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Original Article

Patterns of *Helicobacter pylori* Resistance to Metronidazole, Clarithormycin and Amoxicillin in Saudi Arabia

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There are no generally accepted regimens for the treatment of *H. pylori* infection in patients with gastritis or duodenal ulcers. However, metronidazole based regimens have been reported to be among the most successful. Resistance to metronidazole, clarithromycin, and amoxicillin was determined for 46 clinical isolates of *Helicobacter pylori* in Saudi Arabia and tested by E test. Of these isolates, 69.5% was resistant to metronidazole (MIC > 8 mg/l), 21% to clarithromycin (MIC > 1 mg/l) and 11% were multiresistant. No resistance to amoxicillin was observed. Resistance to metronidazole was more common in isolates from females than in those form males. In conclusion, the present study demonstrates high metronidazole resistance rate of *H. pylori* isolates in Saudi Arabia. Regimens containing metronidazole are best avoided. Trials to test other antimicrobial combinations are recommended.

Key Words: *Helicobacter pylori*, Resistance, Treatment

INTRODUCTION

Helicobacter pylori (*H. pylori*) causes gastritis and ulcer disease and is considered as a risk factor for the development of mucosa-associated lymphoma and gastric cancer (12). Most consensus guidelines now are recommending for the eradication of *H. pylori* infected patients. The most commonly using treatments are triple therapies consisting of a proton pump inhibitor, clarithromycin and metronidazole or amoxicillin (11). The primary and secondary resistance rates reported worldwide vary from 1 to 58% for clarithromycin and from 5 to 76% for metronidazole (6,17). Resistance to one or both of these antibiotics significantly reduces treatment success (19).

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The clinical importance of this resistance makes it necessary for antibiotic treatment decisions to be based on valid and reproducible *in vitro* susceptibility testing results. Reported discrepant susceptibility test results are due to variations in the methods and conditions used for susceptibility testing (18,20). The National Committee for Clinical Laboratory Standards (NCCLS) has recently published guidelines for *in vitro* susceptibility testing of *H. pylori* using an agar dilution method (26). Agar dilution tests are time-consuming and labor-intensive, and a large number of strains should be tested simultaneously since the media containing the antibiotic dilutions must be prepared immediately before use. The procedure is efficient for the testing of large numbers of strains, such as in susceptibility surveys, but it is not readily adaptable for the testing of small numbers of strains on an ongoing basis. The Epsilometer test (E test) method has been approved for use for the susceptibility testing of many organisms, but not for *H. pylori*. However, many studies have been carried out by this method with *H.*

pylori (9,14). The proprietary antibiotic-impregnated E test strips are expensive, but less labor is required relative to the amount of labor required for agar dilution, and the MIC is read directly from the confluence of growth with the strip. Since single tests are easily carried out, E test might be a preferable method for the testing of small numbers of strains (10).

There are limited data on *H. pylori* resistance in Saudi Arabia. In order to find out the pattern of *H. pylori* susceptibility to antimicrobial agents in our community, the present study evaluated the reliability of results obtained by the E test methodology for determining the quantitative susceptibility of *H. pylori* to three antimicrobial agents of currently in use for *H. pylori* infection treatment.

MATERIALS AND METHODS

1. Clinical specimens

Forty-six gastric biopsies of patients with gastritis or duodenal ulcer were underwent endoscopically at Al-Iman General Hospital, Riyadh, Saudi Arabia. Three gastric biopsy specimens were obtained from each patient; one was used for rapid CLO test (*Campylobacter* like organism for determination of urease activity) (Oxoid, Basingstoke, UK) and the remaining two were placed in the sterile screw-capped tubes containing 0.5 ml Brucella broth media (Oxoid) and transported for isolation and antibiotic susceptibility test. The biopsy specimens were ground in a tissue grinder. Using a pasture pipette, about two drops of homogenates were inoculated into *H. pylori* selective agar (Oxoid) and streaked with a bacteriological loop. The inoculated culture media were transferred into microaerophilic condition (jars with CampyGen, Oxoid) at 37℃ for 7 days.

2. Bacterial strains

Forty-six clinical strains of *H. pylori* isolated from patients with gastritis or duodenal ulcer were tested. The strains were identified by Gram staining and oxidase, catalase, and urease reactions. After identification, the bacteria were suspended in the Eppendorf tubes containing Brucella broth media with 30% glycerol and stored at - 70℃ until use.

