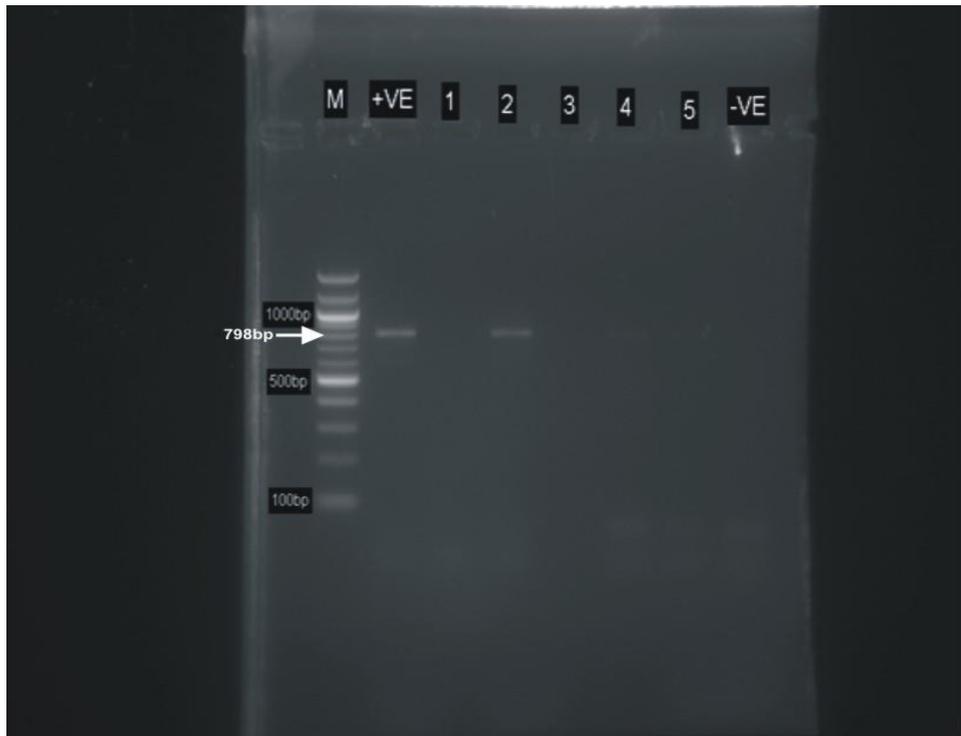


Plate 2: Amplification of Pfatpase-6 gene @ 798bp from DNA of *P. falciparum* infected patients in two hospitals in Kaduna, Nigeria.

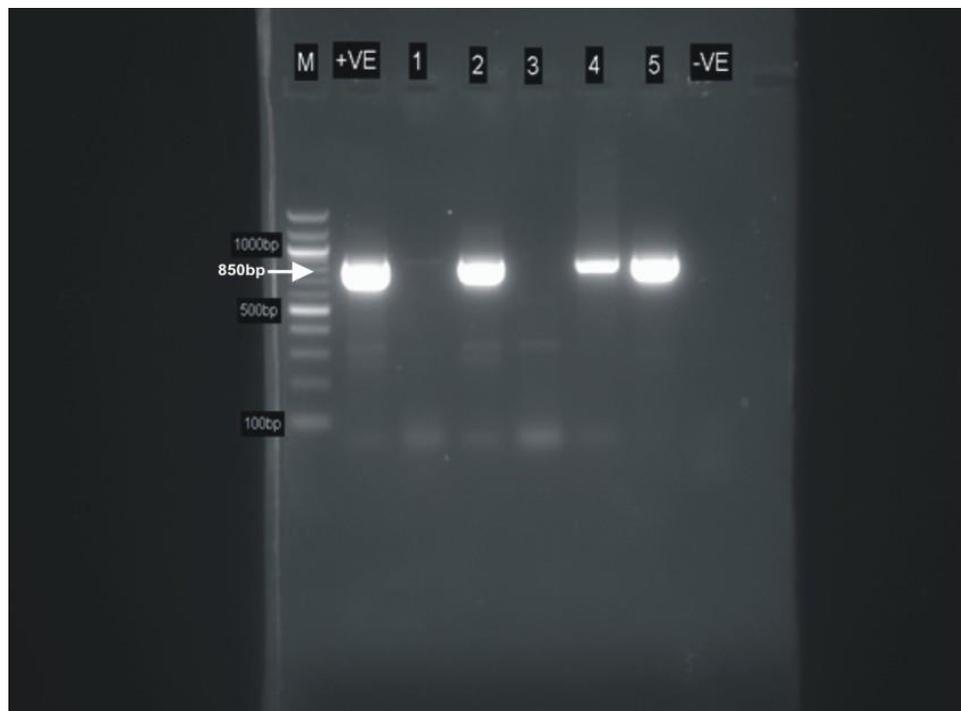


Plate 3: Amplification of Kelch 13 gene @ 850bp from DNA of *P. falciparum* infected patients in two hospitals in Kaduna, Nigeria.

