Antibiotic susceptibilities of *Helicobacter pylori*

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ABSTRACT

Objective: The aim of this study was to evaluate the prevalence of resistance among 83 *Helicobacter pylori* isolates cultured from biopsies taken during routine endoscopies in 1998-1999.

Methods: Minimum Inhibitory Concentration of amoxicillin, tetracycline, clarithromycin and metronidazole were determined by Epsilometer test.

Results: Forty-seven strains (57%) were resistant to metronidazole, and 27 (32.5%) were resistant to clarithromycin. Twenty of the 27 strains resistant to clarithromycin were also resistant to metronidazole. None of the strains were resistant to amoxicillin or tetracycline.

Conclusion: A high percentage of patients from Bahrain were infected with resistant strains of *Helicobacter pylori*. Antibiotic resistance monitoring is very important and unified national treatment policies are needed.

Keywords: Antibiotic resistance, *helicobacter pylori*.

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Helicobacter pylori is the major etiological agent of chronic gastritis and peptic ulcer and a major risk factor for gastric cancer.[1-3] For these reasons, therapy aimed at eradicating the pathogen is given to infected patients. Eradication of *Helicobacter pylori* has been shown to prevent peptic ulcer recurrence and may also decrease the prevalence of gastric cancer in high-risk populations.[3,4] Different therapeutic regimens have been reported to be successful in eradicating the infection.[5] Many of these regimens include metronidazole, amoxicillin, tetracycline and clarithromycin in triple therapies consisting of either a bismuth compound, amoxicillin and tetracycline or metronidazole,[6] or a proton pump inhibitor, erythromycin and metronidazole.[6,7] Thus, resistance to metronidazole, amoxicillin or clarithromycin can have an adverse effect on the success of treatment. Metronidazole resistance is common in developing countries[8,9] and among immigrants in the developed countries.[10,11]

Increasing resistance to several antimicrobial agents has focused attention on the need for reliable methods for determining drug susceptibility in vitro. Current standard methods use either disc diffusion or an agar dilution technique to determine the Minimum Inhibitory Concentration (MIC) of an antimicrobial agent. In comparison with these methods, the Epsilometer test (E-test), a plastic strip carrying an antibiotic gradient, is an accurate and easy to use method of determining MICs, including drugs used in the triple therapy of *Helicobacter pylori*.[12-14] The purpose of this study was to evaluate in vitro the antimicrobial susceptibility of *Helicobacter pylori* strains to amoxicillin, tetracycline, metronidazole and clarithromycin, antibiotics widely used in Bahrain to treat patients with positive *Helicobacter pylori* culture using the E-test.

Methods. ***Patients and strains.*** One hundred and fifty Bahraini patients with dyspeptic complaints and who were undergoing upper gastrointestinal endoscopy were investigated during 1998-1999, none of these patients received any antibiotics treatment

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for prior to the endoscopy. Eighty-three were positive for *Helicobacter pylori* by culture and were enrolled in the present study. The endoscopic findings were retrieved and recorded prior to the sensitivity testing.

Biopsy specimens were transported in Stuart medium (Oxoid) and inoculated within one hour of collection onto Columbia agar plates containing 7% (vol/vol) horse blood, and Dent antibiotic supplement at 37°C for 5 days under microaerophilic conditions (jars with CampyGen, Oxoid, Basingstoke, United Kingdom). Colonies that exhibited characteristic morphologies were identified as *Helicobacter pylori* if they were urease, catalase, and oxidasepositive. Each isolate was then stored in 8% glycerol-peptone at -70°C.

