SHORT COMMUNICATION

Antibiotic resistance pattern of *Helicobacter pylori* strains isolated from patients in Isfahan, Iran

Hajarsadat Sadeghi[1], Tahmineh Narimani[2], Elham Tabesh[3], Fatemeh Shafiee[4], Rasool Soltani[5,6]

[1]Students Research Committee, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, [3]Gastroenterology and Hepatology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [4]Department of Pharmaceutical Biotechnology, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran, [5]Department of Clinical Pharmacy and Pharmacy Practice, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran, [6]Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Background: The objective of this study was to evaluate the antibiotic resistance pattern of *Helicobacter pylori* strains isolated from patients in Isfahan province. **Materials and Methods:** Gastric antrum biopsy specimens of patients undergoing endoscopy were cultured. The samples with the growth of *H. pylori* underwent antibiotic susceptibility test by disk diffusion method. **Reaults:** Of 96 samples, 50 samples (53%) were positive for *H. pylori*. The rates of antibiotic resistance were as follows: amoxicillin, 6%; azithromycin, 20%; furazolidone, 22%; levofloxacin, 16%; metronidazole, 20%; rifampin, 12%; and tetracycline, 22%. **Conclusion:** *H. pylori* strains in our area have high rates of resistance to azithromycin, levofloxacin, metronidazole, tetracycline, and furazolidone.

Key words: Antibiotic, *Helicobacter pylori*, Patient, Resistance

How to cite this article: Sadeghi H, Narimani T, Tabesh E, Shafiee F, Soltani R. Antibiotic resistance pattern of *Helicobacter pylori* strains isolated from patients in Isfahan, Iran. J Res Med Sci 2022;27:39.

INTRODUCTION

Helicobacter pylori is the cause of the most common chronic infection in the world.[1] It plays a key role in gastritis and peptic ulcer, and is one of the factors involved in gastric cancer. The eradication of *H. pylori* reduces the incidence of gastric cancer and treats gastric ulcer.[2]

Several treatment regimens have been suggested for *H. pylori* eradication containing combinations of two to three antibiotics.[3] However, *H. pylori* strains are resistant to a wide range of antibiotics in Iran and other parts of the world.[4] The pattern of resistance to antibiotics changes overtime. Therefore, studies on this issue are necessary at different periods and geographic areas to aid the physicians in better selection of eradication regimens. This study was conducted with the aim of determining the antibiotic resistance pattern of *H. pylori* strains isolated from patients in Isfahan province.

MATERIALS AND METHODS

This was a prospective cross-sectional study conducted from January to August 2020 in Khorshid hospital and School of Medicine, both affiliated to Isfahan University of Medical Sciences (IUMS). The study protocol was approved by the ethics committee of IUMS with the ethics code IR.MUI.RESEARCH.REC.1398.384. Written informed consent was obtained from all participants.

Patients

The patients were selected by convenience sampling from those who were referred to the endoscopy

Access this article online

Quick Response Code:

Website: www.jmsjournal.net

DOI: 10.4103/jrms.jrms_829_21

Address for correspondence: Dr. Rasool Soltani, Department of Clinical Pharmacy and Pharmacy Practice, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Hezar-Jerib Avenue, Isfahan, Iran. E-mail: soltani@pharm.mui.ac.ir
Submitted: 20-Sep-2021; **Revised:** 06-Dec-2021; **Accepted:** 31-Jan-2022; **Published:** 30-May-2022

department of the hospital by gastroenterologist for elective endoscopy.

The inclusion criteria were as follows : (1) age ≥18 years old; (2) the need for endoscopy at the discretion of a gastroenterologist; and (3) no antibiotic intake within the last 2 weeks. Patients with negative culture of gastric biopsy specimens for *H. pylori* were excluded from the study.

Demographic and clinical information of patients including age, gender, final diagnosis following endoscopy, and history of the previous usage of anti-*H. pylori* regimen were recorded.

Tissue sampling

Endoscopic sampling was performed by a gastroenterologist from the gastric antrum of the patients. For this, during the endoscopic examination of the gastrointestinal tract, the appropriate part of sampling was determined by visual examination of mucosal changes such as ulcers or inflammation, and sample was obtained by forceps biopsy.

