Primary and Secondary Antibiotic Resistance of *Helicobacter pylori* in Israeli Children and Adolescents

Michal Kori MD[1,4], Jacob Yahav MD[4], Rita Berdinstein MD[2,4] and Haim Shmuely, MD[3,4]

[1]Department of Pediatrics, Pediatric Gastroenterology Unit, [2]Microbiology Laboratory, and [3]Department of Internal Medicine D, Helicobacter Institute, Kaplan Medical Center, Rehovot, Israel

[4]Faculty of Medicine Hebrew University, Jerusalem, Israel

ABSTRACT: **Background:** Empiric treatment for *Helicobacter pylori* is influenced by antibiotic susceptibility of infecting strains. A rise in the resistance rate to clarithromycin and metronidazole has been reported in pediatric populations.

Objectives: To assess the primary and secondary antibiotic resistance of *H. pylori* isolates in Israeli children and adolescents.

Methods: A retrospective review of *H. pylori* isolates cultured from antral biopsies of consecutive children aged 1 to 18 years, who were referred to the Pediatric Gastroenterology Unit, Kaplan Medical Center, over a 2.8 year period, was performed. Antibiotic susceptibility to clarithromycin, metronidazole, amoxicillin, tetracycline, and levofloxacin was determined by E-test. Data on the age of the patient, indication for endoscopy, and antibiotic treatment for *H. pylori* in previously treated children was collected.

Results: Cultures for *H. pylori* yielded 123 isolates. In children not previously treated (n=95), the primary global resistance was 38% with resistance to clarithromycin 9.5%, metronidazole 32.6 %, and to both 4.2%. Respective rates of resistance in previously treated children (n=28) were 71% (P = 0.002), 29% (P = 0.02), and 61% (P = 0.007). Simultaneous resistance to both drugs was found in 18% (P = 0.02). All *H. pylori* strains were susceptible to amoxicillin, tetracycline, and levofloxacin. Past eradication treatment was the only independent risk factor for antibiotic resistance in multivariate analysis.

Conclusions: Significantly higher resistance rates were found in previously treated patients, stressing the need to refrain from empiric treatment using the "test and treat strategy." Culture-based treatment strategy should be considered in all previously treated children.

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KEY WORDS: antibiotic resistance, primary resistance, secondary resistance, children and adolescents, *Helicobacter pylori*

Recent studies have documented a high *Helicobacter pylori* (*H. pylori*) resistance rate to clarithromycin and metronidazole in pediatric populations [1-4]. The rise in antibiotic resistance has been attributed to resistant strains and poor adherence to treatment protocols. Although *H. pylori* infection is acquired in childhood, compared to adults, most children are asymptomatic. Complications such as peptic ulcers are relatively rare. Mucosa associated lymphoid tissue (MALT) lymphoma is extremely rare and gastric cancer is unknown in children [5,6]. The indications for treatment of *H. pylori* infection in children are limited. The European Society for Paediatric Gastroenterology Hepatology and Nutrition/North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN/NASPGHAN) guidelines recommend treatment only when *H. pylori* is detected by a biopsy-based method, yet many children are still receiving treatment using the test-and-treat strategy. These children receive empiric treatment for *H. pylori* because no information on antibiotic resistance is available that contributes to the rise in antibiotic resistance.

A multinational European study completed in 2002 reported a primary resistance rate to clarithromycin and metronidazole to be 20% and 23%, respectively [7]. In a 10 year study of French children by Kalach and colleagues (1995–2005) [8], clarithromycin and metronidazole resistance rates were 23% and 32%, respectively. Between 2005 and 2007, Israeli children's *H. pylori* resistance rate to clarithromycin and metronidazole was 25% and 19%, respectively [4]. In 2014, Peretz and co-authors [9] reported a resistance rate of 25% to both drugs in Israeli naïve children.

A recent study of Israeli adults reported a high secondary resistance of 57.2% to clarithromycin and 64.4% to metronidazole, which has not increased over the past few years [10]. Respective resistant rates to clarithromycin and metronidazole in children previously treated for *H. pylori* infection were 42% and 52%, respectively, with a simultaneous resistance to both drugs of 13% [4]. Primary *H. pylori* resistance in children adversely affects successful eradication. High secondary resistance following treatment failure suggests a development of resistant strains common in children [6].

