Detection of Molecular Markers of Antimalarial Drug Resistance in Plasmodium Falciparum from South-Western Nigeria

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Abstract: The widespread of drug resistant *Plasmodium falciparum* has led to a rise in malaria-associated mortality most especially in sub-Saharan Africa. *Falciparum* malaria was confirmed by microscopic examination of Giemsa-stained blood samples of patients who presented with fever in selected State Hospitals in Ogun State, Southwestern Nigeria. Molecular methods were employed to detect the markers of resistance of *P. falciparum* to Chloroquine, sulphadoxine/pyrimethamine,and artesunate in Ogun State, Southwestern Nigeria. DNA was extracted from patient blood using the QiaAmp DNA Blood Minikit extraction method. Nested Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphisms (PCR/RFLP) were used for the detection of *P. falciparum* chloroquine resistance transporter (*Pfcrt*), *P. falciparum* multidrug resistance 1 (*pfmdr1*), *P. falciparum* dihydrofolate reductase (*Pfdhfr*), *P. falciparum* dihydropteroate synthase (*Pfdhps*) and *P. falciparum* sarco/endoplasmic reticulum calcium-dependent ATPase (SERCA) *PfATPase*6 genes. *Pfcrt* (K76T) *Pfmdr1 (mdr 1) Pfdhfr (*S108N*)*, and *Pfdhps (K540E)* resistant genes were detected among the isolates while

resistant SERCA*PfATPase*6 gene which codes for artemisinin resistance was not detected in the population.

Keywords: Plasmodium, resistance, molecular markers, genes, detection

INTRODUCTION

The main cause of the worsened malaria situation recorded in recent years has been the spread of drug-resistant parasites. This has led to rising malaria-associated mortality, even though overall child mortality has fallen (Greenwood *et al*., 2005; Adefioye *et. al.,* 2007).

A major breakthrough in the search for the genetic basis of CQR in *P. falciparum* was the identification of PfCRT gene, which encodes a putative transporter or channel protein (Fidock *et al*., 2000). A K76T change on the PfCRT gene appears necessary for the resistance phenotype, and is the most reliable molecular marker of resistance among the various fifteen polymorphic amino acid positions in PfCRT gene associated with CQR in field isolates (Djimde *et al*., 2001; Plowe, 2003; Ojurongbe *et al.*, 2007). PfMDR1, is a homologue of the mammalian multiple drug resistance gene encoding a P-glycoprotein on the chromosome 5 of the *P. falciparum.* Several field studies indicated the positive association between the asparagine to tyrosine change at position 86 (N86Y) and the chloroquine resistance both *in vitro* (Adagu and Warhurst, 1999; Basco and Ringwald, 2001; Pickard *et al*.,

2003) and *in vivo* (Ringwald and Basco, 1999; Basco and Ringwald, 2001; Dorsey *et al*., 2001). However, other studies have cast doubts on this association (Pillai *et al*., 2001; Ojurongbe *et al*., 2007).

Until recently, there has been a reliance on the cheap antimalarial drugs like Chloroquine and Sulphadoxine-Pyrimethamine. In 2001, the World Health Organization (WHO) recommended Artemisinin combination Therapies (ACTs) as the first line of treatment for uncomplicated malaria (WHO, 2001). The ACTs which include Artemether-lumefantrine (AL) and Amodiaquine (AQ) plus artesunate (AS) have been adopted for treatment of *P. falciparum* malaria in many African countries. To protect drugs from resistance, there is now clear evidence that combining them can improve their efficacy without increasing their toxicity (Olliaro and Taylor, 2002) and with the development of highly effective artemisinin derivatives, there is renewed hope for the treatment of malaria in the form of Artemisinin-Based Combination therapy (ACT).

Malaria remains uncontrolled and requires newer drugs and vaccines. Untill the malaria vaccine and newer class of antimalarial drugs become available, the existing drugs need to

be used cautiously. This is because the irrational use of antimalarial drugs can cause the drug resistant malaria. Effective usage of existing antimalarial drugs for malaria control strategies requires continuous input of the drug resistance pattern in the field. Resistance to antimalarial drugs can be assessed *in vivo* and also *in vitro* by parasite susceptibility assays or by the use of molecular techniques including Polymerase Chain Reaction (PCR) methods to detect genetic markers.

