Plasmodium Falciparum Multidrug Resistance Gene-1 N86Y-Y184F-D1246Y Polymorphisms in Northern Nigeria: Implications for the Continued Use of Artemether-Lumefantrine in the Region

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Keywords: Antimalarial drug resistance, prevalence, single nucleotide polymorphisms, pfmdr1, haplotypes

DOI: https://doi.org/10.21203/rs.3.rs-47477/v1
Abstract

Background

The analysis of single nucleotide polymorphism (SNPs) in drug-resistance associated genes is a commonly used strategy for the surveillance of antimalarial drug resistance in populations of parasites. The present study was designed and performed to provide genetic epidemiological data of the prevalence of N86Y-Y184F-D1246Y SNPs in *Plasmodium falciparum* multidrug resistance 1 (*pfmdr1*) in the malaria hotspot of Northern Nigeria.

Methods

*Plasmodium falciparum*-positive blood samples on Whatman-3MM filter papers were collected from 750 symptomatic patients from four states (Kano, Kaduna, Yobe and Adamawa) in Northern Nigeria, and genotyped via BigDye (v3.1) terminator cycle sequencing for the presence of three SNPs in *pfmdr1*. SNPs in *pfmdr1* were used to construct NYD, NYY, NFY, NFD, YYY, YYD, YFD and YFY haplotypes, and all data were analyzed using Pearson Chi-square and Fisher’s exact (FE) tests.

Results

The prevalence of the *pfmdr1* 86Y allele was highest in Kaduna (12.5%, $\chi^2 = 10.47$, $P < 0.05$), whilst the 184F allele was highest in Kano (73.1%, $\chi^2 = 13.20$, $P < 0.05$), and the *pfmdr1* 1246Y allele was highest in Yobe (5.26%, $\chi^2 = 9.18$, $P < 0.05$). The NFD haplotype had the highest prevalence of 69.81% in Kano ($\chi^2 = 36.05$, $P < 0.05$), followed by NYD with a prevalence of 49% in Adamawa, then YFD with prevalence of 11.46% in Kaduna. The YYY haplotype was not observed in any of the studied states.

Conclusion

The present study shows that *P. falciparum* strains with reduced sensitivity to artemether-lumefantrine exist in Northern Nigeria and predominate in the North-West region.

Introduction

Antimalarial drug resistance is a major impediment to malaria chemotherapy in sub-Saharan Africa [9] largely because *Plasmodium falciparum* rapidly develops resistance to drugs [7]. Resistance to antimalarial drugs occurs through drug-selection of spontaneous mutations in *P. falciparum* that confer tolerance to the drug [54]. The selection and spread of drug resistant *P. falciparum* is facilitated by the rapid genome replication rate and by a relatively high mutation rate per generation of the parasite [8, 10]. The speed of selection of mutants within parasite populations depends upon on the pharmacokinetics of the drug itself and its degree of usage within a given host population [9]. For many antimalarial drugs,
molecular markers of parasite resistance are known. Surveillance of these markers in parasite populations can act as a proxy measure of the efficacy of drugs within that population, and can act as early warning signals of the emergence of resistance into new regions. Frequent and thorough molecular surveys of the prevalence of mutations associated with drug resistance can, therefore, inform regional drug policies.

Single nucleotide polymorphisms (SNPs) in the *P. falciparum* multidrug resistance gene (*pfmdr1*) and the *P. falciparum* chloroquine resistance transporter gene (*pfcrt*) are associated with parasite resistance to antimalarial drugs including Artemisinin based Combination Therapies (ACTs) and chloroquine [53]. African *P. falciparum* isolates may carry the resistant allele of *pfcrt* encoding the amino acids CVIET at codons 72–76 as well as a variety of polymorphic *pfmdr1* alleles which have originated and spread within the African continent [3, 33, 40]. The *pfmdr1* gene is a structural homologue of the mammalian multidrug resistance gene encoding a P-glycoprotein homologue-1 (Pgh1) multi-drug resistant transporter [17] and is expressed into a PfMDR1 transporter located in the *P. falciparum* food vacuole.