Microbial culture

Thioglycollate medium (Merck, Germany) was used to transfer biopsy specimens to the microbiology laboratory of the Faculty of Medicine at 4°C within 4 h of sampling. For culturing, the biopsy specimen was crushed by a surgical razor and pulled onto the culture medium. Culture was done three times for each sample. The first culture was performed with the aim of isolating *H. pylori* from biopsy by selective culture. In the second culture, biopsy-derived *H. pylori* colonies were isolated from other grown strains in the first culture. The third culture was performed for antibiotic susceptibility testing.

For culture, the Columbia agar medium (Himedia, India) supplemented with 10% fetal bovine serum (Sigma, USA), defibrinated sheep blood 8% (Himedia, India), Campylobacter supplement (Merck, Germany), amphotericin B, vancomycin, and trimethoprim was used. The plates were subjected to microaerophilic conditions including 5% oxygen, 10% carbon dioxide, and 85% nitrogen at 37°C in an anaerobic jar and incubator. The crushed sample residue was used for Gram staining. The culture media were examined visually after 5–7 days. One-to-2 mm (diameter) gray translucent colonies were identified as *H. pylori*. The colonies were isolated by sterile loop and cultured in the second medium with a combination similar to the previous one without supplement.[5,6]

Antibiotic susceptibility testing

The susceptibility of isolated *H. pylori* strains to the selected antibiotics was evaluated using the disk diffusion (Kirby–Bauer) method. For this, plates containing Columbia agar medium without supplement were inoculated with 1 McFarland microbial suspension and 6-mm antibiotic discs of amoxicillin (25 µg), azithromycin (15 µg), levofloxacin (5 µg), metronidazole (5 µg), tetracycline (30 µg), rifampin (5 µg), and furazolidone (100 µg) were placed at a distance of at least 20 mm from each other (center-to-center) on the surface of the medium. Then, the plates were incubated in microaerophilic conditions at 37°C for 5 days. The result of the disk diffusion test was interpreted as sensitive or resistant based on the inhibition zone diameter around each disk according to the Clinical and Laboratory Standards Institute instructions.[7] Furthermore, the resistance rate of > 15% was considered "high resistance rate" according to the most recent international guidelines.[8,9]

Resistance pattern and history of use of *Helicobacter pylori* eradication regimen

As a secondary objective, the relationship between the resistance to each antibiotic (as the dependent variable) and the history of previous use of *H. pylori* eradication regimen (as the independent variable) was evaluated.

Statistical analysis

Statistical analysis was performed using SPSS software version 24 (SPSS Inc., Chicago, USA). The results were reported as the frequencies and the corresponding percentages. Chi-square test was used to determine the relationship between the resistance to each antibiotic and the history of previous use of *H. pylori* eradication regimen.

RESULTS

Patients

During the study, 94 eligible patients underwent biopsy, of which culture of 50 patients (53.2%, one sample per patient) was positive for *H. pylori*. Of these, 23 (46%) and 27 (54%) samples were obtained from men and women, respectively. The age range of patients was 18–80 years with the mean ± standard deviation of 45.2 ± 17.9 years. The final diagnoses of patients following endoscopy were antral gastropathy (n = 23, 46%), sliding hiatal hernia (n = 7, 14%), erosive gastroduodenitis (n = 6, 12%), duodenal ulcer (n = 5, 10%), antral erosive gastropathy (n = 3, 6%), gastric ulcer (n = 1, 2%), and diverticulum (n = 1, 2%). Of note, four cases (8%) had no pathological finding.

Antibiotic resistance pattern

Table 1 shows the results of the antibiotic susceptibility test. As seen, the isolated strains had the most sensitivity to amoxicillin (94%) and the least sensitivity to tetracycline and furazolidone (78% each). Therefore, the isolates showed high resistance rate to azithromycin, levofloxacin, metronidazole, tetracycline, and furazolidone.

Resistance pattern and history of use of *Helicobacter pylori* eradication regimen

Table 2 shows the relationship between resistance to each antibiotic and history of previous use of *H. pylori* eradication regimen. As shown, the pattern of resistance to any of the studied antibiotics had no significant relationship with the history of previous use of eradication regimen.

