

Antibiotic Susceptibility of *Helicobacter pylori* Clinical Isolates in Hamadan, West of Iran

Amir Majlesi¹, Masood Sayedin Khorasani², Ali Reza Khalilian¹, Mohammad Mehdi Aslani³, Mohammad Jaefari⁴, Mohammad Yousef Alikhani^{2,*}

¹ Department of Gastroenterology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran

² Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran

³ Department of Microbiology, Pasteur Institute of Iran, Tehran, IR Iran

⁴ Department of Pathology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran

*Corresponding author: Mohammad Yousef Alikhani, Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran. Tel: +98-8118276295, Fax: +98-8118276299, E-mail: alikhani43@yahoo.com, E-mail: alikhani@umsha.ac.ir

ABSTRACT

Background: Surveillance data on *Helicobacter pylori* antibiotic susceptibilities are limited in Hamadan, Iran. Since antibiotic resistance is one of the reasons in therapies failure.

Objectives: Thus the resistance patterns of *H. pylori* strains to the antibiotics metronidazole, clarithromycin, amoxicillin and tetracycline were evaluated.

Patients and Methods: Gastric biopsy specimens of 153 patients with nonulcer dyspepsia, peptic ulcer dyspepsia, and peptic cancer collected during May 2010 to February of 2011, and were cultured on Brucella agar (Merck, Germany) under microaerophilic conditions. *H. pylori* isolates were identified using standard biochemical test. Eighty three (54.2 %) specimens had positive results by culture. Antimicrobial susceptibility testing was performed by disc diffusion method.

Results: Totally in vitro resistance rates were 63.8% for metronidazole, 26.5% for clarithromycin, and 7.2% for amoxicillin. Although, 25.3% of strains showed resistance to two antibiotics, and 3.6% to three antibiotics. Tetracycline resistance was identified in only two isolates. Fifty nine percent of the clarithromycin resistant strains also showed resistance to metronidazole. No gender and age associations with resistance were detected.

Conclusion: Our results showed a high incidence of metronidazole resistance (often combined with clarithromycin-resistance) in the isolates. Continuous surveillance is recommended to examine the treatment strategies for *H. pylori* eradication.

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

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The determination of *H. pylori* antibiotic resistance can help clinicians to select a valuable empiric treatment.

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1. Background

Helicobacter pylori (*H. pylori*) colonizes the stomach of about 50% of people around the world. Colonization with *H. pylori* is not a disease, but *H. pylori* is an etiologic agent of acute or chronic gastritis, and a predisposing condition to peptic ulcer disease, gastric lymphoma and gastric carcinoma (1-3). Many studies believe that *H. pylori* eradication leads to curing gastritis and peptic ulcer disease, and possibly as well has an important effect on regression of atrophic gastritis and prevention of gastric cancer (4, 5). In vitro, *H. pylori* is susceptible to most antibiotics, but in vivo only a small number of antibiotics can be used successfully for eradication of *H. pylori*, i.e. amoxicillin, clarithromycin, metronidazole and tetracycline (6). Since none of these drugs is successful enough to eradicate *H. pylori* in monotherapy, successful treatment of *H. pylori* infection requires combination treatment, consisting of two antibiotics, an acid inhibitor and/or a bismuth component.

The extensive use and limited option of the antibiotics have resulted in the raise of antibiotic resistance in *H. pylori*. Resistance to metronidazole is observed in 10 to 50% of the cases in developed countries, but can be as high as 90% in developing worlds (7). The prevalence of macrolide-resistant strains varies among countries and ranges from fewer than 2% of the strains in the Netherlands and Norway (7) to more than 10% in France and some other countries (6, 8, 9). In addition, the prevalence of resistant strains appears to be increasing (9, 10). There are some troubles with antimicrobial susceptibility methods of *H. pylori*. Broth and agar dilution methods usually are not easy to do. Because disk diffusion method is simple, low-cost and easy to perform in many of microbiological labs, it is frequently used (11).

2. Objectives

The occurrence of *H. pylori* resistance varies in different areas, and then requires continuous monitoring. Thus, the aim of this study was to evaluate *H. pylori* resistance to the current antimicrobial agents which used to eradicate this organism in patients.

