

Antibiotic resistance and antibiotic sensitivity based treatment in *Helicobacter pylori* infection: advantages and outcome

M E Street, P Caruana, C Caffarelli, W Magliani, M Manfredi, F Fornaroli, G L de'Angelis

Abstract

Aims—To compare two strategies for the eradication of *Helicobacter pylori* infection.

Methods—Groups 1 and 2 each consisted of 75 consecutive patients. Patients in group 1 were treated with two antibiotics based on antibiotic susceptibility testing; those in group 2 received amoxicillin and clarithromycin for eight days, together with either ranitidine or omeprazole. Eradication rate was assessed in both groups six months after treatment.

Results—In group 1, *H pylori* grew in culture in 63/75 cases. Susceptibility testing showed that 35/63 isolates were resistant to metronidazole, 10/63 to clarithromycin, 2/63 to ampicillin, 1/63 to tetracycline, and 5/63 to both clarithromycin and metronidazole. In group 1 the infection was eradicated in 96% of the initial 75 subjects, and in 98% of the subjects treated according to the antibiotic assay (62/63). As two patients were lost at follow up the overall eradication rate was 99%. In group 2, eradication was achieved in 61/75 subjects (81%). This was significantly lower than the percentage of eradication observed in group 1 (81% versus 99%).

Conclusions—Antibiotic susceptibility tests are useful in childhood as a very high percentage of subjects are cured. This approach is costly, but selective antibiotic treatment contributes to limit further development of antibiotic resistance, and money is saved in terms of reinvestigation and further repeated treatments.

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Keywords: *H pylori*; antibiotic resistance; antibiotic susceptibility assay; eradication

Helicobacter pylori is an important bacterial pathogen. It is widely believed that this common infection should be eradicated because it has a key role in several gastric and duodenal diseases, including peptic ulceration and gastric cancer.^{1 2}

Combined antibiotic therapy is routinely used, but antibiotic resistance is an increasing problem, and *H pylori* may be resistant to commonly used antibiotics such as metronidazole, clarithromycin, and occasionally amoxicillin.³⁻¹³ In 1996-97, the reported eradication rate of *H pylori* in children treated with triple therapy (antisecretory agent and two

antibiotics) for at least two weeks ranged from 70% to 80%.¹⁴ The standard criteria for treatment of adults are those published by the 1996 Digestive Health Initiative International Update Conference¹⁵ and by the 1997 Maastricht Consensus Report.¹⁶ However, these are not entirely applicable to children.

The aim of the present study was to assess the efficacy of combined antibiotic treatment based on the results of antibiotic susceptibility testing using endoscopic gastric biopsy specimens from children with *H pylori* infection, versus standard blind triple therapy.

Materials and methods

A total of 150 children with *H pylori* infection were enrolled consecutively in the study, over a period of two years (January 1997 to December 1998).

The first 75 subjects (group 2) were treated with a standard antibiotic therapy regimen because antibiotic susceptibility testing was not available at that time. The following 75 (group 1) were treated if possible with an antibiotic regimen based on the results of antibiotic susceptibility testing. All patients had symptoms and/or positive serology and/or positive urea breath test at presentation.

Group 1 consisted of 75 patients (age range 2-17 years, 45 males, 30 females). Group 2 consisted of 75 subjects (age range 3-18 years, 49 males, 26 females). The diagnosis of *H pylori* infection was based on the endoscopic appearance of the gastric mucosa, gastric histology, culture of endoscopic biopsy specimens, and rapid urease test. The histology was classified according to the updated Sydney Score System.¹⁷

Endoscopic biopsy specimens were taken in all subjects for: (1) histology (two from the gastric antrum, and one from the gastric fundus); and (2) rapid urease test (one from the antrum) (CP test, Yamanouchi Pharma SpA). From patients in group 1, two further biopsy specimens were taken from the antrum for microbiological culture. The biopsy specimens for histology were fixed in formalin, embedded in paraffin, sectioned, and stained with haematoxylin-eosin. The biopsy specimens for the bacterial culture were immediately placed in an appropriate transport medium (Portagerm-Pylori, bioMérieux, France), then homogenised and cultured on both selective (Pylori agar, bioMérieux) and non-selective (10% horse blood agar, Kima, Italy) media. After seven days of incubation at

Department of Paediatrics, University of Parma, Italy
M E Street
C Caffarelli
M Manfredi
F Fornaroli
G L de'Angelis

Department of Pathology, University of Parma
P Caruana

Department of Microbiology, University of Parma
W Magliani

Correspondence to:
Dr G L de'Angelis, Istituto di Clinica Pediatrica, Via Gramsci, 14, 43100 Parma, Italy
deangelis@pleo.it

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Table 1 Features at diagnosis of the patients in groups 1 and 2

