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High Levels of Resistance to Metronidazole and Clarithromycin in *Helicobacter pylori* Strains in Children

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The aim of the study was to evaluate the prevalence of resistance to amoxicillin, metronidazole, and clarithromycin before treatment of *Helicobacter pylori* infection in children and to assess the evolution of resistance with time. The study was carried out between 1994 and 1999 with 150 *H. pylori*-positive children through gastric culture (antimicrobial susceptibility) and histology. All cultured *H. pylori* strains were sensitive to amoxicillin, 64 (43%) were resistant to metronidazole, 32 (21%) were resistant to clarithromycin, and 14 (9%) were resistant to both metronidazole and clarithromycin. The overall prevalence of resistance to metronidazole and clarithromycin did not change significantly with time. The study highlights the generalized high-level and stable metronidazole and clarithromycin resistance of *H. pylori* strains from children.

The role of *Helicobacter pylori* in the colonization of the stomach in adults and children with chronic gastritis, peptic ulcer, and possibly gastric carcinoma is now documented (16). Eradication of the bacterium has a great effect on prevention of peptic ulcer relapses in both adults (9) and children (23; N. Kalach, J. Raymond, P. H. Benhamou, M. Bergeret, and C. Dupont, Letter, Clin. Microbiol. Infect. **5:**235–236, 1999).

The great jump in the understanding of the diseases mentioned above was accompanied by progressive evidence of the antibiotic resistance phenomenon, first reported for clarithromycin (21), a macrolide that partially carries crossover resistance to other antibiotics belonging to the same class (6). Resistance now involves antibiotics previously devoid of any resistance issues for that bacterium, such as amoxicillin, to which resistance sometimes occurs at a high level (4), largely challenging physicians at the present time (8). One important point is that antibiotic treatment of *H. pylori* involves drugs largely in use for other kinds of infections. For that reason, analysis of the resistance phenomenon must probably take into account a half century of coexistence between antibiotic habits and this parasitic bacterium, emphasizing the importance of analyzing both geographical variations and evolution with time (7).

The purpose of our study was to evaluate the prevalence of resistance to amoxicillin, metronidazole, and clarithromycin before treatment of *H. pylori* infection in children and to assess the evolution of resistance with time compared to those from reference studies with both adults and children.

A prospective study was carried out from January 1994 to July 1999 with 150 *H. pylori*-positive children (76 girls and 74 boys) aged 11.25 ± 3.9 years (mean ± 1 standard deviation [SD]; range, 1.75 to 18 years). Infection was proved by upper gastrointestinal endoscopy for retrieval of gastric antral biopsy specimens in the course of diagnostic evaluation of clinical gastritis, manifested by recurrent abdominal pain for at least 3 months, nausea, and vomiting. Informed consent from the parents was obtained. Children who had already suffered gastric *H. pylori* infection, institutionalized encephalopathic children, or those who had received antibiotics, acid-suppressing medications, or a nonsteroidal anti-inflammatory drug during the 3 months preceding evaluation were excluded from analysis. All children had been living in France for at least 18 months and originated from the following geographical regions: Europe, North Africa and the Middle East, Africa, and the Far East.

Three antral biopsy specimens were taken and analyzed for histology and culture as prescribed previously (18). The organisms were tested for their antimicrobial susceptibilities by growth under microaerophilic conditions for 3 days. MICs were determined by the use of the Etest for amoxicillin, metronidazole, and clarithromycin and the disk diffusion susceptibility test (Kirby-Bauer) for the other antibiotics (other macrolides, tetracyclines, and quinolones). The strains were considered amoxicillin, metronidazole, and clarithromycin resistant when the MICs were greater than 0.5, 8, and 2 mg/liter, respectively [13; XIIth Int. Workshop Gastroduodenal Pathol. *Helicobacter pylori*, 1999; Y. Glupczynski, F. Megraud, L. P. Anderson, and M. Lopez-Brea, Gut **45**(Suppl. III)**:**A105, 1999]. Culture and histologic examination of biopsy samples were carried out in a blinded manner. The presence of both a positive biopsy specimen culture and a positive histologic examination was required for inclusion in the study.

Calculation of the mean, SD, median, and 95% confidence interval (95% CI) for all quantitative parameters was done with the Stat-View system. Differences between groups and the prevalence of resistance in isolates from the different geographical regions were assessed by the chi-square test of homogeneity for categorical variables. All tests performed were two tailed, with P values of <0.05 considered significant.

