Pattern of Primary Resistance of *Helicobacter pylori* to Metronidazole or Clarithromycin in the United States

Michael S. Osato, PhD; Rita Reddy, MS; Siddharta G. Reddy, BS; Rebecca L. Penland, BS; Hoda M. Malaty, MD, PhD; and David Y. Graham, MD

**Background:** Therapy for *Helicobacter pylori* is generally empiric despite the fact that resistance to metronidazole and clarithromycin compromise therapeutic efficacy. The aim of this study was to aid clinicians in choosing a course of therapy for *H pylori* infection in the United States.

**Methods:** The frequency of primary clarithromycin and metronidazole resistance among *H pylori* isolated from patients enrolled in US-based clinical trials between 1993 and 1999 was reviewed in relation to patient age, sex, region of the United States, and test method (Etest and 2 agar dilution procedures).

**Results:** Clarithromycin and metronidazole resistance rates were based on the results of 3439 pretreatment Etest determinations and 3193 agar dilution determinations. Sex and age were available on 900 and 823 individuals, respectively. Metronidazole resistance was 39% by Etest and 21.6% by agar dilution (P < .001). Clarithromycin resistance was 12% by Etest and 10.6% by agar dilution. Amoxicillin or tetracycline resistance was rare. Metronidazole and clarithromycin resistance was more common in women than men (eg, 34.7% vs 22.6% for metronidazole and 14.1% vs 9.7% for clarithromycin (P = .01 and P = .06, respectively). Antibiotic resistance increased gradually up to age 70 years, then declined significantly (P < .05) regardless of test method. Regional differences in antimicrobial resistance did not occur.

**Conclusions:** While age and sex had significant effects on resistance rates, regional differences were not present. The high prevalence of resistance to metronidazole and clarithromycin may soon require the performance of antimicrobial susceptibility testing of *H pylori* isolates prior to initiating treatment.

Arch Intern Med. 2001;161:1217-1220

**RESULTS**

The database consisted of 3439 isolates tested by Etest and 3193 isolates tested by agar dilution. Of the 3193 agar dilution determinations, 2648 were determined by the method approved by the National Committee on Clinical Laboratory Standards. Differences in results between techniques were determined by comparing the responses between single isolates tested by both methods in a subset of samples: 188 specimens tested for metronidazole susceptibility and 332 isolates tested for clarithromycin susceptibility.

**ANTIBIOTIC treatment** of *Helicobacter pylori* infection is complex, requiring multiple antibiotics combined with acid suppressive agents to ensure high cure rates. Treatment failure is attributed generally to the lack of compliance with the drug regimen or to acquired antibiotic resistance of the organism. Emergence of resistant *H pylori* has been documented in various countries and threatens to compromise current treatment regimens. However, data on resistance are generally obtained from unique subsets of populations over a short period. There are few data regarding the resistance rates in the United States.

Investigations have shown that demographic factors play an important role in the reported rates of antibiotic resistance in *H pylori*. Metronidazole resistance is higher in developing countries and is especially high in immigrant populations, in women, and in areas where this agent is used routinely for the treatment of assorted gastrointestinal maladies. There are few reports with regard to the role of demographic factors such as age, sex, and region, on the prevalence of metronidazole and clarithromycin resistance in *H pylori* from the United States.
Patients, Materials, and Methods

Population

The frequency of primary clarithromycin and metronidazole resistance among *H pylori* isolated from patients enrolled in 17 US-based antibiotic treatment trials between 1993 and 1999 was reviewed in relation to patient age, sex, region of the United States, and test method (Etest and 2 agar dilution procedures). During the initial visit, 1 antral and 1 corpus biopsy specimen was obtained from each patient for culture and susceptibility testing. Only 1 isolate per individual was used to calculate the overall resistance rates. The isolate selected was that from the antrum, although the corpus isolate was used in a few instances if the antral site yielded no growth of *H pylori*. Overall rates of clarithromycin and metronidazole resistance were determined by dividing the total number of resistant isolates by the total number of data points for that specific test. Differences in results between techniques were determined by comparing the responses between single isolates tested by both methods.

