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Full Length Research Paper

Prevalence of antimicrobial resistance in *Helicobacter pylori* isolates from Iran

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***Helicobacter pylori*, a gram negative bacterium is capable of being resistant to a wide spectrum of antimicrobial drugs. The prevalence of antibiotic resistance in *H. pylori* strains differs amongst distinct geographical areas and has increased worldwide. Therefore, information concerning the prevalence of antimicrobial-resistant *H. pylori* strains is important in predicting therapeutic response. In this study, drug susceptibility of *H. pylori* in patients was investigated in Laleh hospital, Tehran, Iran from 2007 - 2008. 104 antral biopsies of patients with non ulcer dyspepsia and peptic ulcer were cultured. Susceptibility patterns were determined by disk diffusion method. Minimum inhibitory concentration (MIC) was performed for resistant isolates of *H. pylori*. In our study, 51.5% of clinical isolates showed resistance to metronidazole. All of the isolates were sensitive to other antibiotic disks including clarithromycin, amoxicillin, tetracycline and furazolidone. MIC was determined as 16 µg/ml in 5.8% and 32 µg/ml in 94.1% of isolates for metronidazole resistance isolates. The results indicated that the major drug resisted by *H. pylori* is metronidazole and it should be considered when recommending drugs to patients in this region.**

Key words: *Helicobacter pylori*, antimicrobial resistance, MIC.

INTRODUCTION

Helicobacter pylori, a class I carcinogen, is a main causative agent of chronic gastritis, peptic ulceration and gastric carcinoma (IARC, 1994). The current, combination therapy consists of two antibiotics and a proton pump inhibitor (PPI), and is considered as a treatment of choice to eradicate *H. pylori* infection (Malfertheiner et al., 2002). Treatment of *H. pylori* infection is relatively successful, as the organism is eradicatable from about 80% of patients. According to various studies, with successful treatment of *H. pylori,* the annual relapse rate of duodenal ulcer (80%) and gastritis 60%) is reduced to less than 5% (Nervi et al., 2006). Successful treatment of *H. pylori* infection results both in elimination of the microorganisms and also relieves and inhibits the development of related diseases (Kuipers, 1997). Antimicrobial resistance is a main cause of treatment failure (Megraud, 2004) and resistance rates differ significantly among regions. Reliable regional statistic about the prevalence of antimicrobials resistance has an important role in selecting the appropriate first and second-line treatments for corresponding infection. The evaluation of primary antibiotic resistance should be performed in different areas so that the failure risk can be linked to the antimicrobial resistance (Cavallaro et al., 2006). The prevalence of antibiotic resistant *H. pylori* strains may be variable. So, it seems necessary to investigate the drug resistance of *H. pylori* in different areas to choose the best treatment options in patients. The aim of this study is to assess the prevalence of drug resistance in *H. pylori* clinical isolates from patients in Tehran.

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MATERIALS AND METHODS

Bacterial isolates and culture conditions

H. pylori isolates were obtained from gastric biopsies of patients with upper gastrointestinal tract symptoms in endoscopy section at Laleh hospital in Tehran, Iran. 104 biopsy samples were cultured on brucella agar containing 10% defibrinated sheep blood, 10 mg/l of vancomycin, 5 mg/l of trimethoprim, 20 u/ml of polymyxin and 5 mg/l of amphotericin B under microaerophilic conditions (O_2 5%, Co_2 10%, N_2 85%) at 37°C for 3 - 5 days. Identification of *H. pylori* was confirmed by gram staining and tests for urease, catalase and oxidase activity.

Susceptibility tests

Suspensions from primary plates were prepared in sterile saline solution to obtain the Macfarland opacity standard 2 for performing the susceptibility tests. Aliquots (5 µl) of the suspension were spotted onto Muller Hinton agar containing 5% defibrinated sheep blood and 7% fetal calf serum, and the antibiotic disks were placed on the surface of the medium. Tests for susceptibility to metronidazole, clarithromycin, amoxicillin, tetracycline and furazolidone were performed. Plates were incubated in a microaerobic atmosphere at 37°C for 72 h.

The strains were considered to be resistant to metronidazole when the relative growth inhibition zone around the disk was less than 16 mm (Chaves et al., 1999; McNulty et al., 2001).

