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The Resistance Pattern of Helicobacter Pylori Isolated in the Northeast of Iran, Mashhad

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ABSTRACT

Introduction: Based on serological studies, the prevalence of Helicobacter pylori infection in Iranian adults is up to 80%. Gastritis, peptic ulcer, and gastric adenocarcinoma are common clinical outcomes of this infection in Iran. Since antibiotic resistance patterns of Helicobacter pylori are geographically different, local studies are highly required.

Methods: Eighty isolates of Helicobacter pylori were obtained from patients referred to the endoscopy unit of Ghaem Hospital in Mashhad. Demographic features including age, gender, and symptoms were recorded before the sampling. The antibiotic susceptibility patterns of isolates were determined for the five common antibiotics used for the treatment of Helicobacter pylori infection. The agar dilution method was used to evaluate the antibiotic resistance patterns.

Results: The patterns of antibiotic resistance were determined, and ,%13.7 ,%41.2 %6.6 ,%8.7, and %6.6 of isolates were resistant to metronidazole, clarithromycin, amoxicillin, tetracycline, and furazolidone, respectively.

Conclusion: Our study demonstrates that the overall rate of antibiotic resistance of Helicobacter pylori especially in the case of metronidazole has increased over time. The resistance rates are generally higher in the age range of 60-30 years and in females for the case of metronidazole. This reminds us of the need for a continuous monitoring program of antibiotic susceptibility patterns.

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Introduction

Helicobacter pylori (H. pylori) is a gram-negative bacillus residing in the gastric mucosa. Its prevalence varies in different regions of the world. The prevalence of infection depends on economic and social conditions and increases due to poor hygiene, inadequate water supply, and overcrowding (1). In developed countries, the infection rates are relatively low (0.5-2%).

In developing countries, the rate is much higher in early childhood and up to 20 years of age, and most of the population has a history of infection (70-90%) (2). In Iranian adults, the prevalence of Helicobacter pylori infection is more than 80% (3). H. pylori infection is associated with many digestive disorders, such as gastritis, gastric and duodenal ulcers, gastric cancer, lymphoma, and non-gastrointestinal diseases as well as cardiovascular diseases, skin diseases, autoimmune

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thyroid disease, thrombocytopenic purpura, iron deficiency anemia, Guillain-Barre syndrome, scleroderma, and migraines (4). Several medications are used to treat it as two-drug, triple, and quadruple regimens. Antibiotics that are secreted by infected mucosa are more effective (5).

Amoxicillin, tetracycline, metronidazole, clarithromycin, and furazolidone in combination with proton pump inhibitors (PPIs) and bismuth salts are usually used to treat the H. pylori infection. Obviously, antibiotic resistance limits the success of treatment (6).

The incidence rate of antibiotics resistance is so increasing that some references recommend antibiotics susceptibility tests before any treatment (7). Currently, the Clinical & Laboratory Standards Institute (CLSI) recommends the agar dilution method as the method of choice for antimicrobial susceptibility testing of H. pylori (8).

Since culture and standard antimicrobial susceptibility testing for H. pylori are very demanding, they are not routine, and the epidemiologic data about the resistance pattern of these bacteria are highly valuable. This study aimed to assess the minimum inhibitory concentrations (MICs) of five antibiotics, metronidazole, clarithromycin, amoxicillin, tetracycline, and furazolidone, for H. pylori isolates in Northeast Iran.

Materials and methods

Study population

The study was conducted in 2016-2017 on 80 strains of H. pylori isolated from gastric biopsies of patients who underwent an endoscopic examination to evaluate dyspeptic symptoms at Ghaem Hospital, affiliated with Mashhad University of Medical Science, in the northeast of Iran.

The study was approved by the institutional ethical committee, and informed consent was obtained. For this study, rapid urease tests were initially performed at the endoscopy department, and the urease-positive biopsies were immediately transported to the microbiology laboratory in brain heart infusion (BHI) broth + glycerol (10%) (9). Then gastric biopsy specimens were chopped thoroughly on sterile glass slides, and the homogenized solutions were inoculated on blood agar containing 10% defibrinated horse blood, vancomycin (10 mg/L), trimethoprim (5 mg/L), cefsulodin (5 mg/L), amphotericin B (5 mg/L), and 10% fetal calf serum.

