Reference


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Resistance to Dihydroartemisinin

To the Editor: The emergence of widespread resistance to chloroquine and sulfadoxine-pyrimethamine in Africa has caused a sharp rise in deaths from malaria. The World Health Organization therefore urgently recommends replacement of these drugs, particularly with combinations that include an artemisinin compound (AC) (1). In 2006, although >40 countries have adopted artemisinin-based combination therapies as their first-line treatment for malaria, only a few of these countries actually use these combination therapies because of limiting factors such as high cost (2). When used as monotherapy, ACs are associated with high rates of recrudescence, possibly because of their short elimination half-lives (3). Most artemisinin-based combination therapies contain, in addition to ACs, a partner drug against which resistance has already developed (e.g., mefloquine, amodiaquine, lumefantrine); reports of relatively low efficacy of the combination artesunate-amodiaquine have been recently published (4). In 2005, Jambou et al. claimed to have found the first cases of in vitro Plasmodium falciparum resistance to ACs (5).

We assessed the in vitro susceptibility to dihydroartemisinin (dhART), the biologically active metabolite of artemisinin derivatives, of P. falciparum isolates from travelers returning to France from various African countries during 2004–2006. In addition, we searched for polymorphism in the P. falciparum adenosine triphosphatase-6 (PfATPase6) gene, which was reported to be associated with in vitro artemether resistance (5). We also studied polymorphism (a 3-bp indel) in the gene of the ABC transporter G7, which was reported in 2005 to be associated with in vitro response to artesunate (6).

Determination of in vitro dhART susceptibility by using the isotopic semimicrotest method (7) was successful for 397 isolates. The most represented countries were Cameroon (17%), Côte d’Ivoire (14.5%), Mali (12%), Comoros Islands (8.5%), and Senegal (6.5%). Patients were ≥7 years of age (mean 31, SD 17 years), and the male:female ratio was 1.5:1. The 50% inhibitory concentration (IC50) values ranged from 0.02 to 31.8 nmol/L, with a geometric mean of 1.31 nmol/L and a median of 0.68 nmol/L. IC50 values were <1 nmol/L for 264 isolates, 1–10 nmol/L for 127, and >10 nmol/L for 6. Thus, some isolates showed a diminished susceptibility to dhART, but only 1 isolate had an IC50 >30 nmol/L (31.8 nmol/L).

DNA sequencing of 900-bp and 240-bp PCR products, including the 769 and the 243/263 PfATPase6 codons, respectively, was performed in a subsample of 154 isolates. All isolates had the S769 wild codon except 1 susceptible isolate (IC50 = 0.83 nmol/L), which had a S769N mutant type codon (Table). We found no polymorphism in codon 263. This position may be scrutinized to monitor anticipated artemisinin resistance, according to a recently published structure-function study (8). Conversely, we found 2 isolates that had IC50 values of 4.2 nmol/L and

<table>
<thead>
<tr>
<th>Gene</th>
<th>Predicted products</th>
<th>Position</th>
<th>Amino acid</th>
<th>Nucleotide change</th>
<th>No. isolates</th>
<th>Dihydroartemisinin IC50 (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATPase6</td>
<td>Sarcoplasmic reticulum calcium-transporting ATPases</td>
<td>769</td>
<td>S</td>
<td>AGT</td>
<td>153</td>
<td>0.1–31.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S→N</td>
<td>AAT</td>
<td>1</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>L→S</td>
<td>TCA</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H→Y</td>
<td>TAT</td>
<td>2</td>
<td>4.2; 6.4</td>
</tr>
<tr>
<td>G7</td>
<td>ABC transporter</td>
<td>1,390</td>
<td>Wild (AAT)</td>
<td>69</td>
<td>0.1–25.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mutant (AAT)</td>
<td>85</td>
<td>0.15–31.8</td>
<td></td>
</tr>
</tbody>
</table>

*PfATPase, Plasmodium falciparum adenosine triphosphatase; IC50, 50% inhibitory concentration.
Continued monitoring of the efficacy of their associated partner drugs also appears to be essential.

Acknowledgments

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References


Real-time PCR for Fracisella tularensis Types A and B

To the Editor: Fracisella tularensis, the etiologic agent of tularemia, is highly infectious and considered a potential bioweapon (1–3). Although 4 subspecies of F. tularensis are recognized, most cases of tularemia are due to infection by subsp. tularensis (type A) or holarctica (type B). North America is the only region where both type A and type B cause human disease. Subspecies novicida is also found in North America, but it is of reduced virulence. Disease incidence attributable to either type A or type B is...