Antibacterial Resistance Patterns of *Helicobacter pylori* Clinical Isolates From Gastric Biopsy of Patients in Yazd

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Background: *Helicobacter pylori* related-infections are common in developing countries, including Iran. However, little information is available on the antibiotic susceptibility of *H. pylori* in Yazd.

Objectives: The aim of this study was to investigate the antibiotic resistance patterns of *H. pylori* isolates from gastric biopsy of patients in Yazd.

Materials and method: In this descriptive-analytical study, 651 gastric biopsy specimens were taken from May 2012 to February 2013. Samples were cultured into selective Brucella agar media. After 3 to 10 days of incubation and identification of bacteria by using gram stain and biochemical tests, antibacterial susceptibility assay was performed by disk diffusion method.

Results: All of 651 gastric biopsy specimens were cultured and 144 (22.12%) isolates of *H. pylori* were collected. In total, 76 (52.7%) of 144 *H. pylori* isolates were isolated from females and 68 (47.2%) from males. Rate of antibiotic resistance to metronidazole, tetracycline, clarithromycin, levofloxacin, ciprofloxacin and amoxicillin were 77.8%, 21.5%, 18.8%, 14.6%, 19.4% and 7.6%, respectively. No antibiotic resistance was found against furazolidone. There was no significant association between these isolates antibiotics resistance with sex, age and endoscopic diagnoses (P > 0.05).

Conclusions: *H. pylori* isolates resistance was high to antibiotics, especially metronidazole. Therefore, determining resistance pattern of *H. pylori* isolates is recommended in all parts of the country.

Keywords: *Helicobacter pylori*; Drug Resistance, Multiple, Bacterial; Clarithromycin; Metronidazole

1. Background

*Helicobacter pylori* is a gram-negative bacterium, which plays an important role in the pathogenesis of chronic gastritis, peptic ulcer, duodenal ulcer and gastric carcinoma (1). The bacterium is colonized in the stomach of more than 50% of the world’s population (2). *H. pylori* associated infections are treated with antibiotics such as metronidazole, clarithromycin, amoxicillin, tetracycline, and levofloxacin in addition to an acid secretion inhibitor drug (3). Unfortunately, the recurrence risk is high in the first year after recovery.

Recurrence of *H. pylori* infection occurs further in younger patients and those with inappropriate medication regimens. However, in various studies, it has been found that the recurrence risk is very low following administration of an appropriate drug regimen (4). Today, triple therapy regimens are used in areas with low antibiotic resistance for the treatment of *H. pylori* associated infections, while quadruple and sequential regimens are used in areas with higher antibiotic resistance (5). The main cause of treatment failure is bacterium antibiotic resistance to various antibiotics. However, due to penetration of this bacterium in the mucous layer of stomach and the acidic environment of stomach, the ability of antibiotics is reduced to eliminate the bacterium. Therefore, drugs resistant to stomach acid such as metronidazole and clarithromycin with proton pump inhibitor (PPI) should be used in the treatment regimen (6). Antibiotic resistance to metronidazole, clarithromycin, tetracycline, amoxicillin and levofloxacin had been reported 26.7%, 17.2%, 5.9%, 16.2% and 11.2%, respectively until 2009 around the world. In Asia, resistance to these antibiotics had been 18.9%, 11.6%, 2.4%, 11.6% and 11.6%, respectively until 2009 (7).

Implication for health policy/practice/research/medical education:
In this study, we evaluated the antibacterial resistance patterns of *Helicobacter pylori* clinical isolates from gastric biopsy of patients in Yazd. Rate of antibacterial resistance varied in different regions. Evaluating resistance patterns of common pathogens in different regions helps to better management of the infection by healthcare providers.
2. Objectives
The aim of this study was to determine antibiotic resistance patterns of H. pylori clinical isolates from gastric biopsy of patients in Yazd.

