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Regional Differences in Metronidazole Resistance and Increasing Clarithromycin Resistance among *Helicobacter pylori* Isolates from Japan

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The patterns of antibiotic resistance in *Helicobacter pylori* were assessed in two different regions in Japan. Overall, prevalences of resistance to metronidazole and clarithromycin were 12.4 and 12.9%, respectively. While there was no difference in clarithromycin resistance, the prevalence of metronidazole resistance was significantly higher in Kyoto (23.8%) than in Sapporo (8.1%). From 1996 to 1999, the prevalence of metronidazole resistance did not change but the prevalence of clarithromycin resistance doubled (from 9.1 to 18.7%).

Helicobacter pylori infection is now established as a major cause of peptic ulcer disease (5, 10), and it has been shown that successful treatment of *H. pylori* infection results in the cure of peptic ulcer diseases (6, 17). Although the standard treatment for *H. pylori*-related disease is a combination of two or more antimicrobial agents and acid suppression, no therapy is universally successful. Poor patient compliance with treatment and the presence of resistant organisms are thought to be the most important variables predicting a low cure rate. Antibiotic resistance in *H. pylori* is a growing problem. The reported frequencies of resistance to the antibiotics have varied widely between geographic regions and among subgroups within a study population (1, 3, 4, 7, 14–16, 18). For example, metronidazole resistance varies from 10 to 80% among geographic regions (3, 4, 7, 15, 18). Knowledge of the prevalence of antibiotic resistance of *H. pylori* in any area is important because treatment for *H. pylori* infection is often started on an empirical basis. If the infecting strain is resistant, therapy is likely to be unsuccessful. Macrolide use is widespread in Japan, suggesting that resistance to clarithromycin may be prevalent. The aim of this study was to assess the frequencies of primary resistance to metronidazole, clarithromycin, and amoxicillin among *H. pylori* isolates from two different metropolitan hospitals in Japan.

H. pylori isolates were obtained from Hokkaido University Hospital (Sapporo) and Kyoto Prefectural University of Medicine Hospital (Kyoto) between January 1996 and February 1999. Sapporo is located in Hokkaido, the northern island of Japan, and Kyoto is centrally located in Honshu, the main island of Japan. The population of each city is more than one million people. The population we studied was genetically homogeneous Japanese (so-called modern "Yamato" Japanese). Duodenal ulcers were identified endoscopically, and gastritis was defined as histologic gastritis with no peptic ulcers, gastric cancer, or any esophageal diseases (e.g., gastroesophageal reflux disease or esophageal cancer). No subjects had received treatment for *H. pylori* infection. Informed consent was obtained from all patients, and the protocol was approved by each local ethics committee. Gastric mucosal biopsy specimens obtained from the patients were used to isolate *H. pylori* (multicolonies) as described previously (12). All stock cultures were maintained at −80°C in brain heart infusion broth (Difco, Detroit, Mich.) supplemented with 20% glycerol (Sigma Chemical Co., St. Louis, Mo.).

The recovered *H. pylori* isolates from the stock cultures were tested for susceptibilities to metronidazole, clarithromycin, and amoxicillin by a serial two-fold agar dilution method (11) with a minor modification. Briefly, agar dilution plates were

TABLE 1. Comparison of antibiotic resistance of *H. pylori* isolates between Sapporo and Kyoto

% Prevalence of resistance (no. resistant/total) to[a]:					
Metronidazole			Clarithromycin		
Sapporo	Kyoto	Total	Sapporo	Kyoto	Total
8.1 (23/283)	23.8 (25/105)[b]	12.4 (48/388)	12.0 (34/283)	15.2 (16/105)	12.9 (50/388)

[a] MICs were >8 μg/ml for metronidazole and >1 μg/ml for clarithromycin.
[b] $P < 0.05$ compared to results for Sapporo.

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TABLE 2. Annual prevalences of metronidazole- and clarithromycin-resistant *H. pylori* isolates in Japan

Year	% Prevalence of resistance (no. resistant/total) to[a]:					
	Metronidazole			Clarithromycin		
	Sapporo	Kyoto	Total	Sapporo	Kyoto	Total
1996	5.7 (2/35)	17.0 (9/53)	12.5 (11/88)	5.7 (2/35)	11.3 (6/53)	9.1 (8/88)
1997	5.9 (8/136)	40.0 (12/30)	12.0 (20/166)	9.6 (13/136)	13.3 (4/30)	10.2 (17/166)
1998–1999	11.6 (13/112)	18.2 (4/22)	12.7 (17/134)	17.0 (19/112)	27.3 (6/22)	18.7 (25/134)

[a] MICs were >8 μg/ml for metronidazole and >1 μg/ml for clarithromycin.

