Determination of *Helicobacter pylori* antibiotic resistance patterns in pediatric gastroenterology patients: the Hacettepe experience

Salih Maçin[1], Hülya Demir[2], Hasan Özen[2], Aysel Yüce[2], Yakut Akyön[1]

Departments of [1]Medical Microbiology and [2]Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey.
E-mail: salihmacin@hotmail.com

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In this study, our aim was to show the antibiotic resistance patterns of *Helicobacter pylori* (*H. pylori*) strains isolated from patients who had undergone esophagogastroduodenoscopy at Hacettepe University. Ninety-three culture-positive patients with no history of *H. pylori* treatment were included in the study. MIC values against clarithromycin, metronidazole, amoxicillin and tetracycline were evaluated by gradient strips. In the 93 strains, no resistance against tetracycline and amoxicillin was observed. Clarithromycin resistance was detected in 28 (30.1%) and metronidazole resistance in 45 (48.4%) patients' strains. Resistance to clarithromycin and metronidazole, respectively, was observed in three age groups as follows: in 3 (17.6%) and 5 (29.4%) strains in the 5–9 age group; in 13 (30.9%) and 16 (38.1%) strains in the 10–14 age group; and in 12 (35.3%) and 24 (70.6%) strains in the 15–19 age group. Antibiotic susceptibility testing prior to treatment would prevent the administration of useless treatments. It is therefore recommended that such testing be performed before planning the treatment.

Key words: Helicobacter pylori, antibiotic resistance, pediatrics.

The association between *Helicobacter pylori* and gastroduodenal diseases was established towards the end of the 20th century. This discovery by Warren and Marshall[1] has led to new and successful treatment options for a group of diseases with unknown etiology. *H. pylori* is closely associated with chronic gastritis, peptic ulcer disease, gastric cancer and primary gastric mucosa-associated lymphoid tissue (MALT) lymphoma; current treatment options that eradicate the organism decrease the recurrence of peptic ulcer disease[2].

The prevalence of *H. pylori* positivity is 20-90%, and this Gram-negative bacterium affects the gastric mucosa, leading to asymptomatic colonization and dyspepsia. The frequency of *H. pylori* varies depending on socioeconomic status, geographic region and age. The positivity rate among the general population is 60-80% in developing countries; the prevalence decreases to 5-10% in developed countries, which can be attributed to better sanitation and nutrition, and appropriate antibiotic usage[3].

It is widely accepted that *H. pylori* is acquired during childhood in developing countries and is thus considered a childhood disease. Therefore, appropriate early treatment in children with complaints would decrease serious *H. pylori*-related manifestations[3].

The standard treatment for eradication of *H. pylori* is a combination regimen of clarithromycin, amoxicillin and proton pump inhibitor. Antisecretory treatment aims to decrease stomach acidity in order to increase the efficacy of antibiotics. Another combination regimen used is metronidazole, tetracycline and proton pump inhibitor. Failure of the treatment to eradicate the organism may be due to lack of compliance on the part of patients, but the most important factor is antimicrobial resistance. Clarithromycin and metronidazole resistance have been reported to be in the

0-20% and 10-80% ranges, respectively[5,6]. Resistance to amoxicillin and tetracycline is rare. Antimicrobial susceptibility testing should be done before initiating treatment; this would be beneficial for patient care and also cost effective.

The aim of this study was to determine antibiotic susceptibility (i.e., to clarithromycin, metronidazole, amoxicillin and tetracycline) in *H. pylori* isolates of our pediatric patients using the gradient strip method.

Material and Methods

Patients

The study included 93 *H. pylori* strains isolated from the biopsy samples obtained during esophagogastroduodenoscopy of 93 pediatric patients who had been evaluated in the Hacettepe University Faculty of Medicine Department of Pediatric Gastroenterology for various gastrointestinal symptoms and had not been treated for *H. pylori* before the biopsy. The age range of the study group was 5-19. It was subdivided into three age groups: the 5–9 age group comprised 17 patients, the 10-14 age group, 42, and the 15-19 age group, 34. The mean age of the patients was 12.9 years; 48 (51.6%) patients were male and 45 (48.4%) female.

Between January 2006 and January 2012, 311 biopsy samples were cultured for *H. pylori*, and 167 (53.7%) cases were found to be positive. Ninety-three of the 167 (55.7%) patients, who had not been treated for *H. pylori* prior to esophagogastroduodenoscopy, were included in the study.

Isolation of Helicobacter pylori

Gastric biopsy specimens obtained by esophagogastroduodenoscopy were placed in 0.5 ml brain-heart-infusion (BHI) broth-containing microcentrifuge tubes and sent to the laboratory within four hours. The biopsy specimens were inoculated on 7% blood-containing BHI agar, which also contained vancomycin (10 mg/L), trimethoprim (5 mg/L), cefzulodine (5 mg/L) and amphotericin B (5 mg/L), and incubated at 35-37°C for five to seven days under microaerophilic conditions (8-10% CO_2, 5-6% O_2, 80-85% NO_2, 98% humidity). *H. pylori* colonies were identified based on Gram staining and urease, catalase and oxidase activity.