3. Materials and Methods

3.1. Bacterial Isolates
This study was designed as a descriptive and cross-sectional study. The gastric biopsy specimens were collected during May 2012 to February 2013 from 651 patients not taking any antibiotics or proton pump inhibitor (PPI) at least one week prior to recruitment. All of the samples were obtained from the gastric biopsies of patients with upper gastrointestinal tract symptoms in the endoscopy sections at Shahid Sadoughi and Mortaz hospitals in Yazd, Iran. Then the samples were kept in Stuart transport culture media (Liofilchem, Italy) and were transported to the microbiology laboratory within 4 hours for culture. All of the samples were cultured on selective Brucella agar (Liofilchem- Italy) containing 5% sterile sheep blood, vancomycin (5 mg/L), trimethoprim (5 mg/L), and polymyxin B (2500 U/L). The plates were incubated for 3 to 10 days under microaerophilic conditions (8% CO2) at 37 °C. Identification of H. pylori was based on microscopic colony morphology, positive urease, oxidase and catalase activities (8).

3.2. Antibiotic Susceptibility Test
Antimicrobial susceptibility of H. pylori isolates and the reference strain (ss1, Sydney Strain H. pylori) was determined by disk diffusion method. Bacterial suspensions were prepared in sterile normal saline, with a turbidity adjusted to No.2 McFarland standard. Plates of Brucella agar were supplemented with 5% sterile sheep blood and inoculated with 150 μL of the bacterial suspensions by a glass applicator. Antibiotic disks (Neo-Sensitabs, Denmark) including levofloxacin (5 µg), clarithromycin (15 µg), ciprofloxacin (5 µg), amoxicillin (25 µg), tetracycline (30 µg), furazolidone (100 µg) and metronidazole (5 µg) were deposited on the plates. After 2-3 days of incubation under microaerobic condition, diameter of the growth inhibition zones was measured. The diameters of growth inhibition zones of the antibiotic disks including tetracycline, metronidazole, ciprofloxacin and clarithromycin were interpreted according to Mishara investigation (9). Although, there is still no standard criterion in the CLSI (Clinical and Laboratory Standards Institute) to determine the susceptibility of H. pylori to levofloxacin and amoxicillin disks, the diameters of growth inhibition zones of the antibiotic disks were interpreted according to a standard criterion in the CLSI for Haemophilus influenzae (10).

3.3. Statistically Analysis
SPSS software, version 15 was used for statistical analysis. Statistical tests included Chi-square and Fisher’s exact tests. P value < 0.05 was considered as statistically significant.

4. Results
Of the 651 gastric biopsy samples, 144 (22.11%) H. pylori strains were isolated. In total, 76 (52.7%) of 144 H. pylori isolates were isolated from females and 68 (47.2%) from males. The mean age of patients was 36.25 ± 19 years. In our study, the rates of gastritis, gastric ulcer, duodenitis, duodenum ulcer and other diagnoses were 58% (40.2%) , 19 (22.9%), 33 (13.1%), 20 (13.8%) and 14 (9.7%) respectively. Rates of antibiotic resistance to metronidazole, tetracycline, clarithromycin, levofloxacin, ciprofloxacin and amoxicillin were 77.8%, 21.5%, 18.8%, 14.6%, 19.4% and 7.6% respectively. No antibiotic resistance was detected to furazolidone. The association between antimicrobial resistances of H. pylori isolates with endoscopic diagnoses was shown in Table 1. There was no statistically significant association between antimicrobial resistances and endoscopic diagnoses (P > 0.05). In addition, there was no statistically significant association between antimicrobial resistances and sex and the two age groups of above 20 and below 20 years (P > 0.05) (Tables 2 and 3).

