Prevalence of Primary *Helicobacter pylori* Resistance to Several Antimicrobials in a Saudi Teaching Hospital

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Key Words

Helicobacter pylori · Primary resistance · Metronidazole · Clarithromycin · Amoxicillin · Tetracycline ·

Abstract

Objective: To determine the resistance rate of the most commonly used antimicrobial agents amongst *Helicobacter pylori* isolates. **Methods:** The agar disk diffusion method (Kirby Bauer) was utilized to determine the susceptibility of 223 *H. pylori* strains isolated before treatment. Isolates were tested against metronidazole (5 µg), clarithromycin (15 µg), amoxicillin (10 µg), and tetracycline (30 µg). **Results:** The resistance rate was 80% for metronidazole and 4% for clarithromycin. Tetracycline and amoxicillin showed very low degree of resistance with 1 (0.4%) and 3 (1.3%) of the strains resistant to these antibiotics, respectively. Age, sex and ethnicity had a remarkable effect on the resistance rate. **Conclusion:** The results indicate that metronidazole and clarithromycin should not be used as the only antimicrobial agents in the treatment of *H. pylori* infection. Susceptibility testing using the disk diffusion method is cost-effective in the screening of antimicrobial resistance against *H. pylori*.

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Introduction

Chemotherapy has been documented to be effective in the treatment of *Helicobacter pylori* and associated gastritis and gastroduodenal ulcers [1–3]. However, this species has not always been successfully eradicated. Unsuccessful therapy in patients infected with *H. pylori* is frequently correlated to metronidazole and clarithromycin resistance [4–6]. Most authorities agree that optimal therapy of *H. pylori* infection requires administration of a minimum of two antibiotics in addition to either a proton pump inhibitor or a hydrogen blocker [7]. Among the many antibiotics examined in vitro, only four are clinically useful: amoxicillin, tetracycline, clarithromycin, and metronidazole. Although resistance to amoxicillin has not yet been described, tetracycline and clarithromycin resistance can be found in up to 16% of pretreatment isolates [8–10]. Primary metronidazole resistance is more common and more variable, ranging from 10 to 90% apparently, depending in large part upon the frequency of metronidazole use [8–12]. The correlation between failure to eradicate *H. pylori* with triple antimicrobial therapy (colloidal bismuth subcitrate, metronidazole, amoxicillin or tetracycline) and resistance to metronidazole in pretreatment isolates has been shown worldwide [6, 8, 13, 14]. As a consequence, routine susceptibility testing of *H. pylori* against metronidazole by standardized methods has been advocated by several investigators [9–15]. The

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resistance rate of each of the antimicrobials used in the treatment of *H. pylori* infection should be determined in each geographical area and also among different ethnic groups to guide clinical practice. The aim of this study was to determine the resistance pattern of *H. pylori* isolates at King Abdulaziz University Hospital against agents commonly administered for eradication of *H. pylori* infection.

Material and Methods

Patient Details

A total of 447 consecutive patients with upper abdominal complaints had endoscopy at the Gastroenterology Unit, King Abdulaziz University Hospital, Jeddah, Saudi Arabia from January 1998 to August 2000. Histology, rapid urease, serology testing and culture were used to determine *H. pylori* status. Patients who had antibiotic treatment of *H. pylori* infection, or bismuth salts less than 1 month prior to endoscopy were excluded from the study. Four biopsy specimens were taken from the antral mucosa during endoscopy; two biopsies were used for histological examination, one for rapid urease (CLO) test, and the other for culture and sensitivity.

Culture

Biopsy specimens were cultured onto Columbia blood agar, Columbia agar base (Becton Dickinson, Cockeysville, USA) and chocolate agar both supplemented with 5% defibrinated horse blood (Oxoid, Basingstoke, Hampshire, England). The cultures were incubated in microaerophilic atmosphere (Camp Gas pack, UK) for 5 days. Identification of culture was based on Gram staining, oxidase and catalase activities, and urease positivity. Isolates were stored at -20°C until ready for sensitivity testing.

Antimicrobial Susceptibility

Susceptibility tests were performed on Mueller-Hinton agar plates (BBL, Cockeysville, USA) supplemented with 5% horse blood. A standard inoculum of *H. pylori* culture in saline adjusted to 1 McFarland equivalent was used to inoculate the blood agar plates. All isolates were tested for the following antimicrobial agents using Kirby-Bauer disk diffusion (NCCLS) [16]: amoxycillin (10 μg), tetracycline (30 μg), clarithromycin (15 μg), and metronidazole (5 μg). Inhibition zone diameters were measured after 48–72 h of incubation at 37°C under microaerophilic conditions.

Reproducibility was checked by testing *H. pylori* type strains NCTC 11637 and *H. pylori* NCTC 11638. Since there is no precise cutoff point based on the size of the inhibition zone of tested antibiotics, for this study, a zone diameter of 30 mm or greater was regarded as sensitive, while 29 mm or less was regarded as resistant.