Minimum inhibitory concentration (MIC) determination

The protocols used in this study were based on guidelines of NCCLS (2004). Metronidazole solutions were incorporated in Muller Hinton agar supplemented with 5% defibrinated sheep blood and 7% fetal calf serum to produce serial dilutions ranging from 8 to 256 µg/ml. One plate without antibiotic was used as positive control for each assay. A saline suspension of the strains equivalent to 2 Macfarland (containing 1×10^7 or 1×10^8 CFU/ml), was prepared from a 72 h subculture of a brucella agar plate. 5 µl of each bacterial suspension was inoculated onto the plates and then were incubated under microaerobic conditions at 37°C for 72 h.

MIC was determined as the lowest concentration of any antibiotic that completely inhibited the visible bacterial growth isolates and were considered resistant when the MIC value was >8 µg/ml for metronidazole (Chaves et al., 1999). *H. pylori* 26695 was used as a control in our study.

RESULTS

In the present study, during October 2007 and June 2008, 104 antral biopsies of patients with non ulcer dyspepsia, duodenal ulcer and gastric ulcer were isolated. The patients were between 15 and 75 years old. The samples were cultured on brucella agar medium. Prevalence of *H. pylori* was measured (33.65%) in these samples. Among the patients with positive culture for *H. pylori*, 54.28% were male, 45.71% were female. 25.71% of the patients had peptic ulcer and 74.28% had non ulcer dyspepsia.

Susceptibility tests were performed by disk diffusion method. The disks of metronidazole (5 µg), clarithromycin (2 µg), amoxicillin (10 µg), tetracycline (30 µg) and furazolidone (1 µg) were used. Resistance to metronidazole was detected in 51.5% of isolates. All the isolates were sensitive to other disks. Resistant *H. pylori* isolates are shown in Table 1. The metronidazole resistant *H. pylori* isolates were from 35.29% of males and 64.7% of females and 65% of non ulcer dyspepsia patients and 35% of peptic ulcer patients.

The resistant isolates were used for MIC determination. 5.88% (1/17 isolates) of metronidazole resistant isolates showed 16 µg/ml in MIC test by agar dilution method. Also, 94.11% (16 of 17) of these isolates showed MIC value of 32 µg/ml.

DISCUSSION

Antimicrobial resistance of *H. pylori* is a challenging problem and an important factor in determining treatment outcome. The prevalence of antimicrobial resistance varies in different regions (Debets-Ossenkopp et al., 1999; Toracchio and Marzio, 2003). Culture for the diagnosis of *H. pylori* infection has not been applied for a long time, though many studies have shown that better management results are obtained when antibiotics are selected based on susceptibility testing versus choosing empirically (Lamouliatte et al., 2003; Toracchio et al, 2000).

Recently, one week triple therapy with a proton pump inhibitor (PPI) and two antimicrobial agents such as clarithromycin, amoxicillin or metronidazole was shown to be one of the most effective treatment strategy (Hawkey et al., 2003). Triple therapies using this regimen, twice a day for a week, is the first line therapy in populations where less than 15 – 20% prevalence of clarithromycin resistance is reported. In populations with less than 40% resistance to metronidazole, a PPI with clarithromycin and metronidazole is advised. Quadruple therapy including a PPI, tetracycline, metronidazole and a bismuth salt are alternative first line therapies in areas of high prevalence of antibiotic resistance. *H. pylori* infection control therapies are intricate and their increasing use has resulted in more treatment failures. Antibiotic resistance is the most important agent to be considered (Wolle and Malfertheiner, 2007).

This study shows a high level of the prevalence of metronidazole resistance in our region. Treatment with this antibiotic can lead to eradication failure. Other antimicrobial drugs used including clarithromycin, amoxicillin, tetracycline and furazolidone are appropriate choices for treatment. Rate of positive culture of *H. pylori* in males was higher than in females. Considering the two previous studies in our country, rate of resistance reported 35 and 54.16% for metronidazole, and also 2.4 and 4.16% for clarithromycin, and 2.45 and 8.3% for amoxicillin. In these

Table 1. Resistant isolates of *H .pylori* determined by disk diffusion method.

Antimicrobial	% Resistance rate
Metronidazole	51.5
Clarithromycin	0
Amoxicillin	0
Tetracycline	0
Furazolidone	0

studies, no resistance to tetracycline and furazolidone was reported (Siavoshi et al., 2006; Fallahi and Maleknejad, 2007). Therefore, the major antibiotic resistance would be associated with metronidazole in this area.

