Frequency Distribution of Antimalarial Drug Resistance Alleles among *Plasmodium falciparum* Isolates from Gezira State, Central Sudan, and Gedaref State, Eastern Sudan

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INTRODUCTION

Malaria is still one of the most threatening diseases for people living in tropical and subtropical areas and its control is seriously hampered by the quick development of antimalarial drug resistance by *Plasmodium falciparum*. During the second half of the 20th century, chloroquine (CQ) was the antimalarial treatment of choice, because it was safe, cheap, and highly effective against susceptible malaria parasites. The CQ resistance arose more than 40 years ago in southeast Asia and South America, and increasing rates of CQ resistance contribute to rising morbidity and mortality from malaria in Africa.

The CQ resistance is determined by the major point mutation at codon 76 of the *P. falciparum* CQ resistance transporter (*pfcrt*) gene, which is highly correlated with increased clinical CQ tolerance and treatment failure.⁴ In addition, point mutations in *P. falciparum* multi-drug resistance gene 1 (*pfmdr1*) (e.g., N86Y, Y183F, S1034C, N1042D, and D1246Y) have been shown to modulate CQ resistance and possibly lumeofantrine resistance.⁵

Similarly, resistance of malaria parasites to antifolates is conferred by mutation in dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHP), two enzymes involved in the parasite’s folate synthesis.⁶–¹⁰ In particular, a combination of five point mutations, pfldhfr N51I, C59R, S108N, and pfldhps A437G and K540E, have been shown to be strongly associated with sulfadoxine/pyrimethamine (SP) resistance in Africa.¹¹–¹³

Adding artemisinin derivatives (i.e., artesunate) to standard antimalarial drugs (i.e., SP) is a strategy to reduce treatment failure and transmission potential, because artemisinin derivatives act rapidly and kill malaria parasites that are resistant to other drugs.¹⁴ In fact, many malaria endemic countries have lately adopted artemisinin-based combination therapy (ACT) as the first-line drug for treating uncomplicated malaria, as recommended by the World Health Organization (WHO).¹⁵ Although ACT should represent the best option available to prevent the induction of resistance parasites, the risk of development of resistance in *P. falciparum* to artemisinin compounds is serious, given the recent observation of two cases of clinical resistance in Cambodia¹⁶ and cases of diminished *P. falciparum* susceptibility to artether.¹⁷,¹⁸

Recently, it was observed that variations in susceptibility profiles of malaria parasites to artemisinin derivatives have been associated with single nucleotide polymorphisms (SNPs) in sarcosporic/endoplasmic reticulum calcium ATPase (SERCA)-type of *P. falciparum* ATPase6 (*pfATPase6*) gene, the putative target for artemisinins,¹⁷,¹⁹,²⁰ suggesting a possible role of *pfATPase6* gene as a molecular marker of artemisinin resistance.

Sudan is the largest country in Africa, comprising more than 8% of the entire continent. The total population is estimated to be 39.2 million inhabitants, of whom 75% live in rural areas. Malaria is a leading cause of morbidity and mortality, resulting in 3.1 million cases and 2,500 deaths annually.²¹ The entire country is at risk of malaria, although with different levels of endemicity. In the northern, eastern, and western states malaria endemicity is mainly low to moderate with predominantly seasonal transmission and epidemic outbreaks. In southern Sudan, malaria is moderate to high or highly intense, generally with perennial transmission. *Plasmodium falciparum* is by far the predominant parasite species.

The CQ has been the most used drug as a first line for years in Sudan. Some studies showed that in five hospitals of central Sudan, 85.6% of patients admitted for severe malaria had received CQ before admission²² and that CQ was also the commonly prescribed drug by health care providers.²³ First cases of CQ resistance were reported in 1978,²⁴ and several in vivo and in vitro studies have subsequently documented a
high presence of CQ resistance in different Sudanese areas (central Sudan25–27; eastern Sudan28,29).

Sulfadoxine/pyrimethamine resistance was reported in Sudan since the early 1990s30 and was documented by *in vivo* studies conducted in eastern Sudan just before ACT deployment.31–33 The presence of highly resistant *pfdhfr/pfdhps* quintuple mutants have been previously reported in studies conducted in different areas of southern34 and eastern Sudan.31

In 2004, because of CQ and SP resistance, the national antimalarial drug policy changed from monotherapy to artemisinin-based combination therapy. The National Malaria Control Program decided to replace CQ with artesunate + sulfadoxine/pyrimethamine as the first-line drug and artemether-lumefantrine has replaced SP as the second-line treatment.35 The aim of this study was to assess the effect of the current antimalarial drug policy, i.e., ACT, on prevalence of mutations in target genes, in two malarious areas of Sudan where to the best of our knowledge no recent investigations on the same subject have been carried out.

