Analysis of metronidazole, clarithromycin and tetracycline resistance of *Helicobacter pylori* isolates from Korea

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Antibiotic resistance in *Helicobacter pylori* varies according to geographical region. We studied the primary resistance rates among 652 *H. pylori* isolated from Korea in relation to collection date, disease presentation, age and gender. Resistance rates were 40.6% (metronidazole), 5.9% (clarithromycin), 5.3% (tetracycline), 0% (amoxicillin), 1.5% (furazolidone) and 1.5% (nitrofurantoin). Resistance to metronidazole and clarithromycin increased from 1994 to 1999 (from 33.3 to 47.7% and 4.8 to 7.7%, respectively), but the differences only reached significance when rates of metronidazole resistance in women were compared with those in men (48.6 versus 36.9%).

Introduction

*Helicobacter pylori* infection is recognized as a causal factor in the pathogenesis of chronic gastritis, peptic ulcer and gastric cancer.\(^1\) *H. pylori* eradication treatment is indicated in all patients with active or recurrent peptic ulceration.\(^1\) Numerous regimens combining various antimicrobials and acid-suppressing agents have been used with variable success. Among them, treatment regimens containing a proton pump inhibitor and combination of two or more antibiotics (metronidazole, clarithromycin, amoxicillin or tetracycline) are considered to be most efficacious.\(^2\) However, antibiotic resistance of *H. pylori*, especially to metronidazole and clarithromycin, is increasingly undermining the efficacy of eradication treatment.\(^2\) The reported frequencies of resistance to these antibiotics have varied widely between geographical regions and among subgroups within a study population.\(^3\) It is important to be informed about the local antibiotic resistance of *H. pylori* because treatment for *H. pylori* infection is often started on an empirical basis and, if the infecting strain is resistant, successful eradication is compromised.\(^2\) The prevalence of *H. pylori* infection ranges from 22% (children) to 75% (adults)\(^4\) and the incidence of gastric carcinoma is 34.6 cases per 100 000 in Korea [Annual report of the cancer registry in Korea; Ministry of Health and Welfare (1999)]. There are few data regarding the prevalence of primary antibiotic resistance of *H. pylori* in Asia. The aim of this study was to assess the prevalence of primary antibiotic resistance in *H. pylori* isolates from Korea.

Materials and methods

**Isolation of H. pylori**

*H. pylori* were isolated from gastric mucosal biopsy specimens obtained from Seoul, Korea, from 1994 to 1999 as described previously.\(^5\) One biopsy specimen from antrum or corpus per patient (total 652 strains from 456 patients) was used to isolate *H. pylori*. Some patients provided two separate isolates for study. Briefly, biopsy specimens were ground between the frosted ends of two sterile microscope slides and plated on to 7% horse blood brain–heart infusion (BHI; Difco Laboratories, Detroit, MI, USA) agar plates supplemented with 1% nalidixic acid, 0.5% trimethoprim, 0.3% vancomycin, 0.2% amphotericin (selective media), and the plates incubated under microaerobic conditions at 37°C for up to 14 days. The bacterial growth (multiple colonies) resulting from the primary culture plates was identified as *H. pylori* by colony morphology and Gram’s stain reaction, and by catalase, urease and oxidase reactions. All stock cultures were maintained at −80°C in Brucella broth.

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broth supplemented with 20% glycerol (Sigma Chemical Company, St Louis, MO, USA).

**Determination of MIC**

Measurement of MICs for the recovered *H. pylori* strains was performed by the serial two-fold agar dilution method as described previously. All antibiotics used in this investigation were purchased from Sigma, except for clarithromycin, which was obtained from Abbott Laboratories (Abbott Park, IL, USA).

**Results and discussion**

The resistance breakpoints used for metronidazole and clarithromycin were >8 and ≥1 mg/L, respectively. However, the resistance breakpoints for tetracycline, amoxycillin, furazolidone and nitrofurantoin are not established for *H. pylori*. Thus, we accepted the resistance breakpoints of MIC values >2 mg/L of tetracycline, >8 mg/L of amoxycillin and >2 mg/L of furazolidone and nitrofurantoin. As shown in the Table, the prevalence of antibiotic resistance was 40.5% (185/456) to metronidazole, 5.9% (27/456) to clarithromycin and 5.3% (24/456) to tetracycline. None of the *H. pylori* strains was resistant to amoxycillin. This finding has been reported by others. Furazolidone-resistant *H. pylori* strains isolated from seven patients were also resistant to nitrofurantoin, with an identical MIC value of 4 mg/L.

The prevalence of primary resistance to metronidazole and clarithromycin increased over the study period. Resistance to metronidazole and clarithromycin increased quantitatively between 1994 and 1998–1999 (metronidazole and clarithromycin resistance rates increased from 33.3 to 47.7% and 4.8 to 7.7%, respectively) (the increases did not reach statistical significance) (Table). Dual and/or triple antibiotic resistance was also found among the *H. pylori* strains. Dual antibiotic-resistant strains were found in 9.9% (45/456) of the isolates. Triple antibiotic-resistant strains were rare (0.2%; 1/456).

