

## Treatment Regimens for *Helicobacter pylori* Infection in Children: Is *In Vitro* Susceptibility Testing Helpful?

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### ABSTRACT

**Background:** Treatment regimens for *Helicobacter pylori* have variable success rates, and data comparing effectiveness with respect to strain sensitivity are relatively scarce.

**Objective:** To evaluate the efficacy of two treatment regimens for eradication of *H. pylori* and the impact of bacterial susceptibility testing.

**Study Design:** 265 children endoscopically diagnosed with *H. pylori* infection were randomly assigned to receive omeprazole + amoxicillin with clarithromycin or omeprazole + amoxicillin with metronidazole. Bacterial culture and susceptibility was performed in a subgroup. Eradication was assessed by <sup>13</sup>C-urea breath test.

**Results:** Eradication was achieved in 73.4% by omeprazole + amoxicillin with metronidazole and in 62.6% by omeprazole + amoxicillin with clarithromycin ( $P = 0.078$ ). *H. pylori* was cultured successfully in 105 patients. Resistance to metronidazole was detected in 31.4% of the isolates and resistance to

clarithromycin in 15%. Eradication rate by omeprazole + amoxicillin with metronidazole for metronidazole-susceptible bacteria ( $N = 38$ ) was 90%, and for resistant bacteria ( $N = 19$ ) it was 42%. Only 75% of clarithromycin-sensitive strains were successfully treated by omeprazole + amoxicillin with clarithromycin, and none of the cases with clarithromycin-resistant strains responded to omeprazole + amoxicillin with clarithromycin treatment.

**Conclusion:** There is a trend of greater efficacy of eradication with omeprazole + amoxicillin with metronidazole versus omeprazole + amoxicillin with clarithromycin therapy. Although resistance negatively influences eradication, first-line sensitivity-based treatment would be expected to improve this rate only slightly. Susceptibility testing should probably be reserved only for treatment failures. *JPGN* 40:571–574, 2005. **Key Words:** Gastritis—Abdominal pain—*Helicobacter pylori*—Clarithromycin—Metronidazole. © 2005 Lippincott Williams & Wilkins

### INTRODUCTION

*Helicobacter pylori* infection plays a major role in the etiology of chronic gastritis and duodenal ulcers in children (1). Consensus reports recommend endoscopy as the preferred method for investigation in children with upper digestive tract symptoms suggestive of organic disease. They also propose treatment according to *in vitro* antibiotic sensitivities if the first treatment regimen fails to achieve eradication (2–4). First-line eradication therapy for *H. pylori* infection usually consists of a proton pump inhibitor and amoxicillin plus one of two antibiotics: clarithromycin or metronidazole (5). There are no randomized controlled treatment trials in children comparing clarithromycin to metronidazole, but open studies using adult treatment regimens have reported variable rates of efficacy (6–8). Open trials in children of

treatment regimens over 1 to 2 weeks have shown rates of successful eradication varying from 56% to 96% (6–13). Most of the studies published so far have been small, non-randomized series, and the majority did not assess strain sensitivity, which may be important for successful treatment.

*H. pylori* resistance to commonly used antibiotics, which has been increasing worldwide, varies between 11% and 70% for metronidazole and between 7% and 45% for clarithromycin (10,11,13–17). It has been shown that antimicrobial resistance *in vitro* may predict treatment failure (18–20).

The aim of this prospective randomized study was to assess the efficacy of combined treatment with a proton pump inhibitor and amoxicillin plus either clarithromycin or metronidazole in children and to correlate efficacy with *in vitro* antibiotic resistance of bacterial isolates.

### MATERIALS AND METHODS

Between 1999 and 2002 all children who were evaluated for recurrent abdominal pain by an upper endoscopy had gastric

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biopsy specimens taken for a rapid urease test (CLO test; Ballard Medical Products, Draper, UT) and for histology.

Biopsy specimens for histology were fixed in formalin, embedded in paraffin and sectioned and stained with hematoxylin and eosin and with a polyclonal rabbit anti-*Helicobacter pylori* immunohistochemical stain (DakoCytomation, Denmark).

From the year 2000 specimens were also sent for culture and susceptibility testing. The specimens were transported to the microbiology laboratory within 1 hour. They were homogenized and plated on Skirrow agar medium containing 7% horse blood, vancomycin (10 µg/mL), polymyxin B (2.5 µg/mL) and trimethoprim (5 µg/mL) and on Dent agar medium containing 7% horse blood, vancomycin (10 µg/mL), trimethoprim (5 µg/mL), cefsulodin (5 µg/mL) and amphotericin B (5 µg/mL). The plates were incubated at 37°C under microaerophilic conditions in an atmosphere of 5% to 7% O<sub>2</sub>-5% CO<sub>2</sub> (Campy-Pak Plus; Becton Dickinson Microbiology Systems, Sparks, MD) at 35°C and 100% relative humidity and were examined periodically during days 3 to 7 of incubation (21). The organisms were identified as *H. pylori* by colony morphology, Gram stain and positive urease test. Antimicrobial testing was performed on all isolates identified as *H. pylori*.