**MATERIALS AND METHODS**

**Study sites.** The fieldwork was carried out in health facilities located in and around Wad Medani–Gezira State (central Sudan) and Elfau locality–Gedarif State (eastern Sudan) (Figure 1) between June and December 2007, corresponding to the period of high malaria transmission. These study sites are located in an irrigated agricultural scheme, where transmission is seasonal as identified by epidemiological strata of malaria in Sudan and are inhabited by different ethnic groups and tribes from all regions of Sudan. Wad Medani is the main town of the irrigated area of the Gezira Agricultural Scheme where malaria is classified as mesoendemic to hyperendemic with an unstable transmission pattern. Elfau is located in the west part of Gedarif State neighboring Gezira State. It is the main town of the Erahad irrigation scheme.

**Patients and blood samples.** Participants were enrolled in the study after appropriate informed consent was obtained. Ethical approval for this study was obtained from the Ethical Committee of the Blue Nile National Institute for Communicable Disease/University of Gezira and from the State Health Authority in Gezira.

Blood samples were collected from 212 (central Sudan) and 63 (eastern Sudan) microscopically confirmed *P. falciparum*-infected patients. Of 275 patients screened, 235 patients (42% males, 58% females, age range: 4–45 years) met the criteria for inclusion in the analysis: patients had *P. falciparum* monoinfection, asexual parasitaemias in excess of 1,000 parasites, but less than 80,000 parasites (mean of 29,520 parasites) per μL blood, and had not received quinine and artemisinin within the last 7 days, 4-aminoquinolines within the last 14 days, pyrimethamine and/or sulfonamide within the last 28 days, or mefloquine within the last 56 days.

For molecular analysis, infected blood was collected by finger prick, blotted *in triplicate* on a filter paper, and air dried. Filter papers were wrapped separately in a plastic bag and stored at room temperature. Total DNA was extracted from 149 samples using the phenol/chloroform method9 and from 86 by using PureLink Genomic DNA Kit (Invitrogen, Carlsbad, CA), following the manufacturer’s instructions.

For the *in vitro* test, 2 mL of blood were collected from subjects into a sterile EDTA container after cleaning the site of puncture with 70% ethanol.

**Real-time PCR assay.** All samples were genotyped at *pfcr7* 76 codon, *pfdhfr* 51-59-108 codons, and *pfdhps* 436-437 codons using a 5′-nuclease real-time polymerase chain reaction (PCR) assay. Primers and probes sequences were...
previously described\textsuperscript{46-37} and were synthesized by Roche-Diagnostics (Mannheim, Germany). Wild-type and mutant-type probes were labeled with a FAM and HEX fluorescent reporter at their 5’ ends, respectively. Real-time PCR was performed with a LightCycler 480 System (Roche) according to the following steps: pre-incubation 95°C for 10 min; amplification 95°C for 10 sec, 55°C for 20 sec, and 72°C for 5 sec for 45 cycles. The PCR assays were optimized according to the LightCycler 480 Probes Master Kit, and 0.5 μM of each primer and 0.2 μM of each probe, used in combination, were used in this study. All reactions were done in duplicate, in a final volume of 20 μL. The DNAs from W2 and 3D7 laboratory strains and from characterized field isolates were used as reference standards.

**PCR-restriction fragment length polymorphism (RFLP) analysis.** Detection of \textit{pfmdr1} \textit{86} and \textit{pfldhps} \textit{540} polymorphisms was performed by the PCR-RFLP method. \textit{Pfmdr1} amplification was achieved under conditions described in Duraisingh and others.\textsuperscript{36} The PCR products were digested at 37°C with 1U of \textit{AflIII} (New England Biolabs, Ipswich, MA) restriction enzyme. Detection of \textit{pfldhps540} polymorphism was performed using primers described in Alker and others.\textsuperscript{36} The PCR products were digested at 37°C with 1U of \textit{FokI} (Biolabs) restriction enzyme, as described in Duraisingh and others.\textsuperscript{39}

**Sequencing.** Assessment of single nucleotide polymorphisms for the \textit{pfaTPase6} gene was based on PCR and the sequencing method, as described previously in Menegon and others.\textsuperscript{40}

**Statistical analysis.** Differences in mutation frequency of the studied genotypes by sampling sites or by sampling period were analyzed by the two-sample test of proportions (STATA Statistical Software: Release 8.1, 2003, Stata Corp., College Station, TX). The same statistical test was adopted to compare the 59R mutation frequency observed in 2003\textsuperscript{11} and in this study. Results are showed as z(test) and \(p(z)\) values.

