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ESRA AĞEL

BENGÜL DURMAZ

MEHMET REFİK TEVFİK

NERGİS AŞGIN

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Esra AĞEL
Bengül DURMAZ
Mehmet Refik TEVFIK
Nergis AŞGIN

The Isolation Rate and Antibiotic Resistant Pattern of *Helicobacter Pylori* in Dyspeptic Patients

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Department of Microbiology and Clinical Microbiology, Faculty of Medicine, İnönü University, Malatya-Turkey

Abstract: In order to determine the isolation rates of *Helicobacter pylori* in dyspeptic patients, 167 biopsy specimens were examined using different methods, including culturing, microscopy and the rapid urease test. Resistance to commonly used antimicrobials was investigated using the disk diffusion method. Of the 167 specimens, 82(49.1%) were found to be positive for *Helicobacter pylori* in culture and 72(43 %) were positive in the urease biopsy test. Using Gram staining, *Helicobacter pylori* were

observed in 43 (25.7 %) of the specimens. The resistance rate of the isolates was 53 % for metronidazol, 8% for both clarithromycin and ciprofloxacin, and 4% for tetracycline. The rate of resistance for amoxycillin was not recorded. Our results indicate that a susceptibility test is necessary before initiating therapy for *Helicobacter pylori* infections, otherwise treatment may be ineffective.

Key Words: *Helicobacter pylori*, antimicrobial susceptibility.

Introduction

Helicobacter pylori (*Hp*) is a microaerophilic gram-negative spiral bacillus that infects the gastric mucosa in 20-80 % of humans throughout the world (1,2). Although most *Hp* infections are clinically silent, this microorganism is associated with substantial morbidity and mortality, causing duodenal and gastric ulcers as well as gastric cancers (3,4). There are certain virulence factors important in the pathogenesis of *Hp*: first, a large amount of urease production which helps protect organisms from gastric acid; second, penetration across the mucus layer via rapid motility; and, third, the possession of certain toxins such as vacuolating cytotoxin and cytotoxin-associated antigen (Cag A) (5-7). Because of the lack of an effective vaccine against *Hp*, the diagnosis and cure of patients are of great importance in controlling the spread of *Hp* infections (8).

Diagnosis is performed by using several invasive (histological examination, Gram staining, Leifson staining, culturing, rapid urease test, polymerase chain reaction) and non-invasive techniques (serology, ¹³C, ¹⁴C labelled urea breath test) (9-11). The extensive use of anti-*Hp* antimicrobials in general practice will lead to the emergence of resistance to multiple antibacterial agents among *Hp* isolates (12). Treatment efficacy may be

reduced due to the presence of resistant strains. For these reasons, the designation of antimicrobial sensitivity tests is necessary to choose the most effective drug. Thus, the aims of this study were to determine the isolation rates of *Hp* in dyspeptic patients using three conventional procedures and their susceptibility to antimicrobials.

Materials and Methods

A total of 167 patients who had undergone endoscopies for dyspepsia complaints were included in this study. Biopsy materials taken by a gastroenterologist during endoscopy were first inoculated into freshly prepared Stuart's transport semisolid medium and transferred as soon as possible to the microbiology laboratory. Each of the transport media was subcultured on Brucella blood agar and Christensen urea medium and a direct smear was prepared for Gram staining. The inoculated cultures were incubated under microaerophilic conditions (using a microaerophilic gas generating kit and jars without a catalyst) in a humid environment at 37 °C for 3-4 days. The biopsy smears were dried in air and fixed with methanol for 2 minutes, then routinely stained with gram stain using 4% basic fuchsin instead of safranin. Organisms were identified on the basis of typical

cell morphology, positive oxidase, catalase and urease activities (9,12-14).

The disk diffusion method was used to determine the resistance rate of 49 isolates to amoxycillin (10 µg), clarithromycin (15 µg), metronidazole (16 µg), ciprofloxacin (5 µg) and tetracycline (30 µg). The susceptibility test results were read after 2 days incubation (14,15).

Results

H. pylori was isolated from 82(49 %) of the total of 167 biopsy specimens. The urease test was positive in 72 (43 %) biopsy samples. On direct examination of the gram smears, characteristic gram-negative seagull like organisms distributed in patchy forms were observed in 43 (25.7 %) of the biopsy specimens (Table 1).