The 456 patients studied consisted of 314 men and 142 women, median age 45 years (range 16–82 years). Of the 456 patients, 217 failed previous anti-*H. pylori* therapies containing metronidazole, amoxycillin, clarithromycin or tetracycline, and the others had never received any anti-*H. pylori* therapy. Endoscopic diagnosis of the 456 patients showed that 109 had chronic gastritis, 224 had peptic ulcer diseases (88 gastric ulcer and 136 duodenal ulcer) and 126 had gastric cancer. Statistical analyses revealed that antibiotic resistance was not significantly associated with disease or age. Metronidazole-resistant strains were found more frequently in women compared with men (48.6 versus 36.9%, *P* < 0.05).

According to the literature, metronidazole resistance varies from <10 to >80% between geographical regions. In the present study, the overall rate of primary metronidazole resistance among *H. pylori* isolates in Korea was well within the rates described between geographical regions (i.e., 40%). The prevalence of metronidazole resistance gradually increased from 33.3% in 1994 to 47.7% in 1998–1999. This phenomenon has also been reported in other countries. Metronidazole resistance occurs by functional alterations of nitroreductase encoding genes (*rdxA* and *frxA*) in *H. pylori*, and these alterations can be caused by the mutagenic effect of metronidazole. Therefore, the occurrence of metronidazole-resistant strains may be the consequence of increased consumption of metronidazole in the community. Metronidazole has been widely prescribed for other infections such as parasitic or genital infections in Korea, and increased use or abuse of this inexpensive drug may contribute to the increase in metronidazole resistance. Interestingly, all furazolidone-resistant strains were also resistant to nitrofurantoin with an identical MIC value (4 mg/L of furazolidone or nitrofurantoin). These results indicate that, compared with metronidazole, most *H. pylori* isolates were susceptible to furazolidone and nitrofurantoin. In addition, all furazolidone- and nitrofurantoin-resistant strains were also metronidazole resistant. While all the furazolidone- and nitrofurantoin-resistant strains also showed metronidazole resistance, the converse was not true. This suggests that although the bactericidal mechanism is similar among these related agents, the resistance mechanism of metronidazole is dissimilar to that of furazolidone and nitrofurantoin. Furazolidone and nitrofurantoin are nitrofurans, and metronidazole is a nitrimidazole.

**Table.** Annual prevalence of antibiotic resistance among *H. pylori* isolates from 456 patients (% = number of patients infected with resistant *H. pylori*/total patients)

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<tr>
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<tbody>
<tr>
<td>Metronidazole</td>
<td>33.3 (21/63)</td>
<td>38.5 (50/130)</td>
<td>42.6 (55/129)</td>
<td>40.6 (28/69)</td>
<td>47.7 (31/65)</td>
<td>40.5 (185/456)</td>
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<tr>
<td>Clarithromycin</td>
<td>4.8 (3/63)</td>
<td>4.6 (6/130)</td>
<td>3.9 (5/129)</td>
<td>11.6 (8/69)</td>
<td>7.7 (5/65)</td>
<td>5.9 (27/456)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>3.0 (2/63)</td>
<td>6.9 (9/130)</td>
<td>4.7 (6/129)</td>
<td>2.9 (2/69)</td>
<td>7.7 (5/65)</td>
<td>5.3 (24/456)</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>0 (0/63)</td>
<td>0 (0/130)</td>
<td>0 (0/129)</td>
<td>0 (0/69)</td>
<td>0 (0/65)</td>
<td>0 (0/456)</td>
</tr>
<tr>
<td>Furazolidone</td>
<td>1.6 (1/63)</td>
<td>0 (0/130)</td>
<td>3.9 (5/129)</td>
<td>0 (0/69)</td>
<td>1.5 (1/65)</td>
<td>1.5 (7/456)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>1.6 (1/63)</td>
<td>0 (0/130)</td>
<td>3.9 (5/129)</td>
<td>0 (0/69)</td>
<td>1.5 (1/65)</td>
<td>1.5 (7/456)</td>
</tr>
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Antibiotic resistance of *H. pylori* in Korea

The mechanism of action of these antibiotics is via active nitroreduction of the drugs, which leads to killing of bacteria. However, furazolidone and nitrofurantoin resistance involves additional mechanisms not found in metronidazole-resistant *H. pylori*, which may relate to this differential resistance pattern.

The reported prevalence of primary resistance to clarithromycin varies from 2 to 50%. Primary resistance to tetracycline is rare. The prevalence of clarithromycin-resistant strains was relatively low (5.9%), whereas tetracycline resistance was high (5.3%) compared with other reports. Because primary clarithromycin and tetracycline resistance rates were not very high, the routine pre-treatment testing for clarithromycin and tetracycline susceptibility may not yet be cost effective in Korea. Continuous surveillance of clarithromycin and tetracycline susceptibilities is needed because the prevalence of primary resistance to clarithromycin and tetracycline appears to be increasing in this population. Further increases in antibiotic resistance and the development of dual and/or triple antibiotic resistance among *H. pylori* isolates from Korea would require susceptibility testing before treatment to maximize efficacy of *H. pylori* therapies.

Acknowledgements

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References


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