**In vitro test.** The micro-technique test (Mark III: supplied by WHO Division of Control of Tropical Disease 2001, England) was used to evaluate \textit{in vitro} sensitivity to CQ of \textit{P. falciparum} malaria parasites. Sixty-nine malaria cases were selected for this study, according to the \textit{in vitro} protocol described in Mark III-WHO.\textsuperscript{11} The test was performed in tissue culture plates pre-dosed with drugs in increasing concentrations; briefly, 0.9 mL of RPMI 1640 medium were taken into the sterile Falcon tube and 100 μL from well-mixed blood mentioned previously were added. All wells of appropriate columns were dosed with 50 μL of blood-medium mixture using the 50 μL fixed volume Eppendorf pipette and a disposable sterilizable tip, as provided with the test kit. Plates were incubated at 37.5°C in a candle jar for 24 to 30 h. At the end of incubation, blood from each well was harvested and a thick film was prepared; thick films were stained for 30 min in a Giemsa stain at a dilution of 1% (vol/vol) in buffered water of pH 6.8. After drying, films were examined through a light microscope with oil immersion lens, and the number of schizonts with three or more nuclei of a total of 200 asexual parasites (i.e., schizonts and trophozoites) was counted. For an acceptable test, schizont maturation in control must be 10% or more (i.e., 20 schizonts with three or more nuclei per 200 asexual parasites). Average results for EC50, EC90, EC95, and EC99 (i.e., drug concentrations producing 50%, 90%, 95%, or 99% inhibition of schizont maturation, respectively) were calculated using the WHO log probit program (www.who.int/csr/drugresist/malaria/en/probit.xls) in all isolates.

**RESULTS**

Between June and December 2007, a total of 235 blood samples from \textit{P. falciparum}-infected individuals were examined for the presence of mutations in five genes related to antimalarial resistance: in particular, we investigated one codon of \textit{pfcrt} gene (K76T), one codon of \textit{pfmdr1} (N86Y), three codons of the \textit{pfdhfr} gene (N51I, C59R, S108N), three codons of the \textit{pfldhps} gene (S436A, A437G, K540E), and SNPs in two regions of the \textit{pATPase6} gene, from nucleotide (nt) 652 to 1422 and from nt 1744 to 2402 (nt number is according to the ATG start codon). Among the blood samples examined, 198 samples gave PCR-positive results for each of the genes included in our study, exactly 27 samples from Gedaref State (eastern Sudan), and 171 samples from Gezira State (central Sudan). Thirty-seven patients (16%) with PCR-negative results were excluded from analysis. There were 14 samples (7%) that showed mixed infections of both wild-type and mutant alleles. These mixed wild/mutant infections were considered as mutants. Moreover, a total of 69 samples were analyzed \textit{in vitro} for their susceptibility to CQ, however only 45 (65.2%) isolates with an adequate schizont growth were successfully tested.

**Polymorphism analysis.** Our results showed that 185 samples (93.4%) out of 198 analyzed carried a mutant allele of at least one gene associated with resistance to antimalarial drugs chloroquine and sulfadoxine-pyrimethamine. The results are summarized in Table 1 and Table 2. The majority of the isolates were found to carry the mutant codons \textit{pfldhfr}R\textsubscript{59} (75.3%) and \textit{pfldhfr}N\textsubscript{108} (72.7%), whereas \textit{pfldhfr}R\textsubscript{95} was found in 15 isolates (7.6%). With respect to \textit{pfldhps} gene, \textit{pfldhps}A\textsubscript{130}, \textit{pfldhps}G\textsubscript{437}, and \textit{pfldhps}E\textsubscript{440} were detected in 8.1%, 20.7%, and 16.7% of the isolates, respectively.

Prevalence of the CQ resistance-related mutant alleles was relatively high: \textit{pfcrt}T\textsubscript{76}, allele was present in 72.7% of the isolates, whereas 86Y mutation of the \textit{pfmdr1} gene was detected in 55.5% of the isolates.

