Resistance of Helicobacter pylori to metronidazole, tetracycline and amoxycillin

H. Wu*, X. D. Shi, H. T. Wang and J. X. Liu

Department of Microbiology, Shanghai Second Medical University, Chongqing South Road 280, Shanghai, China 200025

Resistance to metronidazole, tetracycline and amoxycillin, and β-lactamase production were determined for 153 clinical isolates of Helicobacter pylori. Of these isolates, 77.8% were resistant to metronidazole (MIC > 8 mg/L), 58.8% to tetracycline (MIC > 16 mg/L) and 71.9% to amoxycillin (MIC > 0.5 mg/L); 39.2% were multiresistant. Resistance to metronidazole was more common in isolates from females than in those from males (P < 0.05). None of the isolates produced β-lactamase, so the mechanism of amoxycillin resistance was not linked to production of β-lactamase.

Introduction

Triple therapy to eradicate Helicobacter pylori, in which a bismuth preparation or proton pump inhibitor is combined with two drugs from clarithromycin, metronidazole, amoxycillin and tetracycline, is the most widely used regimen. However, H. pylori rapidly acquires resistance to many classes of antibiotics after exposure to them. Resistance is prevalent worldwide, especially in developing countries, and is considered the primary reason for failure to eradicate infection. The purpose of this study was to investigate the prevalence of resistance to metronidazole, amoxycillin and tetracycline in strains isolated from the Shanghai area. In addition, strains were examined for β-lactamase production.

Materials and methods

Bacterial strains

A total of 153 consecutive clinical isolates of H. pylori, 81 from females and 72 from males, were isolated from gastric biopsy specimens taken between January 1998 and February 1999 in the Ninth People’s Hospital of Shanghai. The specimens were inoculated directly on to brain–heart infusion agar supplemented with 7% defibrinated sheep blood and cultures were incubated in microaerobic conditions for 48 h at 37°C. Isolates were identified, and stored at –80°C in brucella broth containing 30% glycerine until use. H. pylori NCTC 11637 (metronidazole MIC, 1 mg/L) was used as a control strain.

Antimicrobial agents

Metronidazole was purchased from Shanghai ChangZheng Pharmaceutical Factory, Shanghai, China. Pure powders of the other two antimicrobial agents were provided by Sichuan Pharmaceutical Co., Sichuan, China.

Determination of MICs

An agar dilution method was used to determine MICs. Bacteria were harvested from plates by suspending them in sterile 0.85% NaCl, yielding a viable count of about 3 × 10^8–3 × 10^9 cfu/mL. Plates containing two-fold dilutions of each antibiotic were inoculated with H. pylori suspensions using a Steer’s replicator to give an inoculum of approximately 10^6 cfu per spot. MICs were determined after 3 days’ incubation. Resistance to metronidazole and amoxycillin was defined based on the European Helicobacter pylori Study Group breakpoints (MICs of >8 and >0.5 mg/L, respectively); tetracycline resistance was defined as an MIC of >16 mg/L.

Determination of β-lactamase production

β-Lactamase production was determined by means of acidometry using β-lactamase detection paper. A penicillinase-producing Staphylococcus aureus strain was used as a positive control.
Results

One hundred and fifty-three clinical isolates of *H. pylori* were tested. The MICs of the three antibiotics tested are shown in Table I. The rates of resistance to metronidazole, amoxycillin and tetracycline were 77.8, 71.9 and 58.8%, respectively. None of the isolates produced β-lactamase.

The MIC50 and MIC90 of each antibiotic are shown in Table II.

Of the 153 isolates, 60 (39.2%) were resistant to all three antibiotics; 119 were resistant to metronidazole (69 from females and 50 from males; \( P = 0.05, \chi^2 = 4.5915 \)). There was no apparent relationship between antibiotic resistance phenotype and disease type or patient age.

Discussion

Among the isolates investigated here, resistance to the three antibiotics tested was surprisingly high: 77.8% of *H. pylori* strains were resistant to metronidazole. A similar rate, 70% (MIC > 25 mg/L) was reported recently by Liu et al.; this is much higher than the resistance rate of 37.3% reported by them 3 years ago, also in the Shanghai area. Ten years ago, our laboratory found that the MIC\(_{50}\) and MIC\(_{90}\) of metronidazole were 1.56 and 100 mg/L, respectively, but in this study we have found higher values, with both the MIC\(_{50}\) and MIC\(_{90}\) being >128 mg/L. The increased rate of resistance could result from increasing use of this drug and other imidazoles, which may create selective pressure for the development of drug resistance. We also found that metronidazole-resistant strains were isolated more frequently from women than from men. The reason for this observation may be linked to the use of these compounds for treatment of genital infection.

