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Screening for *in vitro* susceptibility to pyrimethamine and sequencing of the *pfmdr2* and *pfdhfr* genes were performed in 140 *Plasmodium falciparum* isolates. The risk of *in vitro* resistance to pyrimethamine was analyzed with a logistic regression model. The mutation F423Y in *pfmdr2* (odds ratio [OR] = 2.12 [confidence interval (CI), 1.02 to 4.59]; *P* = 0.0489) and the mutation N51I, C59R, or S108N in *pfdhfr* (OR = 42.34 [CI, 5.52 to 324.61]; *P* = 0.0003) were independently associated with *in vitro* resistance to pyrimethamine.

Sulfadoxine and pyrimethamine (Pyr) (SP) were widely used as first-line drugs for the treatment of malaria until the spread of resistance in *Plasmodium falciparum* isolates all over the world. SP is still recommended in combination with artesunate or chloroquine as a second-line therapy for uncomplicated *P. falciparum* and *P. vivax* malaria (7, 17, 19). SP is also still widely used in Africa for the intermittent preventive treatment (IPT) of malaria in infants (6), children (3), and pregnant women (8). SP resistance has been well characterized at the molecular level. Mutations involving residues 51, 59, 108, and 164 of *P. falciparum* dihydrofolate reductase conferred cross-resistance to the antifolates pyrimethamine and cycloguanil, whereas a mutation in residue 16 specifically conferred resistance to cycloguanil (16). The accumulation of mutations in the enzyme dihydrofolate reductase (in which Gly437 proved to be a key residue) was associated with sulfadoxine resistance in *P. falciparum* (18). Mutations involving other residues in dihydrofolate reductase and dihydropteroate synthase have also been linked to SP resistance in *P. vivax* isolates (1, 9). More recently, other mechanisms of resistance to SP have been proposed. Dahlström et al. (4) have suggested that mutations (specifically, I876V and K1466R) in the *P. falciparum* dihydrofolate reductase conferred cross-resistance to the antifolates pyrimethamine and cycloguanil, whereas a mutation in residue 16 specifically conferred resistance to cycloguanil (16). The accumulation of mutations in the enzyme dihydrofolate reductase (in which Gly437 proved to be a key residue) was associated with sulfadoxine resistance in *P. falciparum* (18). Mutations involving other residues in dihydrofolate reductase and dihydropteroate synthase have also been linked to SP resistance in *P. vivax* isolates (1, 9). More recently, other mechanisms of resistance to SP have been proposed. Dahlström et al. (4) have suggested that mutations (specifically, I876V and K1466R) in the *P. falciparum* dihydrofolate reductase conferred cross-resistance to the antifolates pyrimethamine and cycloguanil, whereas a mutation in residue 16 specifically conferred resistance to cycloguanil (16). The accumulation of mutations in the enzyme dihydrofolate reductase (in which Gly437 proved to be a key residue) was associated with sulfadoxine resistance in *P. falciparum* (18). Mutations involving other residues in dihydrofolate reductase and dihydropteroate synthase have also been linked to SP resistance in *P. vivax* isolates (1, 9). More recently, other mechanisms of resistance to SP have been proposed. Dahlström et al. (4) have suggested that mutations (specifically, I876V and K1466R) in the *P. falciparum* dihydrofolate reductase conferred cross-resistance to the antifolates pyrimethamine and cycloguanil, whereas a mutation in residue 16 specifically conferred resistance to cycloguanil (16). The accumulation of mutations in the enzyme dihydrofolate reductase (in which Gly437 proved to be a key residue) was associated with sulfadoxine resistance in *P. falciparum* (18).
Fisher’s exact test). The difference between the median of the Pyr IC₅₀ (2,598 nM) for isolates harboring the Y substitution at codon 423 (n = 81) and the median of the Pyr IC₅₀ (890 nM) for wild-type isolates (n = 59) was statistically significant (P < 0.02; Mann-Whitney test).

