کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله
Antimicrobial Resistance of *Helicobacter pylori* Isolates from Iranian Adults and Children

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**Background:** *Helicobacter pylori* isolates from 84 adults and 51 children were assessed during the period of 2001 through 2004 to find out whether their susceptibilities to metronidazole, clarithromycin, amoxicillin, tetracycline, and furazolidone differ between the two groups or have changed compared with the results from our previous study done between 1997 – 2000.

**Methods:** Biopsies from 135 dyspeptic patients were cultured on Brucella blood agar. Pure cultures of *H.pylori* isolates were used for antibiotic susceptibility tests. Disk diffusion method was recruited to assess the susceptibility of *H.pylori* isolates to different dilutions of the above mentioned antimicrobials. Results obtained from the two groups were compared and minimum inhibitory concentrations determined.

**Results:** Thirty-five percent of *H.pylori* isolates from adults and 37% from children were resistant to metronidazole. Clarithromycin resistance rate was 2.4% and 5.9% in *H.pylori* isolates from adults and children, respectively. Amoxicillin resistance was 2.4% in isolates from adults and 5.9% in isolates from children. Tetracycline resistance rate was 0% in *H.pylori* isolates from adults and 2.0% in isolates from children. Resistance to furazolidone was not observed.

**Conclusion:** Resistance rates of *H.pylori* isolates from adults and children to metronidazole, clarithromycin, amoxicillin, tetracycline, and furazolidone are similar and not significantly affected by age and time.

**Keywords:** Adults • children • *Helicobacter pylori* • resistance

**Introduction**

Accumulating evidence indicates that most adult infections of *Helicobacter pylori* are contracted during childhood. Since signs and symptoms of the infection do not generally permit identification of the infected cases at the onset of the disease, *H.pylori* infection may develop into a chronic type, which can lead to gastric ulcer or cancer in later stages of life.

In order to prevent such dramatic consequences, *H.pylori* should be eradicated in early stages of life, although eradication of *H.pylori* at a young age is hard mainly due to a low compliance of children, intrafamilial reinfection, and emergence of resistant strains.

On the other hand, most patients referred to endoscopy units are *H.pylori*-positive adults, indicating that persistent *H.pylori* infection establishes in later stages of life as a result of recurrence or recrudescence. Thus, to achieve successful eradication of *H.pylori*, resistance status of the bacterial isolates to the current antimicrobials within a population should be considered.

No strong evidence suggests that *H.pylori* strains, which colonize adults and children, are different in their main features such as genetic fingerprints, virulence factors, or susceptibility to antimicrobials. In this study, we compared the...
susceptibility of \textit{H. pylori} isolates from Iranian adults and children to metronidazole, clarithromycin, amoxicillin, tetracycline, and furazolidone. We also evaluated the change in rates of resistance to the mentioned antimicrobials with time.

**Materials and Methods**

We randomly collected 135 \textit{H. pylori} isolates from dyspeptic patients including 51 children and 84 adults during the period of 2001 – 2004. The children consisted of 33 girls and 18 boys with the age range of 2 – 15 years (mean age of 8 years for girls and 9.7 years for boys) who were referred to Children’s Medical Center of Tehran. The adults consisted of 50 women and 34 men with the age range of 15 – 75 years (mean age of 40.5 years for women and 42.7 years for men) who were referred to the Endoscopy Unit of Shariati Hospital.

Semisolid normal saline was used to transport urease-positive gastric biopsies to microbiology laboratory. Biopsy specimens were cultured on selective Brucella blood agar (Merck) containing defibrinated blood (7%), vancomycin (5 mg/L), trimethoprim (5 mg/L), and polymyxin B (2500 U/L). After microaerobic incubation at 37°C, bacterial cultures were purified and identified as \textit{H. pylori} according to the microscopic observation of spiral morphology and catalase-, oxidase-, and urease-positive reactions.