The primary aim of our study was to assess the current antibiotic susceptibility of *H. pylori* isolates taken from consecutive gastric biopsies of Israeli children. Our secondary aim was to compare antibiotic susceptibility between previously treated

patients and treatment naïve patients. The last goal was to determine whether there has been a change in *H. pylori* antibiotic susceptibility rates in Israel over time.

PATIENTS AND METHODS

This retrospective study was performed on a cohort of consecutive children from central Israel aged 1–18 years old, who had undergone an upper endoscopy at the Pediatric Gastroenterology Unit, Kaplan Medical Center between January 2013 and August 2015.

Children underwent an upper endoscopy for the following indications: abdominal/epigastric pain, anemia, vomiting, suspected celiac disease, suspected inflammatory bowel disease, and other causes. Gastric biopsies were taken from the antrum for histologic examination and *H. pylori* culture. Data regarding the age of the patient and previous treatment for *H. pylori* were collected from the patient's chart. Excluded were patients who had received antibiotic treatment during the previous month or had received H2 blockers or a proton pump inhibitor (PPI) during the previous 2 weeks.

PROCEDURE

The antral biopsy specimen for culture was placed on a Helicobacter plate (Hy Laboratories Ltd, Rehovot, Israel) and processed within 2 hours. The specimen, homogenized with a tissue grinder, was inoculated onto trypticase soy agar plates supplemented with 7.5% sheep blood. The plates were then incubated at 37°C under microaerobic conditions for ≥ 7 days. *H. pylori* isolates were identified by colony morphology, characteristic spiral morphology on gram staining, and positive findings on catalase, urease, and oxidase tests.

MIC DETERMINATION

Susceptibility to five antibiotic agents (amoxicillin, clarithromycin, metronidazole, tetracycline, and levofloxacin) was tested using the E-test (AB Biodisk, Solna, Sweden). The E-test strips were placed on agar plates when the surface was dry. The minimum inhibition concentration (MIC) values were determined after 72 hours of incubation according to the instructions of the E-test. Resistance was defined as follows: amoxicillin, MIC ≥ 1.5 mg/L; tetracycline, MIC ≥ 4 mg/L; clarithromycin, MIC ≥ 2 mg/L; metronidazole, MIC ≥ 8 mg/L; and levofloxacin, MIC ≥ 2 mg/L. The *H. pylori* strain ATCC 43526 was used for quality control of the selective medium. ATCC 43504 was used for quality control of the susceptibility tests.

In our microbiology laboratory, the accuracy of the E-test was: metronidazole, mean = 0.0288, standard deviation (SD) = 0.0067; clarithromycin, mean = 0.0240, SD = 0.0084; tetracycline, mean = 0.0208, SD = 0.0077; and amoxicillin, mean = 0.0256, SD = 0.0083.

ETHICAL CONSIDERATIONS

The study was approved by the hospital's institutional review board.

STATISTICAL ANALYSIS

Categorical variables are reported as frequency and percentages. Age was tested for normal distribution using a histogram and reported as medians and interquartile ranges (IQR). Categorical variables were compared using the chi-square or the Fisher exact test and continuous variables by the Mann–Whitney test. Univariate and multivariate logistic regression were performed to evaluate the association between past treatment and antimicrobial resistance. The multivariate regression included age and gender. A two-tailed $P < 0.05$ was considered statistically significant. Statistical analyses were performed using IBM SPSS statistics software, version 21 (IBM Corp, Armonk, NY, USA).

RESULTS

The study group consisted of 323 children. Culture results were positive in 123 out of 137 patients with histologically documented *H. pylori* infection (89.7%). The indications for endoscopy included epigastric/abdominal pain (162, 50.2%), anemia (14, 4.3%), vomiting (27, 8.4%), suspected celiac disease (74, 22.9%), suspected inflammatory bowel disease (31, 9.6%), and other (15, 4.6%). The median age and IQR for 123 *H. pylori* culture positive patients was 14 years (9.0–16.0); 56 (45.5%) were males.