Results

Of the 447 biopsies, 223 (50%) were positive for *H. pylori*, and 214 (48%) were negative. Ten (2%) of the biopsies, which had positive cultures of *H. pylori* on pri-

Table 1. Distribution of the zone of inhibition diameters (mm) in relation to the antibiotics tested (number of strains)

Zone diameter	Antibiotics			
	metronidazole	clarithromycin	amoxycillin	tetracycline
10–19	170	0	0	0
20–29	9	9	3	1
30–39	10	13	0	0
40–49	7	26	10	6
50–59	3	34	23	15
60–69	9	39	31	28
≥ 70	15	102	156	173

mary isolation, had organisms that died on subculture. Of the 223 isolates tested against the different antimicrobial agents, 179 (80.3%) were resistant to metronidazole agent, 9 (4%) to clarithromycin, 3 (1.3%) to amoxycillin, and 1 strain (0.4%) was found resistant to tetracycline. Table 1 shows the distribution of the antibiotic inhibition zone diameters in relation to the different types of antibiotics tested.

The 9 strains resistant to clarithromycin were also resistant to metronidazole. Metronidazole and clarithromycin resistance was more common in females than males. For females, resistance to metronidazole and clarithromycin was 58 and 78%, respectively, while for males the corresponding resistance rate was 42 and 22.2%. There was a remarkable difference in antimicrobial resistance according to age. The distribution of the resistant organisms ($n = 192$) in relation to age-group was as follows: age-group 0–9 years, 0%; 10–19 years, 2%; 20–29 years, 14.6%; 30–39 years, 35%; 40–49 years, 28%; 50–59 years, 11.5%; 60–69 years, 7.3%, and age-group ≥ 70 years, 1.6%. Antibiotic resistance increased gradually up to age 30–39 years, then declined remarkably. Difference in antimicrobial resistance rates in relation to nationality was also observed in this study. The resistance was higher among patients from Somalia, Pakistan and Sudan (66–70%) than among patients from Yemen, Saudi Arabia and Palestine (50–58%).

Discussion

Eradication of *H. pylori* species brings an improvement in gastritis and prevents the relapse of gastroduodenal ulcers [7]. The efficacy of treatment of gastric infection caused by *H. pylori* can be reduced by the occurrence

of primary or acquired resistance to various drugs, especially metronidazole [6]. This has made susceptibility testing of *H. pylori* increasingly important in the search for effective antimicrobial combinations that eradicate these bacteria from the stomach. Test methods used to investigate antimicrobial resistance in *H. pylori* have included agar dilution, microbroth dilution, disk diffusion and E test. However, until now the methods proposed for in vitro susceptibility testing of *H. pylori* have suffered from a lack of standardization and there is still no consensus as to the break points for agents other than clarithromycin used in the treatment of *H. pylori* infection [17]. While both E test and disk diffusion methods have the advantage of allowing the visualization of resistant subpopulations of bacteria within zones of inhibition, disk diffusion testing is less expensive and results in good agreement reference methods [18].

Resistance to metronidazole has been observed worldwide and presently occurs quite commonly in several countries, ranging from 10 to 90% with the lowest resistance rates in Europe and Australia, the highest rates in Africa whereas in other developing countries, the rate of resistance to metronidazole ranges from 80 to 90% [12]. The 80% resistant rate of this study is consistent with that of the previous report [12] and is attributable to previous treatment of gynecologic infection or to the therapy of intestinal parasitic infections, which occur frequently in these countries [19]. However, even when strains were resistant to metronidazole in vitro, a combination of omeprazole, metronidazole, bismuth, and tetracycline given to 165 patients cured 97.6% of them [20].

The capability of clarithromycin to eradicate *H. pylori* has renewed interest in the macrolides for treating *H.*

pylori infection [21] because these compounds achieve high concentrations in mucus and become concentrated intracellularly. Primary resistance to clarithromycin is up to 16% [5, 9, 22, 23] and is much less common than metronidazole resistance. Based on recent studies, the prevalence of clarithromycin resistance is 10% in France, 16% in Italy, 6.8% in New Zealand and 14.5% in Iran [8, 9, 24, 25]. The low level of resistance (4%) observed in our study is comparable to these data. However, although the prevalence of resistance to clarithromycin is still low, it may rise with the increased use of this compound for treatment of infections due to both *H. pylori* and other organisms. In contrast to clarithromycin and metronidazole, amoxycillin and tetracycline retained excellent activity. Only 1 strain (0.4%) of the 223 *H. pylori* isolates in our study was resistant to tetracycline, and 3 (1%) were resistant to amoxycillin. This high susceptibility of *H. pylori* has also been reported by others [26, 27].

Conclusion

Susceptibility test using the disk diffusion method is cost-effective in the screening of antimicrobial resistance against *H. pylori*. Testing for resistance to metronidazole and clarithromycin will undoubtedly enable the more rational use of these antibiotics to treat *H. pylori* infection and improve treatment success. It is advisable not to include metronidazole in the treatment regimen in localities where the prevalence of metronidazole resistance is high.

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