The disk diffusion method was recruited to assess the susceptibility of \textit{H. pylori} strains to different antimicrobials. Bacterial suspensions were prepared in normal saline, with the turbidity adjusted to No.1 McFarland standard. Surface of Brucella blood agar plates were inoculated with 100 µL of bacterial suspensions. Glass rods were used to spread the inocula. Blank disks were deposited on the plates and impregnated with 10 µL of different dilutions of metronidazole (32, 8, and 4 µg/mL) in methanol (Merck), clarithromycin (2, 1, 0.5, and 0.25 µg/mL) in ethanol (Merck), amoxicillin (1, 0.5, and 0.25 µg/mL) in dimethyl sulfoxide (Merck), tetracycline (1, 0.5, and 0.25 µg/mL) in ethanol, and furazolidone (1, 0.5, 0.25, and 0.12 µg/mL) in N, N-dimethyl formamide (Merck).

After 2 – 3 days of microaerobic incubation, the growth inhibition zone diameters were recorded. \textit{H. pylori} strains were considered as susceptible when exhibited growth inhibition zones of $\geq$20 mm for metronidazole [minimum inhibitory concentration (MIC) 8µg/mL], clarithromycin (MIC 2 µg/mL), amoxicillin (MIC 1 µg/mL) and, tetracycline (MIC 0.5 µg/mL), and $\geq$13 mm for furazolidone (MIC 0.5 µg/mL). No inhibition zone was observed on control plates deposited with blank disks containing solvents of antimicrobials.

SPSS software, version 12 was used for statistical analysis. The statistical tests applied were Student’s $t$ test, Chi-square test, and Fisher’s exact test. Confidence intervals (CI) were calculated using binomial distribution. All tests performed were two tailed, and $P < 0.05$ considered as significant.

**Results**

Out of the 135 \textit{H. pylori} isolates, 55 (40.0%; 95% CI, 31.6 – 48.4%) were resistant to at least one antibiotic. The highest rate of resistance belonged to metronidazole (36.3%, 28.1 – 44.5%). The resistance rates to clarithromycin, amoxicillin, and tetracycline were 3.7% (0.5 – 6.9%), 3.7% (0.5 – 6.9%), and 0.7% (0 – 2.1%), respectively. No resistance to furazolidone was observed.

Resistance rates of \textit{H. pylori} isolates from adults and children to recruited antimicrobials are shown in Table 1. Chi-square and Fisher’s exact tests showed no significant difference between the resistance rates in adults and children ($P > 0.30$).

The distribution of the MICs of metronidazole had an almost identical pattern in \textit{H. pylori} isolates from children and adults (Figure 1). Chi-square test confirmed the similarity between the distributions ($P = 0.98$). There was also no significant difference between the distribution of MICs of other antibiotics in \textit{H. pylori} isolates from adults and children ($P = 0.26$ for amoxicillin, 0.34 for clarithromycin, 0.43 for tetracycline, and 0.64 for furazolidone).

Resistance rates of all \textit{H. pylori} isolates were determined at different dilutions of recruited antimicrobials. The highest rates were obtained at

<table>
<thead>
<tr>
<th>Antimicrobial (MIC µg/mL)</th>
<th>Rates of resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isolates from adults</td>
</tr>
<tr>
<td>Metronidazole (8)</td>
<td>30/84 (35.7%)</td>
</tr>
<tr>
<td>Clarithromycin (2)</td>
<td>2/84 (2.4%)</td>
</tr>
<tr>
<td>Amoxicillin (1)</td>
<td>2/84 (2.4%)</td>
</tr>
<tr>
<td>Tetracycline (0.5)</td>
<td>0/84 (0%)</td>
</tr>
<tr>
<td>Furazolidone (0.5)</td>
<td>0/84 (0%)</td>
</tr>
</tbody>
</table>

*Table 1. Resistance rates of \textit{H. pylori} isolates from 84 adults and 51 children to metronidazole, clarithromycin, amoxicillin, tetracycline, and furazolidone.*
Antimicrobial resistance of *H. pylori* isolates from Iranian adults and children

4 µg/mL of metronidazole (47.4%), 0.25 µg/mL of clarithromycin (9.6%), 0.25 µg/mL of amoxicillin (9.6%), 0.25 µg/mL of tetracycline (3%), and 0.12 µg/mL of furazolidone (17.8%) (Figure 2).