Among all *H. pylori* isolates, the rate of resistance to antibiotics was 45.5%. Resistance to clarithromycin was 13.8% and to metronidazole it was 39%. Simultaneous resistance to both drugs was 7.3%. No resistance was found to amoxicillin, tetracycline, and levofloxacin. The prevalence of *H. pylori* resistance to clarithromycin and metronidazole in the naïve (n=95) and previously treated (n=28) patients are shown in Table 1. Naïve children, 36/95 (38%), demonstrated an antibiotic resistance to at least one drug compared to 20/28 (71%) in previously treated children for *H. pylori* ($P = 0.002$).

Among isolates from the treatment-naïve patients, the rate of resistance to clarithromycin was 9.5% and to metronidazole it was 32.6%. Corresponding rates among the isolates from patients

Table 1. Resistance of *Helicobacter pylori* to clarithromycin and metronidazole

	All strains (n=123)		Naïve patients (n=95)		Patients previously treated for *H. pylori* (n=28)		*P*
Clarithromycin resistance, n (%)	17	(13.8%)	9	(9.5%)	8	(28.6)	0.024
Metronidazole resistance, n (%)	48	(39.0%)	31	(32.6%)	17	(60.7%)	0.007
Clarithromycin and metronidazole resistance, n (%)	9	(7.3%)	4	(4.2%)	5	(17.9%)	0.028
Clarithromycin or metronidazole resistance, n (%)	56	(45.5%)	36	(37.9%)	20	(71.4%)	0.002

Table 2. A multivariate model of risk factors independently associated with an increased risk of *Helicobacter pylori* resistance

Type of analysis	Risk factor	Odds ratio	95% confidence interval	*P*
Univariate	Past treatment	4.09	1.63–10.26	0.003
Multivariate	Male gender	0.89	0.42–1.91	0.782
	Age	1.01	0.93–1.11	0.692
	Past treatment	3.92	1.53–9.99	0.004

previously treated for *H. pylori* were 28.6% ($P = 0.024$) and 60.7% ($P = 0.007$). The prevalence of *H. pylori* resistance to both clarithromycin and metronidazole in the naïve was 4.2% and 17.9% in previously treated patients ($P = 0.028$) [Table 1]. Multivariate logistic regression showed that only past eradication treatment was a risk factor for antibiotic resistance [Table 2].

DISCUSSION

Antibiotic resistance remains an important limiting factor for treatment success. Resistance patterns vary between countries. Surveillance of antibiotic resistance in different geographic areas is recommended. In this retrospective study, we were able to determine the role of previous antibiotic treatment in the development of *H. pylori* resistance.

We demonstrated that in the 28 children who had previously received standard triple *H. pylori* eradication treatment, resistance rates to antibiotics were extremely high (> 70%) compared to 38% in treatment-naïve children. In our study, the primary resistance rates to clarithromycin in naïve children were relatively low (9.5%) compared to previous data, in which the resistance rate in naïve children was close to 20% [4]. The primary metronidazole resistance rate, 33%, found in this study was similar to that detected in Israel [4,9] and reported in other European countries [11-17]. The increasing rates of resistance to clarithromycin worldwide is due to the use of new macrolides in treating upper respiratory infections, acute otitis media, and community-acquired pneumonia by children and adults from the same region. As in other developing countries [18,19], metronidazole resistance rates are relatively high and associated with a greater use of the drug in the treatment of intestinal parasitic and gynecological infections.

In previously treated children, we found a relatively high rate of double resistance (18%) to both clarithromycin and metronidazole. Our findings are consistent with other Israeli reports [4,9] and previous pediatric studies in European centers [7,8,13], thus, reconfirming a low resistance to amoxicillin. As for tetracycline, its use is limited to children < 8 years of age to prevent tooth enamel staining, although it remains a therapeutic option for older adolescents. We found no association between time of culture, gender, or age and resistance to any of the antibiotics tested, in concurrence with previous reports.