Susceptibility testing of *H. pylori* was performed by E-test (AB Biodisk, Solna, Sweden) following the manufacturer's instructions. *H. pylori* strains were classified as resistant to metronidazole when the minimal inhibitory concentration was greater than 8 µg/mL and resistant to clarithromycin if the minimal inhibitory concentration was greater than 1 µg/mL. Amoxicillin resistance was defined as a minimal inhibitory concentration of greater than 0.50 µg/mL. The E-test has been validated as a preferred method to test sensitivity, mainly for metronidazole (21,22).

All patients came through clinics served by Shaare Zedek Medical Center. Patients were allocated to the different treating physicians (ER, JF and MW) on a basis of "first come, first serve" without preference for any physician.

Patients with a positive rapid urease test were assigned for *H. pylori* treatment. Treatment regimens consisted of omeprazole 10 mg twice daily (15 to 30 kg) or 20 mg twice daily (>30 kg) + amoxicillin (25 mg/kg) administered twice daily and one of the following: metronidazole (20 mg/kg) bid (OAM group) or clarithromycin (15 mg/kg) bid (OAC group). Two of the gastroenterologists (JF, ER) preferred OAM as the first-choice treatment, whereas the other (MW) chose OAC as the treatment of choice. Compliance was assessed at a follow-up visit in all patients. The <sup>13</sup>C urea breath test was performed at least 4 weeks after cessation of antibiotic therapy to assess *H. pylori* eradication.

Statistical analysis was performed using Fisher's exact test; a two-tailed *P* value of 5% or less was considered statistically significant.

This study was approved by the Ethics Committee of Shaare Zedek Medical Center.

## RESULTS

In all, 265 patients, aged 4.4 to 18 years, with biopsy-proven *H. pylori* infection were included in the study. There were no significant demographic differences between the two treatment groups (Table 1).

Eradication rate, as defined by a negative <sup>13</sup>C urea breath test, was 73.4% (116 of 158) in the OAM group

**TABLE 1.** Demographic characteristic of study population (n = 265)

	OAM (n = 158)	OAC (n = 107)	<i>P</i> value
Sex (male/female)	77/81	45/62	NS
Median (range) age (years)	12.0 (4.6–18)	12.9 (4.4–18)	NS
Ethnicity (Jewish/Non-Jewish)	138/20	94/13	NS

compared with 62.6% (67 of 107) in the OAC group (*P* = 0.078).

Antimicrobial susceptibility was assessed in 105 isolates; 33 isolates (31.4%) were resistant to metronidazole and 16 (15.2%) were resistant to clarithromycin. These numbers include seven isolates that were resistant to both drugs. Five strains were resistant to amoxicillin, and only one strain was resistant to tetracycline.

In patients with metronidazole-sensitive strains, eradication by metronidazole was more successful than clarithromycin treatment in patients with clarithromycin-sensitive strains. In patients with metronidazole-resistant strains, eradication was successful in almost half of the patients (Table 2).

## DISCUSSION

This prospective randomized study compared efficacy of two *H. pylori* treatment regimens in a large pediatric cohort. We also compared outcome with respect to antibiotic resistance of *H. pylori* isolates in a large subgroup. Table 3 presents a comparison of several previous pediatric studies to our own. Our data show that empirical triple therapies with either metronidazole or clarithromycin achieve comparable eradication rates with a trend towards advantage for metronidazole, without statistical significance.

Triple therapy including clarithromycin achieved only 62.6% eradication in our study, less than the 74.2% to 81% reported in controlled trials in pediatric patients (9,10). This finding probably reflects our relatively high resistance rate to clarithromycin and may be a result of the geographic variation of the *H. pylori* genotype (23). Thus, it is important to perform similar studies in different geographic areas.

**TABLE 2.** *Helicobacter pylori* eradication rate according to antibiotic susceptibility (n = 105)

	Sensitive strain*		Resistant strain*		<i>P</i> value
	N	No. eradicated (%)	N	No. eradicated (%)	
OAM	38	34 (89.4)	19	8 (42)	<0.001
OAC	44	33 (75)	4	0	0.008

\*To metronidazole or to clarithromycin respectively.

OAM, omeprazole, amoxicillin, metronidazole; OAC, omeprazole, amoxicillin, clarithromycin.

TABLE 3. Helicobacter pylori pediatric treatment trials

Author/Study	N	Treatment	Eradication (%)	Eradication in sensitive strains	Eradication in resistant strains
Gottrand et al <sup>9</sup>	63	PPI + amoxicillin + clarithromycin	74.2	NA***	2/2
Tiren et al <sup>7</sup>	32	PPI + amoxicillin + clarithromycin	75	NA	NA
Street et al <sup>10</sup>	75	Susceptibility-based protocol	96	NA	NA
	75	PPI + amoxicillin + clarithromycin	81		
Shashidhar et al <sup>6</sup>	28	PPI + amoxicillin + clarithromycin	56	NA	NA
Kato et al <sup>11</sup>	36	PPI + amoxicillin + clarithromycin	81	89%	56%
Kalach et al <sup>13</sup>	61	PPI + amoxicillin + clarithromycin	83.3	50/50*	0/11
Lopez-Brea et al <sup>8</sup>	57	Bismuth + amoxicillin + metronidazole	78.9	38/43**	7/14
Raymond et al <sup>12</sup>	23	PPI + amoxicillin + metronidazole	83.3	14/17**	1/6
		PPI + macrolide + metronidazole	63.6		
Faber et al (the present study)	158	PPI + amoxicillin + metronidazole	73.4	34/38**	8/19
	107	PPI + amoxicillin + clarithromycin	62.6	33/44*	0/4

PPI, proton pump inhibitor.