DISCUSSION

In the present study, *H. pylori* strains showed low resistance rates (≤15%) to amoxicillin and rifampin, and high resistance rates (>15%) to azithromycin, levofloxacin, metronidazole, tetracycline, and furazolidone.

The observed resistance to amoxicillin (6%) is similar to the resistance rate of 6.4% in the study of Khademi *et al*.[10] and slightly higher than the rate of 4.2% in the study of Mirzaei *et al*.,[11] both conducted in Isfahan and published at 2013. This shows constant rate of amoxicillin resistance among *H. pylori* isolates in Isfahan province.

Table 1: Antibiotic susceptibility of the isolated *Helicobacter pylori* strains

Antibiotic	Susceptibility	
	Sensitive, *n* (%)	Resistant, *n* (%)
Amoxicillin	47 (94)	3 (6)
Azithromycin	40 (80)	10 (20)
Levofloxacin	42 (84)	8 (16)
Metronidazole	40 (80)	10 (20)
Tetracycline	39 (78)	11 (22)
Rifampin	44 (88)	6 (12)
Furazolidone	39 (78)	11 (22)

Table 2: Relationship between antibiotic resistance pattern of isolated *Helicobacter pylori* strains and history of previous use of *Helicobacter pylori* eradication regimen

Antibiotic	Susceptibility	Previous use of eradication regimen		*P**
		Yes, *n* (%)	No, *n* (%)	
Amoxicillin	Sensitive	15 (31.91)	32 (68.09)	0.54
	Resistant	0	2 (100.00)	
Azithromycin	Sensitive	12 (30.00)	28 (70.00)	1.00
	Resistant	3 (30.00)	7 (70.00)	
Levofloxacin	Sensitive	13 (30.95)	29 (69.05)	1.00
	Resistant	2 (25.00)	6 (75.00)	
Metronidazole	Sensitive	14 (32.55)	29 (67.45)	0.24
	Resistant	1 (14.28)	6 (85.72)	
Tetracycline	Sensitive	10 (25.64)	29 (74.36)	0.26
	Resistant	5 (45.45)	6 (54.55)	
Rifampin	Sensitive	14 (31.81)	30 (68.19)	0.65
	Resistant	1 (16.66)	5 (83.34)	

*Chi-Square test

The resistance rate to azithromycin (macrolide) was higher than the rates of 15.3% and 14.6% to clarithromycin in the previous studies of Isfahan,[10,11] showing the increasing trend of resistance to macrolide antibiotics. However, in a review of *H. pylori* eradication regimens, 10-day and 14-day standard triple therapies (clarithromycin + amoxicillin or metronidazole + proton pump inhibitor) still seemed to be appropriate options for first-line *H. pylori* eradication in Iran.[12]

The high rate of furazolidone resistance in our study (22%) and other reports[13] is consistent with the results of a review showing unacceptable eradication rates with the furazolidone-containing triple regimens in Iran.[12]

In this study, the prevalence of *H. pylori* resistance to metronidazole was 20%, which is interestingly lower than the previous mentioned studies in Isfahan (56.3% and 55.1%).[10.11] In the study of Shetty *et al*., the estimated rate of *H. pylori* resistance to metronidazole was 81.4%.[14] In the mentioned study, the dose of metronidazole in the applied test disc was 1 μg, while in this study, we used a dose of 5 μg; this could be a contributing factor to the difference of the results. Overall, given the significant differences between the rates of *H. pylori* resistance to metronidazole in our study and other published works, new clinical studies should be conducted in our region to evaluate the effectiveness of metronidazole-containing regimens for *H. pylori* eradication.

In our study, the rate of *H. pylori* resistance to rifampin was 12%. Due to the relatively low resistance of *H. pylori* to rifampin in Isfahan province, it is worthwhile to evaluate the effect of this antibiotic in eradication therapies, especially for cases of recurrence or treatment failure.

The most important limitations of our study were the small sample size due to the difficult growth of bacteria, and no determination of minimum inhibitory concentration of antibiotics against the bacterial isolates. However, this is the first study in recent years in Isfahan evaluating *H. pylori* resistance to several antibiotics other than amoxicillin, clarithromycin, and metronidazole.

