Helicobacter Pylori Resistance to Metronidazole and Clarithromycin in Dyspeptic Patients in Iran

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Abstract

Background: The resistance of H. pylori to the recently available antibiotic treatment regimens has been a growing problem. The prevalence of high antibiotic resistance of H. pylori is the most common reason of its eradication failure. The purpose of the present study is to determine the prevalence of antibiotic resistance among H. pylori strains isolated from Iranian patients.

Method: We investigated the prevalence of H. pylori resistance to metronidazole, clarithromycin, amoxicillin, and tetracycline among 128 H. pylori isolates from Iranian patients. After the culture of biopsy specimens and identification, susceptibility tests were performed with Modified Disk Diffusion Method (MDDM) and E. test.

Results: Resistance rates to metronidazole, clarithromycin, amoxicillin and tetracycline were 64%, 23%, 2.5% and 0%, respectively. Seventy two percent of the metronidazole resistance strains had MIC>256 mg/ml (High-Level-Resistance).

Discussion: Due to the increasing rate of antibiotic resistance in H. pylori strains and in order to decrease the treatment cost, testing of susceptibility to metronidazole and clarithromycin is recommended.

Keywords: Helicobacter pylori; Metronidazole; Clarithromycin; Dyspeptic patients

Introduction

Helicobacter pylori infect the majority of the adult population in developing countries including Iran. Studies from northern and southern regions of Iran demonstrated high rates of H. pylori infection (>85%) with the frequent rate of development of duodenal ulcer and gastric cancer. The low eradication rate of H. pylori infection in Iran and considerable rates (20%) of reinfection or recrudescence indicate that controlling H. pylori infection should be considered as an important health issue.¹⁴ H. pylori play an important role in the pathogenesis of chronic gastritis, peptic ulcer disease, and possibly, gastric carcinoma.⁵,⁶ Eradication of the organism not only accelerates the healing of the ulcer but also prevents long-term ulcer relapse.⁷

The current treatment for H. pylori infections includes anti-secretory agents or bismuth citrate plus two or more antimicrobial agents.⁵ Clarithromycin and metronidazole are the most frequently used antibiotics for the treatment of H. pylori infection.⁹ However, the treatment of H. pylori infection does not always eradicate the organism and antibiotic resistance is increasingly recognized as a contributing factor in the 10-15% of patients who fail H. pylori eradication therapy.¹⁰ The prevalence of resistance to metronidazole differs in different countries; a lower prevalence has been reported for industrialized countries ranging from 10 to 50%, whereas up to 90% of H. pylori isolates from developing countries have been estimated to be resistant.¹¹ Although the rates of clarithromycin
were prepared in 500 ml Brucella blood agar with 5% fetal calf serum. The isolates were from dyspeptic patients (74 females and 54 males, aged 17-65 years) among whom the strains were obtained.

The isolates were subcultured on the Brucella blood agar for 48-72 hrs and stored at -70°C in aliquots of Brucella broth supplemented with 20% (v/v) glycerol and 10% (v/v) fetal calf serum. The susceptibility of the H. pylori isolates to metronidazole, clarithromycin, amoxicillin and tetracycline was examined by Modified Disk Diffusion Method (MDDM) and E. tests. MDDM was performed with Brucella blood agar with 5% fetal calf serum. For this purpose, suspensions from the primary plates were prepared in 500 µl Brucella broth to a Mc Farland opacity standard 4 (approximately 108 CFU/ml) and then 100 µl of the suspension was spread on the medium. The plates were briefly dried and then disks containing 5µg metronidazole, 2µg clarithromycin, 10 µg amoxicillin, 30 µg tetracycline were placed on the plate surface. The plates were incubated microaerophilically at 37°C for 5 days. The diameters of the zones of complete growth inhibition were finally measured in millimeters. Reference strain 26695 was included as a quality control.

Susceptibility results were recorded as resistant according to the following interpretive criteria: for clarithromycin, no zone of growth inhibition; for metronidazole, a growth inhibition zone <16 mm; for amoxicillin, a zone <11 mm and for tetracycline, a growth inhibition zone <20 mm.

Brucella blood agar with 5% fetal calf serum was used as the base medium. The plates were streaked in three directions as described for the MDDM with each inoculum and then metronidazole E. test strips (0.016-256 µg/ml) were aseptically placed on to the dried surface of the inoculated plates. The plates were incubated under microaerophilic condition at 37°C for 5 days.

E. test MIC values were defined as the intercept of the elliptical zone of inhibition with the graded E. test strip according to the instructions of the manufacturer.