3. Patients and Methods

Bacterial strains: In this cross-sectional descriptive study from the May 2010 to February of 2011, a total of 153 adult patients [4 gastric cancer (GC), 42 peptic ulcer dyspepsia (PUD) and 107 nonulcer dyspepsia (NUD)] undergoing upper gastroduodenal endoscopy for diagnosis and treatment purposes in the gastroenterology departments of Shahid Beheshti Hospital (Hamedan Province, Western of Iran) were included in this study. We used routine diagnostic methods for isolation of *H. pylori* strains from gastric biopsies according to standard laboratory procedures (11).

Biopsy specimens for culture were taken into screw capped bottle containing Thioglycolate Broth (Merck, Germany) with 0.5% agar and 3% yeast extract, and promptly transported to the microbiology lab at Faculty of Medicine, Hamadan University of Medical Sciences. Gastric biopsy specimens were ground with tissue homogenizer and then inoculated onto Brucella Agar (Merck, Germany) with 10% sheep blood and 10% fetal bovine serum (GIPCO), and Campylobacter Selective Supplement (Merck, Germany), and incubated under microaerophilic (5% O₂, 10% CO₂, and 85% N₂) conditions at 37° C for 3 to 5 days. Organisms were identified as *H. pylori* on the basis of morphology on Gram stain examination and by oxidase, catalase, and urease tests (11).

Antibiotic susceptibility testing: *H. pylori* isolates were grown on Brucella Agar (Merck, Germany) plates supplemented with 10% sheep blood, and incubated under microaerophilic (5% O₂, 10% CO₂, and 85% N₂) conditions for 3 days. The bacterial suspension was adjusted to a final concentration of 3×10⁸ CFU/ml in 1.0 ml sterile saline solution. The suspensions were spread on Mueller-Hinton agar plates (Merck, Germany) supplemented with 10% fetal bovine serum (GIPCO) by cotton swabs and then disks containing metronidazole (5 µg), amoxicillin (10 µg), clarithromycin (15 µg), and tetracycline (30 µg) (HiMedia Laboratories Co., India), were placed on the agar surface. The plates were incubated under microaerophilic conditions for 3 days at 37°C. Then, the inhibition zone diameters were considered as resistant (R), intermediate (I) or susceptible (S). The *E. coli* strain ATCC 25922 was included as a quality control in all assays. A inhibition zone size ≤16 mm was consider resistant for metronidazole, ≤25 mm for amoxicillin resistance, and ≤30 mm for clarithromycin, and tetracycline resistance. An inhibition zone larger than those sizes was determined to be susceptible. The *E. coli* strain ATCC 25922 was included as a quality control in all assays (12).

Statistical analysis: Statistical analysis was performed by the two-tailed Fisher or χ^2 tests. P-value of < 0.05 was considered statistically significant.

4. Results

Individual antibiotic susceptibilities: Over the 9 months from May 2010 to February of 2011, 153 patients 64 females and 89 males (Mean age 53 years; age range 16 to 88 years) included in this study. Of 153 biopsy specimens, 83 (54.2%) patient samples were confirmed to have positive results for *H. pylori* by culture [3 gastric cancer (GC), 27 peptic ulcer dyspepsia (PUD), and 53 non ulcer dyspepsia (NUD)].

Overall resistance rates were: 26.5% (22/83) for clarithromycin and 63.8% (53/83) for metronidazole. Tetracycline resistance was identified in only two isolates (2.4%), and 6 (7.3%) isolates showed resistance to amoxicillin (Table 1).

Table 1. Number of Isolates of *H. pylori* From Gastric Biopsies Resistant to Each Antibiotic

Sensitive, No(%)	Intermediate, No(%)	Resistance, No(%)	Antibiotic
18(21.6)	12(14.4)	53(63.8)	Metronidazole (Mt)
38(45.7)	23(27.7)	22 (26.5)	Clarithromycin (Cla)
72(86.7)	5 (6.1)	6 (7.2)	Amoxicillin (AM)
79 (95.1)	2 (2.4)	2 (2.4)	Tetracycline (Tet)

Combined antibiotic susceptibilities: All isolates of *H. pylori* were characterized by the assignment of a susceptibility pattern based on combined susceptibilities to metronidazole, clarithromycin, amoxicillin and Tetracycline (Table 2). Most strains (60%) were resistant (MtR/ClaS) or intermediate (Mtl/ClaS), whereas sixteen strains (19.3%) were resistant to both antibiotics (MtR/ClaR), and 21(25.3%) resistant to two different antibiotics (MtR/TetR, MtR/AmR, MtR/ClaR) and 3.6% to three antibiotics (MtR/ClaR/TetR, MtR/ClaR/AmR). Fifty nine percent of the clarithromycin resistant strains also showed resistance in metronidazole. Twenty four and fifty percent of those strains resistant to metronidazole showed resistance to clarithromycin too.