Etest and Agar Dilution Procedures

Mueller-Hinton agar (Remel Laboratories, Lenexa, Kan) with 5% sheep blood (BBL Becton Dickinson, Cockeysville, Md) was used as the base medium. Metronidazole and clarithromycin Etest strips were aseptically placed onto the dried surfaces of the inoculated plates. The Etest plates were incubated under 12% carbon dioxide at 37°C for 4 days. The agar dilution procedure was performed using Mueller-Hinton agar as the base medium to which 5% aged (>2 weeks old) sheep blood and serial dilutions of metronidazole (Sigma Chemical Co, St Louis, Mo) and clarithromycin (Abbott Laboratories, Abbott Park, Ill) were added. The plates were poured on the day of testing. Fresh bacterial suspensions were prepared in sterile saline and adjusted to an optical density (OD) of 0.38 to 0.40 at 625 nm (approximately 6 × 10⁸ cells/mL). Five microliters of the adjusted inoculum was delivered to each plate by a Steers type replicate plating device. The plates were incubated in anaerobic jars with CampyPak Plus (BBL Becton Dickinson) gas generating envelopes for 72 hours at 37°C.

MIC Determinations

Etest minimum inhibitory concentration (MIC) values were defined as the intercept of the elliptical zone of inhibition with the graded Etest strip per the instructions of the manufacturer. The MIC value for the agar dilution method was defined as the lowest concentration of antibiotic that completely inhibited visible growth.

MIC Interpretive Criteria and Comparisons

Isolates were considered resistant if the MIC value was greater than 8 µg/mL for metronidazole and greater than 1 µg/mL for clarithromycin. Differences are reported in relation to a variance in the respective MIC values of 2 log₂ or higher between the antral and corpus isolates, and as a change in the susceptibility pattern from sensitive to resistant or vice-versa.

Statistical Analysis

The prevalence rates of clarithromycin and metronidazole resistance were calculated for the total isolates. In addition, the age- and sex-specific prevalence, as well as regional distribution and the year of the isolate collection were calculated using each test independently. Mantel-Haenszel χ² statistics were applied to compare the magnitude of the difference between rates of resistance to different antibiotics and within the same antibiotics using different techniques. The data were analyzed using the SAS program, version 3 (SAS Institute, Cary, NC, 1985).

Effect of Test Method

The test method greatly affected the results of susceptibility testing for metronidazole (Table 1). Forty-two percent of the metronidazole MIC values differed by 2 log₂ or more and resulted in a difference in susceptibility pattern in 17.6%. In contrast, the difference in susceptibility pattern was only 3% with clarithromycin, and thus the results with either method were combined for overall susceptibility. The data with metronidazole are presented separately for both methods except where noted.

Table 1. Effect of Test Method on Frequency of Metronidazole Resistance: Comparison of Etest vs Agar Dilution*

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Percent Within ± 2 Log₂ Difference</th>
<th>Percent With a Change in Susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>42.0 (79/188)</td>
<td>17.6 (33/188)</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>28.6 (95/332)</td>
<td>3.0 (10/332)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses are number of patients affected/total number of patients tested.

Figure 1. Frequency of clarithromycin resistance rates by year of collection.

Annual rate of clarithromycin resistance as determined by combined Etest and agar dilution results. Data were combined because intratest analysis revealed only a 3% change in susceptibility results between test methods.
RESISTANCE OVER A 7-YEAR PERIOD

The rates of resistance to clarithromycin varied among the years of evaluation ($\chi^2=12.855; P=.05$) (Figure 1). The overall prevalence of clarithromycin resistance was 11.1% varying from 6.1% to 14.5% among the years of evaluation.