Mutations in PfMDR1 are associated with enhanced efflux of diverse antimalarial drugs reducing their intracellular accumulation [17, 42]. Single nucleotide polymorphisms (SNPs) in *pfmdr1* are associated with resistance to multiple antimalarial drugs [12, 44]. Several codons in *pfmdr1* have been putatively linked with changes in the parasite’s sensitivity to antimalarial drugs, but codons N86Y, Y184F and D1246Y are uniquely associated with changes in artemether-lumefantrine (AL) and artesunate-amodiaquine (AS-AQ) efficacies in sub-Saharan Africa [14]. While the *pfmdr1* 86Y allele was strongly associated with chloroquine (CQ) and amodiaquine (AQ) resistance [16, 49], 1246Y alleles were shown to confer resistance to quinine (QN) and possess the capacity to increase parasite susceptibility to mefloquine (MQ), halofantrine (HF) and artemisinin (ART) [20, 24].

The mutant *pfmdr1* 86Y and 1246Y alleles have also been linked to reduced sensitivity to AS-AQ, whereas the wild-type *pfmdr1* N86 and D1246 alleles are linked to resistance against AL [34, 41]. In Africa, the common use of AL and AS-AQ in the treatment of uncomplicated malaria has been linked with the emergence of *pfmdr1* N86Y, Y184F and D1246Y SNPs [31], and the prevalence of these mutations are frequently used for evaluating AL and AS-AQ efficacies [36]. Several studies have shown that parasites carrying a combination of *pfmdr1* N86, 184F, and D1246 (the “NFD” haplotype) display decreased susceptibility to AL and that treatment with AL can select for such a haplotype [4, 46].

Nigeria accounts for 25% of global cases of malaria and an estimated 50% of the country’s population suffer at least one episode of malaria every year while under-five children experience an average of 2–4 attacks in a year [55]. *Plasmodium falciparum* is stably and perennially transmitted in all parts of the country [39], with transmission increased during the wet season compared to the dry [18, 43].

North-West and North-East Nigeria have so far been identified as hotspots of malaria in relation to the southern parts of the country due primarily to climatic and environmental conditions [37]. However, the North-West region of the country suffers a much higher *P. falciparum* transmission rate than the other regions including North-East Nigeria [28].
The frontline drug for malaria chemotherapy in the country was chloroquine until 2005 when it was withdrawn as a result of resistance. Subsequently, ACTs especially artemether-lumefantrine (AL) were recommended in all parts of the country as the frontline chemotherapy for uncomplicated malaria. Unfortunately, several reports investigating molecular markers of antimalarial resistance have suggested an increased risk of parasite tolerance to AL [13, 20, 30–31]. However, there is no baseline data involving pfmdr1 SNPs in both North-West and North-East Nigeria since the withdrawal of CQ and adoption of AL in Northern Nigeria. In this study, the distributions of the pfmdr1 N86Y, Y184F and D1246Y SNPs across the North-West and-East Nigeria were investigated.

**Methods**

**Description of study sites**

Nigeria’s North-West and North–East are two out of the six geo-political zones of Nigeria. The North-West is made up of seven states and is home to a population of over 35 million people whilst the North-East comprises six states with a population of over 18 million [52]. Two states from the North-West; Kano (longitude 7° 10´E, 10° 35´E and latitude 10° 25´N, 13° 53´N) and Kaduna (longitudes 7° 23´E and 7° 29´E and latitudes 10° 25´N and 10° 36´N) with a combined population of 15,450,244 were randomly selected for inclusion in this study while Yobe (longitude 13.5° E and latitude 11° N) and Adamawa (longitude 11° and 14°E and latitude 7° and 11°N ) states with a combined population of 5,489,692 were similarly selected from the North-Eastern region [52]. Other relevant details about the study sites are indicated in Fig. 1.