Table 1. The Association Between Antibiotic Resistances and Endoscopic Diagnoses 

<table>
<thead>
<tr>
<th>endoscopic diagnoses</th>
<th>Tet</th>
<th>Amx</th>
<th>Cla</th>
<th>Met</th>
<th>Lev</th>
<th>Cip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastritis</td>
<td>12</td>
<td>04</td>
<td>14</td>
<td>47</td>
<td>01</td>
<td>14</td>
</tr>
<tr>
<td>peptic ulcer</td>
<td>07</td>
<td>02</td>
<td>06</td>
<td>24</td>
<td>02</td>
<td>07</td>
</tr>
<tr>
<td>duodenitis</td>
<td>03</td>
<td>01</td>
<td>03</td>
<td>16</td>
<td>01</td>
<td>03</td>
</tr>
<tr>
<td>duodenum ulcer</td>
<td>07</td>
<td>02</td>
<td>03</td>
<td>18</td>
<td>03</td>
<td>04</td>
</tr>
<tr>
<td>Other diagnoses</td>
<td>02</td>
<td>02</td>
<td>01</td>
<td>07</td>
<td>04</td>
<td>00</td>
</tr>
</tbody>
</table>

P value 0.56 0.85 0.61 0.84 0.17 0.35

a Abbreviations: Amx, amoxicillin; Cip, ciprofloxacin; Cla, clarithromycin; Lev, levofloxacin; Met, metronidazole; Tet, tetracycline.
b Data are presented as No. (%).
Table 2. The Association Between Antibiotic Resistances and Sex \(^{a,b}\)

<table>
<thead>
<tr>
<th></th>
<th>Lev</th>
<th>Cip</th>
<th>Tet</th>
<th>Amx</th>
<th>Cla</th>
<th>Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
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<td></td>
<td></td>
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</tr>
<tr>
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<td>12</td>
<td>17</td>
<td>15</td>
<td>05</td>
<td>15</td>
<td>58</td>
</tr>
<tr>
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<td>09</td>
<td>11</td>
<td>16</td>
<td>06</td>
<td>12</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>0.81</td>
<td>0.4</td>
<td>0.68</td>
<td>0.75</td>
<td>0.79</td>
<td>0.39</td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations: Amx, amoxicillin; Cip, ciprofloxacin; Cla, clarithromycin; Lev, levofloxacin; Met, metronidazole; Tet, tetracycline.

\(^b\) Data are presented as No. (%).

Table 3. The Association Between Antibiotic Resistances and Age \(^{a,b}\)

<table>
<thead>
<tr>
<th></th>
<th>Lev</th>
<th>Cip</th>
<th>Tet</th>
<th>Amx</th>
<th>Cla</th>
<th>Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>&gt; 20</td>
<td>18</td>
<td>26</td>
<td>101</td>
<td>11</td>
<td>1.6</td>
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<td>&lt; 20</td>
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<td>00</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.98</td>
<td>0.29</td>
<td>0.59</td>
<td>0.98</td>
<td>0.4</td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations: Amx, amoxicillin; Cip, ciprofloxacin; Cla, clarithromycin; Lev, levofloxacin; Met, metronidazole; Tet, tetracycline.

\(^b\) Data are presented as No. (%).