Molecular studies using various markers can provide the advance information on the emergence of drug resistance pattern in the field and such can be used to design malaria control strategies. Using molecular studies, point mutations on the *P. falciparum* Chloroquine Resistant Transporter (PfCRT) and *P. falciparum* Multi-drug Resistant1 (PfMDR1) genes have been reported to play an additional role for the chloroquine resistance in *P. falciparum* isolates while dhfr and dhps were associated with resistance to sulfadoxine-pyrimethamine (Ittarat *et al*., 1994; Adagu and Warhurst, 1999).

MATERIALS AND METHODS
Sample Collection
This study was carried out in Ogun State, located in the tropical zone of Southwestern Nigeria. Children between 1-15 years, pregnant women and other adults were included in this study. The blood samples were collected and analyzed between April 2008 and June 2009. Samples were collected from the four geopolitical zones of Ogun state viz: Sango – Ota (Yewa), Abeokuta (Egba), Ijebu Ode (Ijebu) and Sagamu (Remo). Blood samples were collected for malaria screening from both fingerprick and venepuncture. About 2-5 ml of blood was then drawn (venepuncture) with a sterile disposable syringe and transferred to a heparinised centrifuge tube. The blood samples were transported to the laboratory at 4^0C. Drops of peripheral blood were placed on 1.5 x 7.0-cm strips of Whatman (Brentford, United Kingdom) 3MM filter paper so that the blood covered half the length of the strip. The strips were air-dried and kept in plastic bags until use.

Molecular Methods
Polymerase Chain Reaction/Restriction fragment Length Polymorphism (PCR/RFLP) was used to determine the resistant genes and study the genetic diversity/genetic variation of antimalarial resistant *Plasmodium falciparum.* DNA was extracted from patient blood spotted on the filter paper ("pre-culture") and cultured parasites ("post-culture") using the QiaAmp DNA Blood Mini kit Blood and Body Fluid Spin Protocol (Qiagen, Valencia, CA). The protocol for the extraction was

carried out according to manufacturer's instruction.

PCR for detection of *Pfcrt* gene
The oligonucleotides primers were designed from published sequences. For amplification of the 1.6-kb fragment of *pfcrt,* the lower primer was 5'-CCGTTAATAATAAATACAGGC-3'.The upper primer was 5'-CTTTTAAAAATGGAAGGGTGT-3', (Dorsey *et al.,* 2001). The primary PCR components, in a final volume of 20µL, was 2.5mM $MgCl_2$, 640µM deoxynucleotide triphosphate (dNTPs), buffer 10x, 10pM of each primer, 1U of Taq polymerase (Ampli Taq Gold; Applied Biosystems, Foster City, CA, USA) and 2µL of DNA samples. The cycling protocol was as follows: 95°C for 5 min for initial denaturation; 40 cycles of 94°C for 30 s, 52°C for 40 s and 72°C for 30 s; and a final extension of 72°C for 5 min.

Nested PCR and RFLP for *Pfcrt* mutation-specific detection
For amplification of the 1.6kb fragment of *pfcrt,* a primary PCR was set up using the primers *PFC*F 5'-CCGTTAATAATAAATACAGGC-3' and *PFC*R 5'-CTTTTAAAATGGAAGGGTGT-3'. Product from primary PCR (2µl of 10x dilution) was used in a follow-up, nested, allele-specific PCR amplifications to detect the codons for *pfcrt* 76K or 76T. These diagnostic PCR amplifications used a common inner primer pair 5'-GGCTCACGTTTAGGTGGA-3' and 5'-TGAATTTCCCTTTTTATTTCCAAA-3' (detects the 76T codon) or 5'-GTTCTTTTAGC AAAAATCT-3' (detects the 76K codon). The PCR stages for these diagnostic amplifications were at 94°C for 5 minutes, followed by 40 cycles at 94°C for 30 seconds, 52°C for 40 seconds, and 72°C for 45 seconds and a final extension of 72°C for 5 minutes. Purified genomic DNA from *P. falciparum* clones HB3 (chloroquine sensitive) and Dd2 (chloroquine resistant) were used as positive controls, and water, extracted uninfected blood smears, and uninfected blood spots on filter paper were used as negative controls. The PCR products from the amplification reactions were evaluated by electrophoresis on 2% agarose gels containing ethidium bromide. 10µl of the nested PCR product reaction mixture were treated directly with 3U of the restriction enzyme *Apo* I for 6 to 16 hours at 50°C as recommended by the manufacturer (New England Biolabs, Beverly, MA). The enzyme *Apo* I recognise and cut the 76K codon, releasing fragment from product. It does not cut the product containing the 76T codon found in chloroquine-resistant *P. falciparum*.

