Tetracycline and Amoxicillin as Promising Antibiotics in First-Line Treatment of *Helicobacter Pylori* Infection

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ABSTRACT

Background:
Helicobacter pylori (H. pylori) infection is associated with many gastroduodenal disorders. So far, few updated reports exist on the pattern of susceptibility to antibiotics that are mainly used in Iran. Our main purpose of this study was to investigate the resistance of *H. pylori* to tetracycline, amoxicillin, metronidazole, and clarithromycin in Iranian patients with dyspepsia. Due to the increasing resistance of *H. pylori* against the current first-choice antibiotics, alternative drugs including tetracycline may be the focus of attention by clinicians.

Materials and Methods:
In this survey, after the bacterial culture of 200 gastric biopsy specimens obtained from consecutive patients, *H. pylori* strains were identified and confirmed using universal standard methods. Minimal inhibitory concentration (MIC) values of tetracycline were determined by E-test method.

Results:
Of 200 taken biopsy specimens, 73 (36%) samples were positive for *H. pylori* infection. Overall, the resistance to metronidazole was detected (46%), to amoxicillin (6%), to clarithromycin (16%), and to tetracycline (13%). In our examination, no significant association was found between the *H. pylori* antibiotic-resistant infections and age or sex. Our findings confirm the urgent need for susceptibility testing to find the best anti-*H. pylori* therapeutic regimen in our geographical region.

Conclusion:
Our results support the higher importance of tetracycline and amoxicillin as alternatives and options in first-line therapy.

Keywords: *Helicobacter pylori*, Tetracycline, Amoxicillin, Antibiotic resistance, MIC

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INTRODUCTION

Helicobacter pylori (H. pylori) is a gram-negative spiral microorganism causing superficial acute gastritis, peptic ulcer diseases (PUD), and gastric cancer [1,2]. The prevalence of *H. pylori* in developed countries ranged from 20% to 40%, while in developing countries the rates are normally more than 70% [3]. Recently, the World Health Organization (WHO) called the clarithromycin (CLR)-resistant *H. pylori* as a high priority for antibiotic research, which should be under intense consideration by clinicians worldwide. The first exposure to *H. pylori* mainly happens in childhood and the carriers remain

colonized if antibiotic therapy will not be correctly recommended (4). The main reason for eliminating *H. pylori* infection is to reduce the risk of occurrence of severe gastroduodenal diseases (5,6). Following the discovery of *H. pylori*, clinicians tried to find the best therapeutic regimen against this chronic infection. So far, many studies have been conducted to design and optimize the formulation of the therapy, but it is still a challenging issue (7,8). To now, many antibiotics have been used to provide an effective therapeutic regimen against *H. pylori* including amoxicillin, clarithromycin, tetracycline, and metronidazole (9-12). The purpose of this cross-sectional study was to examine the prevalence of four conventional antibiotics resistance among the *H. pylori* clinical strains retrieved from Iranian patients with dyspepsia.

MATERIALS AND METHODS

Sample collection

A total of 200 patients suffering from gastritis, gastric ulcer, duodenal ulcer, and gastric cancer, admitted to Labafi-Nejad, Mehrad, and Imam Khomeini Hospitals (Tehran, Iran) for routine endoscopy during 2016-2019, were entered into the study. All patients signed the written informed consent for our research. The demographic data and pathological findings were coded and kept confidential in a known box under control of the supervisor. Patients who reported the use of antibiotics within 3 months prior to entering the study and with the record of abdominal surgery were immediately excluded from our survey. Endoscopic findings and pathology data were used as criteria for the clinical diagnosis of these conditions (13,14). This study was approved by the Ethics Committee of Tarbiat Modares University, Iran (IR.TMU.REC.1395.466).

H. pylori culture

Three antral biopsy specimens were taken from the patient during endoscopy; the first specimen was used for a rapid urease test (RUT), (Shim-Anzim, Tehran, Iran), the second one was shipped to the Pathology Department and the last specimen was placed into a sterile thioglycollate broth medium (Merck, Germany), then sent to the *Helicobacter* laboratory within 3-4 hours. In order to culture *H. pylori*, after mild homogenization, 100 microliters of homogenate was inoculated onto the surface of Brucella agar (Merck, Germany) plate supplemented with 7% defibrinated sheep blood (Bahar-Azma, Tehran, Iran), 7% fetal calf serum (FCS) (Gibco, CA, USA); and Selectab Skirrow (MAST, UK) per 250 mL agar was added to prevent contamination. In order to provide optimal bacterial growth, microaerophilic conditions (10% CO_2, 5% O_2, and 85% N_2) were provided by anaerobic jar (Merck, Germany). The culture plates were incubated at 37°C for 7–8 days. Bacterial identification was done based on classic positive biochemical tests including catalase, oxidase, and urease reactions, as well as gram-staining and typical colony morphology (15). All the isolates were stored at -80°C in brain heart infusion broth supplemented with 15% glycerol and 7% FCS until further analysis (16).