Isolates from eastern Sudan (Gedarif) showed a higher prevalence of all but one mutation when compared with isolates from central Sudan (Gezira). This difference has been confirmed as significant by statistical analysis for \textit{pfcrt}R\textsubscript{59}, \textit{pfmdr1}B\textsubscript{186Y}, \textit{pfldhfr}T\textsubscript{108N}, and \textit{pfldhps}A\textsubscript{436} mutated codons (Table 1A). None of the isolates from eastern Sudan had \textit{pfldhfr}S\textsubscript{95R} mutation.

Moreover, when we compared samples from central Sudan collected during June–July (\(N = 71\)) and those collected during November–December (\(N = 100\)), we observed that the rate of almost all mutations is lower in the isolates collected in November–December. However, statistical analysis showed that there were no significant differences between \textit{Plasmodium} genotype frequencies from parasite populations collected in two different periods within the same sampling area (Table 1B).

By the analysis of allele combinations of these four resistance markers, we observed 41 different haplotypic conformations in our isolates (Table 2). In particular, we found 5 distinct haplotypes in isolates that carried a single point mutation (8.6% of the isolates analyzed), 8 haplotypes in double mutant isolates (14.7%), 12 haplotypes in triple mutant isolates (22.7%), 4 haplotypes in mutants isolates carrying quadruple mutations (25.3%), 8 haplotypes in quintuple mutant isolates (13%), and
2 and 1 haplotypes in mutant isolates that carried 6 (7.6%) and 7 (1.5%) point mutations, respectively (Table 2, Figure 2). The wild-type sequence K76N, N86K, S108S, S436A, K540E, and K59R was present in 6.6% of isolates (Table 2, Figure 2).

With regard to the haplotypes, isolates from eastern Sudan did not exhibit wild-type, single mutant type, and 7-fold mutant genotypes but they displayed a high prevalence of mutant alleles with four mutations (33.3%), whereas the majority of isolates from central Sudan were found to carry the triple mutant haplotype (25.1%) (Figure 2).

P. falciparum ATPase6 SNPs. In this study, we analyzed the prevalence of single nucleotide polymorphisms in two regions of the pfATPase6 gene. Most of the Sudanese parasite population harbored wild-type allele. Only two previously reported mutations were detected: 431K in 35 isolates (17.7%) and 402V in 8 isolates (4%) (Table 1).

Chloroquine susceptibility. In vitro activity of CQ was successfully evaluated in 45 isolates. The majority (55.6%) of tested isolates were fully sensitive to CQ, 37.8% had a low resistance level, and 6.7% were marked resistant (Table 3). The test results processed by log concentration–response probit analysis for determination of 50%, 90%, 95%, and 99% effective concentrations (EC_{50}, EC_{90}, EC_{95}, and EC_{99}, respectively) were: EC_{50} = 312.1808 nmol/L, EC_{90} = 930.0186 nmol/L, EC_{95} = 1287.3088 nmol/L, and EC_{99} = 363.1583 nmol/L.

Polymorphism analysis of this group of isolates showed high prevalence of single and double mutations pfcrT76K/pfmdr1Y863C related to CQ resistance (Table 3), however we found that isolates showed low correlation between molecular profile of both genes and in vitro susceptibility. In fact, two (66.7%) of three marked resistant isolates were fully sensitive to CQ, 37.8% had a low resistance level, and 6.7% were marked resistant (Table 3).