The prevalence of metronidazole resistance differed depending on whether the Etest or agar dilution was used. The prevalence of metronidazole resistance with Etest was 39% (690/1768) compared with 25.2% (367/1459) determined by the agar dilution methods ($P<.001$) (Table 2). The Etest consistently provided a higher estimate of the prevalence of metronidazole resistance in all years of evaluation in which more than 10 isolates were tested.

EFFECT OF SEX

Women were more likely to have metronidazole-resistant H pylori than men (63% vs 35.1%, respectively, as determined by Etest [$P=.01$] and 34.7% vs 22.6%, respectively, as determined by agar dilution [$P=.03$]) (Table 2). The test method affected the overall rate of metronidazole resistance for both men and women, but did not affect the comparative outcome (ie, that the rate of metronidazole-resistant H pylori was higher for women than for men).

Isolates from women were also more apt to be resistant to clarithromycin than isolates from men (14.1% vs 9.7%), although the results were not significantly different ($P=.06$) (Table 2).

EFFECT OF AGE

The age of the patient at the time of H pylori recovery from the gastric biopsy specimens had a significant effect on both the metronidazole and clarithromycin resistance rates. From ages older than 20 years, metronidazole resistance as determined by Etest increased to 50% of the isolates tested until age 71 years or older, when the rate dropped to 31% ($P<.05$). A similar pattern of increasing resistance to metronidazole was seen with the agar dilution method (Figure 2). Helicobacter pylori isolates from patients older than 70 years were also less likely to be resistant to clarithromycin by either test method ($P<.05$).

EFFECT OF GEOGRAPHIC REGION

We evaluated the effect of living in different regions of the United States, and marked regional differences were not detected (Figure 3). The highest level of metronidazole resistance occurred in the Southeast (27.5%; 88/320) and the lowest in the Northeast (22.1%; 91/412) ($P>.20$). The highest rate of clarithromycin resistance (13%; 67/516) occurred in the Northeast, while the lowest rate of clarithromycin resistance was found in the West (8.3%; 44/533). However, none of the comparisons was statistically significantly different.

COMMENT

Treatment outcomes of anti–H pylori therapies are primarily dependent on compliance with the regimen and the presence of antibiotic-resistant H pylori. Because antimicrobial testing is not widely available for H pylori in most regions, empiric therapy has been the rule. We used the...

Table 2. Frequency of Metronidazole and Clarithromycin Resistance Associated With the Sex of the Patient

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Test Method</th>
<th>Sex</th>
<th>No. of Isolates</th>
<th>No. Resistant</th>
<th>Percent Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>Agar dilution</td>
<td>F</td>
<td>167</td>
<td>58</td>
<td>34.7</td>
</tr>
<tr>
<td></td>
<td>Agar dilution</td>
<td>M</td>
<td>301</td>
<td>68</td>
<td>22.6</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>Etest</td>
<td>F</td>
<td>92</td>
<td>58</td>
<td>63.0</td>
</tr>
<tr>
<td></td>
<td>Etest</td>
<td>M</td>
<td>188</td>
<td>66</td>
<td>35.1</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>Combined</td>
<td>F</td>
<td>347</td>
<td>49</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>M</td>
<td>544</td>
<td>53</td>
<td>9.7</td>
</tr>
</tbody>
</table>

Figure 2. Frequency of metronidazole and clarithromycin resistance as determined by agar dilution associated with the age of the patient. The frequency of metronidazole and clarithromycin resistance as determined by agar dilution with respect to each successive decade until age 70 years. After age 70 years, there was a significant decrease in the level of metronidazole and clarithromycin resistance.

Figure 3. Regional resistance patterns for Helicobacter pylori isolates. CR indicates clarithromycin resistance; MR, metronidazole resistance. All data are given as percentages. Regional information was available for 1741 isolates for clarithromycin resistance and 1165 isolates for metronidazole resistance. No significant regional differences in resistance to metronidazole or clarithromycin occurred.


©2001 American Medical Association. All rights reserved.