5. Discussion

Antibiotic-resistant *H. pylori* is the most important factor in treatment failure (6). Antibiotic resistance of *H. pylori* to antibiotics varies in different geographical regions and depends on local use of antibiotics (5). In our study, the prevalence of resistance to metronidazole was 77.8%. Furthermore, rate of high resistance to metronidazole had been reported in three other studies in Iran. Haghi (11) in Tehran, reported a 64% resistance rate to metronidazole in *H. pylori* isolates. In a study by Sirous (12) in Tehran and Milani (13) in Tabriz, resistance rates to metronidazole were reported 51.5% and 76.8%, respectively. In Italy, the prevalence of resistance to this antibiotic was reported 59.3%. Similar to our study result, in the studies of Siavoshi (8) in Tehran and Saracino (14) in Italy, no significant association was found between resistance to metronidazole and sex and age (P > 0.05). Moreover, similar to our study result, Milani (13) in Tabriz showed no significant association between metronidazole resistance and endoscopy diagnoses. However, in some studies, there was a direct correlation between sex and metronidazole resistance, so that rate of metronidazole resistance in females was higher than males, because this antibiotic might be used for the treatment of related diseases to women (15). Clarithromycin can be used only when resistance is below 15-20%, which is in accordance with the study of Boer (16). Relatively similar results were reported in Tabriz by Milani (13) and in Netherlands by Loffeld (17). Clarithromycin resistance rates in these regions were 14.3% and 20.5%, respectively. However, unlike our study results, Siavoshi (8) and Fallahi (18) in Tehran reported a low resistance to this antibiotic. In their study, clarithromycin resistance rates were 8.3% and 4.6%, respectively. Moreover, in Malaysia (19) a very low prevalence of clarithromycin resistance (2.1%) was reported. Unlike our study results, the prevalence of clarithromycin resistance in some studies was reported high. In our study, there was no direct association between sex and age and endoscopy diagnoses with clarithromycin resistance (P > 0.05), but Siavoshi (8) in Tehran and Kato (20) in Japan reported a statistically significant association between clarithromycin resistance and childhood age group (P < 0.05). It might be due to using antibiotics to treat ear infections in this age group. In our study, the prevalence of tetracycline resistance was 21.5%, which was in line with a study performed in Tabriz (about 18.7%) (13). Unlike the results of our study, in the studies of Siavoshi (8) and Oleastro (2), tetracycline resistance was not observed. In our study, there were no furazolidone-resistant *H. pylori* isolates, which was similar to the results of Sirous (12) and Fallahi (18) in Tehran. It might be due to low utilization of furazolidone to treat associated infections with *H. pylori* in Yazd and its side effects. These side effects include sister chromatid exchanges and carcinogenic nature of the drug. But nowadays, furazolidone is used in some parts of the world due to its low cost, especially in developing countries, as a first-line antibiotic of *H. pylori* treatment in triple-based regimens (21). In our study, the prevalence of amoxicillin resistance was 7.6%. In the study of Siavoshi (8) similar to our study results, resistance to amoxicillin in *H. pylori* isolates was low. Resistance rates were 5.9% in children and 2.4% in adults. In a study in South Korea (22), 8.8% of *H. pylori* isolates were amoxicillin resistant. But, the high prevalence of resistance to amoxicillin was reported (85.6%) in Cameroon (23). Unlike other studies, in a study in Tehran (12), no amoxicillin resistance was observed. In our study, the prevalence of resistance to le-
voloxacin was 14.6%. Resistance rates to levofloxacin in \textit{H. pylori} isolates in Japan (24), Taiwan (25) and Hong Kong (26) were 14.9%, 11.9% and 2.6% respectively. Although, no study has yet surveyed resistance to levofloxacin in \textit{H. pylori} isolates in Iran. In our study, 19.4% of \textit{H. pylori} isolates were resistant to ciprofloxacin. The prevalence of resistance to ciprofloxacin in \textit{H. pylori} isolates in Tabriz (13) and Portugal (2) were 33% and 4.6%, respectively. Resistance to levofloxacin and ciprofloxacin in Taiwan (25) increased from 2.8% during 1998-2003 to 11.8% during 2004-2007. In our study, 80% of metronidazole-resistant \textit{H. pylori} isolates were susceptible to clarithromycin. Moreover, 88% of metronidazole-resistant \textit{H. pylori} isolates were susceptible to levofloxacin. It seems that these two antibiotics are effective in the treatment of these patients. In this study, the prevalence of \textit{H. pylori} associated-infections was 22.12%. The frequency of metronidazole resistance was high; whereas, resistance rate to amoxicillin was very low. Moreover, all of \textit{H. pylori} isolates were sensitive to furazolidone. Finally, there was no statistically significant association between antibiotic resistances with age, sex and endoscopic diagnoses ($P > 0.05$).

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Authors’ Contribution

All authors participated equally in the present study.

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