DISCUSSION

Observations from this study showed artemisinin combination therapy (ACT) as the most widely prescribed antimalarial; 93.7% in the public health facility and 92.3% in the private facility. Thus implying strong adherence to the antimalarial policy which had been in place in Nigeria since 2004 (FMOH, 2005). Of the ACTs, artemether-lumefantrine was mostly prescribed in both facilities (71.90/59.10%). This is expected because the combination is a first line among all the ACT combinations adopted by the government of Nigeria for the treatment of malaria (FMOH, 2005). Artesunate-amodiaquine combination was the least prescribed ACT and was recommended only in the private health facility. However, its use was very low and this may not be unrelated to factors such as adverse effects such as described by Ekong et al. (2008) who reported effects of amodiaquine on the brain macromolecules of Wistar rats. ACTs became the mainstay of antimalarial therapy following global reports of resistance to previously used antimalarial drugs such as chloroquine and sulphadoxine-pyrimethamine.

Other antimalarial drugs prescribed in order of decreasing average frequency were artesunate (2.7-3.0%), sulphadoxine-pyrimethamine (2.4-2.6%) and chloroquine (1.1-2.1%). This is an indication that oral monotherapy is still practised. Availability of these drugs as monotherapy may constitute a continuous challenge towards eradicating drug resistant *Plasmodium falciparum* malaria in this region (WHO, 2014). According to WHO, availability of oral artesunate monotherapy in the private sector was highest (35%) next to DR Congo (45.5%) (WHO, 2014). Sulphadoxine-pyrimethamine has also been reported as commonly available over-the-counter in both rural and urban areas in Nigeria and it is the drug of choice for intermittent preventive therapy in pregnancy (IPTp) (Okeke et al., 2006; Ikpa et al., 2014).

From this study, *in vitro* sensitivity of *Plasmodium falciparum* isolates to chloroquine was remarkably high with susceptibility of 89.3% with a median IC₅₀ of 1.85µM and similar to the first report of decline in prevalence of chloroquine resistant

parasites in Africa was in Malawi, 12 years after chloroquine was withdrawn with clinical efficacy going from less than 50% in 1993 to 100% sensitivity by 2001 (Laufer et al., 2006). Lucchi et al. (2015) reported an increase in the *in vitro* sensitivity *Plasmodium falciparum* isolates to chloroquine following a decline in the IC₅₀ from 2010-2013 in Western Kenya. In China, *in vivo* resistance to chloroquine decreased over 5-8 years from >84% to 40% (WHO, 2014). Legrand et al (2012) reported a decline in *in vitro* resistance of chloroquine to an approximate 50% in French Guiana following years of chloroquine withdrawal. Kwansa-Bentum et al. (2011) found *Plasmodium falciparum* showed no improvement in susceptibility to chloroquine but exhibited satisfactory response to artesunate. Peletiri et al. (2012) reported 88.9% of *Plasmodium falciparum* isolates resistance to chloroquine of isolates in Abuja, Nigeria with a median IC₅₀ of 0.6µM (0.36-1.32) while Basco and Ringwald (2002) reported 59% *in vitro* resistance in Cameroon. There appears to be a gradual reversal in chloroquine resistance following change in treatment policy and exclusion of chloroquine from the current antimalarial regimen. Frequent drug usage has been observed as the driving force for selection of antimalarial resistance single nucleotide polymorphisms (SNPs). Therefore, a decrease in drug pressure, e.g. chloroquine, following policy change could have helped to reduce and can further reduce resistance. Decrease in drug pressure has been reported to increase fitness cost of resistant strains thereby causing such mutant strains to lose their survival advantage (Petersen et al., 2011).