Downloaded From: http://archinte.jamanetwork.com/pdfaccess.ashx?url=/data/journals/intemed/11984/ on 04/01/2017
antibiotic susceptibility data from clinical trials in patients with duodenal ulcers performed in the United States between 1993 and 1999 to evaluate the prevalence and rate of emergence of resistance to clarithromycin and metronidazole in the United States. Interlaboratory variation was eliminated as a variable because all testing was performed in a single laboratory using standard procedures and established end points.

Metronidazole resistance was present and stable in approximately 35% of the isolates tested, while clarithromycin resistance was present in approximately 11% (higher in some populations, eg, women). It seems unlikely that the United States is not involved in the worldwide trend of increasing clarithromycin resistance.13-17

Women and the young adults were found to harbor resistant H pylori more often than men and older patients. This may be attributed to the use of metronidazole-containing treatment regimens used by women to treat gynecologic infections. The frequency of either metronidazole or clarithromycin resistance was lower in those older than 70 years than in those of middle age. We can only conjecture as to the reason for this finding, but confounding demographic factors are suspected. There were no significant regional differences in antibiotic resistance, suggesting that such differences can be ignored currently when choosing an antibiotic regimen to treat H pylori infection.

While the proportion of samples with resistant H pylori was higher with the Etest than with agar dilution, the difference was particularly striking only with metronidazole. It is important to emphasize that neither of these test methods (Etest and agar dilution) has been clinically verified with metronidazole, and only the presence of clarithromycin resistance generally predicts a treatment failure. As a general rule, clarithromycin resistance results in that antibiotic effectively “dropping out,” with the results approximating those expected with the other components of the therapy (eg, a proton pump inhibitor plus clarithromycin treatment would yield almost no cures with clarithromycin-resistant H pylori, and the combination of a proton pump inhibitor, amoxicillin, and clarithromycin would yield what would be expected with the proton pump inhibitor and amoxicillin alone).19 In contrast, high-dose metronidazole therapy can often overcome resistance when part of a multidrug regimen.19

Although not part of this investigation, 3 clinical H pylori isolates were found to be amoxicillin resistant (Etest MIC >16 µg/mL).20 The rates for amoxicillin and tetracycline resistance among H pylori in the United States are sufficiently low (<0.005% of 6470 isolates; unpublished observation, 1999) that antimicrobial susceptibility testing for these drugs is currently not needed clinically. However, data should be gathered to monitor the level of emergent resistance in the general population.

Triple and quadruple antibiotic treatment regimens used for initial therapy still achieve eradication in more than 80% of infected patients. However, pretreatment resistance is a growing problem and is the main factor responsible for treatment failure with regimens using these compounds. Continued increases in the level of pretherapy antibiotic resistance might necessitate pretreatment antibiotic susceptibility testing in many regions. Retreatment owing to failed initial therapy is associated with higher costs and possibly higher levels of adverse effects related to salvage therapies.21 Knowledge of the antibiotic of the infecting H pylori strain, especially for metronidazole and clarithromycin, will have an immediate beneficial outcome on the type and course of treatment.

Accepted for publication January 23, 2000.

Research was supported in part by Hilda Schwartz and the Veterans Affairs Medical Center, Houston, Tex.

Data were obtained from clinical trials supported by the following pharmaceutical companies: Abbott Laboratories, Abbott Park, Ill; Tap Pharmaceuticals Inc, Deerfield, Ill; Pfizer Inc, New York, NY; Astra-Merck Inc, Wayne, Pa; Wyeth-Ayerst Pharmaceuticals, Philadelphia, Pa; and GlaxoWellcome Inc, Research Triangle Park, NC.

Reprints: Michael S. Osato, PhD, Gastroenterology Microbiology Laboratory, Department of Medicine, Veterans Affairs Medical Center, MS 111D, 2002 Holcombe Boulevard, Houston, TX 77030 (e-mail: mosato@bcm.tmc.edu).

REFERENCES