Resistance to multiple drugs was found in 6 isolates (4.4%; 0.9 – 7.9%), of them four (3.0%) were resistant to two antibiotics, and two isolates (1.5%) were resistant to three antibiotics. The prevalence of resistance to multiple drugs was 1.2% (1/84) in adults and 9.8% (5/51) in children (*P* = 0.029).

Of the four isolates resistant to two antibiotics, three were resistant to metronidazole and clarithromycin and one was resistant to metronidazole and amoxicillin.

Of the two isolates resistant to three antibiotics, one was resistant to metronidazole, amoxicillin, and clarithromycin, and the other one was resistant to metronidazole, amoxicillin, and tetracycline.

*H. pylori* isolates from boys compared to girls, had a significantly higher resistance rate to metronidazole (55.5% vs. 27.3%, *P* = 0.046) and clarithromycin (16.6% vs. 0.0%, *P* = 0.039). A higher rate of resistance to both metronidazole and clarithromycin was also observed in boys (3/18, 16.6% vs. 0/33, 0.0%, *P* = 0.039).

The resistance rates to amoxicillin and tetracycline were 16.6% and 5.55% in boys as compared to 0.0% and 0.0% in girls, respectively. The difference was significant for amoxicillin (*P* = 0.039) but not for tetracycline (*P* = 0.39). Resistance to furazolidone was not observed.

In *H. pylori* isolates obtained from women, the resistance rates to metronidazole, clarithromycin, and amoxicillin were 36.7%, 4.1%, and 2.1%, respectively, while in men these rates were 36.6%, 0.0%, and 3.4%, respectively. The differences between resistance rates in men and women were not significant.

The mean age of adults was 41.2 years and that of children was 8.6 years. The mean age of adults and children of whom resistant and susceptible strains were isolated did not differ significantly for metronidazole, clarithromycin, amoxicillin, and tetracycline (*all* *P* > 0.1).

**Discussion**

Susceptibility of *H. pylori* isolates from 84 adults and 51 children to metronidazole, amoxicillin, clarithromycin, and tetracycline were 52.6%, 16.6%, 4.4%, and 5.55%, respectively. The differences were significant for metronidazole (*P* = 0.046) and clarithromycin (*P* = 0.039) but not for amoxicillin and tetracycline (*P* = 0.39 and 0.1). The resistance rates to furazolidone were negligible (*P* = 0.5).

The prevalence of resistance to multiple antibiotics was higher in children than in adults (9.8% vs. 1.5%, *P* = 0.029). The resistance rates to metronidazole and clarithromycin were significantly higher in boys than in girls (55.5% vs. 27.3%, *P* = 0.046 and 16.6% vs. 0.0%, *P* = 0.039). The resistance rate to amoxicillin was also significantly higher in boys (16.6%) than in girls (0.0%), *P* = 0.039). A higher rate of resistance to amoxicillin and tetracycline was observed in boys (16.6% vs. 0.0%, *P* = 0.039). The resistance rates to furazolidone were negligible in both boys and girls (0.0%).

In *H. pylori* isolates obtained from women, the resistance rates to metronidazole, clarithromycin, and amoxicillin were 36.7%, 4.1%, and 2.1%, respectively, while in men these rates were 36.6%, 0.0%, and 3.4%, respectively. The differences between resistance rates in men and women were not significant.

The mean age of adults was 41.2 years and that of children was 8.6 years. The mean age of adults and children of whom resistant and susceptible strains were isolated did not differ significantly for metronidazole, clarithromycin, amoxicillin, and tetracycline (*all* *P* > 0.1).

**Discussion**

Susceptibility of *H. pylori* isolates from 84 adults and 51 children to metronidazole,
clarithromycin, amoxicillin, tetracycline, and furazolidone was assessed during the period of 2001 – 2004.