All culture media were incubated at 37 °C in the microaerophilic atmosphere for 5 days. H. pylori isolates were confirmed based on colony morphology, positive urease, oxidase, catalase reaction, and gram staining.

After the identification of grown colonies as H.

Inclusion and exclusion criteria

Inclusion criteria were the dyspeptic patients referred to the Ghaem Hospital endoscopy department. Exclusion criteria were patients who had received antibiotics in the preceding months or proton pump inhibitors drugs two weeks before being referred to the endoscopy department.

Laboratory methods

Urease test: A number of bacterial colonies are transferred to the urea broth medium (Stuart's urea broth), and in less than one minute, the color of the environment changes from yellow to purple due to the presence of a very strong urease enzyme of this bacterium (10). Antibiotic susceptibility testing: The antibiotic resistance of H. pylori isolates and clinical breakpoints for H. pylori were evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

For this purpose, all Mueller Hinton agar media were incubated at 37 °C under microaerophilic conditions for 48 hours. The resistance breakpoints for amoxicillin, clarithromycin, metronidazole, levofloxacin, and tetracycline were defined as > 0.125, > 0.5, > 8, > 1, and > 1 mg/L, respectively. The final concentrations of clarithromycin (Biaxin XL), tetracycline (®Achromycin), furazolidone (®FURABEN), and amoxicillin (Amoxill®) ranged from 0.015 to 64 µg/mL, while metronidazole concentration ranged from 0.015 to 256 µg/mL. The MICs values were the lowest antibiotic concentrations that completely inhibited the visible growth of the bacteria.

Minimum inhibitory concentrations (MICs) interpretative criteria: There is no standardized MIC breakpoint for H. pylori, except for clarithromycin (MIC ≥ 1 µg/mL and intermediary if MIC = 0.5 µg/mL). The MIC breakpoint for other antimicrobials was based on the literature (MIC ≥ 2 µg/mL for amoxicillin and furazolidone, ≥ 4 µg/mL for tetracycline, and ≥ 8 µg/mL for metronidazole). Statistical analysis was performed using SPSS 16.0 software. We describe the MICs of H. pylori isolates in both MIC50, which means the minimum inhibitory concentration required to inhibit the growth of 50% of organisms, and MIC90, which shows the minimum inhibitory concentration required to inhibit the growth of 90% of organisms (11).

Result

This cross-sectional study was conducted on 80 isolates of H. pylori obtained from patients in the northeast of Iran. The study included 42 women (%52.5) with a mean age of 18.37 ± 48.96 years and 38 men (%47.5) with an average age of 14.72 ± 44.6 years. The youngest patient was 16 years and the oldest was 85 years old. The resistance rate of these isolates is shown in Table 1.

Table 1: The resistance rate of Helicobacter pylori isolates

Antibiotics	Resistance rate N (%)		MIC50 µg/mL	MIC90 µg/mL
	Female (n=42)	Male (n=38)		
Metronidazole	19 (45.2%)	14 (36.8%)	8	32
Clarithromycin	4 (9.5%)	7 (18.4%)	0.25	1
Amoxicillin	2 (4.8%)	5 (13.2%)	0.25	1
Tetracycline	2 (4.8%)	3 (7.9%)	0.5	1
Furazolidone	4 (9.5%)	1 (2.6%)	0.5	1

The overall resistance rate of metronidazole, clarithromycin, amoxicillin, tetracycline, and fura zolidone was 41.2%, 13.7%, 8.7%, 6.6%, and 6.6%, respectively. Metronidazole showed the highest resistance level of 41.2%.

Table 2: The frequency of antibiotic resistance in three age groups

Age	Frequency	Metronidazole	Clarithromycin	Tetracycline	Amoxicillin	Furazolidone
30 >	15	3 (3.75%)	0	1 (1.25%)	2 (2.5%)	1 (1.25%)
30-60	51	23 (28.75%)	9 (11.25%)	3 (3.75%)	6 (7.5%)	3 (3.75%)
60 <	14	10 (12.5%)	5 (6.25%)	1 (1.25%)	1 (1.25%)	1 (1.25%)

Table 2 shows the prevalence of resistance to metronidazole, clarithromycin, tetracycline, amoxicillin, and furazolidone in different age groups. As can be seen, the highest resistance was observed among the age group of 30 to 60 years old, for all antibiotics.