Prevalence of point mutations in isolates analyzed in this study

<table>
<thead>
<tr>
<th>Table 1A</th>
<th>Total</th>
<th>Gezira</th>
<th>Gedarif</th>
<th>Total</th>
<th>Gezira</th>
<th>Gedarif</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>June–December</td>
<td>November–December</td>
<td>June–December</td>
<td>No. (%)</td>
<td>June–December</td>
<td>November–December</td>
</tr>
<tr>
<td>Pfcrt76T</td>
<td>144 (72.7)</td>
<td>44 (28.8)</td>
<td>100 (71.0)</td>
<td>2.03</td>
<td>0.043</td>
<td>54 (76)</td>
</tr>
<tr>
<td>Pfmdr186Y</td>
<td>110 (55.5)</td>
<td>33 (49.9)</td>
<td>77 (55.6)</td>
<td>3.34</td>
<td>0.037</td>
<td>36 (50.7)</td>
</tr>
<tr>
<td>Pfddhfr511</td>
<td>149 (75.3)</td>
<td>49 (87.3)</td>
<td>100 (71.0)</td>
<td>0.32</td>
<td>0.0746</td>
<td>58 (81.6)</td>
</tr>
<tr>
<td>Pfddhfr599R</td>
<td>15 (7.6)</td>
<td>0</td>
<td>15 (8.8)</td>
<td>1.42</td>
<td>0.014</td>
<td>7 (9.9)</td>
</tr>
<tr>
<td>Pfddhfr108SN</td>
<td>114 (72.7)</td>
<td>30 (46.9)</td>
<td>84 (62.4)</td>
<td>2.03</td>
<td>0.037</td>
<td>52 (73.3)</td>
</tr>
<tr>
<td>Pfddhps436A</td>
<td>16 (8.1)</td>
<td>5 (18.5)</td>
<td>11 (6.4)</td>
<td>2.15</td>
<td>0.0133</td>
<td>4 (5.6)</td>
</tr>
<tr>
<td>Pfddhps437G</td>
<td>41 (20.7)</td>
<td>8 (29.6)</td>
<td>33 (19.3)</td>
<td>1.24</td>
<td>0.214</td>
<td>16 (22.5)</td>
</tr>
<tr>
<td>Pfddhps540E</td>
<td>33 (16.7)</td>
<td>6 (22.2)</td>
<td>27 (15.7)</td>
<td>0.84</td>
<td>0.399</td>
<td>15 (21.2)</td>
</tr>
<tr>
<td>PfATPase6-402V</td>
<td>8 (4)</td>
<td>1 (3.7)</td>
<td>7 (4)</td>
<td>0.07</td>
<td>0.941</td>
<td>7 (9.8)</td>
</tr>
<tr>
<td>PfATPase6-431K</td>
<td>35 (17.7)</td>
<td>12 (44.5)</td>
<td>23 (13.4)</td>
<td>3.94</td>
<td>0.001</td>
<td>13 (18.3)</td>
</tr>
</tbody>
</table>

DISCUSSION

We believe that the current study is one of the few recent reports of drug resistance molecular markers in these malarious areas of Sudan. In this view, it is interesting to compare our results with those obtained in studies previously carried out in this malaria endemic region.

In Sudan, malaria has a scattered distribution and varies a lot according to the different regions of the country: there are regions with high, stable malaria transmission (southern Sudan), others with a high seasonal transmission pattern (central/eastern Sudan), and finally, in the northern part of the country, spotted areas characterized by riverine and urban transmission. Furthermore, the level of falciparum resistance to the most used antimalarials differs from region to region, making a countrywide drug policy difficult.

In 2004, the National Control Malaria Program decided to adopt artesunate + sulfadoxine/pyrimethamine as the first-line drug and artemether-lumefantrine as the second line. However, the implementation of this decision and consequent real withdrawal of CQ from the local market were still in agenda in the year 2005. As reported by Malik and others, manufacturing and importation of CQ tablets and vials was stopped completely in January 2006 only. As a consequence, we might expect that CQ has been available in the local market for a long period after that date. This is an important consideration in evaluating the result of the molecular marker investigation.

In fact, frequency of pfcrt76T mutation observed in our study is still relatively high (72.7%, N = 144/198) and it is consistent with a current or recent presence of drug pressure, even if it is lower than previously reported from other Sudanese studies. A-Elbasit and others, for example, reported a pfcrt76T frequency close to fixation of mutated allele (pfcrt76T = 93%). In this scenario, the results of the in vitro tests are quite surprising and the observed high percentage of isolates showing full sensitivity to CQ is difficult to explain. Recent data from literature have already discussed the potential inaccuracies of in vitro drug susceptibility tests and also some conflicting results between the frequency of pfcrt mutations and in vitro/in vivo tests. However, we can speculate that in vitro tests data obtained in our study could have resulted from the limited number of tests performed since, according to the WHO protocol, a correct MARK III outcome can be obtained by performing at least 100 tests. If we take into account the recognized strong link between presence of pfcrt mutations...
and CQ resistance and we look at the frequency of 
*pfcrt* 76T mutation from this study, we can assume that CQ is definitely
an unsuitable drug for this area. These data suggested that
prevalence of molecular markers for CQ should be monitored
(while this drug is being replaced by combination therapies)
to verify if the level of CQ resistance is decreasing in this area.
In fact, as previously reported, 25, 26 recession of chloroquine-
resistant parasites preceded the complete withdrawal of CQ
in areas with high malaria transmission.