All cultured *H. pylori* strains were sensitive to amoxicillin (median MIC, 0.01 mg/liter; 95% CI, 0.011 to 0.018 mg/liter).

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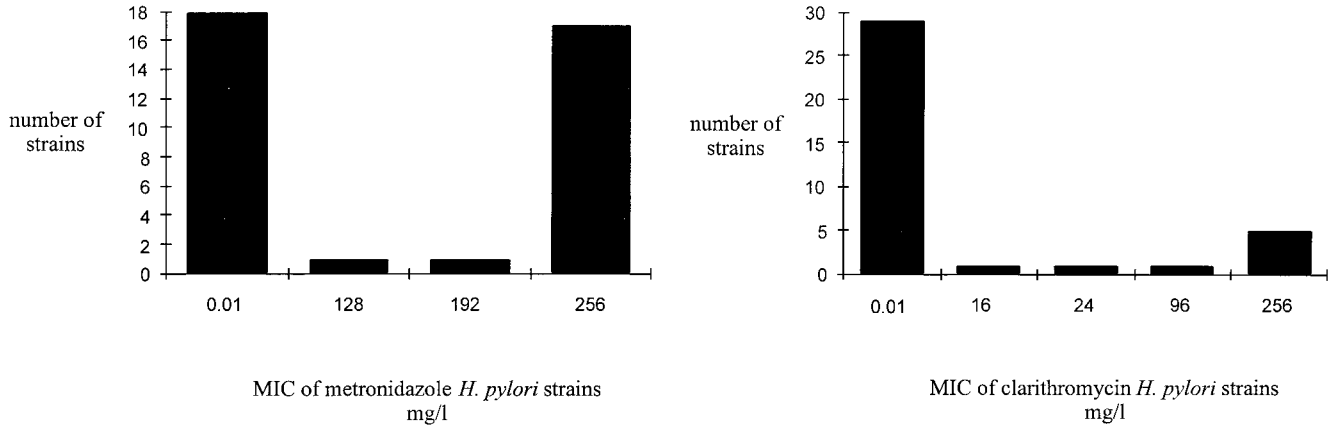

FIG. 1. Distribution of metronidazole and clarithromycin MICs for *H. pylori* strains ($n = 37$) isolated in 1998.

There were 86 (57%) metronidazole-susceptible strains (median MIC, 1 mg/liter; 95% CI, 1.33 to 2.21 mg/liter) and 64 (43%) metronidazole-resistant ones (median MIC, 256 mg/liter; 95% CI, 234.1 to 256.9 mg/liter). One hundred eighteen children (79%) were infected or colonized with clarithromycin-susceptible to isolates (median MIC, 0.01 mg/liter; 95% CI, 0.03 to 0.09 mg/liter), and 32 (21%) were infected or colonized with clarithromycin-resistant isolates (median MIC, 256 mg/liter; 95% CI, 160.2 to 230.3 mg/liter). Fourteen children (9%) were infected or colonized with both metronidazole- and clarithromycin-resistant strains. The distribution of MICs of metronidazole and clarithromycin for *H. pylori* strains isolated from children in 1998 is reported in Fig. 1.

The prevalence of the resistance to amoxicillin, metronidazole, and clarithromycin before treatment of *H. pylori* infection among the isolates from the different geographical regions is reported in Table 1.

The highest rates of resistance were for isolates from children originating from Africa for metronidazole (50%) and from Europe for clarithromycin (29%), but with no statistically significant difference compared with the rates of resistance for isolates from the other geographical regions. The only significant difference in rates of resistance was found between metronidazole-resistant *H. pylori* strains from children living in France but originating from North Africa and the Middle East (31 of 68 [46%]) and those from children from Europe (17 of 59 [29%]) ($P = 0.05$).

The overall prevalence of resistance did not change significantly with time either for metronidazole or for clarithromycin. The highest resistance rates were observed for metronidazole in 1995 (56%) and for clarithromycin in 1997 (28%) (Fig. 2).

The rate of resistance to metronidazole was higher for girls (60%) than for boys (40%) ($P < 0.05$), whereas no significant difference by sex was found for clarithromycin-resistant *H. pylori* strains. There was no significant difference in rates of metronidazole and clarithromycin-resistant *H. pylori* strains according to age.