Tetracycline resistance in *H. pylori* was not reported until 1996, when Midolo and colleagues reported tetracycline-resistant strains from Australia. Piccolomini et al. reported that 6% of strains were tetracycline resistant in 1997 in Italy. We found a surprisingly high prevalence of tetracycline resistance, ≥58.8%; the tetracycline MIC for many strains was >32 mg/L, although the reasons for this are unknown. Tetracycline was a component of the bismuth-based triple-therapy regimen recommended in the 1990s for treating *H. pylori* infection, and has also been used extensively as therapy in other types of infection, such as non-gonococcal urethritis, in recent years. Whether the mechanism of tetracycline resistance is related to carriage of resistance plasmids, originating from other bacterial species, remains to be determined.

Amoxycillin resistance was not considered important until recently, when amoxycillin resistance in *H. pylori* isolates was identified in the USA, Canada and Italy. Resistance rates of 31%\(^5\) and 45%\(^6\) have been reported from Italy. Similarly, 41.2% of the isolates we tested were resistant to amoxycillin defined by the same breakpoints (MIC > 8 mg/L), or 71.9% as defined by European breakpoints (MIC > 0.5 mg/L).

In 1999, rates of resistance for other bacteria were also high in China: 81.8% of *S. aureus*\(^7\) and 60.2% of *E. coli*\(^8\) were resistant to amoxycillin. The prevalence of amoxycillin resistance was probably a result of indiscriminate use of this antimicrobial agent, because of the lack of clearly defined guidelines for the management of *H. pylori*-associated dyspepsia and other infections. Amoxycillin has also been one of the most commonly used antimicrobial agents in the community in China in recent years. Though the MICs for many resistant strains were high, no strain produced β-lactamase. Dore et al. suggested that the

### Table I. Distribution of MICs (mg/L) of three antibiotics for 153 *H. pylori* isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>128</th>
<th>64</th>
<th>32</th>
<th>16</th>
<th>8</th>
<th>4</th>
<th>2</th>
<th>1</th>
<th>0.5</th>
<th>0.25</th>
<th>0.125</th>
<th>0.06</th>
<th>0.03</th>
<th>0.015</th>
<th>0.008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>107(^a)</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>18</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>63(^b)</td>
<td>32</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>11</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>90(^d)</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>10</td>
<td>3</td>
<td>17</td>
<td>12(^e)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)MIC > 128 mg/L for 98 strains.
\(^b\)MIC > 16 mg/L for 44 strains.
\(^c\)MIC < 0.008 mg/L for nine strains.
\(^d\)MIC > 32 mg/L for 79 strains.
\(^e\)MIC < 0.06 mg/L for 10 strains.

### Table II. MIC\(_{50}\) and MIC\(_{90}\) (mg/L) of three antibiotics for 153 *H. pylori* isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC(_{50})</th>
<th>MIC(_{90})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>8</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
</tbody>
</table>
mechanism of amoxycillin resistance may be related to the development of tolerance and that the antibiotic resistance phenotype may be lost upon freezing or storage.\textsuperscript{5} Amoxycillin MICs of all the strains we examined on multiple occasions, however, were stable before and after freezing for storage. We assume, therefore, that amoxycillin resistance is probably a result of alterations in penicillin-binding proteins.

Surprisingly, 39.2\% of \textit{H. pylori} isolates were resistant to all three antibiotics tested. The reason that so many multiresistant strains were identified may reflect extensive use of these three antibiotics in this area. This suggests that therapy regimens should be adjusted to include one of these three antibiotics combined with another agent for which rates of resistance are low, such as clarithromycin, which has good efficacy \textit{in vitro}. To avoid increasing resistance, it is now probably essential to test antibiotic sensitivity of bacteria before treating patients, rather than giving empirical treatment.

Acknowledgement

This study was supported financially by a fund of the Bacterial Laboratory in the Department of Microbiology, Shanghai Second University, Shanghai, China.

References


Received 8 October 1999; returned 25 November 1999; revised 4 January 2000; accepted 23 February 2000