In the antimicrobial susceptibility test, 26(53%) of the 49 isolates were found to be resistant to metronidazole. The other antimicrobial resistance rates were 8% for both clarithromycin and ciprofloxacin, and 4% percent for tetracycline. No resistance to amoxycillin was detected (Table 2).

Discussion

Culturing remains the most sensitive technique in the detection of *Helicobacter pylori* from clinical samples (12,13,16). It yields a positive result even if only a small number of organisms exists in the sample. However, there are some difficulties in achieving successful and proper isolation by bacterial culture. The reasons for this may be patchy distribution of the organism in the gastric mucosa, contamination of biopsy forceps, ingestion of anaesthetic, presence of oropharyngeal flora or loss of viability of organisms during transportation (11). The sensitivity of direct Gram staining is greatly affected by

the quality and the accuracy of sampling and the presence of other urease-producing bacteria in the gastric sample, such as *H. heilmannii*, *Proteus* spp. which may reduce the specificity of the biopsy urease test (11). Our findings indicated that the positivity rate of the culture was found to be higher than those of both the rapid urease test and direct Gram staining (49% versus 43% and 25.7% respectively). These results are in agreement with those of other studies (6,17).

It has been widely reported that the majority of children in developing countries acquire *Hp* infection earlier in life and more frequently than those in developed countries. The prevalence rate increases with age and may reach 90 % in adulthood in some populations (4,18).

In the present study, the isolation rate of *Hp* in dyspeptic adult patients was 49%. In other studies performed in Turkey, the prevalence has been found to be within the range of 48 % to 70 % (19-24). The positivity rate of *Hp* infection can be affected by sampling errors and the processing and transportation of specimens (12,16,17). Ethnicity, race and environmental factors such as general hygiene level, water supply, sanitation and crowding in the household as well as socio economic status also have important effects on the prevalence of *Hp* infection. (4).

In our study, the resistance rates of *Hp* strains isolated before commencing antimicrobial treatment were: 53% for metronidazole, 8% for clarithromycin, 8% for ciprofloxacin and 4% for tetracycline. All the strains were susceptible to amoxycillin. In another study from Turkey, the metronidazole resistance rate was reported to be 48 %. To our knowledge, no resistance to clarithromycin or amoxycillin has been noted yet in Turkey.

In the world, the rates of resistance of *Hp* to metronidazole are as follows: < 10-50 % in Europe (14,25,26), 25-30 % in North America, 10-20% in Australia, 30-70 % in South America, and 70-90 % in

Table 1. *H. pylori* according to applied methods in total of 167 biopsy specimens.

	<i>H. pylori</i> (+)	<i>H. pylori</i> (-)
Microscopy	43(25.7)*	124
Urease activity	72(43)	95
Culture	82(49.1)	85

*Numbers in parentheses show percentages.

Table 2. Resistance rates of *H. pylori* strains to antimicrobial agents.

Antimicrobial agents	Resistant strains	
	Numbers	Percentages
Metronidazole	26	53
Clarithromycin	4	8
Ciprofloxacin	4	8
Tetracycline	2	4
Amoxycillin	0	0

Central Africa (14). The rate in Turkey is similar to that in South America and is nearly as high as the rate in Europe. A possible explanation for metronidazole resistance may be the wide use of this drug in the prophylaxis of protozoal diseases.

The resistance rates of *Hp* are within the ranges: less than 5% to 15 % for clarithromycin in Europe (14,27), and < 1 % for ciprofloxacin in Europe and the USA (14). Our findings demonstrate that the rate of resistance to clarithromycin is almost the same as in Europe whereas ciprofloxacin resistance is higher. A reason for this high resistance might be the fact that ciprofloxacin is

commonly used as an empirical drug in the treatment of urinary tract infections. (28).

In conclusion, the data given above reinforce the importance of routine susceptibility testing in the effective treatment of *Hp* infections. Further investigations are necessary to overcome the difficulties in culturing and antimicrobial sensitivity testing.

Corresponding author:

Bengül DURMAZ

Inönü Üniversitesi Tıp Fakültesi

Mikrobiyoloji ve Klinik Mikrobiyoloji ABD.

Malatya

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