The indications for treatment of *H. pylori* infection in the pediatric population are more limited than in adults, thus high *H. pylori* resistance rates in previously treated children are of concern. The ESPGHAN/NASPGHAN guidelines recommend treatment only when *H. pylori* is detected by a biopsy-based method. They also recommend susceptibility testing for clarithromycin before initial clarithromycin-based triple therapy in areas/populations with a known high resistance rate of > 20% [6]. Nonetheless, many primary physicians still recommend empiric treatment for *H. pylori* in children after a confirmed diagnosis by C13 urease breath tests or stool antigen testing. The test-and-treat strategy is presently being utilized in many children. This strategy may not be effective in high-prevalence, low-income settings, especially among children, due to high recurrence rates (20% < 10 years) as shown in Bolivia [19]. Most children diagnosed by a pediatric gastroenterologist via endoscopy receive empiric treatment since most laboratories worldwide do not perform cultures. In settings where antibiotic susceptibility testing is unavailable, epidemiologic and regional susceptibility data, such as shown in our study, are essential.

All children in this study received triple standard treatment. A marked decrease in the eradication rates of *H. pylori* infection with standard triple therapy has been observed worldwide. In a prospective, randomized study, the efficacy of sequential versus standard triple therapy in the context of clarithromycin resistance was compared. Sequential therapy offered only a small advantage over standard triple therapy in the eradication of clarithromycin-resistant strains [20].

The present study suggests that the development of secondary antibiotic resistance is common in Israeli children, thus, we recommend that treatment be given only when *H. pylori* is detected by a biopsy-based method using an *H. pylori* culture with antibiotic sensitivity testing, whenever possible.

The primary clarithromycin resistance rate in our study was relatively low, only 9%, thus in cases in which antibiotic sensitivity testing canot be performed, we recommend clarithromycin-based triple therapy as an empiric first-line therapy, which is still recommended in areas where clarithromycin resistance rates are < 20% [6].

Based on our results, resistance to metronidazole and clarithromycin in previously treated children was significantly higher compared to naïve children, thereby demonstrating that the choice of empiric second-line therapy must take into account the initial therapy administered and avoid readministering an antibiotic previously provided [21]. Despite a high rate of metronidazole resistance, no significant differences were reported between metronidazole-sensitive strains and metronidazole-resistant strains after quadruple therapy [22]; therefore, quadruple therapy (PPI + metronidazole + amoxicillin + bismuth) may be recommended as second-line therapy in concordance with most guidelines [5,23]. Levofloxacin regimen

as a second-line therapy in children is limited and thus is not recommended.

STUDY LIMITATIONS

Since only antral biopsies for *H. pylori* susceptibility testing was performed in this retrospective study, we might have missed 10–15 % of resistant *H. pylori* strains, as the infection may consist of more than one strain with different antibiotic susceptibility between the antrum and corpus [24].

CONCLUSIONS

In conclusion, significantly high rates of resistance to antibiotics were found in children who were previously empirically treated for *H. pylori*, stressing the need to refrain from the test-and-treat strategy. According to our findings, a cultured-based treatment strategy should be considered for all children. If primary culture and sensitivity testing is unavailable for naïve children, we recommend using clarithromycin-based triple therapy as a first-line therapy. Prospective *H. pylori* culture studies in Israeli children are warrented to confirm our results.

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Correspondence

Dr. H. Shmuely

Dept. of Medicine D, Kaplan Medical Center, Rehovot 76100, Israel.

Phone: (972-8) 944-1996

Fax: (972-8) 944-1866

e-mail: hshmuely@zahav.net.il

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Capsule

Fetal protection from maternal immunity

Recurrent miscarriages can be caused by a breakdown in immune tolerance between the mother and the fetus. **Li** et al. found that the surface levels of the receptor Tim-3 were increased on circulating natural killer (NK) cells in the first trimester of pregnancy. In contrast to NK cells from women with normal pregnancies, those from patients with recurrent miscarriages had decreased Tim-3 levels and were defective in immune suppression. Abortion-prone mice were protected from fetal loss if given Tim-3-positive NK cells, but not if given NK cells lacking Tim-3.

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