	Group 1 (n = 75)	Group 2 (n = 75)	p value
Symptoms			
Epigastric pain	29 (39%)	39 (52%)	NS
Recurrent abdominal pain (RAP)	41 (55%)	49 (65%)	NS
RAP with vomiting	28 (37%)	18 (24%)	NS
Gastric pyrosis	9 (12%)	7 (16%)	NS
Anorexia	6 (8%)	5 (7%)	NS
Epigastric bloating	9 (12%)	4 (5%)	NS
Bleeding of the upper GI tract	2 (3%)	1 (1%)	NS
Melaena	1 (1%)	1 (1%)	NS
Histology			
Mild gastritis	63 (84%)	60 (80%)	NS
Moderate gastritis	6 (8%)	10 (13%)	NS
Pronounced gastritis	6 (8%)	5 (7%)	NS
<i>H. pylori</i> positive	70 (93%)	75 (100%)	NS
Positive urease tests	70 (93%)	64 (85%)	NS
Positive cultures	63 (84%)	Not done	
Endoscopic appearance of the mucosa			
Antral nodularity and/or hyperaemia	58 (77%)	53 (71%)	NS
Duodenal ulcer	3 (4%)	2 (3%)	NS

Table 2 Antibiotic resistance to *H. pylori*, based on the E test performed on the positive cultures (63/75) from the endoscopic biopsy specimens from patients in group 1

Antibiotic	MIC interpretative values ($\mu\text{g/ml}$) R	Resistance n (%)
Metronidazole	≥ 16	35 (56%)
Clarithromycin	≥ 4	10 (16%)
Ampicillin	≥ 4	2 (3%)
Tetracyclines	≥ 16	1 (2%)
Metronidazole + clarithromycin		5 (8%)
Total		53 (84%)

The isolates with resistance to both metronidazole and clarithromycin are in addition to those with resistance to metronidazole or clarithromycin alone.

Table 3 *H. pylori* eradication rate in groups 1 and 2

	Group 1 (n = 75)	Group 2 (n = 75)	p value OR (95% CI)
Subjects successfully treated	72 (96%)	61 (81%)	0.010* 5.5 (1.51 to 20.06)
Subjects in whom treatment failed	3 (4%)	14 (19%)	

*p < 0.05 eradication rate of group 1 versus group 2; OR: 5.5, 95% CI: 1.51 to 20.06.

37°C under microaerophilic conditions, typical oxidase and catalase positive colonies were identified by API Campy strips (bioMérieux), and subsequently tested for antibiotic sensitivity (E test).¹⁸ The following antibiotics were tested: ampicillin, tetracycline, metronidazole, and clarithromycin (AB Biodisk, Sweden). The minimum inhibitory concentration (MIC) interpretative values used to define resistance (R) to each antibiotic are reported in table 2.

Patients in group 1 were treated with ranitidine (6 mg/kg/day, twice daily) if there was mild gastritis, or omeprazole (1 mg/kg/day, once daily) if there was moderate or pronounced gastritis, or if there was an ulcer. Subjects with a positive culture also received two antibiotics chosen according to susceptibility at the standard dose for eight days (metronidazole 20 mg/kg/day; tetracycline 30 mg/kg/day if over 9 years of age; amoxicillin 50 mg/kg/day; clarithromycin 30 mg/kg/day).¹⁴

The group 1 subjects with a negative culture, and the patients in group 2 were treated with ranitidine or omeprazole according to the same criteria, and with amoxicillin (50 mg/kg/day) and clarithromycin (30 mg/kg/day) for eight days.¹⁴ The antisecretory treatment was continued for up to eight weeks in both groups, if symptoms persisted.

All patients were re-evaluated six months after finishing treatment, using the same investigation protocol as at diagnosis.

The main outcome of the study was evaluation of the efficacy of standard antibiotic treatment versus an antibiotic sensitivity assay based treatment.

STATISTICS

Statistical analysis was done using the χ^2 test with Yates's correction. The odds ratio (OR) and the 95% confidence interval (CI) were calculated; p < 0.05 was considered significant.

Results

There was no significant difference between the ages of the two groups of subjects. The number of males and females was also similar in the two groups.

Table 1 details symptoms at presentation, endoscopic findings, histology, and Giemsa staining results of the sections for histology. In group 1, five subjects with negative Giemsa staining had positive cultures and urease tests. Seventy of 75 patients (93%) had a positive urease test, but the five subjects with a negative test had a positive culture and positive histology. The 12/75 (16%) patients with a negative culture had positive histology and urease test.

In the patients in whom endoscopic biopsy specimens were taken for the culture and antibiotic susceptibility testing, *H. pylori* grew in culture in 63/75 (84%). Antibiotic resistance was detected in 53/63 isolates (84%), with the highest percentage of resistance being observed towards metronidazole (56%) (table 2).

Six months after treatment in 62/63 (98%) subjects with a positive culture, all tests were negative (macroscopic appearance at endoscopy, urease test, histology, and culture).

Of the 12/75 (16%) patients with an unsuccessful culture, 2/12 were lost at follow up. *H. pylori* was eradicated in the remaining 10 using the standard antibiotic combination of amoxicillin/clarithromycin. The infection was thus eradicated in 96% of the initial 75 subjects, in 99% of the 73 patients followed up, and in 98% of the subjects with a positive culture treated according to the antibiotic assays (62/63) (table 3).