Antibiotic susceptibility testing

Antibiotic resistance pattern of *H. pylori* strains to four antibiotics; metronidazole (MAST, UK), clarithromycin (MAST, UK), amoxicillin (MAST, UK), and tetracycline (MAST, UK) were determined in this study based on disk diffusion method (DDM) by Muller Hinton Agar (MHA) supplemented with 7% defibrinated sheep blood and 7% FCS Serum (16, 17). Then, the plates were incubated at microaerophilic conditions at 37°C for 1-2 weeks. Susceptibility results were recorded as resistant according to the following interpretive criteria: for clarithromycin, a growth inhibition zone < 22 mm; for metronidazole, a growth inhibition zone < 16 mm; for amoxicillin, a zone < 25 mm, and for tetracycline, a growth inhibition zone ≤ 30 mm (16,18-21). Tetracycline MIC of the isolates with reduced susceptibility was determined by the E-test method. For this purpose, suspensions from tetracycline-resistant isolates determined by DDM were prepared in 500µL brucella broth adjusted to the McFarland standard turbidity 3 (~9.0×108 CFU/mL) and then 100 µL of the suspension was lawn on the medium (MHA supplemented with 7% defibrinated sheep blood and 7% FCS). After the plates were dried, E-test strips were placed onto the media and they were incubated for 48 h in microaerophilic conditions (jars with Gaspack). According to the CLSI (Clinical & Laboratory Standards Institute) guidelines, *H. pylori* isolates were classified as susceptible (MIC ≤

2μg), intermediate (MIC: 2-4 μg), or resistant (MIC ≥ 4μg). *H. pylori* reference strain ATCC43504 and characterized clinical strains were used as positive controls (22-24).

RESULTS

Patients

A total of 73 strains of *H. pylori* were isolated from 200 biopsies samples taken for culture and antibiotic susceptibility tests from patients with different gastroduodenal disorders (with a prevalence of 36%). Of the 73 clinical isolates of *H. pylori*, 46 isolates (63%) were obtained from female patients, 44 isolates (60%) were from patients with gastritis, 8 isolates (10%) from gastric ulcer, 10 isolates (13%) from duodenal ulcer, and 3 isolates (4%) were from gastric cancer; however 8 isolates (10%) did not show any clinical manifestation. Our subjects' age range was from 19 to 88 years. Our study showed that no significant association was found between *H. pylori* antibiotic-resistant infections and age or sex.

Prevalence of antibiotic resistance rates

The prevalence of antibiotic resistance among 73 isolates measured by DDM was 47% for metronidazole, 16% for clarithromycin, 6% for amoxicillin, and 13% for tetracycline. In this survey, based on the minimum inhibitory concentrations (MICs) determined using E-test for 10 strains of tetracycline-resistant *H. pylori*, four strains (5%) were tetracycline-insusceptible and six were susceptible strains (8%), while no resistant strains were found for tetracycline (0%). The prevalence of antibiotic resistance in these ten strains (susceptible and insusceptible to tetracycline) was 100% for metronidazole, 100% for clarithromycin, and 50% for amoxicillin.

DISCUSSION

H. pylori is a microaerophilic bacillus that colonizes the human gastric mucosa for lifelong. An important implication for human health is accompanied by this chronic infection after several years of colonization. The current evidence suggests that successful *H. pylori* eradication leads to reduce the occurrence of severe gastroduodenal diseases and can be even beneficial for affected individuals. Since1998, there is a general

agreement about the consideration of *H. pylori* as a pathogenic microbe, which deserves to receive sufficient antibiotics (25-27). The increasing trend in the treatment failure of *H. pylori* and the emergence of antibiotic resistance among these strains disclose the rationale for the continuous survey on antimicrobial susceptibility profile, to achieve efficient infection control. According to the Maastricht III Consensus Report, successful elimination of *H. pylori* infection is recommended for patients with peptic ulcer using triple-therapy consists of clarithromycin, amoxicillin, and a proton pump inhibitor (PPI) (28). During this study, we aimed to examine the prevalence of antibiotic resistance for *H. pylori* isolated from two hospitals in Tehran. Resistance to clarithromycin, as the most effective antibiotic in the treatment of *H. pylori* infections, varies from 30% to 50% in different parts of the world (29). Our findings show that the prevalence of clarithromycin resistance is about 16% among the *H. pylori* strains isolated from the patients with dyspepsia (7). In Asia, the mean rate of resistance to clarithromycin is about 27%, while our reported rate is almost half of it. The current report is showing that clarithromycin is still an option for gastroenterologists. To now, Malaysia reported the lowest rate for clarithromycin (2%), similar to Europe where Norway has 5% as the lowest rate (27,29). Resistance to clarithromycin in developed countries is about 10% and in developing countries is higher and about 25-50% (30). For example, resistance to clarithromycin in Asian countries are as follows; Japan (18.19%), Vietnam (33%), Iran (14.3%), and Pakistan (37%) and among the European countries are; Finland (2%), Sweden (1.5%), Germany (7.5%), Spain (15-20%), France (17.5%) and in African countries such as Egypt the rate is 40% (31). It is noteworthy that an increase in resistance to clarithromycin in Asia from 15.28% in 2009 to 32.46% in 2014 was reported, possibly due to the increased use of macrolide drugs in Asian countries. It can be stated that resistance to clarithromycin is slowly increasing worldwide (32). This increase in resistance can be associated with a reduction in the effectiveness of the treatment regimen containing clarithromycin in the successful eradication of this organism. Another possible explanation for this high rate of resistance to clarithromycin can be due to the almost freely utilization of this antibiotic by

clinicians mainly without susceptibility profile.