**Selection Criteria**

In this study, patients who presented with symptoms of uncomplicated malaria across all ages and did not take any antimalarial drug prior to arrival to the facilities were included whilst those who presented with severe malaria were excluded.

**Sample Collection**

Between June and November 2017, thick and thin film microscopy was used to confirm *P. falciparum* positivity of malaria symptomatic patients that attended selected health facilities within the study sites. The total number of samples collected from Kano, Kaduna, Adamawa and Yobe were 250, 150, 150 and 200 respectively. 10 µL of microscopically confirmed *P. falciparum* parasitized blood samples were spotted on four different positions onto Whatman-3MM filter papers and allowed to dry at room temperature. Each sample was placed in sachets containing desiccant, and was preserved in a refrigerator at 4 °C.

**Genomic DNA isolation, amplification and genotyping of pfmdr1**
Three discs (3 mm/disc) were punched from the *P. falciparum*-positive dried blood spots and the punch sterilized between punches. The discs were used to extract genomic DNA using a QIAamp DNA Mini Kit (Qiagen Inc, Japan) according to the manufacturer's instructions. Nested polymerase chain reaction (PCR) was carried out as described by Humphreys *et al.* [20] with slight modifications to the PCR cycle programs (Table 1). The nested PCR runs for the amplification of two separate *pfmdr1* segments S1 and S2, spanning codons 86–184, and −1246 respectively were performed with a 10 µL final master mixture and 200 nM primers, and 1U ExTaq polymerase (Takara-Bio, Japan). Two µL of isolated genomic DNA was added to each of the first PCR master mixtures and run in the thermocycler. At the completion of the first PCR runs, 1 µL of each of the resulting amplified fragments were further used as templates in 10 µL of secondary PCR reactions. The PCR reactions were run along with parasite free genomic DNA (negative) and 3D7 clone *P. falciparum* genomic DNA (positive) controls. Two µL of each PCR product was evaluated by electrophoresis on 1.5% agarose gels that were stained with Midori Green Advance and visualized under ultraviolet light. The remaining 8 µL nested PCR products were stored at -30 °C. All nested amplicons were subsequently purified using the one step ExoSAP-IT (ThermoFisher Scientific, Japan) purification kit and the resulting products subjected to BigDye Terminator (v3.1) Cycle Sequencing (ThermoFisher Scientific, Japan). Sequences were analyzed using BioEdit Sequence Alignment Editor (v7.0.5.3) while *pfmdr1* SNPs were determined using MEGA5 software (Build#:5130611) in reference to the *pfmdr1* sequence of *P. falciparum* deposited at the NCBI database [Accession Number X56851]. Consequently, the prevalences of wild and mutant *pfmdr1* across the three codons were calculated using the following formula:

\[
\text{Prevalence} = \frac{\text{Frequency of wild or mutant alleles}}{\text{Total number of contiguous sequences analyzed}} \times 100
\]
Table 1
Primers for Nested PCR of \( pfmdr1 \) Long and Short Fragments and Cycling Programs adapted and modified from the work of Humphreys et al. (2007).