PCR and RFLP for detection of *Pfmdr1* gene

Gene segments spanning codon 86 of the *Pfmdr*1 gene were amplified in 20µl of standard PCR mixture containing 5µl of extracted DNA and primers MDR1 5'-ATGGGTAAA GAGCAGAAAGA-3' and MDR2 5'-AACGCAAGTAATACATAAAGT CA-3'. The PCR amplification stages were at 94°C for 2 minutes, followed by 35 cycles at 94°C for 20 seconds, 52°C for 10 seconds, 48°C for 10 seconds, and 60°C for 1.5 minutes. A second, nested amplification from this segment was then performed under the same PCR conditions using 1µl of the product solution and primers MDR3 5'-TGGTAACCTCAG-TATCAAAGAA-3' and MDR4 5'-ATAAACCTAAAAGGAACTGG-3'.

Presence of the mutant 86Y codon was detected by digestion of 8µl of the second amplification product solution with 1.5U of *Afl* III according to the manufacturer's instruction (New England Biolabs). The products of restriction digestion were separated by electrophoresis on a 2% agarose gel and detected by staining with ethidium bromide.

PCR assays for the detection of *Pfdhfr* and *Pfdhps* genes

Pfdhfr, and *Pfdhps* PCRs were performed as described by May and Meyer (2003a) and Marks *et al* (2004). For the *Pfdhps* PCR, two primers (primer *Pfdhps*-F 5-ATGATTCTTTT TCAGATG-3_ and primer *Pfdhps*-R 5-CCAATTGTGTGATTGTCCAC-3 were designed to amplify 747 bp of the region exhibiting mutations relevant to Sulphadoxine resistance. PCR was performed with a volume of 20µl (each primer at 0.2M, dNTPs at 200M, and 1U of Hotstar-*Taq* with the appropriate buffer [Qiagen, Valencia, Calif.] and $MgCl_2$ at a final concentration of 2.0 mM) and approximately 80ng of template genomic human DNA, with parasite DNA concentrations corresponding to the parasite burden in the individual. After an initial denaturation (15 min at 95°C), 31 cycles of 30s at 94°C, 40s at 53°C, and 1min at 72°C were run. Elongation of the amplicons was completed by a final cycle of 10min at 72°C. Subsequently, a nested PCR was performed to increase the yields of the specific amplicons using primers primer *pfdhps*-F1 (5-GTTGAACCTAAACGTGCTG-3) and *pfdhps*-R1 (5-ATTACAACATTTTGATCATTC-3). 3µl of the primary PCR product was used in a reaction volume of 25µl containing 0.2M of each primer, dNTPs at 200M, reaction buffer with $MgCl_2$ at a final concentration of 2.0 mM, and 1U of Hotstar-*Taq*). In the nested PCR, a high initial annealing temperature (AT), which ensures a high level of specificity of initial primer binding,

is followed by a gradual decrease in the AT toward the pre-calculated optimal AT. The parameters consisted of an initial denaturation step (15 min at 95°C) and 43 cycles of 30s at 94°C, the AT for 40 s, and 72°C for 1 min, in which the ATs were 65°C (5 cycles), 60°C (5 cycles), 56°C (7 cycles), 54°C (13 cycles), and 53°C (13 cycles). Fragment elongation was performed by use of a cycle of 10 min at 72°C. The amplicons were monitored for quality and the expected size on 1% ethidium bromide-stained agarose gels. Statistical analysis (χ^2 tests) was performed by the use of STATA software (version 8.2; Corp., College Station, Tex.).