3. Control strains

H. pylori ATCC 43504 was the reference strain used for quality control and was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA).

4. Antimicrobial agents

The antimicrobial agents tested against *H. pylori* included metronidazole, clarithromycin, and amoxicillin. The E test strips of each antibiotic were purchased from AB Biodisk (Solna, Dalvagen, Sweden).

5. Antimicrobial susceptibility testing

Thawed isolates were inoculated onto Mueller-Hinton agar supplemented with 5% sheep blood and were incubated under a microaerophilic atmosphere (CampyGen, Basingstoke, UK) for 72 h at 37℃. Colonies were suspended in 2 ml of Dulbecco's modified Eagle medium (ICN Biomedicals, Livermore, CA, USA) to achieve a turbidity equivalent to that of the no. 3 McFarland opacity standard.

6. E test

Plates containing Mueller-Hinton agar supplemented with 5% heparinized horse blood were used for the E test. All antimicrobial agents except metronidazole were tested at concentrations ranging from 0.016 to 128 mg/l; Clarithromycin and amoxicillin were tested at 0.016 to 8 mg/l. The agar plates were inoculated by confluent swabbing of the surface with the adjusted inoculum suspensions. After the surface of the inoculated plates had dried at 37℃ inside a microaerophilic chamber, E test strips were applied onto the surface of each agar plate. The plates were incubated at 37℃ under microaerophilic conditions (CampyGen). MICs were read after 72 h of incubation on the basis of the intersection of the elliptical zone of growth inhibition with the MIC scale on the E test strip. Strains were considered as resistant when the MIC was > 8 mg/l for metronidazole, > 1 mg/l for clarithromycin and > 0.5 mg/l for amoxicillin. These breakpoints were used based on the recommendations from the National Committee for Clinical Laboratory Standards (NCCLS) (25,29).

Table 1. Minimum inhibitory concentrations (MICs) of metronidazole, clarithromycin, and amoxicillin for 46 isolates of *H. pylori*

MICs No. (%)			
MICs mg/l	Metronidazole	Clarithromycin	Amoxicillin
0.016	2 (4)	7 (16)	10 (21)
0.032	2 (4)	10 (21)	13 (28)
0.064	2 (4)	4 (8)	17 (37)
0.125	1 (2)	3 (7)	3 (7)
0.25	1 (2)	6 (13)	3 (7)
0.5	1 (2)	3 (7)	0
1	0	3 (7)	0
2	3 (7)	10 (21)	0
4	0	0	0
8	2 (4)	0	0
16	9 (20)	0	0
32	9 (20)	0	0
64	8 (18)	0	0
128	6 (13)	0	0
	46	46	46

RESULTS

The antimicrobial susceptibility test results of all *H. pylori* strains are presented in Table 1. Resistance to metronidazole was found in 32/46 isolates (69.5%) (MIC > 8 mg/l) and resistance to clarithromycin in 10 (21.7%) (MIC > 1 mg/l). No resistance to amoxicillin was observed.

Examination data in the male patients of known age (n= 22) demonstrated rates of metronidazole and clarithromycin resistance of 68.2% (n=15) and 18.2% (n=4), respectively. In contrast, resistance rates were higher in female patients (n=24) for metronidazole (70.8%, n=17) and for clarithromycin (25%, n=6).

For patients aged < 40 years (n=14), 57.1% of isolates (n=8) was metronidazole-resistant and 28.5% (n=4) clarithromycin-resistant. In the >=40 years age group (n=32), resistance rates were higher for metronidazole (75%, n=24) and to a lesser extent for clarithromycin (18.8%, n=6), compared with the younger adult age group. Clarithromycin resistance rates were higher in female than male patients in both the < 40 age group (33.3% vs 25%) and in the >=40 years age group (22.2% vs 14%). In contrast, it was only in the >=40 years group that metronidazole resistance was higher in females (78% vs 71%) (Table 2).

Table 2. Rates of metronidazole and clarithromycin resistance in 46 isolates of *H. pylori* in relation to patient age and sex

Age	Sex	No.	No. of isolates (% of resistance)		
			[a]MTZ	[b]CLA	MTZ+ CLA
>=18 ~ < 40	Male	8	5 (62.5)	2 (25)	1 (13)
	Female	6	3 (50)	2 (33.3)	1 (17)
>= 40	Male	14	10 (71)	2 (14)	1 (7)
	Female	18	14 (78)	4 (22.2)	2 (11.1)
Total		46	32 (70)	10 (21)	5 (11)

[a] MTZ, metronidazole, [b] CLA, clarithromycin.

