Antimicrobial Susceptibility and Mutations Involved in Clarithromycin Resistance in Helicobacter pylori Isolates from Patients in the Western Central Region of Colombia

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Received 30 January 2009/Returned for modification 8 May 2009/Accepted 14 June 2009

Resistance to clarithromycin, and amoxicillin (amoxicilline), clarithromycin, and metronidazole (11). The main reason for resistance varies geographically (16). In Colombia, only metronidazole resistance was found. In all of the clarithromycin-resistant isolates, only one point mutation was present, either A2143G or A2142G. Our results indicate that metronidazole should not be included in the treatment of empirical treatment of H. pylori infection in this region.

Helicobacter pylori is the major cause of chronic gastritis and peptic ulcer disease (21). The prevalence of H. pylori infection in dyspepsia patients in Colombia is higher than 80% (20).

Resistance to antimicrobials is of particular concern as a major cause of the failure to eradicate this pathogen (5, 23).

Treatment consists of triple therapy with a proton pump inhibitor plus two antibiotics (1, 27). In Colombia, the most commonly prescribed antibiotics are amoxicillin (amoxicilline), clarithromycin, and metronidazole (11).

Many reports have indicated that the prevalence of resistance varies geographically (16). In Colombia, only metronidazole resistance has been reported (11). The main reason for treatment failure has been H. pylori resistance to clarithromycin (16, 17). Monitoring of antimicrobial susceptibility patterns can provide general guidelines in the effort to eradicate H. pylori (6).

The usual phenotypic methods for susceptibility testing can be applied to H. pylori (3, 10). Numerous genotypic methods have been developed to detect clarithromycin resistance (16); the common methods used are PCR-restriction fragment length polymorphism (RFLP) and real-time PCR (17). Clarithromycin resistance is due to point mutations lying within the peptidyltransferase-encoding region of the 23S rRNA (4, 16, 21).

We conducted the present study to evaluate the prevalence of resistance to commonly used antimicrobial agents, the distribution of MICs, and the detection of the A2143G and A2142G mutations in H. pylori clinical isolates from patients living in the western central region of Colombia (cities of Pereira, Armenia, and Manizales) who were part of a study to detect the prevalence of infection (unpublished data). The isolates were stored in Trypticase soy broth plus 20% glycerol at −80°C until used for MIC determination and detection of the A2143G and A2142G mutations.

Susceptibility to metronidazole, clarithromycin, amoxicillin, and tetracycline was determined by Etest according to the manufacturer’s recommendations (EAS 013; AB Biodisk). The plates were incubated for 3 days at 37°C in a microaerobic atmosphere (5% O2, 10% CO2, and 85% N2) in a TS Autolow CO2/O2 water-jacketed incubator (NuAire). H. pylori ATCC 43504 was used as the control strain.

Chromosomal DNA was extracted from 48-h-old confluent cells with the Wizard genomic DNA purification kit (Promega) according to the manufacturer’s recommendations. The point mutations of H. pylori were detected by a PCR assay that targeted a 267-bp 23S rRNA fragment with primers HPYS and HPY (GenBank accession no. 881379) (18), followed by RFLP. The mutations A2143G and A2142G were detected with the BsaI and BbsI (New England BioLabs) restriction enzymes, respectively.

PCR amplification of DNA was performed in a Perkin-Elmer GeneAmp 9700 in a final volume of 50 μl as described by Ménard et al. (18). The 267-bp amplicon was analyzed by electrophoresis on a 2% agarose gel (Fisher), and bands were purified with Wizard PCR Preps (Promega). The purified amplicon was digested with BsaI for 1 h at 50°C and with BbsI for 1 h at 37°C. The restriction products were analyzed by electrophoresis on a 2.5% agarose gel. The amplicon was sequenced in all of the mutated isolates with the BigDye Terminator V3.1 kit (Applied Biosystems) in a ABI Prism 3100 genetic analyzer (Applied Biosystems).

All isolates were susceptible to tetracycline. Table 1 gives an overview of the distribution of the MICs and the number of resistant isolates.

The information on in vitro H. pylori susceptibility tests in Colombia is limited, except for one study reported in 1998...
TABLE 1. Distribution of antibiotic MICs for 106 *H. pylori* isolates

<table>
<thead>
<tr>
<th>Etest MIC range (µg/liter)</th>
<th>No. (%) of isolates resistant to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Metronidazole</td>
</tr>
<tr>
<td>&lt;0.016</td>
<td>2 (1.9)</td>
</tr>
<tr>
<td>0.023–0.094</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>0.125–0.419</td>
<td>9 (8.5)</td>
</tr>
<tr>
<td>0.5–0.75</td>
<td>3 (2.8)</td>
</tr>
<tr>
<td>1–2</td>
<td>2 (1.9)</td>
</tr>
<tr>
<td>6–8</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>16–32</td>
<td>8 (7.5)</td>
</tr>
<tr>
<td>48–128</td>
<td>4 (3.8)</td>
</tr>
<tr>
<td>&gt;256</td>
<td>7 (57.0)</td>
</tr>
</tbody>
</table>

This work was supported by the Vicinoria de Investigaciones y Extensión of the Universidad Tecnológica de Pereira, Colombia.

We thank the Universidad Tecnológica de Pereira, the Center of Molecular Biology and Biotechnology, the Laboratory of Medical Genetics, and Arcángel Mesa for their assistance with the present study. We also thank Gloria Isabel Mejía, Alvaro H. Alegria, and Juan Carlos Sepulveda-Arias for reviewing the manuscript.

REFERENCES