PCR and RPLP assay for (SERCA) *PfATPase*6

P. falciparum positive samples were amplified by PCR using *PfATPase6*-specific primary and nested primer pairs. DNA extract for each sample was subjected to nested PCR amplification with primers flanking nucleotide codon 2307of the *PfATPase6* gene. Both the primary and secondary reactions comprised 2μl template, 0.25μM primer, 1.5mM $MgCl_2$, 200μM dNTP's, 1x PCR buffer and 1U Taq DNA polymerase, in 25μl reactions. Amplification cycles for both primary and secondary reactions consisted of an initial denaturation at 94°C for 2 minutes, followed by 25 cycles of denaturation at 94°C for 45 seconds, annealing at 46°C for 45 seconds and extension at 65°C for 1 minute, final extension was run at 65°C for 2 minutes. For primary amplification, the primers are 2307FW 5'-TGA GCA TGG CAC AAG TIT 3'; 2307RV- 5'TCA ATA ATA CCT AAT CCA CCT AAA TA-3'.For nested PCR the primers are FW-EN 5'-TGA GCA TGG TAG AAG TTT T-3' and RV-EN 5'-TCA TCT GTA TTC TTA ATA ATA TTT AAA TCT GTA CTA-3' (Zhang *et al*., 2008). Internal primers for the nested PCR amplification (2307FW-EN and 2307RV-EN, were engineered to create Csp6 I restriction sites. One site, at *PfATPase6* nucleotide codon 1916, serves as the internal control for the restriction digestion assay, which is always cut by the enzyme. Csp6 I digestion assay comprised 4μl of secondary PCR amplicon (432bp), 1x buffer and 1.5U of Csp6 I restriction enzyme, in 30μl reactions. Digestion assays were incubated for 12 hours at 37 °C. PCR amplicon and restriction digests were analyzed by electrophoresis on a 2% ethidium bromide-stained agarose gel and visualized under UV transillumination. Restriction digests were loaded in 15 μl volumes per lane. Band sizes were measured using Syngene gel imaging analysis software.

RESULTS
Prevalence of drug resistant molecular markers

Table 1: Zonewise Prevalence of molecular markers of resistance to antimalarial drugs in *P. falciparum* from Ogun State, Southwestern Nigeria.

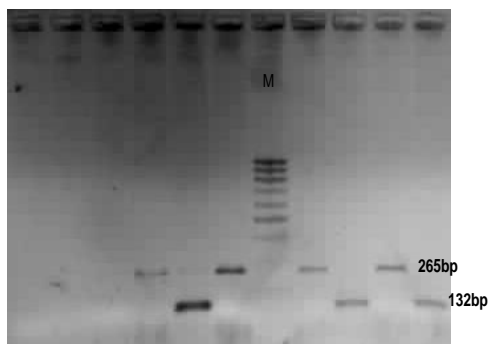| Zone	No. of Isolates	Prevalence of resistance genes				
		pfcrtK76T (%)	Pfmdr1Y86N (%)	PfdhfrS108N (%)	pfdhpsK540E (%)	pfATPaseS769N (%)
Ijebu	20	9 (45)	10 (50)	3 (15)	0 (0)	0 (0)
Yewa	43	28 (65.1)	30(69.8)	4 (9.3)	1 (2.3)	0 (0)
Egba	25	5 (20.0)	12 (48)	4 (16)	1 (4)	0 (0)
Remo	12	6 (50.0)	8 (66.7)	1 (8.3)	0 (0)	0 (0)
Total	100	48 (48)	60 (60)	12 (12.0)	2 (2)	0 (0)

Plate 4.1: DNA bands of wild type and mutated *P. falciparum* chloroquine resistance genes
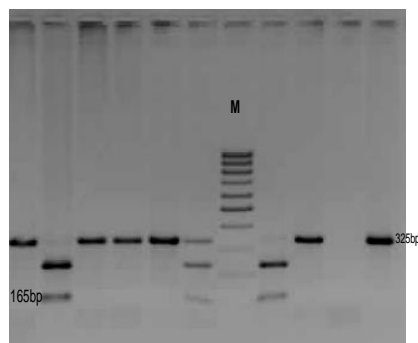

Plate 4.2: *P. falciparum* Multidrug Resistance Genes showing the wild type and mutated genes
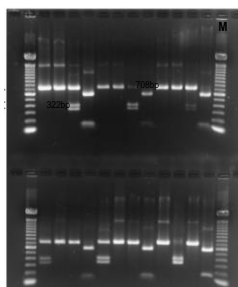

