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Helicobacter Pylori Resistance to Metronidazole and Clarithromycin in Dyspeptic Patients in Iran

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Abstract

Background: The resistance of *H. pylori* to the recently available antibiotic treatment regimens has been a growing problem. The prevalence of high antibiotic resistance of *H. pylori* is the most common reason of its eradication failure. The purpose of the present study is to determine the prevalence of antibiotic resistance among *H. pylori* strains isolated from Iranian patients.

Method: We investigated the prevalence of *H. pylori* resistance to metronidazole, clarithromycin, amoxicillin, and tetracycline among 128 *H. pylori* isolates from Iranian patients. After the culture of biopsy specimens and identification, susceptibility tests was performed with Modified Disk Diffusion Method (MDDM) and E. test.

Results: Resistance rates to metronidazole, clarithromycin, amoxicillin and tetracycline were 64%, 23%, 2.5% and 0%, respectively. Seventy two percent of the metronidazole resistance strains had MIC>256μg/ml (High-Level-Resistance).

Discussion: Due to the increasing rate of antibiotic resistance in *H. pylori* strains and in order to decrease the treatment cost, testing of susceptibility to metronidazole and clarithromycin is recommended.

Keywords: *Helicobacter pylori*; Metronidazole; Clarithromycin; Dyspeptic patients

Introduction

Helicobacter pylori infect the majority of the adult population in developing countries including Iran. Studies from northern and southern regions of Iran demonstrated high rates of *H. pylori infection* (>85%) with the frequent rate of development of duodenal ulcer and gastric cancer. The low eradication rate of *H. pylori* infection in Iran and considerable rates (20%) of reinfection or recrudescence indicate that controlling *H. pylori* infection should be considered as an important health issue.[1-4]

H. pylori play an important role in the pathogenesis of chronic gastritis, peptic ulcer disease, and possibly, gastric carcinoma.[5,6] Eradication of the organism not only accelerates the healing of the ulcer but also prevents long-term ulcer relapse.[7]

The current treatment for *H. pylori* infections includes anti-secretory agents or bismuth citrate plus two or more antimicrobial agents.[8] Clarithromycin and metronidazole are the most frequently used antibiotics for the treatment of *H. pylori* infection.[9] However, the treatment of *H. pylori* infection does not always eradicate the organism and antibiotic resistance is increasingly recognized as a contributing factor in the 10-15% of patients who fail *H. pylori* eradication therapy.[10] The prevalence of resistance to metronidazole differs in different countries; a lower prevalence has been reported for industrialized countries ranging from 10 to 50%, whereas up to 90% of *H. pylori* isolates from developing countries have been estimated to be resistant.[11] Although the rates of clarithromycin

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resistance are relatively low, the rate of clarithromycin resistance has been increased. Studies have shown that 7-12% of the patients with *H. pylori* infection have isolates with a primary resistance to clarithromycin.[12] Incidence of primary resistance to amoxicillin and tetracycline is still rare.[13] Determination of antimicrobial susceptibility is therefore important, particularly when treatment has failed.[14]

The purpose of the present study was to determine the prevalence of metronidazole and clarithromycin resistance among the *H. pylori* strains isolated from Iranian dyspeptic patients.

Materials and Methods

A total of 128 clinical isolates of *H. pylori* of antrum biopsies were collected during *2006* to *2007* at the Endoscopy Center of Baqiyatollah Hospital in Tehran, Iran. The isolates were from dyspeptic patients (74 females and 54 males, aged 17-65 years) among who 32 suffered from peptic ulcers and the other 96 had only gastritis. Also, 56 patients had no prior history of eradication therapy while the other 72 had already received eradication therapy.

After primary recognition with rapid urease test, the biopsy specimens were immediately placed in a transport medium (thioglicollate) and sent to the microbiology laboratory. The specimens were placed on the Brucella agar supplemented with *10%* defibrinated sheep blood, *5%* fetal calf serum, *10 mg/l* of vancomycin, *5 mg/l* of trimethoprim and *20 μg/ml* of polymyxin B. The plates were incubated in a microaerophilic atmosphere (10% CO_2, 6% O_2, and 84 % N_2) and relative humidity at *37°C* for 8 to 10 days. Bacterial growth was identified as *H. pylori* on the basis of colony morphology, Gram staining and positive biochemical reaction to catalase, oxidase and urease tests.

The isolates were subcultured on the Brucella blood agar for *48-72 h*rs and stored at *-70°C* in aliquots of Brucella broth supplemented with *20%* (v/v) glycerol and *10%* (v/v) fetal calf serum.

The susceptibility of the *H. pylori* isolates to metronidazole, clarithromycin, amoxicillin and tetracycline was examined by Modified Disk Diffusion Method (MDDM) and E. tests. MDDM was performed with Brucella blood agar with *5%* fetal calf serum. For this purpose, suspensions from the primary plates were prepared in *500 μl* Brucella broth to a Mc Farland opacity standard 4 (approximately *108 CFU/ml*) , and then 100 μl of the suspension was spread on the medium. The plates were briefly dried and then disks containing *5μg* metronidazole, *2μg* clarithromycin, *10 μg* amoxicillin, *30 μg* tetracycline were placed on the plate surface. The plates were incubated microaerophically at *37 ^{0} C* for *5* days. The diameters of the zones of complete growth inhibition were finally measured in millimeters. Reference strain 26695 was included as a quality control.