prepared using Mueller-Hinton agar as the base medium. Aged sheep blood (2 weeks old) was added to the Mueller-Hinton base medium at a concentration of 5%. The ranges for the antibiotics were the following: metronidazole, 1 to 256 μg; and clarithromycin and amoxicillin, 0.5 to 16 μg. Fresh *H. pylori* isolates (cultured for 2 to 3 days) were prepared in sterile saline and adjusted to an optical density at 625 nm of 0.38 to 0.4. Using a Steers-type replicating device, 1 to 5 μl of the adjusted inocula was delivered to the agar plates. All plates were incubated under CampyPak Plus conditions (Becton Dickinson BBL, Cockeysville, Md.) at 37°C for 3 days. The MIC was defined as the lowest concentration of antibiotic that completely inhibited the growth of the inoculum. *H. pylori* isolates were considered resistant when the MIC of metronidazole was >8 μg/ml (12) and the MIC for clarithromycin was >1 μg/ml (9). Breakpoints for amoxicillin have not been established, and the data are presented in relation to the MICs. Metronidazole-resistant *H. pylori* ATCC 43504 was used as a quality-control organism. Any test in which the MIC for the quality-control organism was outside the approved range (64 to 256 μg of metronidazole/ml) was completely discarded.

We examined 388 *H. pylori* isolates: 283 strains from Sapporo, Hokkaido (53 from patients with histological gastritis only [gastritis], 100 from patients with gastric ulcer, 93 from patients with duodenal ulcer, and 37 from patients with gastric cancer), and 105 strains from Kyoto, Honshu (27 from patients with gastritis, 39 from patients with gastric ulcer, 25 from patients with duodenal ulcer, and 14 from patients with gastric cancer). Patients from Sapporo consisted of 180 men and 103 women (median age, 50 years; range, 21 to 85 years), and patients from Kyoto consisted of 67 men and 38 women (median age, 53 years; range, 21 to 84 years). There were no correlations between gender or age and any disease group in either Sapporo or Kyoto.

Overall, the rates of primary resistance to metronidazole and clarithromycin were 12.4 and 12.9%, respectively (Table 1). Dual metronidazole and clarithromycin resistance was present in 2.3% of isolates (9 of 388) (5 from Sapporo and 4 from Kyoto). For all isolates, MICs of amoxicillin were ≤0.5 μg/ml. The prevalence of metronidazole resistance was significantly higher in Kyoto than in Sapporo (23.8 versus 8.1%; $P < 0.05$). There was no difference in the prevalence of clarithromycin resistance between the two areas. There were no significant differences in metronidazole and clarithromycin resistance rates according to age, gender, and disease presentation among the patients. However, the prevalence of primary metronidazole resistance increased recently in Sapporo, and there was a marked increase in the prevalence of primary metronidazole resistance in 1997 in Kyoto (Table 2). Interestingly, the prevalence of primary clarithromycin resistance increased in both regions. Overall, the prevalence of clarithromycin doubled (9.1 to 18.7%) during the study period ($P < 0.05$) (Table 2).

Antimicrobial resistance of *H. pylori* is thought to be the consequence of antibiotic consumption in the community (8). In Japan, the government (The Ministry of Health and Welfare) has not allowed the use of antibiotics for *H. pylori* therapies in the study period. For this reason, Japan should be the ideal country for examining primary resistance rates of *H. pylori* to various antibiotics. The primary resistances to clarithromycin and metronidazole observed in this study are probably due to the previous use of these antimicrobial agents for other infections. The reported prevalence of primary resistance of *H. pylori* to clarithromycin varies between 1 and 17% in other countries (1, 14, 16, 18). The prevalence of clarithromycin resistance in *H. pylori* was high and increasing despite the fact that *H. pylori* therapy is not used in Japan. Clarithromycin was developed in Japan and is very popular for the treatment of respiratory tract infections. Reducing the use of clarithromycin might reduce the prevalence of clarithromycin resistance. For example, a 50% decrease in macrolide consumption between 1988 and 1992 in Finland led to a decrease in resistance of group A streptococci from 19 to 9%, but after a 5-year lag phase (13).

The overall prevalence of metronidazole resistance in *H. pylori* (12.4%) was within the range reported from other countries (less than 10% to greater than to 80% between geographic regions) (2, 3, 4, 7, 14). Metronidazole-resistant *H. pylori* isolates were significantly more prevalent in Kyoto than in Sapporo (23.8 versus 8.1%; $P < 0.05$). Our preliminary observation for multicenter analysis indicates that there is a north-to-south gradient in metronidazole resistance in Japan. There was a similar tendency for macrolide resistance rates in Europe, high in the south and low in the north, possibly due to a difference in consumption of the antibiotic (8). Debets-Ossenkopp et al. examined metronidazole resistance in three different parts of The Netherlands and found that resistance rates were different (south, 11.6%; west, 24.3%) although the rates of consumption of metronidazole were similar (2). There was no significant difference in clarithromycin and metronidazole resistance in relation to age, sex, or disease presentation in this study. Other studies have shown a higher frequency of metronidazole-resistant isolates from women, possibly due to the use of this agent for the treatment of genitourinary tract infections.

In summary, we found regional differences in metronidazole resistance and increasing clarithromycin resistance among *H. pylori* isolates from Japan. These results suggest that differential factors may be involved in the development of metronidazole resistance in the two areas. In addition, the high rate of clarithromycin resistance suggests that the effectiveness of clarithromycin-based anti-*H. pylori* therapies may soon be compromised in Japan.

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