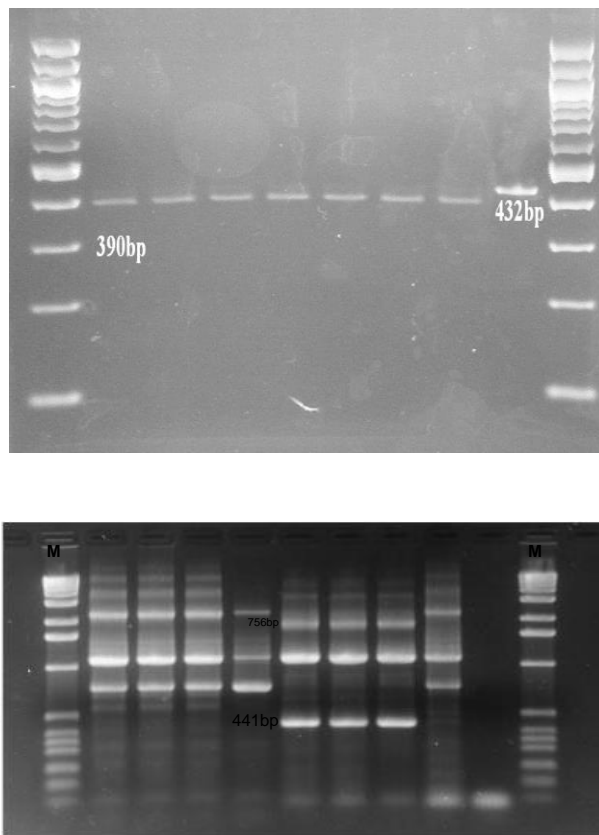Plate 4.3: DNA band of Dihydrofolate reductase gene (DHFR 108)

Plate 4.4: DNA band of Di hydropteroate synthase gene (DHPS 540)

DISCUSSION

One of the factors to be considered in the prophylaxis, treatment, and control of *Plasmodium falciparum* malaria is the resistance of parasite strains that may arise against virtually every drug available. Identification of *Pfcrt* as the central determinant of chloroquine-resistant *P. falciparum* malaria provides a molecular marker that can be used for surveillance of resistance and to evaluate drug treatment and prophylaxis policies. The present results further support this role of the *Pfcrt* gene. Amplification products from all the chloroquine-sensitive samples carried the codon for *Pfcrt* 76K. Out of the 51 samples that were chloroquine-resistant by *in vitro* testing carried out in earlier studies, 48 were found to carry the 76T codon, 5 were found to contain mixed 76T/76K codons, and 3 were discordantly found to contain the 76K codon. This finding corroborates with earlier findings where molecular markers of resistance were found in samples that gave *in vivo* resistance/drug failure (Djimde *et al.*, 2001; Chen *et al.*, 2001; Happi *et al.*, 2004).

The high prevalence of mutations at codons 76T, that code for chloroquine resistance in Nigerian *P. falciparum* isolates suggests that the population of *P. falciparum* that circulates in South-Western Nigeria has been selected by the long use of CQ. The overall picture emerging from this study is that resistance to this drug is abundant in south-Western Nigeria, and this finding strongly supports withdrawal of CQ as the first-line drug for treatment of *falciparum* malaria in Nigeria. This observation also supports the view that the *Pfcrt* polymorphism at position 76 is in fact a significant factor of CQ resistance, as shown in previous studies from Cameroon (Basco and Ringwald, 2001.), Mali (Djimde *et al*., 2001), Mozambique (Mayor *et al.,* 2001), Nigeria (Adagu and Warhurst, 2001), Sudan (Babiker *et al*., 2001), Uganda (Kyosiimire-Lugemwa et al., 2002), Madagascar (Ariey *et al*., 2002), Laos (Labbe *et al*., 2001), Papua New Guinea (Maguire *et al*., 2001), and Thailand (Chen *et al*., 2001; Jürgen and Christian, 2003). CQ resistance has been attributed to a single mutation at codon 76 in the *Pfcrt* gene (Djimde *et al*., 2001; Basco and Ringwald, 2001).

It has been observed that the *Pfcrt* K76 and the *Pfmdr1* Y86 alleles are closely associated in chloroquine resistant strains (Adagu and Warhurst, 1999). A similar observation was made in this study as all isolates carrying *Pfcrt* mutated allele were also positive for *Pfmdr1* mutated allele. However, studies comparing the associations of the *Pfmdr* 1 variant and the *Pfcrt* K76 variant have shown that the impact of the *pfcrt* gene was stronger than that of the *pfmdr* gene (Djimde *et al*., 2001; Dorsey *et al*., 2001; Ojurongbe *et al.*, 2007). It has been suggested that the degree of chloroquine resistance is further modulated by factors linked to genes other than *Pfcrt* or *Pfmdr* (Chen *et al*., 2001).