The results showed similar rates of resistance to metronidazole among *H. pylori* isolates from adults (35.7%) and children (37.3%). Studies from Portugal showed a higher rate of metronidazole resistance in *H. pylori* isolates obtained from adults (32.3%), compared to those from children (19%). The rates of metronidazole resistance in this study were intermediate, compared to the range of rates reported worldwide for adults’ isolates, from 10 – 50% in developed countries to 80 – >90% in developing countries. In contrast, no resistance has been reported from Canada. The rates of metronidazole resistance in this study were close to the results of our previous study on *H. pylori* isolates from adults (32.8%), which was performed with the same MIC (8 µg/mL) of metronidazole, between the years 1997 to 2000. These results indicated that metronidazole resistance among *H. pylori* isolates in Iran did not change significantly with time. Rates of resistance also remained constant among *H. pylori* isolates from Portugal and Germany. Although a considerable increment was observed in other European countries, Belgium, and Shanghai (Table 2). Distribution of different metronidazole MICs among *H. pylori* isolates from adults and children showed similar patterns, and the MIC for the majority (52.6%) of isolates from adults and children was 4 µg/mL. This finding indicates that most of *H. pylori* isolates are highly sensitive to metronidazole (MIC≤4 µg/mL), although a considerable number (25.2%) are highly resistant to this antibiotic (MIC≥32 µg/mL).

The rate of resistance to metronidazole in *H. pylori* isolates from boys was significantly higher than those from girls, although a report from France was different (Table 3). The rate of resistance to metronidazole was similar among *H. pylori* isolates from men and women. Similar results were reported from Portugal, whereas isolates from women in Germany and China showed a higher resistance to metronidazole (Table 3).

The rate of resistance to clarithromycin in *H. pylori* isolates from adults (2.4%) and children (5.9%) were not significantly different. In Portugal clarithromycin resistance rate was lower in *H. pylori* isolates from adults (14.6%), compared to those from children (44.8%). The range of clarithromycin resistance in *H. pylori* isolates from adults varies between 2 and 15% in developed countries (Table 4). The results from this study, compared to the results from our previous study (1.43%, MIC 0.25 µg/mL), showed no significant changes with time. Similarly, resistance rates of *H. pylori* isolates from adults in Germany and France did not show a significant change with time. Although reports from Belgium and Portugal describe significant change with time (Table 4).

Similar to metronidazole, the clarithromycin resistance rate was higher in *H. pylori* isolates from boys (16.6%) than in those from girls (0%). There was no significant differences between clarithromycin resistance in isolates from men (0%) and women (4.1%). Similar results have been reported from Germany and Portugal (Table 5).

The amoxicillin resistance rate was not

### Table 2. Metronidazole resistance rates of *H. pylori* isolates reported from Iran and other countries.

<table>
<thead>
<tr>
<th>Countries</th>
<th>Iran (1997 – 2000)</th>
<th>Iran (2001 – 4)</th>
<th>Developed countries</th>
<th>Developing countries</th>
<th>Canada</th>
<th>Portugal</th>
<th>Germany</th>
<th>Belgium</th>
<th>Europe</th>
<th>Shanghai</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rates of resistance</td>
<td>32.8%</td>
<td>35-37%</td>
<td>10 – 50%</td>
<td>80 – &gt;90%</td>
<td>0%</td>
<td>32.3%</td>
<td>26.2%</td>
<td>30%</td>
<td>20 – 30%</td>
<td>37.3%</td>
</tr>
<tr>
<td>Change with time</td>
<td>NSCh</td>
<td>NSCh</td>
<td>NSCh</td>
<td>NSCh</td>
<td>NSCh</td>
<td>++</td>
<td>NSCh</td>
<td>NSCh</td>
<td>NSCh</td>
<td></td>
</tr>
</tbody>
</table>

NSCh = no significant change.

### Table 3. Metronidazole resistance rates of *H. pylori* isolates reported from Iran and other countries, based on the age and gender.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Iran</th>
<th>France</th>
<th>Portugal</th>
<th>China</th>
<th>Germany</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>55%</td>
<td>40%</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Girls</td>
<td>27.3%</td>
<td>60%</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Men</td>
<td>36.6%</td>
<td>–</td>
<td>31.7%</td>
<td>69%</td>
<td>20.8%</td>
</tr>
<tr>
<td>Women</td>
<td>36.7%</td>
<td>–</td>
<td>30.8%</td>
<td>85%</td>
<td>32.2%</td>
</tr>
</tbody>
</table>
Antimicrobial resistance of *H. pylori* isolates from Iranian adults and children

### Table 4. Clarithromycin resistance rates of *H. pylori* isolates reported from Iran and other countries.

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Rates of resistance</td>
<td>1.43%</td>
<td>2.4%</td>
<td>2%</td>
<td>21%</td>
<td>1.7%</td>
<td>4.6%</td>
</tr>
<tr>
<td>Change with time</td>
<td>NSCh</td>
<td>NSCh</td>
<td>10.5% (1995–2000)</td>
<td>NSCh</td>
<td>NSCh</td>
<td>NSCh</td>
</tr>
</tbody>
</table>

NSCh = no significant change.