| Primers                        | Amplicon Sizes (bp) | PCR Cycling Programs                                                                 |
|--------------------------------|---------------------|---------------------------------------------------------------------------------------|
| Outer forward FN1/1 5_-        | 578                 | 34 cycles of 94 °C for 30 s; 55 °C for 30 s; and 65 °C for 1 min; then 65 °C for 5 min and 4 °C forever. |
| AGGTGAAAAAGAGTTGAAC            |                     |                                                                                       |
| Outer reverse REV/C1 5_-       | 958                 | 34 cycles of 94 °C for 30 s; 55 °C for 1 min; and 65 °C for 1.5 min; then 65 °C for 5 min and 4 °C forever. |
| ATGACACCACAACATAAAAT           |                     |                                                                                       |
| Outer forward MDRFR2F1 5_-     |                     | 29 cycles of 94 °C 30 s; 60 °C for 30 s; and 65 °C for 1 min; then 65 °C for 5 min and 4 °C forever. |
| GTGTATTTGCTGTAAGAGCT           |                     |                                                                                       |
| Outer reverse MDRFR2R1 5_-     |                     | 28 cycles of 94 °C for 30 s; 60 °C for 30 s; and 65 °C for 1 min; then 65 °C for 5 min and 4 °C forever. |
| GACATATTTAAATAACATGGGTTC       |                     |                                                                                       |
| Nested forward MDR2/1 5_-      | 534                 |                                                                                        |
| AAAAAAAGAGTACCGCTGAAT          | 864                 |                                                                                        |
| Nested reverse NEWREV1 5_-     |                     |                                                                                        |
| AAACGCAAGTAATACATAAAAGTC       |                     |                                                                                        |
| Nested forward MDRFR2F2 5_-    |                     |                                                                                        |
| CAGATGATGAAATGTTTAAGATC        |                     |                                                                                        |
| Nested reverse MDRFR2R2 5_-    |                     |                                                                                        |
| TAAATAACATGGGTTCCTTGACT        |                     |                                                                                        |

Each PCR run was preceded by an initial denaturation at 94 °C for 3 minutes.

\( Pfmdr1 \) \textbf{N86Y-Y184F-D1246Y Haplotypes}

The \( pfmdr1 \) haplotypes used in this study were based on eight previously reported haplotypes associated with artemether lumefantrine-tolerance; \( pfmdr1 \) N86Y, Y184F and D1246Y single nucleotide polymorphisms in different \( P. falciparum \) populations from Africa [22, 27].

\section*{Statistical analysis}

All data were statistically analyzed using Pearson Chi-square and Fisher’s exact (FE) tests of Graph-Pad Prism (8.1.0) and P values \( \leq 0.05 \) were considered to be statistically significant.

\section*{Results}

\textbf{Prevalence of SNPs in \( pfmdr1 \) codons 86, 186 and 1246}

Of the 750 \( P. falciparum \) positive samples collected from the four states in northern Nigeria, 500 were successfully genotyped for the \( pfmdr1 \) N86Y, Y184F and D1246Y alleles. Six of the genotyped \( pfmdr1 \) sequences were deposited in the GenBank with accession numbers MT472640, MT472641, MT472642,
MT495456, MT495458, and MT495459 on the basis of presence or absence of mutations in the three codons of the gene. The prevalence of the mutant \textit{pfmdr1} 86Y allele was observed to be significantly (\(\chi^2 = 10.47, P < 0.05\)) different across the states, with highest prevalence of 12.5% obtained in Kaduna state, followed by Kano with 4.68% and 2% in Adamawa state (Fig. 2A). The prevalence of the mutant \textit{pfmdr1} 184F allele was 73.1% in Kano state, 61.46% in Kaduna state and 48% in Adamawa state (\(\chi^2 = 13.20, P < 0.05\)), Fig. 2B. Yobe state had the highest prevalence of the mutant \textit{pfmdr1} 1246Y allele (5.26%) while Kaduna and Adamawa states had zero prevalence of this mutation (\(\chi^2 = 9.18, P < 0.05\)) (Fig. 2C).

The regional distributions of the mutant \textit{pfmdr1} 86Y, 184F and 1246Y alleles are shown in Fig. 3. Based on the results, an overall regional prevalence of 7.49% and 3% for the \textit{pfmdr1} 86Y allele was recorded in North-West and North–East Nigeria, respectively. However, the observed difference was not significant (\(\chi^2 = 1.68, P > 0.05\)), Fig. 3A. Similarly, the prevalence of the \textit{pfmdr1}184F and 1246Y mutants were not significantly different between the North-West and North–East Nigeria, whereas the prevalence of the 184F allele differed significantly between these two regions; 68.91% and 56.22%, in the North-West and North-East respectively (\(\chi^2 = 3.60, P > 0.05\)) (Fig. 3B). The prevalence of the \textit{pfmdr1} 1246Y allele in the North-West and North–East Nigeria was 1.93% and 3% respectively (\(\chi^2 = 0.21, P > 0.05\)) (Fig. 3C).