Metronidazole is a prodrug activated by nitroreductases in the bacterial cell, and absence or the inactivation of these enzymes leads to metronidazole resistance (Edwards, 1993), which is the prevalent type of resistance, with the worldwide rates of 10 to 90% (Debets-Ossenkopp et al., 1999; Toracchio and Marzio, 2003).

The main factor determining clarithromycin resistance is previous consumption of macrolides which induces cross resistance to clarithromycin (Megraud, 2004; Wolle and Malfertheiner, 2007). Mechanism of clarithromycin resistance is due to the presence of gene mutations in a few positions, especially in domain V of the 23S rRNA (Megraud, 2004). The reported prevalence of primary resistance to clarithromycin, ranges between 0 and 15% in most countries (Debets-Ossenkopp et al., 1999; Toracchio and Marzio, 2003).

Resistance to amoxicillin is either null or less than 1%, indicating that it is not yet a problem. The primary cause of amoxicillin resistance is a mutation in the *pbp*-1A gene. The main cause of resistance to tetracycline is associated with mutations in the 16S rRNA. Resistance to tetracycline is also very low or even absent in most countries (Megraud, 2004).

Resistance to metronidazole was found to be 51.5% in the current study and is higher than the previous report from Tehran (35%) but similar to another from Gilan province, Iran (54.16%). It is also similar to other Asian country (Taiwan) (51.9%) and less than Bangladesh (77.5%). Resistance to clarithromycin was not detected in this survey and was also reported to have low prevalence in previous studies in Tehran (2.4%), Gilan (4.16), Taiwan (13.5%) and Bangladesh (10%) which is probably, due to the less use of clarithromycin in gastritis treatment in our region. Moreover, resistance to amoxicillin was not detected in our study and was very low in other investigations in Tehran (2.4%) and less than Taiwan (36%) too (Siavoshi et al., 2006; Fallahi and Maleknejad, 2007; Hu et al., 2007; Nahar et al., 2004).

In our investigation, metronidazole resistances of *H. pylori* isolates in women were more than in men. It is probably because of the use of nitroimidazole drugs for treatment of gynecological infections (Megraud, 2004). Metronidazole resistant isolates were found to be more frequent among non ulcer dyspepsia patients than in peptic ulcer patients in the current study. This is similar to the findings in Singapore, that the metronidazole resistance in non ulcer dyspepsia patients was more than that in peptic ulcer patients (Lui et al., 2003).

Our results showed that metronidazole resistant isolates of *H. pylori* were more than other antibiotics analyzed and remained in high prevalence (51.5%) for *H. pylori* isolates. Using metronidazole in our region can lead to eradication failure in clinical therapies. Therefore, it is advisable to use triple therapy with clarithromycin and amoxicillin for recovery in treatments in order to prevent induction of double resistance to both metronidazole and clarithromycin.

As the antibiotic susceptibility testing is not routine for *H. pylori* isolates in our region, the empirical treatments are usually used in clinical therapies and there is a risk of increase in drug resistance in the future. It is important for specialized centers to perform the monitoring of antibiotic resistance to define appropriate and specific treatment patterns in our country.

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REFERENCES

Cavallaro LG, Egan B, O'Morain C, Di Mario F (2006). Treatment of *Helicobacter pylori* infection. Helicobacter, 11: 36-39.

Chaves S, Gadanho M, Tenreiro R, Cabrita J (1999). Assessment of Metronidazole Susceptibility in *Helicobacter pylori*: Statistical validation and error rate analysis of breakpoints determined by the disk diffusion test. J. Clin. Microbiol. 37: 1628-1631.

Debets-Ossenkopp YJ, Herscheid AJ, Pot RG, Kuipers EJ, Kusters JG, Vandenbroucke-Grauls CM (1999). Prevalence of *Helicobacter pylori* resistance to metronidazole, clarithromycin, amoxicillin, tetracycline and trovafloxacin in The Netherlands. J. Antimicrob. Chemother. 43: 511-515.

Edwards DI (1993). Nitroimidazole drugs-action and resistance mechanisms. I. Mechanisms of action. J. Antimicrob. Chemother. 31: 9-20.

Fallahi GH, Maleknejad S (2007). *Helicobacter pylori* culture and antimicrobial resistance in Iran. Indian J. pediatr. 74: 127-130.