In vitro resistance of test *P.falciparum* to artesunate observed in this study was 39.3% with a median IC₅₀ of 2.4µM. This is similar to Na-Bangchang et al. (2013) who reported declining sensitivity of *P. falciparum* isolates (36.7%) in the Thai-Myanmar border. Continued use of oral artesunate monotherapy could pose a source of resistance to the ACTs because of the presence of artesunate in the combination therapy. Such usage without a partner antimalarial agent is against WHO recommendation and has been highly discouraged. The use of oral artesunate

monotherapy could be as a result of cost compared to the ACTs. However, continued use of oral artesunate monotherapy poses a threat to the therapeutic life of the ACTs and malarial control (WHO, 2014).

Resistance of test *P. falciparum* isolates to artemether *in vitro* was 25% with a median IC₅₀ of 2.69µM. This is quite similar to Pradines et al. (1998) who reported 14% decline in *P. falciparum* susceptibility to artemether while Badru (2017) reported 100% sensitivity. The antimalarial, artemether is recommended in combination with lumefantrine in fixed dose as drug of choice for treatment of uncomplicated malaria. It is also available as oily injection for intramuscular administration, the form in which it is often used as monotherapy.

The degree of *in vitro* resistance of test *P. falciparum* isolates to mefloquine observed in this study was 17.86% with a median IC₅₀ of 25µM. This result is comparable with that of Yavo et al. (2010) where in Cote d'Ivoire a 15.2% *in vitro* resistance was observed. In Senegal, Fall et al. (2011) reported reduced *P. falciparum* isolates susceptibility of 50% to mefloquine even though the drug was not in the ACT combination in Senegal at the time of the study and in 2015 prevalence of *P. falciparum* isolates with decreased susceptibility to mefloquine remained high at 55.6%. Although mefloquine is part of the ACT recommended for treatment of uncomplicated malaria, its use is low. The low level of use of mefloquine could be the reason for increased susceptibility to mefloquine probably as a result of reduced drug pressure.

Five of the *P. falciparum* isolates which were found to be resistant to all the classes of test antimalarial agents in this study were subjected to Polymerase Chain Reaction (PCR) to determine the presence of molecular markers responsible for resistance to the drugs. One of the *P. falciparum* isolates (20%) clearly displayed Pfmdr-1 which amplified at 603bp. Lucchi et al. (2015) reported a prevalence of Pfmdr-1 of 18% in 2010 and 0% in 2013 in Western Kenya. Antony et al. (2016) also found low association (degree of association not specified) of Pfmdr-1 to chloroquine resistance following PCR and sequencing. In this study, the proportion of

Pfmdr-1 may not be considered as low because of the size of sample used.

In this study, PfATPase6 was clearly amplified in 20% of the isolates around 798bp. Jambou et al. (2005) in a study in three countries, reported presence of PfATPase-6 mutation exclusively in French Guiana but none was reported in Senegal and Cambodia while Saha et al. (2013) in India observed a pattern of PfATPase-6 inconsistent with that associated with decrease *in vitro* susceptibility to artemether of field isolates from French Guiana. The role of PfATPase6 with respect to resistance of *P. falciparum* to artemisinin will require further research.

Kelch 13 propeller gene was amplified in 60% of *P. falciparum* isolates at around 850bp. This shows that most the isolates carried the k-13 gene. However, Dama et al. (2017) reported a low frequency of K13 propeller mutations of <2% while Ariey et al. (2014) reported that the mutations associated with prolonged parasite clearance time following the use of ACTs in Cambodia and other South East (SE) Asian countries were not observed in Mali. From recent studies in Africa, only one has reported the presence of K13 propeller SNPs associated with artemisinin resistance found in SE Asia (JID, 2016).

Conclusion

From this study, there was increased susceptibility of test *Plasmodium falciparum* isolates to chloroquine while sensitivity of the test *P. falciparum* isolates to the artemisinins used in the study showed reduced sensitivity. Also, oral artesunate and chloroquine as well as sulphadoxine-pyrimethamine monotherapy were still prescribed as observed from this study. Molecular markers for resistance to the antimalarial drugs to which *P. falciparum* isolates were tested were amplified among resistant isolates. However, this study involved a rather small sample size upon which results obtained are based. We therefore encourage further surveillance involving larger sample size and sequencing of the resistant genes that may be observed. Periodic surveillance using therapeutic efficacy tests and *in vitro* susceptibility tests

should be carried out to obtain early signal of impending antimalarial drug resistance.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Study was designed and supervised by JOE and co-supervised by ROB while the experiments were carried out and the write up and analysis done by OIA.

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