\*Clarithromycin sensitive; \*\*metronidazole sensitive; \*\*\*not available.

In the 105 patients in whom *in vitro* sensitivity was examined, metronidazole was effective in 90% of those with a metronidazole-sensitive strain. However, even with resistant strains, eradication was achieved in more than 40% of the patients. This is consistent with several studies that have found 83% to 88% eradication using metronidazole in sensitive strains and 17% to 50% eradication in resistant strains (8,12).

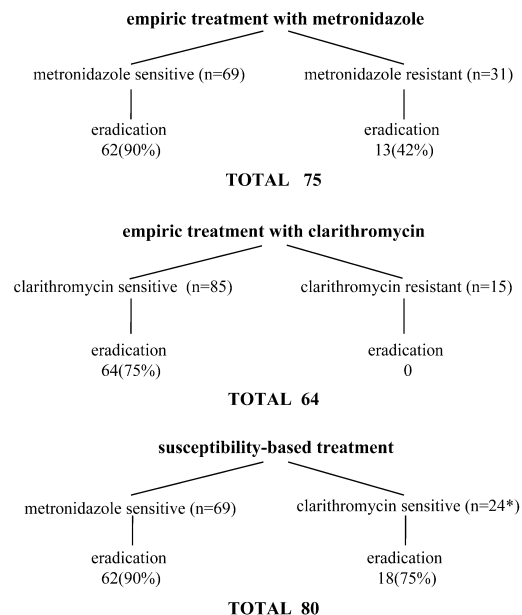
Clarithromycin was effective in 75% of clarithromycin-sensitive strains and failed to achieve eradication in all cases of clarithromycin resistance. This finding concurs with Kalach et al. (13), who reported complete treatment failure when clarithromycin was used against resistant strains. It thus appears that the effect of resistance on treatment outcome is less pronounced for metronidazole than for clarithromycin. Dore et al. (18) also showed that clarithromycin resistance reduced efficacy by an average of 55%, compared with metronidazole resistance, which reduced efficacy by only 37.7%.

A possible explanation for our observation may lie in the mechanisms of resistance of *H. pylori* to clarithromycin and metronidazole. Resistance to clarithromycin is the result of a mutation in the 23S ribosome gene impairing clarithromycin binding and rendering the drug totally ineffective. Metronidazole resistance, on the other hand, is much more heterogeneous, with various mutations in the rdx gene, and shows a much wider range of minimal inhibitory concentrations (24). Thus, *in vitro* resistance may not always be predictive of *in vivo* treatment failure.

We suggest another intriguing possibility, based on the observation by Olson et al. (25) that *H. pylori* uses molecular hydrogen as an energy-yielding substrate. They postulated that the source of the hydrogen in gastric mucosa is a product of carbohydrate metabolism by normal colonic flora. It is possible that metronidazole, by reducing colonic anaerobic flora, will decrease the availability of hydrogen, which is an important growth

factor for *H. pylori*. It may be worthwhile studying colonic hydrogen production in humans before and after treatment with metronidazole and to correlate it with *H. pylori* eradication.

Based on the current results we must question whether *in vitro* susceptibility testing is mandatory in first-line treatment. We constructed a hypothetical arithmetical model based on our observation (Figure 1). Of 100 *H. pylori*-positive patients who would be treated initially with OAM, we would expect eradication in 75 patients. If the same 100 patients would be treated with either OAM or OAC, based on microbial sensitivity, eradication



\* of the 31% of the patients with metronidazole resistant strain 6.6% are also resistant to clarithromycin

FIG. 1. Hypothetical treatment strategies and outcome of 100 *H. pylori* positive patients.

would be expected in 80 patients. The few patients with resistance to both drugs are not likely to benefit from either drug. Thus, only 5% of the patients are expected to benefit from a sensitivity-based treatment protocol. The cost-effectiveness of this approach is, therefore, questionable. If, however, OAC treatment is preferred as the initial treatment, a case could be made for susceptibility testing, as only 64 patients of the 100 would be cured (75% of 85 clarithromycin-sensitive strains and none of the 15 clarithromycin-resistant strains versus 80 in the sensitivity-based protocol).

In conclusion, efficacy of treatment protocols for eradication of *H. pylori* is very variable and certainly depends upon a diversity of factors including host, environmental and microbial. Our results demonstrate a trend of greater efficacy with a metronidazole-based regimen versus a clarithromycin-based regimen. Also, the benefit of a sensitivity-based protocol, as opposed to empiric metronidazole therapy, would be marginal but might be justified if one chooses a clarithromycin-based protocol.

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