\textbf{Analysis of pfmdr1 Haplotypes}

The distribution of \textit{pfmdr1} haplotypes in the four Northern Nigerian states is shown in Fig. 4A. The \textit{pfmdr1} N86Y, Y184F and D1246Y mutations were constructed into NYD, NYY, NFY, NFD, YYY, YYD, YFD and YFY haplotypes. Out of the 500 \textit{pfmdr1} samples genotyped, a total of 492 haplotypes were constructed (sub-divided into seven different types). As shown in the Fig. 3, there was a significant difference in the prevalences of all \textit{pfmdr1} haplotypes across the locations (\(\chi^2 = 36.05, P < 0.05\)); the NFD \textit{pfmdr1} haplotype was highest in Kano state with a prevalence of 69.81%, the NYD haplotype was highest in Adamawa with a prevalence of 49%, whilst the \textit{pfmdr1} YFD haplotype predominated in Kaduna with a prevalence of 11.46%. The \textit{pfmdr1} YYY haplotype was not detected (Fig. 4A). Figure 4B showed the distribution of the \textit{pfmdr1} haplotypes across North-West and North–East Nigeria, but in contrast to the states distribution, no significant difference was observed (\(\chi^2 = 4.26, P > 0.05\)). The \textit{pfmdr1} NFD haplotype was highest in the North-West with a prevalence of 61.96%, the NYD haplotype was highest in the North-East with a prevalence of 41.63%, and the YFD haplotype was highest in the North-West with a prevalence of 5.88%.

\textbf{Discussion}

There has been recent report of AL failure in Northern Nigeria [5]. Mutations in several genes, including \textit{pfmdr1}, \textit{pfcrt} and \textit{pfk13} are associated with variation in parasite sensitivity to a range of drugs [6, 15, 26]. The \textit{pfmdr1} mutations N86Y, Y184F and D1246Y SNPs are thought to modulate susceptibility to CQ, AL and AS-AQ [53]. We found that, \textit{pfmdr1} 184F and 86Y alleles predominated in North-West Nigeria while 1246Y was higher in the North-East.
Alleles of pfmdr1 carrying the wild type N86 residue are associated with higher IC$_{50}$ and IC$_{90}$ values for LMF, MFQ and DHA, while the alternative 86Y residue seems to confer increased resistance against CQ and AQ [53]. Similarly, there are varying epidemiological reports on the prevalence and consequences of pfmdr1 N86Y polymorphisms from different parts of the world. For example, Ibraheem et al. [21] reported that pfmdr1 mutations are geographically confined and have inconsistent distributions from one geographic region to another.

Following adoption of ACTs in many African countries, some studies from West Africa have linked the prevalence of the pfmdr1 N86 allele to selection by AL [36]. Therefore, the high prevalence of pfmdr1 N86 allele observed in the present study might be suggestive of possible AL pressure in all the states. In addition, the finding might also suggest that the efficacy of the LMF component of ACTs is susceptible to the emergence of decreased tolerance in the local P. falciparum populations, as the presence of pfmdr1 N86 is critical in the initiation of resistance to LMF in vivo and that its selection primarily follows reinfection and recrudescence events associated with the elimination stage of LMF, 4–5 days after artemether clearance [45].