As far as ACT implementation is concerned, unfortunately
falciparum tolerance/resistance to artemisinin derivatives
has been recently reported in Cambodia. 16 This recent report
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**Molecular Investigation on Antimalarial Resistance in Sudan**

In vivo tests assessing falciparum sensitivity to pyrimetamine and sulfadoxine drug combination are needed, because the lack of efficacy of SP will adversely affect the outcome of ACT treatment.

We have also taken into consideration the frequency of each mutated codon by sampling areas (Gedarif versus Gezira) and by sampling periods (June/July versus November/December, Gezira). The results of statistical analysis showed that the differences in mutations frequencies between the two sampling periods are not significant, thus indicating that probably the sampling period encompassed the same malaria transmission season. Conversely, when comparing the two sampling areas, there are significant differences in frequency for four mutated codons (**pfcr**T76, **pfmdr**186Y, **pfdhfr**108N, and **pfdhps**436A). However, we have to be cautious in drawing conclusions if we consider that there are some differences between the two batches of samples (samples size and sampling period) and that information about the malaria treatment implementation in the two study areas is lacking. Therefore, we can only speculate that falciparum populations in Gedarif are exposed to a higher drug pressure than parasite populations from Gezira.

Finally, interesting issues arise from analysis of the different haplotypes generated by the association of all identified mutations in four analyzed genes. In fact, the most prevalent haplotype is quadruple mutant **pfcr**T76Y**pfmdr**186Y**pfdhfr**108N**pfdhps**436 (23.8%), but also haplotypes showing five or six mutations are well represented in the falciparum isolates circulating in this study area, in particular sextuple mutant **pfcr**T76Y**pfmdr**186Y**pfdhfr**51N**pfdhfr**108G**pfdhps**436E**pfdhps**540 that accounts for 7.1%. Moreover, we found also three isolates showing seven mutations **pfcr**T76Y**pfmdr**186Y**pfdhfr**108N**pfdhps**436E**pfdhps**540. The presence of multiple haplotypes in African falciparum isolates has been already described and discussed in previous studies. The reason for these observed patterns of mutations in specific genes scattered on four different chromosomes and linked to the resistance to a different class of antimalarials is still to be elucidated. We can speculate that this association could occur in endemic areas where the probably high fitness cost of multiple mutations is balanced by simultaneous pressure of different drugs. The current situation in Sudan could represent an example: as stated previously, because in this country CQ is probably still available and used (or has been used until sometime ago) especially in rural setting and, as a part of ACT, SP combination is used as well. This could explain the detection in the isolates analyzed in our study of the TYIN, TYINGE, and TYIRNGE haplotypes. The circulation of these isolates harboring multiple CQ and SP resistance mutations in an endemic area is not to be underestimated, because, as previously reported, selection of a high degree of resistance toward a given antimalarial, in this case the pyrimethamine, could be accompanied by increase of a total number of mutations in *P. falciparum*. Hence, the Sudanese isolates of *P. falciparum* carrying multiple mutations are expected to be somewhat resistant and in case of accumulation of further mutations could become really difficult to be treated.

In conclusion, this study supports the usefulness of molecular marker screening to be carried out in the frame of country surveillance activities. Monitoring evolution of *P. falciparum*

**Figure 2.** Overall prevalence of Sudanese isolates carrying point mutations observed in a total of eight codons in the **pfcr**, **pfmdr**, **pfdhfr**, and **pfdhps** genes.

**Table 3**

<table>
<thead>
<tr>
<th>In vitro test result</th>
<th><strong>pfcr</strong> T76</th>
<th><strong>pfmdr</strong>186Y</th>
<th><strong>pfdhfr</strong>108N</th>
<th><strong>pfdhps</strong>436E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive (N = 25)</td>
<td>12 (48%)</td>
<td>13 (52%)</td>
<td>14 (56%)</td>
<td>11 (44%)</td>
</tr>
<tr>
<td>Low resistant (N = 17)</td>
<td>3 (17%)</td>
<td>14 (82.4%)</td>
<td>3 (17.6%)</td>
<td>14 (82.4%)</td>
</tr>
<tr>
<td>Marked resistant (N = 3)</td>
<td>2 (66.7%)</td>
<td>1 (33.3%)</td>
<td>3 (100%)</td>
<td>0</td>
</tr>
</tbody>
</table>

* N = no. isolates; wt = wild-type codon; mut = mutant codon.
drug resistance by analyzing the prevalence of drug resistance mutations in an endemic area as Sudan, where both logistical and political problems make the implementation of the Efficacy Therapeutic Tests difficult, could provide key information for the deployment of an effective countrywide drug policy.

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