Discussion

H. pylori infection is a common infection worldwide with geographical variations. The prevalence of antibiotic resistance in H. pylori is increasing rapidly (12-14). The results of the present study demonstrate higher rates of resistance to metronidazole and furazolidone in females than in males (metronidazole: 45.2% vs 36.8%, and furazolidone: 9.5% vs 2.6%). Moreover, there were higher rates of resistance to clarithromycin, amoxicillin, and tetracycline among males than among females, i.e., clarithromycin: 18.4% vs 9.5%, amoxicillin: 13.2% vs 4.8%, and tetracycline: 7.9% vs 4.8% (males vs females respectively).

In line with our results, another study also reported significantly higher rates of clarithromycin resistance in males than in females (clarithromycin: 44.4% vs 15.2%, males vs females, P-value < 0.001) (15). The results of another study showed that resistance to clarithromycin and metronidazole was significantly associated with sex and higher in females (P< 0.001) (16).

Furthermore, our results demonstrate that the age group between 30 to 60 years old has a higher rate of antibiotic resistance, as provided in Table 1. Similar to our findings, a study in China also indicated a higher resistance rate to clarithromycin in patients aged 31-50 years (17).

Metronidazole is one of the most commonly used antibiotics to treat H. pylori (18, 19). The metronidazole resistance rate was 41.2% in our study. In line with our results, another study also reported the metronidazole resistance rate as 68% (20). According to reports from other areas of Iran, the highest metronidazole resistance rate was 95% reported in Tabriz, while the lowest resistance to this antibiotic was 30% in Isfahan (3).

Moreover, resistance to metronidazole is considered the most prevalent antibiotic resistance in H. pylori worldwide. The overall resistance rate to this antibiotic is 75.02% in Africa, while it is 52.85% in South America, 46.57% in Asia, 31.19% in Europe, and 30.5% in North America, which shows a higher rate of resistance in developing countries (21-23).

As observed in our study and other studies, due to excessive use of this antibiotic in several bacterial and parasitic infections, the resistance rate of this antibiotic is higher than the other antibiotics.

The tetracycline resistance rate in this study was 6.6%. Considering the average rate of tetracycline resistance reported in Iran (11.5%), our rate was not very high. However, there are other studies that reported no resistance to tetracycline for H. pylori in Tehran in 2001 and 2005 (24-26).

The overall rate of tetracycline resistance for H. pylori is 11.7% in the world. Moreover, it shows a higher incidence in Africa (50%) and lower risk in some Asian countries, for example, 0.6% in China and 0.01% in South Korea. However, the H. pylori resistance to tetracycline was found to be

%53.8 in India (28 ,27).

The clarithromycin resistance rate in this study was %13.7. Regarding the widespread use of clarithromycin in the treatment of several infections, especially respiratory tract infections, such a high resistance rate is expected (29 ,12).

The clarithromycin resistance rate found in our study is relatively low compared with other studies in Iran. The present study showed a resistance rate of %8.7 for amoxicillin. Some previous studies have reported zero or very low amoxicillin resistance rates (30 ,25 ,12). In contrast, Milani et al. reported a %28.6 amoxicillin resistance rate in Tabriz, Iran (31). Evidence suggests a low prevalence of resistance to amoxicillin in European countries and North America, ranging from %1.4 to %2. However, it has a higher prevalence in Asia, Africa, and South America ranging from %20.5 in Colombia to %97.5 in South Africa (28 ,27).

The furazolidone resistance rate was %6.6 in this study. While this antibiotic seems effective for H. pylori, it is not widely recommended because some evidence of carcinogenesis has been reported (,32 33). Studies on furazolidone resistance have not been done widely, and no extensive study in Europe, North America, and Africa in the last six years was found. However, according to the reports for Asian countries, the furazolidone resistance rate was reported to be %61.4 in Iran, %16.8 in China, and %13.8 in India (28 ,27).

Conclusion

all these findings reveal that resistance rates to metronidazole have increased over time, and females are at higher risk. Age is another factor that affects the incidence rate of metronidazole resistance, and according to our results, the risk is higher with ages between 30 and 60 years. These results further confirm the essence of the continuous program for monitoring the antibiotic susceptibility patterns according to the influencing factors to reduce the rate of treatment failure in the northeast of Iran.

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Conflict of interest

The authors declare no conflict of interest.

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