There is no established NCCLS metronidazole breakpoint for H. pylori. In this study; the isolates were considered resistant when the MIC value was 8mg/l for metronidazole. High level resistance was defined as MIC≥256mg/l.

Results

Table 1 shows the characteristics of patients from whom the strains were obtained.

Table 1: Characteristics of the patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No of patients=128</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td>17-65</td>
</tr>
<tr>
<td>Male/Female ratio</td>
<td>42/86</td>
</tr>
<tr>
<td>Peptic ulcer disease</td>
<td>32 (25%)</td>
</tr>
<tr>
<td>Gastritis</td>
<td>96 (75%)</td>
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</table>

As shown in Table 2, the prevalence of antibiotic resistance according to the MDDM was 64% to metronidazole, 23% to clarithromycin, and 2.5% to amoxicillin (p<0.001, McNemar test). All the isolates (128) were susceptible to tetracycline. Inhibition zone...
diameters for disk diffusion ranged from 8 to 20 mm for amoxicillin. No zones of growth inhibition to 10mm for clarithromycin, 22 to 34 mm for tetracycline and 18mm for metronidazole were obtained. The MIC of 128 H. pylori isolates for metronidazole was determined using the E. test, a simple but relatively expensive routine method, that shows a consistent reproducibility and excellent correlation with agar dilution, which is widely used as reference method for H. pylori. The lowest and highest concentration of metronidazole on the E. test strips was 0.016 and 256 mg/l.

Table 2: The susceptibility rates to antibiotics by MDDM

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Disk Zone size</th>
<th>Resistance No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>5 16</td>
<td>82 (64.1)</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>2 Any zone</td>
<td>29 (23.5)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>10 11</td>
<td>3 (2.5)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30 20</td>
<td>0</td>
</tr>
</tbody>
</table>

*p value < 0.001

Thus, the isolates with MIC lower than 0.016 and higher than 256 could not be detected. According to E. test, resistance rate to metronidazole was 64%. The range of metronidazole MIC varied from 2 to >256 μg/ml. The overall agreement between the results of MDDM and E. tests for metronidazole was found in 128 strains, of which 82 (64%) isolates were found resistant to 8 mg/l metronidazole by the E. test and 82 (64%) of them exhibited inhibitory zones of 16mm or less by MDDM tests. Furthermore, 60 (73.1%) out of the 82 resistant isolates (using E. test) had a high level of resistance to metronidazole (MIC>256 μg/ml).

Of 128 H. pylori isolates, 29 (23%) were resistant to both metronidazole and clarithromycin.

Discussion

The importance of H. pylori, as a human pathogen, is well established due to its increasing resistance to various antimicrobial agents.4,16 The emergence of metronidazole and clarithromycin resistant H. pylori strains has resulted in decreased success of the current therapies using this antibiotic.4,16 Surveillance of H. pylori susceptibility tests in the pre-treatment population is difficult since there are only a few centers which offer gastroendoscopy and culture as a routine primary diagnostic test;1 the sensitivity of H. pylori to antibiotics is only checked occasionally.18

Metronidazole resistance varies from <10% to >80% in different geographical regions.19 In the present study, the overall rate of metronidazole resistance among the H. pylori isolates was 64%. Metronidazole resistance occurs by rdxA gene, which encodes an oxygen-insensitive NADPH nitroreductase. Recent evidence has suggested that inactivation of frxA (NADPH flavin oxidoreductase), fdxB (ferredoxin-like protein) and possibly other reductase-encoding genes may also contribute to the resistant phenotype.20 Another possible mechanism of intrinsic metronidazole resistance involves decreased drug uptake or increased drug efflux.21 Clarithromycin resistance is known to be associated with the point mutations of 23S rRNA gene (A2143G or A2144G mutation) of the bacterium which inhibit the binding of clarithromycin to the ribosome.22 Elviss et al. reported a resistance rate of 11% for clarithromycin.22

In our study, the overall rate of clarithromycin resistance was 23%. The prevalence of resistance to both metronidazole and clarithromycin among the 128 isolates was 23%. Several investigators have noted that the rates of resistance to these two antibiotics among the patients, previously treated for H. pylori infections, are generally higher than those observed among the untreated patients.23 Resistance to tetracycline and amoxicillin is rare. In our study, amoxicillin resistance was 2.5% and no tetracycline resistant was observed. There have been reports of 7% resistance in Brazil24 and 4.9 and 6.7% in Japan and Korea, respectively.25 This study highlights the importance of antibiotics susceptibility tests to guide treatment plans for H. pylori infections. Accordingly, high rates of resistance to metronidazole and clarithromycin might require the introduction of new antibiotics, culture and susceptibility testing prior to selecting a therapy.

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Conflict of interest: None declared.
References