The proportion of female patients in the >= 40 group (n=18) infected with metronidazole-resistant *H. pylori* was higher (78%) than in females < 40 years (50%, n=6) (Table 2). There was no difference in clarithromycin resistance rates in females from either age group (Table 2). Similarly, antibiotic resistance rates did not differ significantly between age groups for male patients (Table 2).

DISCUSSION

The efficacy of the treatment of gastric infection caused by *H. pylori* could be reduced by the occurrence of primary or acquired resistance to various drugs, especially to metronidazole (4). This had made the susceptibility testing of *H. pylori* increasingly important for the search for efficient antimicrobial combinations that allow for the eradication of this bacterium from the stomach. Many studies had used an agar dilution method (5,16), but it is difficult to perform this routinely and impractical for clinical use when testing individual isolates. The E test is now widely accepted as an alternative, providing results comparable with those of agar dilution (8,27). Moreover, E test is very easy to perform and not labor intensive (2). In this study this point was addressed by using a control strain (ATCC 43504) with

known MIC values. All the MIC values of the reference strain were maintained throughout the study. Metronidazole is the main antibiotic used in several eradication therapies for *H. pylori*, but resistance is well documented in developing and developed countries (5,22). In the present study the incidence of metronidazole resistance was 69.5%, a high percentage when compared to the developed countries (13).

It has been documented that metronidazole resistance is higher in female patients (7,31) This is most likely to be due to the use of metronidazole to treat gynaecological infections. Interestingly, the clarithromycin resistance rate was higher in female compared with male patients in both age groups. Higher clarithromycin resistance rate in females was documented in a multi-centre study in the United States (23). Mutations (A2142G and A2143G) in the 23S rRNA gene associated with clarithromycin resistance in *H. pylori* confer cross-resistance to other agents in the macrolide, lincosamide antibiotic group, including clindamycin. The higher clarithromycin resistance rates in females may again be due to treatment of gynaecological infections, as clindamycin is used as an alternative to metronidazole in the treatment of bacterial vaginosis (30). Continued monitoring of resistance rates in larger study populations over a longer period would be essential in order to identify potential trends.

The limitations of existing therapies has led to the development of alternative "rescue therapies" involving agents not used in first or second-line therapies, such as the rifamycin and fluoroquinolones (15). Resistance to these agents has not been examined here, in Saudi Arabia, as few validation studies to determine appropriate cut-off for resistance had been conducted.

Data obtained in this investigation revealed that 11% of *H. pylori* strains was resistant to two antibacterial agents. Torres *et al*. from Mexico had reported 30.7% double resistance among *H. pylor*i isolates (28). *H. pylori* resistance to these antimicrobials may be partially explained by the high prevalence of these bacteria in our population. Metronidazole and amoxicillin in combination with a proton pump-inhibitor were used for treatment of *H. pylori* infection with eradication rate of 80% in Europe (21). In this study, 69.5% of *H. pylori* isolates was resistant to metronidazole and therefore, recurrence of the infection with *H. pylori* could be expected among patients receiving the above triple therapy.

Furthermore, countries in the Middle East such as Saudi Arabia have high prevalence of *H. pylori* and this could be the cause of high resistance to metronidazole and clarithromycin. High prevalence of *H. pylori* was reported by many authors previously. In 1990, Al Moagel et al, reported that 40% of the Saudi population in the age group of 5~10 years and 70% of people ≥20 years of age had *H. pylori*, which makes it one of the highest endemic areas in the world (3). In 1994, Mohammed et al, from Saudi Arabia reported that 145 (74%) of 196 dyspeptic patients were found infected with *H. pylori* (24). Moreover, the other reason of high prevalence of *H. pylori* resistance to metronidazole and clarithromycin is that Saudi Arabia has a large community of expatriates from the Far East and the Indian subcontinent where metronidazole and clarithromycin resistance are prevalent due to frequent usage of the drug for parasitic diseases and for common ailments such as diarrhea (1).

The present study suggests that culture and antimicrobial susceptibility testing of *H. pylori* must be performed in patients who have failed in the metronidazole based triple therapy. In conclusion, this study demonstrates high metronidazole resistance of *H. pylori* isolates in Saudi Arabia as tested by E test. Regimens containing metronidazole are best avoided. Trials to test other antimicrobial combinations are recommended.

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