Hawkey CJ, Atherton JC, Treichel HC, Thjodleifsson B, Ravic M (2003). Safety and efficacy of 7-day rabeprazole and omeprazole based triple therapy regimens for the eradication of *Helicobacter pylori* in patients with documented peptic ulcer disease. Aliment Pharmacol. Ther. 17: 1065-1074.

Hu CT, Wu CC, Lin CY, Cheng CC, Su SC, Tseng YH and Lin NT (2007). Resistance rate to antibiotics of *Helicobacter pylori* isolates in eastern Taiwan. J. Gastroenterol. Hepatol. 22: 720-3.

IARC (International Agency for Research on Cancer) (1994). IARC Monograph on the Evaluation of Carcinogenic Risks to Humans: Schitosomes, Liver Flukes and *Helicobacter Pylori*. Lyon: IARC; Vol.

61.
Kuipers EJ (1997). *Helicobacter pylori* and the risk and management of associated diseases: gastritis, ulcer disease, atrophic gastritis and gastric cancer. Aliment Pharmacol. Ther. 11: 71-88.
Lamouliatte H, Mégraud F, Delchier JC, Bretagne JF, Courillon-Mallet A, De Korwin JD, Fauchère JL, Labigne A, Fléjou JF, Barthelemy P, Multicentre Study Group (2003). Second-line treatment for failure to eradicate *Helicobacter pylori*. a randomized trial comparing four treatment strategies. Aliment. Pharmacol. Ther. 18: 791-797.
Lui SY, Yeoh KG, Ho B. (2003). Metronidazole-resistant *Helicobacter pylori* is more prevalent in patients with nonulcer dyspepsia than in peptic ulcer patients in a multiethnic Asian population. J. Clin. Microbiol. 41: 5011-5014.
Malfertheiner P, Megraud F, O'Morain C, Hungin AP, Jones R, Axon A, Graham DY, Tytgat G (2002). European *Helicobacter pylori* study group. Current concepts in the management of *Helicobacter pylori* infection-the Maastricht 2-2000 Consensus Report. Aliment Pharmacol Ther. 16: 167-180.
McNulty C, the PHLS Helicobacter working group: Owen R, Tompkins D, Howtin P, McColl K, Price A, Smith G, Teare L (2001). Helicobacter pylori susceptibility testing by disk siffusion. J. Antimicrob. Chemother. 49: 601-609.
Megraud F (2004). *Helicobacter pylori* antibiotic resistance: prevalence, importance, and advances in testing. Gut. 53: 1374-84.
Nahar S, Mukhopadhyay AK, Khan R, Ahmad MM, Datta S, Chattopadhyay S et al. (2004). Antimicrobial susceptibility of *Helicobacter pylori* strains isolated in Bangladesh. J. Clin. Microbiol. 42: 4856-4858.
NCCLS (2004). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard, 6th edition. NCCLS document M7-A6. Wayne, PA: National Committee for Clinical Laboratory Standards 2004.
Nervi G, Liatopoulou S, Cavallaro LG, Gnocchi A, Dal-Bo N, Rugge M, Iori V, Cavestro GM, Maino M, Colla G, Franze A, Di Mario F (2006). Does *Helicobacter pylori* infection eradication modify peptic ulcer prevalence? A 10 years endoscopical survey. World J. Gastroenterol. 12: 2398-2401.
Siavoshi F, Safari F, Doratotaj D, Khatami GR, Fallahi GH, Mirnaseri MM (2006). Antimicrobial resistance of *Helicobacter pylori* isolates from Iranian adults and children. Arch. Iranian Med. 9: 308-314.
Toracchio S, Cellini L, Di Campli E, Cappello G, Malatesta MG, Ferri A, Ciccaglione AF, Grossi L, Marzio L (2000). Role of antimicrobial susceptibility testing on efficacy of triple therapy in *Helicobacter pylori* eradication. Aliment Pharmacol. Ther. 14: 1639-1643.
Toracchio S, Marzio L (2003). Primary and secondary antibiotic resistance of *Helicobacter pylori* strains isolated in central Italy during the years 1998-2002. Dig. Liver Dis. 35: 541-545.
Wolle K, Malfertheiner P (2007). Treatment of *Helicobacter pylori*. Best Pract. Res. Clin. Gastroenterol. 21: 315-324.