There was no statistically significant correlation between the F423Y mutation in pfmdr2 and the mutation in pfdhfr (P = 0.14; Fisher’s exact test). As illustrated in Fig. 1, 16 isolates were wild type for both the pfmdr2 and pfdhfr genes; the mean Pyr IC₅₀ for these isolates was 434.6 nM (95% CI, 0 to 1,014; minimum and maximum, 10 and 9,421 nM). A total of 13 isolates had the F423Y mutation in pfmdr2 and were wild type for pfdhfr; the mean Pyr IC₅₀ for these isolates was 170.1 nM (95% CI, 0 to 459.3; minimum and maximum, 10 and 1,762 nM). A total of 43 isolates were wild type for pfmdr2 and mutant type for pfdhfr (with at least one of the mutations N51I, C59R, or S108N); the mean Pyr IC₅₀ for these isolates was 3,229 nM (95% CI, 2,186 to 4,272; minimum and maximum, 9.43 and 13,294 nM). A total of 68 isolates were mutant type for both the pfmdr2 and pfdhfr genes; the mean Pyr IC₅₀ for these isolates was 4,192 nM (95% CI, 3,362 to 5,022; minimum and maximum, 50 and 19,654 nM). There was no statistically significant association between the median of the Pyr IC₅₀ (50 nM) for isolates with both pfmdr2 and the pfdhfr wild type and the median of the Pyr IC₅₀ (50 nM) for isolates with the mutant type for pfmdr2 and the wild type for pfdhfr (P = 0.25; Mann-Whitney test). The difference between the median of the Pyr IC₅₀ (1,993 nM) of pfmdr2 wild-type and pfdhfr mutant-type isolates and the median of the Pyr IC₅₀ (3,255 nM) for isolates harboring the Y substitution at codon 423 and a pfdhfr mutation was statistically significant (P = 0.0438; Mann-Whitney test).

The in vitro resistance to Pyr (Pyr IC₅₀ > 2,000 nM) was analyzed using a logistic regression model (Table 1). The mutation F423Y in pfmdr2 (OR = 2.12 [95% CI, 1.02 to 4.59]; P = 0.0489) and the mutation N51I, C59R, or S108N in pfdhfr (OR = 42.34 [95% CI, 5.52 to 324.61]; P = 0.0003) (Hosmer-Lemeshow goodness-of-fit test; P = 0.77) were independently associated with in vitro resistance to Pyr.

MDR2 is associated with heavy metal ion efflux and resistance to cadmium (14). MDR2 may be involved in the transport of organic anions, such as folate or pABA, as has been suggested for

![FIG 1 Pyrimethamine IC₅₀s (in nanomoles) of the 140 Plasmodium falciparum isolates according to their genotypes.](image-url)

### TABLE 1 Multivariate logistic regression model

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. (%) of Pyr-susceptible isolates</th>
<th>No. (%) of Pyr-resistant isolates</th>
<th>Crude OR (95% CI)</th>
<th>P value</th>
<th>Adjusted OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pfmdr2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F423</td>
<td>37 (26.4)</td>
<td>22 (15.7)</td>
<td>1 (reference)</td>
<td></td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>Y423</td>
<td>34 (24.3)</td>
<td>47 (33.6)</td>
<td>2.31 (1.11–4.91)</td>
<td>0.017</td>
<td>2.12 (1.02–4.59)</td>
<td>0.0489</td>
</tr>
<tr>
<td>pfdhfr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>28 (20)</td>
<td>1 (0.7)</td>
<td>1 (reference)</td>
<td></td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>Mutant type</td>
<td>43 (30.7)</td>
<td>68 (48.6)</td>
<td>43.36 (6.67–1,819.1)</td>
<td>&lt;0.0001</td>
<td>42.34 (5.52–324.61)</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

*a* OR, odds ratio; CI, 95% confidence interval.

*b* Fisher’s exact test.

*c* Multivariate logistic regression model.
MRP1 (4); it may be also implicated in the transport of sulfadoxine, as suggested by Martinelli et al. (10). Interestingly, in the latter study, an association between the in vivo resistance of *Plasmodium chabaudi* to sulfadoxine and a mutation in the *mdr2* gene has been shown. The same investigation performed with *Plasmodium falciparum* would be really interesting, but no laboratory is able to shown. The same investigation performed with this particular and also to evaluate the potential association between this *mdr2* mutation and the resistance of *P. falciparum* to sulfadoxine.

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**REFERENCES**