Table 2. Number of Isolates of *H. pylori* from Gastric Biopsies Resistant to Combine Antibiotics

Resistance, No(%)	Antibiotic
2 (2.4)	(Mt) + (Cla) + (AM)
1 (1.2)	(Mt) + (Cla) + (Tet)
13 (15.6)	(Mt) + (Cla)
6 (7.2)	(Mt) + (AM)
2 (2.4)	(Mt) + (Tet)

Abbreviations: Mt, Metronidazole; Cla, Clarithromycin; Tet, Tetracycline; AM, Amoxicillin

5. Discussion

H. pylori-associated disorders such as peptic ulcer disease generally treat completely after eradication of *H. pylori* with antibiotics. Antimicrobial resistance is an increasing difficulty in *H. pylori* treatment. Multidrug resistance in *H. pylori* is important, as the efficiency of the present therapeutic regimens may be compromised (13, 14). Metronidazole, clarithromycin, amoxicillin and tetracycline are the most extensively used antimicrobial drugs to cure *H. pylori* infection, but antimicrobial resistance rates have been reported (13, 15). There is slight information on antimicrobial resistance of *H. pylori* in west of Iran, therefore we evaluated antimicrobial resistance in this region. We evaluated 83 *H. pylori* isolates from patients. Of the 83 *H. pylori* isolates, 24 (28.9%) exhibited resistance to at least one of the four antimicrobial agents. Antimicrobial resistances to metronidazole, clarithromycin, amoxicillin, and tet-

racycline were 63.8, 26.5, 7.2, and 2.4%, respectively. The antimicrobial resistance of *H. pylori* isolates to two, and multiple antimicrobial agents was found in 25.3% and 3.6%, respectively. A previous study reported antimicrobial resistance rate to metronidazole in Tehran was 51.5%, and all the isolates were sensitive to clarithromycin, amoxicillin and tetracycline (16). In the other study in Isfahan of Iran, resistance were 30.0%, 6.25%, 3.75%, and 2.50% for metronidazole, clarithromycin, tetracycline, and amoxicillin, respectively. Multiple antibiotic resistances were observed in 8 of 27(29.6%) resistant isolates (17). In Shiraz, South of Iran, resistances of *H. pylori* were 44%, 20%, 3%, and 5% for metronidazole, clarithromycin, tetracycline, and amoxicillin, respectively (18). The prevalence rate of metronidazole resistance between *H. pylori* strains is greatly variable, and is higher in developing countries than developed worlds (19). This variation may be caused by the wide use of metronidazole in developing countries for other infectious diseases, such as protozoal diseases (17). The resistance rate to metronidazole (63.8%) in our research is comparable with the earlier studies (20-23), but was higher than what was reported by others (17, 24-26). Our results suggest that metronidazole combined with other antimicrobial agents can be still used for *H. pylori* treatment, because there is moderately low resistance when it was experienced in combination with other antimicrobial agents. Clarithromycin resistance rates have been reported more than 20% in some areas of the world (27-30). Clarithromycin has been used for the management of respiratory tract infections in some area, and maybe over use of this antibiotic is the reason for the high clarithromycin resistance in our study. Additional examination should be considered since the use of clarithromycin combined with other antimicrobial agents for *H. pylori* treatment has been increased (31). The resistance rates to amoxicillin and tetracycline were low in this study (7.2 and 2.4%, respectively), which were higher than previously reported (16). In Thailand, the resistance rates to amoxicillin and tetracycline were 13.9% and 5.1%, respectively (29). Our results suggest that these antimicrobial agents may be used to reduce *H. pylori* infection in our area.

In conclusion, the determination of *H. pylori* antibiotic resistance can help clinicians to select a valuable empiric treatment. Surveillance of antimicrobial resistance is required to be performed frequently to identify antimicro-

bial resistance.

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Authors' Contribution

All authors participated equally in the present study

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There is no conflict of interest.

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