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Clarithromycin Resistance among *Helicobacter pylori* Strains Isolated from Children: Prevalence and Study of Mechanism of Resistance by PCR-Restriction Fragment Length Polymorphism Analysis

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Clarithromycin and metronidazole resistance was 29.1 and 23.9%, respectively, in 96 *Helicobacter pylori* strains obtained from pediatric patients. No resistance to amoxicillin was observed. Resistance according to patients' ages to clarithromycin and metronidazole was 45.4 and 18.2% in 22 patients from 4 to 8 years old, 30.2 and 20.7% in 53 patients from 9 to 13 years old, and 9.5 and 38.1% in 21 patients from 14 to 18 years old, respectively. The A2143G mutation was the most prevalent (82.1%) among clarithromycin-resistant strains.

Helicobacter pylori is a gram-negative microaerophilic rod found in the human gastric mucosa associated with different digestive diseases, such as peptic ulcer, gastritis, and mucosa-associated lymphoid tissue lymphoma (7), and it is considered a risk factor in the development of gastric cancer (24). *H. pylori* infection is frequently acquired during childhood, and symptoms such as vomiting and epigastric or recurrent abdominal pain are associated with *H. pylori* infection (6).

Amoxicillin, tetracycline, metronidazole, and clarithromycin are frequently used, combined with proton pump inhibitors or bismuth salts, for the treatment of *H. pylori* infections (22). However, side effects, poor compliance, and resistance to antibiotics are causes of treatment failure (4, 16). Resistance to metronidazole and clarithromycin is population dependent, and several studies suggest that clarithromycin resistance is higher in strains obtained from children than in those from adults (9).

The aim of this study was to determine the rate of resistance to clarithromycin in *H. pylori* strains obtained from pediatric patients according to the age of the patients. Metronidazole and amoxicillin resistance was also studied. The mutation involved in clarithromycin resistance was detected by PCR-restriction fragment length polymorphism analysis.

Ninety-six pediatric patients, aged from 4 to 18 years (mean age ± standard deviation, 10.86 ± 3.3 years), attending the Gastroenterology Unit at the Hospital del Niño Jesus during 1999 and 2000, were included in this study. Patients were referred for endoscopy due to different symptoms, with epigastric pain (74.2%), vomiting (33.3%), and recurrent abdominal pain (25.8%) being the most prevalent. Parents signed an informed consent form for the endoscopy, and the Ethical Committee supervised the study. Patients previously treated for *H. pylori* infections were not included. Strains were grouped according to the age of the patient at the time of endoscopy. Only culture-positive patients were included. *H. pylori* clinical isolates were obtained from gastric biopsy specimens according to standard procedures.

Clarithromycin was obtained from Abbott Laboratories SA, Madrid, Spain, and metronidazole and amoxicillin were obtained from Sigma-Aldrich, Madrid, Spain. MICs were determined by an agar dilution technique with Mueller-Hinton agar plus 7% horse blood according to NCCLS recommendations (17). Plates with twofold dilutions of each antibiotic were inoculated with 1 to 2 μl of 10^9 CFU/ml by using a Steers replicator and incubated for 3 to 5 days. A strain was considered resistant to clarithromycin when the MIC was ≥1 mg/liter (17), resistant to metronidazole when the MIC was ≥8 mg/liter, and resistant to amoxicillin when the MIC was ≥1 mg/liter (9). A strain was considered intermediate to clarithromycin when the MIC was 0.5 mg/liter (17). Few data are available concerning the type of mutations associated with clarithromycin resistance in strains from pediatric patients. So, mutations involved in clarithromycin resistance (A2142G or A2143G) were detected by a previously reported PCR-restriction fragment length polymorphism analysis method (2), with *Mbo*II (Amersham Pharmacia) or *Bsa*I (New England Biolabs, Inc., Beverly, Mass.).

Data were analyzed with the computer software program EpiInfo 6.04 (Centers for Disease Control and Prevention, Atlanta, Ga.). Ninety-five percent confidence intervals (95% CI) of prevalence rates were calculated, and the chi-square test was used to compare resistance percentages in the different age groups (two-by-three matrix) for both clarithromycin and metronidazole. Mean ages of patients infected with clarithromycin-susceptible and -resistant strains were also compared. A P value of <0.05 was considered statistically significant.

Table 1 shows the MIC_{50}s (MICs at which 50% of the isolates tested are inhibited), MIC_{90}s, and MIC ranges of, and percentages of resistance to, the three antimicrobial agents tested against the total number of strains. A high percentage of clarithromycin-resistant strains was detected among our pediatric strains, similar to other studies (12, 25). In some countries resistance is higher in children than in adults (9), although not

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TABLE 1. MICs of drugs and percentages of resistance among strains

Drug	MIC (mg/liter)			% Resistance
	MIC_{50}	MIC_{90}	Range	
Clarithromycin	0.032	16	0.008–64	23.9
Metronidazole	1	64	0.008–128	29.1
Amoxicillin	0.016	0.125	0.008–0.25	0

TABLE 3. Distribution of 28 clarithromycin-resistant strains according to mutation and MIC

Mutation	MIC (mg/liter)							Total no. of strains	% of strains with each mutation (95% CI)
	1	2	4	8	16	32	64		
A2143G	0	0	5	7	6	4	1	23	82.1 (63.1–93.9)
A2142G	0	0	0	0	0	1	1	2	7.1 (0.8–23.5)
No mutation	0	1	0	1	0	1	0	3	10.7 (2.3–28.2)

in others (21). An increase of resistance to clarithromycin and metronidazole in children has also been described elsewhere (3, 8, 15).