Some reports have associated a rise in the prevalence of pfmdr1 86Y alleles with increasing CQ resistance [11–12, 25]. The low prevalence of pfmdr1 86Y in Adamawa and Yobe raises the possibility that CQ may be effective against P. falciparum malaria in North-Eastern Nigeria once again, although this would be presumably tempered by CQ-resistance associated mutations in pfcr, which we have not assayed here. It is possible that the selection of pfmdr1 86Y allele in this region was aided by the cessation of CQ usage due to the emergence of resistance. The high prevalence of this mutation across Northern Nigeria may indicate that the efficacy of AL is at risk in this region, but raises the possibility that CQ may be effective in the chemotherapy of uncomplicated malaria here.

The effect of pfmdr1 Y184F polymorphisms on the efficacy of antimalarial drugs has been shown by various in vitro studies to be insignificant [15, 53]. Variations in the IC$_{50}$ of antimalarial drugs between parasite lines expressing wild type pfmdr1-Y184 or mutant 184F were shown to be closely linked to either of pfmdr1 N86 or 86Y alleles and not the 184F allele [53]. However, several epidemiological studies on the prevalence of pfmdr1 Y184F polymorphisms have shown that the Y184 allele is predominantly confined to East and Central Africa while the mutant 184F allele predominates in West Africa [1, 32, 36, 48]. Indeed, reports of the high occurrence of the mutant pfmdr1 184F in West Africa were corroborated by the present findings in which we show that the prevalence of pfmdr1 184F was high in all the states, and especially in Kano, and that its prevalence is higher in North-West compared to North-East Nigeria. This mutation has been previously linked to a reduction in susceptibility to LMF and/or ART [19]. It is perhaps unsurprising, given this, that we find a relatively high prevalence of this mutation in regions where AL is first line intervention against uncomplicated malaria.

Despite the fact that most in vitro and in vivo studies have not strongly associated changes in mutant pfmdr1 184F with alterations in antimalarial drug response [47], the high prevalence of mutant pfmdr1 184F obtained in the present study might indicate an increased propensity towards AL treatment. In
agreement with this is the fact that there is a lower prevalence of mutant pfmdr1 184F alleles in those East African countries that use AS-AQ or AL/AS-AQ as first line treatments compared to West African countries strictly managing uncomplicated malaria with AL [36]. The observed high prevalence of pfmdr1 184F across the region suggests that AS-AQ or DHAP should be used as an alternative to AL as first line treatments for uncomplicated falciparum malaria here.

The pfmdr1 D1246Y mutation affects P. falciparum susceptibility to various antimalarials including QN, MFQ, (HF), CQ and ART, with the latter two drugs affected in a strain specific manner [14, 42]. The observed low prevalence of mutant pfmdr1 1246Y alleles compared to the wild type in this study is consistent with reports from Southern Nigeria [38] as well as other West and East African countries that adopted AL as a front-line antimalarial therapy for uncomplicated malaria [1, 36]. Countries in Central Africa have observed an unsteady increase in the prevalence of the pfmdr1 D1246 allele, possibly due to the selective pressure of AS-AQ [2, 6].

Several reports from Africa have suggested that linkage between pfmdr1 N86Y/Y184F/D1246Y results in haplotypes with particular phenotypic characteristics that may be selected depending on the particular drugs that the population is exposed to [50–51]. The occurrence of pfmdr1 NFD and NYD haplotypes, for example, may result from AL selection while the pfmdr1 YYY haplotype may be favoured in regions where parasites are exposed to AS-AQ, DHAP and CQ [15, 32]. The treatment of uncomplicated malaria with AL often selects pfmdr1 haplotypes bearing the N86 allele [29, 48]. We found a predominance of the pfmdr1 NFD haplotype in Northern Nigeria with Kano, and a complete absence of the pfmdr1 YYY haplotype. These findings are line with the selective effect of AL on the NFD haplotype, and may indicate a loss of susceptibility to AL treatment by parasites in this region.

Conclusions

In conclusion, Kaduna and Kano States had higher prevalences of the pfmdr1 86Y allele than Yobe and Adamawa indicating differential selection in North-East and North-West Nigeria, possibly due to differing population density of these regions. Furthermore, there was a very high prevalence of pfmdr1 NFD and NYD haplotypes which suggests that AL efficacy may be reduced in both regions. Overall, the scarcity of pfmdr1 YYY with a high prevalence of NFD haplotypes could inform a rational antimalarial drug policy shift from AL to either AS-AQ or DHAP and CQ in this region.