Determination of antimicrobial susceptibility by Epsilometer test. The antibiotic susceptibilities to metronidazole, amoxicillin, tetracycline and clarithromycin were determined by the E test (AB Biodisk, Solna, Sweden). The frozen suspensions were inoculated on fresh Columbia agar plates containing 7% (vol/vol) horse blood and cultured under microaerophilic condition (jars with CampyGen, Oxoid, Basingstoke, United Kingdom) at 37°C. After 3 days, the *Helicobacter pylori* strains that had grown were collected with cotton swabs and resuspended in 2 ml of Dulbecco's modified Eagle medium (ICN Biomedicals. Inc). Columbia agar plates containing 7% (vol/vol) horse blood were flooded with 100ml of this suspension, at a turbidity equivalent to 2 to 3 on the McFarland scale, verified spectrophotometrically O.D.625 = 0.38-40 (corresponding to approximately 108 cfu/ml). When the plate surface was dry, an E test strip was placed on the plate according to the manufacturer's instructions. All plates were incubated for 3-5 days at 37°C under microaerophilic conditions. A control strain (ATCC 43504) with known susceptibility patterns was used in duplicate with each run. The MIC was defined as the concentration indicated on the E test strip that was closest to the point of intersection with growth on the plate. Where there was a haze of growth that could not be distinguished from the inoculum, the haze was discounted. Where the growth ended between MIC values, the next

Table 1 - Minimal inhibitory concentration of metronidazole, clarithromycin, tetracycline and amoxycilliin for 83 clinical isolate of Helicobacter pylori.

MIC* Mg/L	Antibiotics N (%)			
	Metronidazole	**Clarithromycin**	**Tetracycline**	**Amoxicillin**
<0.016	5 (6)	12 (14.5)	36 (43)	22 (26)
0.032	2 (2)	12 (14.5)	15 (18)	38 (46)
0.064	5 (6)	9 (11)	29 (35)	19 (23)
0.125	2 (2)	6 (7)	3 (4)	4 (5)
0.25	0	8 (10)	0	0
0.5	3 (4)	3 (4)	0	0
1	2 (2)	6 (7)	0	0
2	2 (2)	20 (24)	0	0
4	4 (5)	7 (8)	0	0
8	11 (13)	0	0	0
16	15 (18)	0	0	0
32	18 (22)	0	0	0
64	11 (13)	0	0	0
128	3 (4)	0	0	0
Total	83	83	83	83

*Resistance break point: >8 mg/L for Metronidazole and > 1 for Clarithromycin[15,16]
MIC-Minimum Inhibitory Concentration

higher value was recorded. In the absence of standard breakpoints, the breakpoints used to define a resistant strain in this study were based on a previous publication.[15,16] They were the following: metronidazole, >8 μg/ml; clarithromycin >1 μg/ml (0.25 to 1 μg/ml, intermediate). No breakpoint predefined for amoxicillin or tetracycline.

Results. A total of 83 *Helicobacter pylori* isolates were investigated; 52 (63%) from male and 31 (37%) from females. All the 83 strains of *Helicobacter pylori* were recovered from –70°C storage for inclusion in this study. All the MIC were read after 72 hours, there were no discrepancies between MIC values read at 3 days and 5 days of incubation. The antimicrobial susceptibility test results of all *Helicobacter pylori* strains are presented in Table 1. Resistance to metronidazole was found in 47 (57%) (MIC > 8 mg/L) isolates and resistance to clarithromycin in 27 (32.5%) (MIC > 1 mg/L) as seen from Table 2. Twenty of the 27 strains resistant to clarithromycin were also resistant to metronidazole, two of these exhibited high-level resistance (MIC 128 mg/L). No resistance to amoxicillin or tetracycline was observed. In an attempt to evaluate whether resistance to metronidazole affected the overall outcome of the disease and the severity, we investigated the endoscopy findings, the number of visits to the hospital and treatment given to those patients infected with metronidazole resistant *Helicobacter pylori*. Out of 47 patients infected by metronidazole-resistant *Helicobacter pylori* strains, 39 (83%) had a duodenal ulcer documented by endoscopy and attended the hospital with a mean of 6 visits, and complained of the same symptoms at the time of their first visit. None of the patients were given any antibiotics for their treatment, they were given H2 receptor antagonist only.