Antibiotic susceptibility tests

After identification, *H. pylori* colonies were subcultured onto antibiotic-free BHI agar and incubated at 35°C for 72 hours under microaerophilic conditions. Mueller-Hinton agar (MHA) containing 5% horse blood was used for antibiotic susceptibility testing. For bacterial inoculation, a 3 McFarland suspension was prepared in BHI broth. This suspension was spread over the agar plate, and a single gradient strip (Etest, bioMérieux, France) was placed on each 90 mm Petri dish. Plates were incubated under microaerophilic conditions at 35°C for three days. For testing amoxicillin susceptibility, a *H. pylori*-specific gradient strip with a broader gradient spectrum (0.002–32 μg/ml) was used. Strains with a clarithromycin minimum inhibitory concentration (MIC) value $\geq$1 μg/ml, a metronidazole MIC value $\geq$8 μg/ml, an amoxicillin MIC value $\geq$0.5 μg/ml and a tetracycline MIC value $\geq$1 μg/ml were considered resistant according to the *European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards*.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 15.0. A chi-square test was used to analyze between-group differences of the antimicrobial resistance of *H. pylori* for the three age groups. A p-value <0.05 was considered statistically significant.

Results

Among the 93 isolates included in this study, none were resistant to amoxicillin and tetracycline. Clarithromycin resistance was found in 30.1% and metronidazole resistance was found in 48.4% of the isolates. Eighteen strains (19.4%) were found to be resistant to both clarithromycin and metronidazole. Resistance to clarithromycin and metronidazole, respectively, was observed in the three age groups as follows: in 3 (17.6%) and 5 (29.4%) isolates in the 5–9 age group; in 13 (30.9%) and 16 (38.1%) isolates in the 10–14 age group; and in 12 (35.3%) and 24 (70.6%) isolates in the 15–19 age group.

When clarithromycin and metronidazole resistance were examined separately according

Table I. Clarithromycin and Metronidazole Resistance of *Helicobacter pylori* Strains According to Patient Age Groups

Age	Positive n (%)	Cl resistance n (%)	Met resistance n (%)	Cl+Met resistance n (%)
5-9	17 (18.3)	3 (3/17=17.6)	5 (5/17=29.4)	0
10-14	42 (45.2)	13 (13/42=30.9)	16 (16/42=38.1)	7 (7/18=38.9)
15-19	34 (36.5)	12 (12/34=35.3)	24 (24/34=70.6)	11 (11/18=61.1)
Total	93	28 (28/93=30.1)	45 (45/93=48.4)	18 (18/93=19.4)
p		0.61	0.15	0.015

$p<0.05$ was considered statistically significant.
Cl: Clarithromycin
Met: Metronidazole

to age group, the percentage of resistance increased with age, but this difference was not statistically significant (p=0.61 and p=0.15, respectively). Conversely, when clarithromycin and metronidazole resistance were evaluated together according to age group, the percentage of resistance also increased by age and in this case, the difference was statistically significant (p=0.015). The rate of antibiotic resistance among the isolates according to patient age is presented in Table I.

Discussion

After the association between *H. pylori* infection and gastric cancer/MALT lymphomas was established, the World Health Organization classified *H. pylori* as a class 1 carcinogen[4].

The standard regimen for *H. pylori* eradication is clarithromycin, amoxicillin and proton pump inhibitor. Antisecretory drugs are given to increase gastric pH in order to increase the efficacy of the antibiotics. Another treatment option is metronidazole, tetracycline and proton pump inhibitor. Reasons for the failure of such treatments include noncompliance on the part of the patient and resistance of *H. pylori* strains to antimicrobial agents. Correlation between antimicrobial drug use and the prevalence of antimicrobial resistance is reported worldwide[5].

The frequency of metronidazole resistance in *H. pylori* is reported to be 15.9-77.9% and varies in different geographic locations[6-8]. Due to the widespread use of metronidazole, the frequency of resistance has also increased in Turkey. Studies conducted in Turkey have reported 36.4–62.5% resistance rates[9,10]. In this study, metronidazole resistance was 48.4%; this high prevalence was not unexpected, and was compatible with the literature.

Resistance to amoxicillin and tetracycline are rare. Antibiotic susceptibility testing before initiating treatment would be beneficial for patient care and would enhance cost effectiveness. A study conducted by Aguda et al.[11] revealed no resistance of *H. pylori* to amoxicillin, rifampicin and tetracycline. In another study, by Vécsei et al.[6], there was likewise no finding of amoxicillin resistance; however, tetracycline resistance was found to be 0.9%. The present study was conducted in pediatric patients who had not had *H. pylori* treatment and found no amoxicillin and tetracycline resistance in *H. pylori*.

Clarithromycin is the antibiotic most frequently used to eradicate *H. pylori*. Clarithromycin resistance was reported to be 34% in Austria, 23.7% in Belgium, 54.6% in Spain and 13.5% in Taiwan[5,7,11,12]. In two recent reports from Turkey, clarithromycin resistance was found to be 18.7% in a study by Goral et al.[13] and 54.5% in a study by Baglan et al.[14]. Clarithromycin resistance was also found to be high in our study (30.1%).

Performing susceptibility tests before treatment would prevent unnecessary treatment efforts, and patients would benefit from more accurate therapy planning. Such testing would also prevent unwanted side effects from inappropriate antibiotic therapy. Resistance to clarithromycin and metronidazole is increasing rapidly in developing countries, including Turkey, thereby decreasing the eradication rate in patients treated with these regimens. In patients in whom eradication therapy is unsuccessful, a

quadruple regimen including bismuth salts, proton pump inhibitor and two antibiotics may be tried[15]. In addition, quinolones are recommended for treatment of *H. pylori* in adults. However, the utility of these drugs in pediatric *H. pylori* eradication therapy is limited[16]. Unnecessary antibiotherapy should be avoided to slow the development of resistance. Frequent checks of *H. pylori* resistance rates in index centers can lead to the development of specific treatment protocols for each region or country.

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