Abbreviations

SNPs
Single nucleotide polymorphisms; pfmdr1:P. falciparum multidrug resistance gene-1; pfcrt: P. falciparum chloroquine resistance transporter gene; Pgh-1:P-glycoprotein homologue-1; PfMDR1: P. falciparum multidrug resistance protein-1; DNA:Deoxyribonucleic acid, PCR:Polymerase chain reaction; CIOMS:Council for international organizations of medical sciences; ACTs:Artemisinin based combination therapies; CQ:Chloroquine; AQ:Amodiaquine; QN:quinine; HF:halofantrine; ART:artemisinin,
MQ: mefloquine; AL: artemether-lumefantrine, AS-AQ: artesunate-amodiaquine; DHAP: dihydroartemisinin-piperaquine.

**Declarations**

**Authors’ contributions**

MNS, AA, MSJ, BOY and HMSG conceived the idea, designed and performed the experiments, analyzed the data and wrote the manuscript. MAI and EOB participated in the design of the experiment, supervised samples collection/preparation in the field and revised the manuscript. KK, KH, RC and DKI supervised the laboratory experiments, participated in the overall interpretation of data and revision of the manuscript. The authors read and affirmed the final version of the manuscript.

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**Acknowledgments:**

We are thankful to the authorities of Ahmadu Bello University, Zaria, Nigeria and deanery of School of Tropical Medicine and Global Health, Nagasaki University, Japan who were highly instrumental to the success and completion of this study.

**Availability of data and materials**

Authors assure that data will be available upon request following acceptance and publication of the article.

**Consent for publication**

Authors received the consent of patients or their guardians to use data for publication.
Ethics and consent to participate

The consent of patients or their guardians attending public health facilities was sought following explanation of the voluntary nature of participation. Prior to sample collection, ethical clearance was granted from the Ministries of Health of Kano, Adamawa, Yobe and Kaduna with references MOH/Off/797/T.1/285, S/MOH/1131/V.1, MOH/GEN/704/VOL.1 and MOH/ADM/744/VOL.1/537 respectively. The study was conducted in accordance with the 2016 international ethical guidelines for health-related research involving human subjects developed by the Council for International Organizations of Medical Sciences (CIOMS) in collaboration with the World Health Organization.

Competing interests

The authors of this study have no competing interest to declare.

Funding

The study was funded by the University Board of Research of Ahmadu Bello University, Zaria, Nigeria.

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Figures
Figure 1

A map of Nigeria showing the study sites for the surveillance of pfmdr1 N86Y-Y184F-D1246Y polymorphisms
Figure 2

Prevalence of pfmdr1 Single Nucleotide Polymorphisms (SNPs) across Four States of Northern Nigeria. The State-wise Distribution of pfmdr1 SNPs at codons 86, 184 and 1246 are shown in A, B and C respectively. Shown in green and red color are bars representing pfmdr1 wild and pfmdr1 mutant alleles, respectively.
Figure 3

Prevalence of pfmdr1 Single Nucleotide Polymorphisms (SNPs) across north-east and -west Nigeria. The Regional Distribution of pfmdr1 SNPs at codons 86, 184 and 1246 are shown in A, B and C respectively. Shown in green are bars representing pfmdr1 wild alleles while red color bars represent pfmdr1 mutant alleles.
Figure 4

Prevalence of pfmdr1 86-184-1246 Haplotypes across Four States and Two Geopolitical Regions of Nigeria. The Distribution of pfmdr1 Haplotypes across the States and Regions are shown in A and B respectively. Shown in red color are bars representing the most prevalent haplotype whilst ash color and empty bars are the least and unavailable haplotypes respectively.

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