CONCLUSION

H. pylori strains isolated from patients in Isfahan province have high rates of resistance to azithromycin, levofloxacin, metronidazole, tetracycline, and furazolidone, and acceptable susceptibility rates to amoxicillin and rifampin. Controlled clinical trials are mandatory to determine the effectiveness of the combination of these drugs in the form of multidrug regimens for eradication of *H. pylori*.

Acknowledgments

This study was financially supported by the Vice-Chancellery for Research and Technology of Isfahan University of Medical Sciences. We would like to acknowledge the staff of Endoscopy Department of Khorshid Hospital and Microbiology Laboratory of Faculty of Medicine for their assistance.

Financial support and sponsorship

This work was supported by the Vice-Chancellery for Research and Technology, Isfahan University of Medical Sciences (grant number: 398451).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Pabla BS, Shah SC, Corral JE, Morgan DR. Increased incidence and mortality of gastric cancer in immigrant populations from high to low regions of incidence: A systematic review and meta-analysis. Clin Gastroenterol Hepatol 2020;18:347-59.e5.
2. Sung JK. Diagnosis and management of gastric dysplasia. Korean J Int Med 2016;31:201-9.
3. Chiba N, Van Zanten SJ, Sinclair P, Ferguson RA, Escobedo S, Grace E. Treating *Helicobacter pylori* infection in primary care patients with uninvestigated dyspepsia: The Canadian adult dyspepsia empiric treatment-*Helicobacter pylori* positive (CADET-Hp) randomised controlled trial. BMJ 2002;324:1012-6.
4. Mousavi S, Toussy J, Yaghmaie S, Zahmatkesh M. Azithromycin in one week quadruple therapy for H pylori eradication in Iran. World J Gastroenterol 2006;12:4553-6.
5. Castro-Fernández M, Sánchez-Muñoz D, García-Díaz E, Miralles-Sanchiz J, Vargas-Romero J. Diagnosis of *Helicobacter pylori* infection in patients with bleeding ulcer disease: Rapid urease test and histology. Rev Esp Enferm Dig 2004;96:395-8.
6. van Doorn LJ, Henskens Y, Nouhan N, Verschuuren A, Vreede R, Herbink P, *et al.* The efficacy of laboratory diagnosis of *Helicobacter pylori* infections in gastric biopsy specimens is related to bacterial density and vacA, cagA, and iceA genotypes. J Clin Microbiol 2000;38:13-7.
7. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100-S28. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
8. Ndip RN, Takang AE, Ojongokpoko JE, Luma HN, Malongue A, Akoachere JF, *et al. Helicobacter pylori* isolates recovered from gastric biopsies of patients with gastro-duodenal pathologies in Cameroon: Current status of antibiogram. Tropic Med Int Health 2008;13:848-54.
9. Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, *et al.* Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. Gut 2017;66:6-30.
10. Khademi F, Faghri J, Poursina F, Nasr Esfahani B, Moghim S, Fazeli H, *et al.* Resistance pattern of *Helicobacter pylori* strains to clarithromycin, metronidazole, and amoxicillin in Isfahan. Iran J Res Med Sci 2013;18:1056-60.
11. Mirzaei N, Poursina F, Faghri J, Talebi M, Khataminezhad MR, Hasanzadehet A, *et al.* Prevalence of resistance of *Helicobacter pylori* strains to selected antibiotics in Isfahan, Iran. Jundishapur J Microbiol 2013;6:e6342.
12. Fakheri H, Saberi Firoozi M, Bari Z. Eradication of *Helicobacter pylori* in Iran: A Review. Middle East J Dig Dis 2018;10:5-17.
13. Khademi F, Sahebkar A. An updated systematic review and meta-analysis on the *Helicobacter pylori* antibiotic resistance in Iran (2010–2020). Microb Drug Resist 2020;26:1186-94.
14. Shetty V, Lamichhane B, Tay CY, Pai GC, Lingadakai R, Balaraju G, *et al.* High primary resistance to metronidazole and levofloxacin, and a moderate resistance to clarithromycin in *Helicobacter pylori* isolated from Karnataka patients. Gut Pathog 2019;11:21.