Susceptibility results were recorded as resistant according to the following interpretive criteria: for clarithromycin, no zone of growth inhibition; for metronidazole, a growth inhibition zone <16 mm; for amoxicillin, a zone <11 mm and for tetracycline, a growth inhibition zone <20 mm.

Brucella blood agar with *5%* fetal calf serum was used as the base medium. The plates were streaked in three directions as described for the MDDM with each inoculum and then metronidazole E. test strips (*0.016-256 μg/m*l) were aseptically placed on to the dried surface of the inoculated plates. The plates were incubated under microaerophilic condition at *37^0 C* for *5* days.

E. test MIC values were defined as the intercept of the elliptical zone of inhibition with the graded E. test strip according to the instructions of the manufacturer.

There is no established NCCLS metronidazole breakpoint for *H. pylori*. In this study; the isolates were considered resistant when the MIC value was 8mg/l for metronidazole. High level resistance was defined as MIC≥256mg/l.

Results

Table 1 shows the characteristics of patients from whom the strains were obtained.

Table 1: Characteristics of the patients

Characteristic	No of patients=128
Age	17-65
Male/Female ratio	42/86
Peptic ulcer disease	32 (25%)
Gastritis	96 (75%)

As shown in Table 2, the prevalence of antibiotic resistance according to the MDDM was 64% to metronidazole, 23% to clarithromycin, and 2.5% to amoxicillin (p<0.001, McNemar test). All the isolates (128) were susceptible to tetracycline. Inhibition zone

diameters for disk diffusion ranged from 8 to 20 mm for amoxicillin. No zones of growth inhibition to 10mm for clarithromycin, 22 to 34 mm for tetracycline and 18mm for metronidazole were obtained. The MIC of 128 *H. pylori* isolates for metronidazole was determined using the E. test, a simple but relatively expensive routine method, that shows a consistent reproducibility and excellent correlation with agar dilution, which is widely used as reference method for *H. pylori*. The lowest and highest concentration of metronidazole on the E. test strips was 0.016 and 256 mg/l.

Table 2: The susceptibility rates to antibiotics by MDDM

Antibiotics	Disk	Zone size breakpoint	Resistance No. (%)
Metronidazole	5	16	82 (64.1)
Clarithromycin	2	Any zone	29 (23.5)
Amoxicillin	10	11	3 (2.5)
Tetracycline	30	20	0

*p value < 0.001

Thus, the isolates with MIC lower than 0.016 and higher than 256 could not be detected. According to E. test, resistance rate to metronidazole was 64%. The range of metronidazole MIC varied from 2 to >256 μg/ml. The overall agreement between the results of MDDM and E. tests for metronidazole was found in 128 strains, of which 82 (64%) isolates were found resistant to 8 mg/l metronidazole by the E. test and 82 (64%) of them exhibited inhibitory zones of 16mm or less by MDDM tests. Furthermore, 60 (73.1%) out of the 82 resistant isolates (using E. test) had a high level of resistance to metronidazole (MIC>256 μg/ml).

Of 128 *H. pylori* isolates, 29 (23%) were resistant to both metronidazole and clarithromycin.

Discussion

The importance of *H. pylori*, as a human pathogen, is well established due to its increasing resistance to various antimicrobial agents.[4,16] The emergence of metronidazole and clarithromycin resistant *H. pylori* strains has resulted in decreased success of the current therapies using this antibiotic.[4,16] Surveillance of *H. pylori* susceptibility tests in the pre-treatment population is difficult since there are only a few centers which offer gastroendoscopy and culture as a routine primary diagnostic test;[17] the sensitivity of *H. pylori* to antibiotics is only checked occasionally.[18]

Metronidazole resistance varies from <10% to >80% in different geographical regions.[19] In the present study, the overall rate of metronidazole resistance among the *H. pylori* isolates was 64%. Metronidazole resistance occurs by *rdxA* gene, which encodes an oxygen-insensitive NADPH nitroreductase. Recent evidence has suggested that inactivation of *frxA* (NADPH flavin oxidoreductase), *fdxB* (ferrodoxin-like protein) and possibly other reductase-encoding genes may also contribute to the resistant phenotype.[20] Another possible mechanism of intrinsic metronidazole resistance involves decreased drug uptake or increased drug efflux.[21] Clarithromycin resistance is known to be associated with the point mutations of *23S rRNA* gene (A2143G or A2144G mutation) of the bacterium which inhibit the binding of clarithromycin to the ribosome.[22] Elviss *et al.* reported a resistance rate of 11% for clarithromycin.[22]

In our study, the overall rate of clarithromycin resistance was 23%. The prevalence of resistance to both metronidazole and clarithromycin among the 128 isolates was 23%. Several investigators have noted that the rates of resistance to these two antibiotics among the patients, previously treated for *H. pylori* infections, are generally higher than those observed among the untreated patients.[23]

Resistance to tetracycline and amoxicillin is rare. In our study, amoxicillin resistance was 2.5% and no tetracycline resistant was observed. There have been reports of 7% resistance in Brazil[24] and 4.9 and 6.7% in Japan and Korea, respectively.[25] This study highlights the importance of antibiotics susceptibility tests to guide treatment plans for *H. pylori* infections. Accordingly, high rates of resistance to metronidazole and clarithromycin might require the introduction of new antibiotics, culture and susceptibility testing prior to selecting a therapy.

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Conflict of interest: None declared.

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