Molecular methods that detect genetic markers of drug resistance are potentially powerful tools for tracking drug-resistant malaria. In this study, the combination of *Pfcrt* and *Pfmdr1* mutations in isolates associated with *in vitro* amodiaquine resistance was observed. Mutant *PfcrtT76* and *Pfmdr1Y86* alleles were observed in 48% and 60% of the samples, respectively. A previous study in Sudan (Babiker *et al*., 2001) found that the mutant *PfcrtT76* allele is associated with amodiaquine treatment failure. The high prevalence of the mutant *PfcrtT76* allele (48%) and *Pfmdr*1 allele (60%) observed in Ogun State, Nigeria confirms recent reports of the high prevalence rate of this alleles in parasites obtained from similar studies (Happi *et al*., 2006) and is also consistent with rates ranging from 60% to 100% reported in other malaria-endemic regions (Maguire *et al*., 2001; Basco and Ringwald, 2001; Djimde *et al* 2001). The non-significant selection of the mutant *PfcrtT76* by amodiaquine

may be due to the high prevalence of this allele in the *P. falciparum* population from South Western Nigeria. Selection of *Pfmdr1Y86* by amodiaquine has also been reported previously in the Gambia (Happi *et al.*, 2003). Although the importance of point mutations in *Pfcrt* in producing chloroquine resistance is beyond dispute (Basco and Ringwald, 2001; Maguire *et al.*, 2001; Chen *et al.*, 2001) recent transfection studies of *Pfcrt* have shown that isolates expressing the mutant *PfcrtT76* allele retain sensitivity to amodiaquine while showing a reduced susceptibility to monodesethyl amodiaquine, the active metabolite of amodiaquine (Happi *et al.*, 2003). The selection of the mutant *PfcrtT76* and *Pfmdr1Y86* alleles indicates the primary involvement of these two genes in the mediation of amodiaquine resistance. Thus, similar to chloroquine resistance, amodiaquine resistance in *P. falciparum* may depend primarily on mutation(s) in *Pfcrt* and additional mutations in *Pfmdr1* or other *Plasmodium* genes may also have significant roles in increasing resistance to the drug.

The combination of *pfcrtT76* and *pfmdr1Y86* mutations was associated with amodiaquine treatment failure. These two alleles have been shown to be in linkage disequilibrium in chloroquine-resistant isolates of *P. falciparum* from The Gambia and Nigeria (Happi *et al.*, 2003). The similarity in the chemical structures of chloroquine and amodiaquine and their possible likely common mode of action suggests that the molecular basis of resistance to these two drugs may be similar.

Resistance against Sulphadoxine and Pyrimethamine was observed in this study. Among the isolates analyzed for resistance markers, *PfdhfrS108N* and *PfdhpsK540E* genes coding for Pyrimethamine and Sulphadoxine respectively were detected. It has been earlier observed that Resistance to pyrimethamine is primarily conferred by a non-synonymous point mutation at codon 108 and is consecutively enhanced by mutations at codons 51 and 59 of the *P. falciparum pfdhfr* gene located on chromosome 4 (Bruce-Chwatt, 1985; Kublin *et al.,* 2002). The enzyme is part of the folate pathway and, thus, of DNA replication. In this study genes that code for sulphadoxine and pyrimethamine resistance were detected among the isolates screened for markers of resistance against these drugs. *PfdhfrS108N* and *PfdhpsK540E* alleles were detected in 12 and 2 isolates respectively.

On the basis of evolutionary theories, biological disadvantages are expected for parasites carrying resistance-mediating mutations in the absence of drug pressure. The fitness deficit conferred by the *PfdhfrS108N* mutation in the absence of pyrimethamine use is considered quite low. Enduring resistance in the absence of strong drug pressure implies that the expected decline in

the prevalence of resistant parasites is balanced by mechanisms that confer biological advantages with regard to survival fitness, replication and transmission probability, invasion, reproduction, and vector properties that favor transmission. As Sub-Saharan African countries are confronted with the rapid emergence of resistance against virtually every drug that is used for the treatment of *P. falciparum,* malaria drug pressure is considered to essentially promote the emergence of SP resistance, which is now widespread in East Africa, but also well recognized in West Africa.