The mean age of patients infected with a clarithromycin-resistant strain was 9.3 ± 3.2 years, and the mean age of the patients infected with a clarithromycin-susceptible strain was 11.48 ± 3.17 years ($P = 0.002$). The percentages of resistance to clarithromycin according to the different age groups are shown in Table 2. We observed that, while metronidazole resistance increased with the age of the patients, clarithromycin resistance decreased. Kalach et al. reported that the mean age of children with a clarithromycin-susceptible strain was 139.8 ± 43.2 months, versus 125.6 ± 52.2 months for children with a clarithromycin-resistant strain, although the differences were not statistically significant (13). Another study found results similar to ours: a higher level of clarithromycin resistance in children (16%) than in adults (2 to 3%) [I. Jesch et al., abstr. from the 12th International Workshop on Gastroduodenal Pathology and Helicobacter pylori, Helsinki, Finland, 1999. Gut **45**(Suppl. 111)**:**A93, 1999]. In a European study, the percentage of resistance to clarithromycin in children (17.3%) was higher than those in teenagers (13.6%) and in adults (8.2%). The resistance to metronidazole, however, increased with age (9).

Eight out of the 96 patients (8.3%) were infected with a multiresistant strain (resistant to clarithromycin and metronidazole simultaneously), and the percentages were 13.6% in group I, 5.6% in group II, and 9.5% in group III ($P = 0.5$, not significant).

Resistance to metronidazole was 28.5% in girls and 19.2% in boys. Clarithromycin resistance was 33.3% in girls and 26.4% in boys. These differences were not statistically significant.

For all the strains, the amoxicillin MICs were lower than 1 mg/liter. Resistance to amoxicillin is very infrequent, despite the wide use of this antibiotic, either alone or combined with clavulanic acid, to treat *H. pylori* or respiratory tract infections in both children and adults, although resistance has appeared in some parts of the world (1, 9).

Mutations detected in the 28 clarithromycin-resistant strains are summarized in Table 3. In 23 out of the 28 strains studied, A2143G was detected. A2142G was very infrequent, appearing in only 2 out of the 28 strains (7.1%). In three strains no mutation was detected by this method. For strains with the A2143G mutation, the MIC range was 4 to 64 mg/liter; for the two strains with the A2142G mutation, the MIC range was 32 to 64 mg/liter; and for the three strains without mutations, the MIC range was 2 to 32 mg/liter. Some papers showed that A2142G was the most frequently detected (48 or 52.5%) (20, 23), although some others found that A2143G was more prevalent (55.6 or 56.5%) (5, 18). Only a few groups had studied the mutation involved in clarithromycin resistance in strains obtained from children; however, they found results similar to those obtained in our study (8, 14, 25).

The percentage of resistance found in children younger than 8 years was very high. New macrolides were marketed in Spain at the beginning of the 1990s: roxithromycin in 1990, clarithromycin in 1991, and azithromycin in 1992 (data obtained from http://www.portalfarma.com), suggesting that younger children have been more exposed to new macrolides than have older children. Macrolides are very often used nowadays to treat respiratory infections in young children. Ruiz Bremón et al. studied the nonhospital use of antibiotics in Spain from 1987 to 1997 and reported that macrolides composed 8% of antibiotic use in 1987 and increased to 12% in 1990, 15% in 1993, and 18% in 1997. Moreover, in the first period of study, erythromycin was the main macrolide used, while in 1997 clarithromycin and azithromycin accounted for 50% of use of macrolides (19). Similar data were found by Granizo et al., studying resistance to macrolides in *Streptococcus pyogenes* (11) and *Streptococcus pneumoniae* (10).

It is important to know the resistance to clarithromycin and metronidazole in each population, especially when data for susceptibility are not available at the time of applying treatment. In our pediatric population, clarithromycin should not be used as an empirical treatment for *H. pylori* infection in children younger than 8 years, in whom 45% of strains are resistant to this antibiotic.

TABLE 2. Numbers of resistant strains, percentages of resistance, and 95% CI according to age group for clarithromycin and metronidazole[a]

Antibiotic	Group[b]	Total no. of strains	No. of resistant strains	% Resistance[c]	95% CI
Clarithromycin	I	22	10	45.4*	24.3–67.7
	II	53	16	30.2*	18.3–44.3
	III	21	2	9.5*	1.17–30.3
Metronidazole	I	22	4	18.2**	5.18–40.2
	II	53	11	20.7**	10.8–34.1
	III	21	8	38.1**	18.1–61.5

[a] A chi-square test (two-by-three matrix) was used to compare resistances among the three age groups.
[b] Group I, 4 to 8 years old; group II, 9 to 13 years old; group III, 14 to 18 years old.
[c] Significance: *, $P = 0.03$; **, $P = 0.2$ (not significant).

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