Resistance to metronidazole, which has been widely used in the treatment of *H. pylori* infection, varies from 10% to 90% in many regions (33,34). Resistance to metronidazole has been reported in Asia (46%), Europe (31%), Africa (75%), North America (30%), and South America (52.85%) (29). Metronidazole resistance in developed countries is approximately 30%, whereas in developing countries is much higher (60%)(35). Resistance to metronidazole in Asian countries is as follows; China (56-95%), Japan (57%), Malaysia (75%), Vietnam (69%), Saudi Arabia (69%), Kuwait (70%), Pakistan (64%), and Iran (76%). In European countries the rate is as follows; Norway (22%), France (34%), and Germany (37.2%). In American countries, the rate is; Mexico (76%), Colombia (75%), and Brazil (40%) (31). Accordingly, it can be concluded that resistance to metronidazole has remained remarkably high in Asian, European, and American countries, but in African countries, metronidazole resistance has increased from 51% in 2010 to 85% in 2013 (29). Comparing the resistance level to metronidazole (47%) and the average resistance to this antibiotic in Iran (69%), it can be concluded that the resistance of *H. pylori* strains to metronidazole is still high in Iran. There is a correlation between high levels of metronidazole resistance and the economic conditions of the community, which can be due to the high-level consumption of this antibiotic in the treatment of infectious diseases of women, oral, and dental, as well as parasitic infections (36,37).

Global resistance to amoxicillin, which is a common antibiotic in the treatment of *H. pylori* infection, has not been common and has been reported at a low level (14%) (29). Resistance to amoxicillin has been determined in Asia (23%), Europe (0.35%), Africa (40%), North America (2%), and South America (6%) (29). Resistance to amoxicillin is different in various areas; it has been reported in Asian countries such as Malaysia, Taiwan, Japan at 15% and in India 72%, and in Bangladesh at 6%, but was not observed in Vietnam (0%) (29,31). The prevalence of resistance to amoxicillin in Africa was 40%, South America 97%, Nigeria 66%, Colombia 20%, Germany, Norway, and the Netherlands (1%), Spain 2%, and Italy 0%. Noticeably, in European and

North American countries it seems to be about zero (38). Considering the resistance level of *H. pylori* to amoxicillin in this study (6%) and its comparison with the mean resistance to this antibiotic in Iran (20%), it can primarily be concluded that *H. pylori* resistance in Iran remained at a low level. The potential explanation for this decreased rate may be referred to the reduction in the consumption of amoxicillin over the last decade. However, this rate is still higher than countries from Europe, Canada, and the United States (30). Tetracycline is now mainly applied as a second-line treatment if our regimen failed at the first hit by the *H. pylori* infection. The mean resistance rate to tetracycline is about 10% worldwide. Fortunately, among the four common antibiotics used in the eradication of *H. pylori* infection, tetracycline resistance is at the lowest level. This suggests a novel drug likely to be replaced in current therapeutic regimens against *H. pylori*. Resistance to tetracycline has been reported in China (0.6%), South Korea (0%), India (58%), and Iran (12%) (26,29). Studies have revealed that *H. pylori* resistance to tetracycline has increased from (6%) in 2009 to (26%) in 2014 (29). According to the tetracycline resistance rate in the current study (13%) and the average resistance to this antibiotic in Iran (34.51%), it can be concluded that it has been changed slightly. Comparing Iran with other Asian countries, resistance to tetracycline and amoxicillin is lower, but resistance to metronidazole, clarithromycin, and rifamycin is significantly higher (39,40). This difference in resistance indicates the need for replacing new drugs. Tetracycline in parallel with amoxicillin can be the newest suggestion to be included in novel therapeutic regimens against *H. pylori* infections in Iran.

CONCLUSION

The prevalence of antibiotic resistance varies in different geographic regions. It seems that the global trend of resistance is increasing over the last years. According to this study, an increasing rate of antibiotic resistance of *H. pylori*, particularly against clarithromycin and metronidazole antibiotics were determined. Considering the clinical importance and wide consumption of both antibiotics in the eradication of this bacterium, the use of first-line regimens containing amoxicillin instead of metronidazole or the second-line regimen therapy containing tetracycline

would confer a promising effect on the eradication of this bacterium in Iran, owing to the lower resistance rate as compared with other antibiotics used in the eradication of *H. pylori* infection. Last but not least, according to the available susceptibility profile of antibiotic resistance, there is an ongoing demand for antibiotic susceptibility testing for better management of patients carrying *H. pylori* in their stomachs.

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CONFLICT OF INTEREST

None of the authors declare any conflict of interest.

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