Selection for the Ser to Asn substitution at codon 108 of the *Pfdhfr* gene has been shown to be linked to parasite survival after treatment with pyrimethamine - containing regimens (Plowe *et al.,* 1998; Marks *et al.*, 2005). Accordingly, the high frequencies of resistant parasite populations have been attributed to increased pyrimethamine consumption (Marks *et al.*, 2005). An Asp to Ile substitution at codon *Pfdhfr*51 (*Pfdhfr*N51I) and/or a Cys to Arg exchange at codon *pfdhfr*59 (*pfdhfr*C59R) appears to enhance pyrimethamine resistance if one or both of these occur concurrently with *Pfdhfr*S108N. *Pfdhfr*S108N-N51I-C59R is the combination of mutations most strongly associated with pyrimethamine resistance. Point mutations at codons 437 and 540 of the *Pfdhps* gene located on chromosome 8 of *P. falciparum* are considered responsible for sulphadoxine resistance. *Pfdhps* encodes a key enzyme in the folate pathway, as does *Pfdhfr*. The Ala to Gly substitution at position 437 (*Pfdhps*A437G) is, in general, the first mutation to occur. In Africa this is followed by the Lys to Glu substitution at position 540 (*Pfdhps*K540E), which confers higher levels of resistance. It was recently shown that the presence of the three *Pfdhfr* mutations combined with the two *Pfdhps* mutations (quintuple mutation) is strongly associated with SP resistance (Marks *et al.*, 2005).

Geographical clustering was reported for chloroquine-resistant *Pfcrt* (Wootton *et al.*, 2002) and anti folate resistant *Pfdhfr* and *Pfdhps* haplotypes (Cortese *et al.*, 2003), indicating distinct ancestral selection events in different areas. One important aspect in the possible setting leading to drug resistant parasites in Western Nigeria is the genetic background of the local parasite population. Multidrug-resistant *P falciparum* is common, with a high rate of *Pfcrt, Pfmdr1, Pfdhfr* and *Pfdhps* resistance haplotypes. Thus, selection pressures were exerted on a heavily mutated genetic background. The several *PfATPase6* haplotypes harbouring the S769N mutation indicate that selections of such mutants are therefore not impossible in the nearest future. As chloroquine and

SP are replaced by more effective artemisinin-based combination therapies (ACTs), strategies for monitoring (and, if possible, deterring) drug-resistant malaria must be updated and optimized. *In vitro* methods for measuring resistance will be critical for confirming and characterizing resistance to ACTs. Molecular markers are useful for tracking the emergence and dissemination of resistance and guiding treatment policy where resistance is low or moderate.

One hundred samples that were positive for *P. falciparum* by microscopy were subjected to PCR genotyping for estimating prevalence of the *PfATPase* codon S769N mutation. All the 100 (100%) isolates carried the artemisinin sensitive wild type allele, S769. This observation is similar to the ones observed in several African countries where artemisinin and its derivatives are used as first line of treatment of uncomplicated malaria (Basco and Ringwald, 2001; Djimde *et al*., 2001; Mayor *et al.,* 2001; Adagu and Warhurst, 2001; Babiker *et al*., 2001; Kyosiimire-Lugemwa et al., 2002).

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However Artemisinin resistant genes have been detected by some workers especially in South Asia (Labbe *et al*., 2001; Maguire *et al*., 2001; Chen *et al*., 2001; Jürgen and Christian, 2003). Artemisinin derivatives are an essential component of treatment against multidrug-resistant *falciparum* malaria. The genes that code for artemisinin resistance was not detected in any of the isolates screened for antimalarial resistance genes. Widespread multidrug-resistant *falciparum* malaria led WHO to recommend combination drug therapy as first-line treatment, with formulations containing an artemisinin compound as policy standard. Artemisinin and its derivatives are the most potent and rapidly acting antimalarials. However, artemisinin resistance has been reported in murine models of malaria (Ferrer-Rodiriguez *et al.,* 2004; ambou *et al*., 2005), in the Thai-Cambodia border (Noedl et al., 2009; Dondorp et al., 2009) and the western border of Thailand (Aung et al., 2012). Diligent surveillance is needed to monitor continued susceptibility to artemisinin derivatives in endemic areas.

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