In all of the subjects in group 2, Giemsa staining of the biopsy specimens was positive for *H. pylori*. Eradication, verified six months later by means of endoscopy, urease test, and histology, was achieved in 61/75 subjects (81%) (table 3). This was significantly lower than the percentage of eradication observed in group 1 (81% v 96%, p < 0.05; OR: 5.50, CI: 1.51 to 20.06).

According to eradication these patients were divided into two subgroups, and compared in order to assess if there were any differences between the two subpopulations. Patient age, gender, and histological severity did not differ between those in whom eradication was or was not successful. The percentage of positive urease tests was significantly greater in those in whom eradication was successful (57% v 92%, p < 0.05; OR: 8.40, CI: 2.07 to 34.03; table 4).

Table 4 Features at diagnosis of the two subgroups in group 2

	Patients successfully treated (n = 61)	Patients in whom treatment failed (n = 14)
Chronological age (y)†	9.29 (7.60)	13.10 (7.60)
Males/females	38/23	11/3
Positive urease tests	56*	8
Histology		
Mild gastritis	50	10
Moderate gastritis	8	2
Pronounced gastritis	3	2
Duodenal ulcer	2	0

*p < 0.05 versus the subjects who did not eradicate; OR: 8.40, 95% CI: 2.07 to 34.03. †Mean (SD).

In an analysis of the eradication rate in all patients for whom antibiotic susceptibility results were available compared with those treated blindly, the cure rate was significantly higher in the former group (83% v 98%, p < 0.05; OR: 12.58, CI: 1.60 to 98.45; table 3).

Discussion

The data reported in this study show that successful *H pylori* eradication is obtained in a greater percentage of subjects, when an antibiotic susceptibility test is available and treatment is adjusted accordingly.

Antibiotic susceptibility was assessed using the E test, which is considered to be appropriate for testing *H pylori*, and better than agar dilution and disc diffusion tests.¹⁸

Antibiotic resistance is a complex topic and has been discussed elsewhere.⁸ Dore and colleagues¹⁹ reported unexpected antibiotic resistance in genotypically identical *H pylori* isolates, based on the E test.¹⁹ There is uncertainty as to whether *H pylori* should be eradicated given the existence of many mutants, and the fact that these are associated with clinically significant diseases in less than 20% of the population.²⁰ Many would consider that children should be treated in order to prevent long term complications.^{1, 2} Our policy is to treat children with *H pylori* for eight days with combined therapy based on antibiotic susceptibility testing. We achieved eradication in 99% of cases. An additional advantage of susceptibility testing is that it may reduce the risk of creating *H pylori* resistance.²¹

In the literature, to date, the percentage of antibiotic resistance varies between the continents, and within Europe. A significant proportion of our isolates were resistant to metronidazole, clarithromycin, ampicillin, or tetracycline, and a few were resistant to both clarithromycin and metronidazole, with the resistance to metronidazole being the most frequent. These ratios of resistance are higher than those published by most authors. Metronidazole resistance has been reported in up to 50% of isolates,^{3, 7, 9-11} clarithromycin resistance in 1-15%,^{3, 5-7, 9-11, 13} amoxicillin resistance in 0.3-6%,^{10, 11, 13} but resistance to tetracycline has not generally been reported.¹⁰ However, our results are comparable with those recently reported in adults with gastric ulcers.²²

The success of treatment in *H pylori* infection depends mainly on antibiotic sensitivity. With regard to the isolates tested in this study during the two time periods, sensitivity

should have been similar as the patients were enrolled over a relatively short time interval and came from the same geographical area.

Treatment is generally more effective in children than in adults in whom the eradication rate has been reported at approximately 70-80% with triple therapy for two weeks.¹⁴ Combined treatment with amoxicillin and clarithromycin is considered a treatment of proven efficacy,¹⁴ and therefore represents a good comparison.

Among patients in whom antibiotic susceptibility testing was available, treatment failed in only one case. This patient also had Crohn's disease and was receiving long term steroid treatment.

We believe that treatment with amoxicillin is important, even with susceptibility testing, as it reduces the risk of antibiotic resistance as shown in subjects with persistent *H pylori* infection previously treated with antibiotic regimens including clarithromycin.²³

In this study, eradication was assessed six months after completion of treatment in order to reduce the possibility of *H pylori* recurrence, although most authors consider assessment after four weeks, and some suggest 12 months.²⁴

The only distinguishing feature of the patients in whom *H pylori* was successfully eradicated in group 2 was the higher proportion of positive urease tests. This could reflect a better response to treatment in patients with a larger number of organisms. However, positive urease tests were found in both subgroups and this type of assay does not allow a quantitative analysis.

In conclusion, antibiotic susceptibility testing is worthwhile in childhood as a greater proportion of subjects are successfully cured. Testing may help to limit the development of antibiotic resistance and may avoid the need for repeated courses of treatment and reinvestigation. The extra cost of susceptibility testing is offset by the consequent financial savings.^{4, 15, 25}

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