Our study highlights the generalized high-level primary and stable resistance to metronidazole and clarithromycin of *H. pylori* strains from children. It also shows the difference (not statistically significant) in rates of resistance according to the geographical origin of the children, with the highest resistance rate found for metronidazole (50%) for children from Africa and for clarithromycin (29%) for those from Europe. Resistance to amoxicillin (Glupczynski et al., Gut **45**(Suppl. III)**:** A105, 1999) was not observed in the present series of isolates. Whether resistance allows prediction of the clinical outcome and the bacterial eradication rate deserves extensive discussion (18).

The cutoff values used for metronidazole resistance vary among investigators. Bouchard et al. [IXth Int. Workshop Gastroduodenal Pathol. *Helicobacter pylori*, 1996; S. Bouchard, C. Birac, H. Lamouliatte, S. Forestier, and F. Megraud, Gut **39**(Suppl. I)**:**A5, 1996] state that a breakpoint of 8 mg/liter is valid and in accordance with those used in other studies (14). Our previous results (18) obtained by using this breakpoint suggest clinical relevance when metronidazole is included as part of a triple-drug therapy. In adults, the resistance of *H. pylori* to metronidazole before treatment depends on the geographical region, with average rates of resistance of 20% in North America and 30% in Europe. In Europe, a multicenter study yielded a mean metronidazole resistance rate of 27%, but the rate varied among countries, from 7% in Spain to 49% in Greece (7). Rates of resistance in tropical regions, especially South America, are higher (80 to 90%). In Europe, the average rate of resistance to metronidazole increased progressively with time, from 20 to 30% in the period from 1990 to 1993 to

TABLE 1. Prevalence of resistance to amoxicillin, metronidazole, and clarithromycin before treatment of *H. pylori* infection by different geographical regions[a]

Region	No. (%) of isolates resistant to:			
	Amoxicillin	Metronidazole	Clarithromycin	Metronidazole and clarithromycin
Europe (Caucasian) ($n = 59$)	0	17 (29)	17 (29)	7 (12)
North Africa and Middle East ($n = 68$)	0	31 (46)[b]	11 (17)	5 (7)
Africa ($n = 16$)	0	8 (50)	2 (13)	1 (6)
Far East ($n = 7$)	0	3 (43)	2 (28)	1 (14)

[a] A total of 150 isolates were tested.
[b] Significant difference for metronidazole-resistant *H. pylori* strains from North Africa and Middle East versus Europe (Caucasians) by chi-square test ($P = 0.05$).

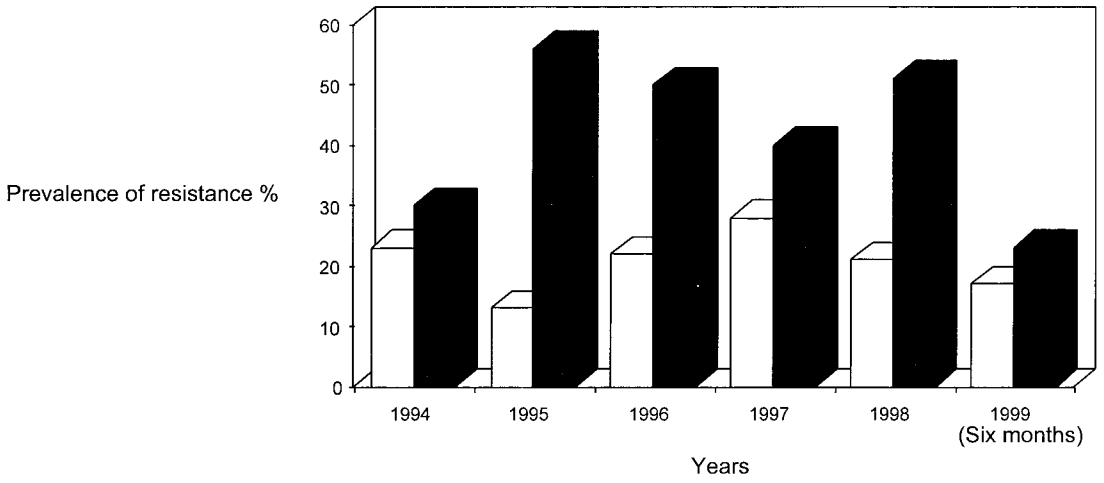

FIG. 2. Evolution of metronidazole- and clarithromycin-resistant *H. pylori* strains ($n = 150$) in children from 1994 to 1999. □, clarithromycin; ■, metronidazole.