- Resistance to multiple drugs was higher in *H. pylori* isolates from children compared to adults. In children, the highest rate of resistance (50%) was to metronidazole and clarithromycin. Resistance to combination of metronidazole and clarithromycin has been reported from France (9%), Japan (10%), Bulgaria (2.8%), and Germany (58.3%).

- Resistance to combination of metronidazole, amoxicillin, and tetracycline was exhibited by one strain. However, 39.2% of isolates from Chinese children exhibited combined resistance to these antibiotics.

Results from this study showed that age, sex, and time do not significantly affect the resistance of *H. pylori* isolates to different antibiotics. It appears that in spite of the current use of metronidazole and macrolides against parasitic, gynecologic, and respiratory infections, the rates of resistance to these antibiotics in many regions of the world have remained constant. This finding indicates that antibiotic resistance in *H. pylori*, like in other Gram-negative bacteria, is part of the evolutionary designed adaptation machinery. This includes poorly permeable outer membrane, beta-lactamases, and active efflux pumps, which confer to bacteria the intrinsic resistance to environmental stresses. In other word, antibiotic resistance in bacteria is not necessarily the consequence of frequent exposure to certain antibiotics, although it could be due to the selection of resistant survivors and spread of resistant genes. Furthermore, lines of evidence indicate that efflux pumps in different bacteria have evolved long before the antibiotic era.

Mutations either leading to over-expression of efflux pumps, with an additive increase in MICs of antibiotics, or to change in the target site(s) of resistance to antibiotics.

### Table 6. Resistance rates of *H. pylori* isolates reported from Iran and other countries to amoxicillin, tetracycline, and furazolidone.

<table>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Amoxicillin</strong></td>
<td>1.43%</td>
<td>2.4%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>71.9%</td>
<td>31%</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Tetracycline</strong></td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>–</td>
<td>1.9%</td>
<td>58.8%</td>
<td>6%</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Furazolidone</strong></td>
<td>0%</td>
<td>0%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2%</td>
<td>4%</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
antibiotics could also confer resistance. These mutations are also the common consequences of bacterial responses to environmental stresses such as osmotic shock and starvation. Concurrence of both types of mutations can lead to highly refractory bacterial infections.

Since beta-lactamases are not present in H. pylori, it has been proposed that intrinsic resistance of H. pylori to antibiotics is due to the low permeability of bacterial lipopolysaccharide and recently-recognized active efflux. However, point mutations in the peptidyl transferase domain of the 23S rRNA lead to resistance to clarithromycin, and mutations in rdxA or fdxA genes encoding NAD(P)H nitroreductase and NAD(P)H flavin oxoreductase, respectively, could result in metronidazole resistance.

In Iran, a considerable number of individuals (~90%) are infected with H. pylori and there are regions in which the prevalence of peptic ulcers and gastric cancer is high. Since the use of antibiotics and eradication of H. pylori significantly decrease the recurrence rate of peptic diseases, effective H. pylori eradication should be initiated especially in patients at risk. Regarding the considerable rate of metronidazole resistance among H. pylori isolates (32.8–37%) in Iran, gastroenterologists recruit quadruple regimens in which metronidazole has been substituted with clarithromycin or furazolidone. Based on the results of this study and several other reports, and having considered that metronidazole is an antibiotic of choice against susceptible strains of H. pylori, performance of susceptibility tests is recommended for H. pylori isolates, in order to design an appropriate therapeutic regimen. Furthermore, comparison of geographic regions with low and high rates of resistance to metronidazole might reveal the cultural or environmental factors that play a role in the prevalence of resistance to metronidazole.

References

20. Kalach N, Bergeret M, Benhamou PH, Dupont C, Raymond J. High levels of resistance to metronidazole
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کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله