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Annals of Biological Research, 2013, 4 (1):99-103
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Evaluation of antimicrobial susceptibility of *Helicobacter pylori* to common antibiotics in Tabriz, northwest of Iran

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ABSTRACT

Background: *The aim of the present study was to investigate the prevalence of resistance rate in Helicobacter pylori against commonly used antibiotics including tetracycline, metronidazole, clarithromycin, amoxicillin, ciprofloxacin, nitrofurantoin, erythromycin and rifampin in Iranian patients.* ***Methods:*** *H. pylori isolates were obtained from gastric biopsies from patients referred for upper gastrointestinal endoscopy at Emam Reza hospital of Tabriz. H. pylori was identified based on gram staining and biochemical tests (catalase, oxidase, and urease activity). All positive cultures were tested for antimicrobial susceptibility by used of disk agar diffusion for all antibiotics and Etest method for tetracycline and metronidazole.* ***Results:*** *One hundred and four H. pylori isolates were obtained from 322 patients, mean age 39 ±17 years, 145 were women and 177 were men. The prevalence of resistance of H. pylori isolates were 10.6% for tetracycline, 77.9% for metronidazole, 15.4% for clarithromycin, 22.1% for amoxicillin, 39.4% for ciprofloxacin, 4.8% for nitrofurantoin, 24% for erythromycin and 26.9% for rifampin by used of disk agar diffusion.* ***Conclusions:*** *The prevalence of H. pylori antibiotic resistance to metronidazole was high, while resistance to tetracycline was low in this area. There was an increasing trend in antibiotics clarithromycin, metronidazole and ciprofloxacin. Therefore, surveillance of antibiotic susceptibility test for H. pylori is essential.*

Key word: *H. pylori*, Tetracycline, Metronidazole, antibiotic susceptibility

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INTRODUCTION

Helicobacter pylori is a spiral bacterium which lives in the environment of the human stomach. The association of *H pylori* infection with chronic gastritis, peptic ulcers and gastric lymphoma and adenocarcinoma of the body and antrum of the stomach is recognized worldwide. This bacterium is a Class I human carcinogen according to the World Health Organization and infection occurs more frequently in developing countries than in industrialized countries where up to 10% of children and 80% of adults can have laboratory evidence of an *H. pylori* infection usually without having symptoms (1). Eradication of *H. pylori* prevents peptic ulcer recurrence and may also decrease the prevalence of gastric cancer in high-risk populations (2). Over time, the success rates of eradication therapy have fallen as the prevalence of antibiotic resistant *H. pylori* has increased. Eradication treatment of *H. pylori* infection usually consists of various combinations 'quadruple therapy' of tetracycline, metronidazole, bismuth citrate and a proton pump inhibitor is employed as a second-line after failure of initial 'triple therapy' treatment (typically amoxicillin or metronidazole together with clarithromycin, and a proton pump inhibitor) (3). The

combination of two antibiotics can increase the success of eradication therapy and decrease the possibility of secondary antibiotic resistance (4). Such dual, triple or quadruple treatments have been found to be more effective than administering one antibiotic alone.

Antibiotic resistance in *H. pylori* is the major cause of eradication failure. Growing resistance often parallels the patterns of antibiotic consumption, and may according to geographical region (5). Resistance to metronidazole is common in developing countries, leading to very frequent failure of metronidazole-based anti-*H. Pylori* therapy (2) besides, Worldwide tetracycline resistance in *H. pylori* remains relatively uncommon. However, evidence has emerged of increasing resistance in regions where tetracycline is more widely used in *H. pylori* therapy.

For example, reported resistance levels were 5.3%in Korea, 58.8% in China, 3.3% in Bulgaria and 9% in Brazil (3). Therefore, it is important to know about the antibiotic resistance pattern of local *H. pylori* strains (6) and the resistance rates of isolates to antibiotics commonly used in the treatment. The aim of this study was to assess the susceptibility of clinical *H. pylori* isolated from gastric biopsies of patients with ulcer to antibiotics commonly used in therapeutic procedures in east Azerbaijan, Iran and to evaluate minimum inhibitory concentrations (MICs) of metronidazole and tetracycline by use of the Etest, and to find the best antibiotic therapy procedure for the eradication of *H. pylori* infections in our region.

MATERIALS AND METHODS

1. Clinical specimens

A total of 104 clinical isolates of *H. pylori*, 47 (45.2%) from female and 57 (54.8%) from male patients (sex ratio F/M: 0.82); the average age was 39 ±17 years were obtained from December 2011 to November 2012 in eastern Azerbaijan, Iran and was included in this study.

H. pylori isolates were cultured from gastric biopsies (either from the antrum or the corpus) of patients who underwent a diagnostic gastroduodenoscopy, at Emam Reza Hospitals in Tabriz. Isolates were from patients with gastritis, duodenal ulcer, peptic ulcer, and gastro esophageal reflux disease. At least 1 week before endoscopy the patients had not received antimicrobial agents. The specimens were transported to the laboratory in Stuart medium (Merck, Germany) instantly (Max 4h). Biopsy samples were gently homogenized and cultured on Brucella agar (Pronadisa, Spain) containing 5-10 % sheep blood and antibiotics supplement (vancomycin 6 µg/ml, amphotericin B 2.5 µg/ml and trimethoprim 20 µg/ml). Cultures were kept in a microaerophilic atmosphere (Anoxomat; Mart, Lichtenvoorde, The Netherlands; O2 5 %, CO2 8%, H2 8%), at 37 ºC and high humidity, for 5–7 days. After incubation the plates were examined for growth and small rounded colonies subculture to obtain a pure culture.

2. Identification of *H. pylori* isolates

H. pylori isolates were identified by colonial morphology, characteristic spiral morphology on Gram staining, and positive findings for catalase, urease and oxidase tests.

3. Antimicrobial susceptibility testing

Suspensions of *H.pylori* (3-day-old) cultures were adjusted to a McFarland standard no. 4 as the inoculums on Mueller Hinton agar plates were supplemented with 5 % sheep blood then the disks of different antibiotics (Mast, UK) including nitrofurantoin (300 µg), tetracycline (30 µg), clarithromycin (15 µg), ciprofloxacin (5 µg), erythromycin (15 µg), amoxicillin (25 µg), rifampin (5 µg), and metronidazole (5 µg) were placed on the plates and were incubated for 72 h then examined for inhibition zones which were measured in millimeters. The zones of inhibition were interpreted by according previous studies (7, 8). In our study *H. pylori* ATCC 26695 was the reference strain as quality control.

4. Determination of MIC by E-test

Plates containing Mueller-Hinton agar supplemented with 5% sheep blood were used for the E test method (Epsilometer test; Biomeriux, Solna, Sweden). The Etest strips were concentrations ranging from 0.016 to 256 µg/ml. The agar plates were inoculated by prepared confluent swabbing of the surface with adjusted inoculums suspensions (McFarland no. 4). Etest strips were applied onto the surface of each agar plate and incubated at microaerophilic conditions for 3 days. According by published studies, strains were considered as resistant when the MIC was > 8 mg/l for Metronidazole, and ≥ 1 mg/l for tetracycline (6, 9).

RESULTS

In this study from the total of 322 biopsies samples, 104 *H. pylori* were isolated (32.29 %), which 57 (54.8 %) were from males and 47 (45.2 %) from females (sex ratio M/F: 0.82). The antimicrobial susceptibility test results of all *H. pylori* isolates are presented in Table 1.

Table 1: Pattern of 104 *H. pylori* isolates resistance to different antibiotics by disk-diffusion method according to gender.

patients	Tet (%)	Mz (%)	Cl (%)	Am (%)	Cip (%)	Nit (%)	E (%)	Rif (%)
Male	7 (63.7)	37 (45.7)	5 (31.2)	12 (52.2)	21 (51.2)	4 (80)	12 (48)	15 (53.6)
Female	4 (36.3)	44 (54.3)	11 (68.8)	11 (47.8)	20 (48.8)	1 (20)	13 (52)	13 (46.4)
Total	11 (10.6)	81 (77.9)	16 (15.4)	23 (22.1)	41 (39.4)	5 (4.8)	25 (24)	28 (26.9)

Tet (Tetracycline), Mz (metronidazole), Cl (clarithromycin), Am (amoxicillin), Cip (ciprofloxacin), Nit (nitrofurantoin), E (erythromycin), Rif (rifampin)

In this study the range of MIC (µg/ml) of *H. pylori* isolates for tetracycline and metronidazole were 0.016 to 1.5 and 0.016 to 256 µg/ml respectively. When the tetracycline MIC for the 104 isolates were measured by use of the Etest, 8 (7.7 %) of the isolates were found to be resistant, however, when tested by disk diffusion, 11 (10.6%) strains were found to be resistant to clarithromycin. Table 2 shows the bimodal distribution of metronidazole and tetracycline MICs for *H. pylori* isolates.

Table 2. Comparison of disk diffusion and Etest results in tetracycline and metronidazole.

	Etest				**Disk diffusion**	
	MIC(µg/ml)	Isolates (%)			Isolates (%)	
Antibiotics	Range	Sensitive	Resistance		Sensitive	Resistance
Tetracycline	0.016-1.5	97(93)	8(7.7)		94(90.38)	11(10.6)
Metronidazole	0.016–256	23(22.12)	81(77.88)		23(22.12)	81(77.88)

Out of 104 *H. pylori* isolates, five isolates were resistance to metronidazole/tetracycline by Etest method and eight isolates were resistance by disk diffusion simultaneously. Also twenty eight (27%) isolates were resistance to two antibiotics and ten isolates (9.61 %) were resistant to four antibiotics and 4 isolates (3.85 %) were resistant to five antibiotics and 1 (0.96%) isolates was resistant to more than 5 antibiotics.

DISCUSSION

Failure of therapy for *H. pylori* infection has been related to poor compliance with the treatment regimen and development of antibiotic resistance. The antibiotics most widely used in the treatment of *H. pylori* infection include metronidazole, clarithromycin, amoxycillin and tetracycline (10). Over the last few years, many papers from different parts of the world dealing with antimicrobial resistance rates in *H. pylori* have been published. Comparing the results from different studies can be difficult, as in many instances a different methodology has been used. Some articles do not clarify whether the reported numbers reflect primary resistance or not (11).

In this study, the same of previous study (12), resistance rate of metronidazole was high (77.9 %). This high prevalence of *H. pylori* resistance to this antibiotic has been reported from our country and some other Asian countries (13-15) which may be related to the high utilize of this antibiotic in these countries (12), but metronidazole resistance is uncommon in developed countries (16). Also our results revealed that metronidazole resistance was more prevalent in females than in males. Similar results were reported by Fallahi et al. (17) from Tehran. They observed resistance to metronidazole in females (54%) than males (46%). Metronidazole resistance varies from <10% to >80% in different geographical regions (18).

Our present data indicate a clarithromycin, metronidazole and ciprofloxacin resistance of 15.4%, 77.9% and 39.4% for 2012, respectively. While resistance rates for this antibiotics were reported 14.3%, 76.8% and 33% respectively, in previous study from this area (12). Therefore this study was showed that the resistance of clarithromycin, metronidazole and ciprofloxacin in compared with previous study in this area (12) was increased, while other antibiotics in contrast.

There have been reports of 7% tetracycline resistance in Brazil (19), 4.9% in Japan and 6.7% in Korea (20), accordingly, the result of our study are agreement with these studies. Also this correlates well with the reported rate

of resistance of 5.9% in the worldwide multicenter study (21). In our study, like the studies that have been done in worldwide, the prevalence of tetracycline resistance for *H. pylori* is very low, and the most isolates have MICs from 0.016 to 0.125 µg/ml with maximum MIC of tetracycline for our resistance isolates were 1.5 µg/ml. In our country, according to three previous studies, rate of resistance reported 3%, 0% and 9% for tetracycline, 44%, 57.5% and 73.4% for metronidazole and also 2.4%, 16.7% and 30% for clarithromycin and 20%, 1.6% and 6.8% for amoxicillin (4, 13, 22). In most cases results are agreement with our results. Our results revealed that resistant rate to clarithromycin, metronidazole and erythromycin in females more than males, while about other antibiotics resistant rate in contrast. In our study, correlation between disk-diffusion method and Etest results was good, as Mishra et al.(6) were reported same results with us. The disk diffusion test is cheap than the E-test, and is simple method for testing *H. pylori* susceptibility to antimicrobial agents.

In conclusion the basis of our findings, metronidazole should not be used therapeutically among patients in first line therapy. Clarithromycin should be the first choice antibiotic and tetracycline should replace amoxicillin in the eradication treatment of *H. pylori*. Considering the increasing resistance rate in many countries, monitoring of susceptibility of *H. pylori* to antibiotics appears to be necessary in the choice of effective therapy in order to eradicate *H. pylori* infectious and to optimized the regime in case of treatment failure.

Acknowledgments
We would likes to thanks the staff of hospitals and microbiology laboratories for their help in conducting this study. This article was written based on a dataset of PhD thesis, registered in Oloom Tahghighat Tehran University.

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