Discussion. The prevalence of *Helicobacter pylori* infection in Bahrain is very consistent with that of developing Countries.[17] We believe that the incidence is very high and further epidemiological studies are needed, especially among children, to assess the true incidence rate of infection. This has been the first study in Bahrain to determine the MIC for isolates of *Helicobacter pylori* using E tests. We evaluated the antibiotic susceptibility to four major antibiotics used in the treatment of *Helicobacter pylori*. The susceptibility testing of *Helicobacter pylori* is important in finding an effective antimicrobial agent that permits the eradication of this pathogen. The efficacy of treatment of gastric infection by *Helicobacter pylori* can be reduced by the occurrence of primary or acquired resistance to various drugs, especially metronidazole. Many studies have used an agar dilution method,[18,19] but this is difficult to perform 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.[13,14,16] We found the E test very easy to perform and not labor intensive. Although some workers showed discrepencies between E-test and agar dilution method for testing Metronidazole susceptibility of *Helicobacter pylori*,[20,21] 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 *Helicobacter pylori*, but resistance is well documented in western countries[16,18,22] and also in some developing countries.[8,9] In the present study the incidence of metronidazole resistance was 56.5%, a high percentage when compared to the developed countries. All the strains tested were fresh clinical isolates of *Helicobacter pylori* which were freshly subcultured as recommended by Weel et al.[23] We believe that the reason for this alarming pattern is the uncontrolled use of metronidazole, which is widely used in our country and may be obtained in drug stores without a prescription.

Two types of resistance to metronidazole must be considered: primary resistance supposedly linked to previous contact of *Helicobacter pylori* strains to nitroimidazoles during treatment for another infection unrelated to *Helicobacter pylori*, and secondary (acquired) resistance resulting from treatment regimen aimed to eradicate *Helicobacter pylori*. Our results clearly indicate that the resistance type in the strains tested is primary resistance, emphasized by the wide use of nitroimidazoles for the treatment of parasitic and gynecological infections in Bahrain, and the fact that none of our patients received any *Helicobacter pylori* eradication therapy.

The prevalence of clarithromycin resistance (32.5%) among *Helicobacter pylori* was high when compared with previous studies in developed countries.[16,22,24] In this study, 87% of clarithromycin-

Table 2 - Antibiotics resistance of Helicobacter pylori strains isolated from 83 patients.

Antibiotics	No. of resistance isolate	Percentage of resistant isolates
Amoxycillin	0	0
Clarithromycin	27	32.5
Tetracycline	0	0
Metronidazole	47	57

resistant strains were also resistant to metronidazole. The reasons for such double resistance are not clear, but it has been reported by another investigator.[25] Although antibiotic treatment was not reported in the clinical records of the patients infected by such strains, we believe that these finding may be related to inappropriate previous treatment. This might lead to the selection of strains with dual resistance that are difficult to eradicate and may contribute to the increase in the prevalence of clarithromycin resistance. Furthermore in our study resistance to metronidazole as well as clarithromycin was higher in patients with peptic ulcer.

Our data indicates that treatment outcome would benefit from susceptibility testing before starting therapy, especially when prescribing metronidazole. In comparison to clarithromycin and metronidazole, amoxicillin and tetracycline maintained their activity; none of the strains were resistant to these antibiotics. It may however may be only a matter of time until we see the first case of tetracycline resistance in Bahrain, as was demonstrated before by Midolo et al[26] in Australia.

There is a high risk of resistance development in *Helicobacter pylori* with increase duration of therapy. The recent triple or dual therapy for the eradication of Helicobacter pylori are highly effective and well tolerated, however therapeutic metronidazole resistance decreases the success of eradication significantly and reduces the eradication rate to only 50%.[27] In conclusion, susceptibility testing for therapy evaluation may be extremely helpful, particularly for patients in whom previous treatment to eradicate Helicobacter pylori has failed. Unified national treatment guideline for physicians who treat patients with Helicobacter pylori infection in Bahrain is needed. It is also deemed very important to institute national surveillance programs to follow the evolution of Helicobacter pylori resistance and to enable treatment regimens to be adapted in accordance with changes in resistance patterns.

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