50% in 1998 (Glupczynski et al., Gut **45**(Suppl. III):A105, 1999), with 32% being the more recent rate in a German study with children (I. Jesch, I., A. Kindermann, S. Krauss-Etschmann, I. Autenrieth, N. Lehn, and S. Koletzko, Gut **45**(Suppl. III):A93, 1999). Our rates for children are similar to those for adults, with a metronidazole resistance rate stable at about 56% during the period from 1994 to 1999 (Fig. 2). This stable metronidazole resistance rate for isolates from children for the last 5 years could also result from a progressive increase in the rate of resistance before 1994, as has been described for adults (Glupczynski et al., Gut **45**(Suppl. III):A105, 1999), related either to a previous repeated treatment for parasitic diseases or to intrafamilial transmission of the infection, especially for children originating from Africa [19; XIth Int. Workshop Gastroduodenal Pathol. *Helicobacter pylori*, 1998; J. Raymond, C. Chevalier, N. Kalach, M. Bergeret, and A. Labigne, Gut **43**(Suppl. 2):A39, 1998]. As a matter of fact, this high rate of metronidazole resistance for isolates from children originating from Africa could be related to the contraction of resistant strains of *H. pylori*, most likely from their parents, which is strongly supported by some new data suggesting intrafamilial transmission of *H. pylori* infection (1, 5) and evidence that early childhood is the critical period of *H. pylori* infection (15).

The eradication rates obtained by triple-drug therapy regimens that include metronidazole are lower for adults (2) and children (18) infected with metronidazole-resistant strains than for those infected with susceptible ones.

In adults, the rate of resistance of *H. pylori* to clarithromycin before treatment is largely less, amounting to 0 to 10% in European and North American countries, averaging 1% in The Netherlands, 3.5% in Spain, 5% in Ireland, and 6% in the United States, with the highest rate (10%) occurring in France (3, 12, 20). This disparity in resistance rates seems to be correlated to the national level of macrolide consumption, since a crossover resistance mechanism among different types of macrolides develops rapidly (3). More clarithromycin-resistant strains are found in children aged <10 years (16 to 19%) than in older children (9%) [XIIth Int. Workshop Gastroduodenal Pathol. *Helicobacter pylori* 1999; Glupczynski et al., Gut **45**(Suppl. III):A105, 1999; Jesch et al., Gut **45**(Suppl. III):A93, 1999], in agreement with our own results that indicate that 13 to 28% of strains are clarithromycin resistant. The high level of clarithromycin resistance among *H. pylori* strains from children compared to that among strains from adults suggests the importance of macrolide use in children, especially in Europe.

The development of metronidazole resistance in *H. pylori* strains is frequently associated with mutational inactivation of the *rdxA* gene, even though other mechanisms of resistance are likely to be implicated (10). Versalovic et al. (21, 22) found that clarithromycin resistance is associated with a mutation, A→G, at position 2142 or 2143 within a conserved loop of 23S rRNA (A2142G, formerly A2143G). Recently, Occhialini et al. (17) isolated another new mutation (A→C instead of A→G at position 2143 [formerly A2144G]). These alterations might result in a decreased affinity of clarithromycin for the 23S ribosome components and could thus result in diminished antimicrobial activity (11).

Finally, in adults (20) as well as in the children in our study, there was a significantly higher rate of resistance to metronidazole before treatment of *H. pylori* infection in females than in males. The idea that the resistance of *H. pylori* to metronidazole is partly due to the use of metronidazole for the treatment of unrelated infections such as gynecological ones is not valid in children. The occurrence of parasitic infections does not vary by sex in children (20), so another mechanism must be envisioned.

In conclusion, this study shows the generalized high level of resistance to metronidazole and clarithromycin but not to amoxicillin before treatment of *H. pylori* infection in children, highlights the stable resistance rates with time over 5 years, and highlights the fact that resistance rates differ according to the patient's native country. Resistance rates in the pediatric population need to be monitored in order to provide guidelines for therapeutic recommendations.

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