High Prevalence of Clarithromycin Resistance and cagA, vacA, iceA2, and babA2 Genotypes of *Helicobacter pylori* in Brazilian Children

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We isolated 45 *Helicobacter pylori* strains from 217 child patients. Resistance to clarithromycin, metronidazole, amoxicillin, and tetracycline was detected in 27%, 13%, 4%, and 0% of strains, respectively. The A2143G mutation was the most prevalent (67%) among clarithromycin-resistant strains. In addition, strain genotyping revealed a significant association between gastritis severity and the simultaneous presence of *cagA*, *vacA s1m1*, *iceA2*, and *babA2* genes.
Helicobacter pylori infection is found worldwide and constitutes a public health concern in many countries. Previous epidemiological studies have shown a high prevalence of H. pylori infection in Brazil (2, 21, 25). H. pylori infection, generally acquired in childhood, persists asymptptomatically for decades in most individuals.

Amoxicillin, tetracycline, metronidazole, and clarithromycin are frequently used, combined with proton pump inhibitors or bismuth salts, for the treatment of H. pylori infections (26). However, antibiotic resistance is frequently associated with eradication failure (3, 17). Resistance to metronidazole and clarithromycin is population dependent, and several studies suggest that clarithromycin resistance is higher in strains isolated from children than from adults (11). In Brazil, the prevalence of clarithromycin-resistant strains in adults is reported to be from 7 to 10% (16, 19). However, little is known about the prevalence of clarithromycin-resistant H. pylori infection in Brazilian children.

The primary aim of this study was to determine the prevalence of clarithromycin-resistant H. pylori strains in children, to identify those isolates via rapid methodology, and to examine the severity of gastritis caused by the antibiotic-resistant H. pylori isolates. Metronidazole, amoxicillin and tetracycline resistance were also studied. Furthermore, the study aimed to genotype the vacA and iceA and detect the cagA gene in gastric biopsy specimens, since recent studies found a high frequency of cagA-positive, iceA2-positive, and the vacA signal region genotype s1 and middle region sequence m1 strains among pediatric H. pylori isolates in Brazil (7, 8, 12, 24). This is also the first investigation of babA2 gene prevalence in Brazilian children.

A total of 217 consecutive child patients, aged from 1-18 years (mean age, 10 years) (105 girls and 112 boys) who underwent upper gastrointestinal endoscopy for evaluation of dyspeptic symptoms at the outpatient clinic of Pediatric Gastroenterology at the Child Institute of the Faculdade de Medicina da Universidade de São Paulo during 2008 and 2009, were included. The study was
approved by the Ethics Committee of the University Hospital. Patients previously treated for *H. pylori* infections were not included.

Gastric biopsy specimens were processed for histological examination and evaluated according to the updated Sydney System of classification and grading of gastritis (4).

Antral gastric specimens were transported in sodium thioglycolate broth (Difco, Detroit, Mich.) in an ice bath and ground before submission to DNA extraction and PCR-restriction fragment length polymorphism analysis with primers specific to the *H. pylori* 23S rRNA gene (18). QIAmp Tissue Kit (Qiagen) was used for DNA extraction. Point mutations related to clarithromycin resistance in the 23S rRNA amplicon were investigated in all *H. pylori* isolates by PCR-restriction fragment length polymorphism using *Bsa*I and *Mbo*II enzymes (27). The *vacA*, *cagA*, *iceA*, and *babA2* genotypes were detected by PCR, as described elsewhere (1, 5, 10, 22, 28). In each experiment, *H. pylori* 26695 strain (ATCC 700392) was used as the positive control strain.

*H. pylori* strains were cultured on Belo Horizonte medium (23) under microaerophilic atmosphere at 37°C for 3 to 7 days and the isolates were identified by Gram staining and biochemical tests for oxidase, catalase, and urease production. Resistance to clarithromycin, metronidazole, amoxicillin, and tetracycline was determined by disc diffusion method (Oxoid, USA) and MICs were determined by the E-test according to the manufacturer’s recommendations (AB Biodisk, Solna, Sweden). An isolate was considered resistant to clarithromycin or tetracycline if the MIC was >1 mg/l and to metronidazole or amoxicillin if the MIC was >4 mg/l (20).

Data were analyzed by the 2-tailed χ² test and Fisher exact test. *P* value < 0.05 was considered statistically significant.

*H. pylori* was isolated in 45 (20.7%) of the 217 children; 12 (26.7%) of the 45 strains were clarithromycin-resistant, 6 (13.3%) were metronidazole-resistant, and 2 (4.4%) were amoxicillin-resistant. All cultured *H. pylori* strains were susceptible to tetracycline (Figure 1). No histological differences were observed between biopsies with antibiotic-resistant strains and those with susceptible...
strains. PCR-RFLP was performed with all 12 clarithromycin-resistant isolates: 8 had the 23S rRNA A2143G point mutation, and 4 had the 23S rRNA A2142G mutation.

Among the 45 *H. pylori*-infected children, 13 had mild chronic gastritis, 28 had moderate chronic gastritis, 2 had marked chronic gastritis, and 2 had normal gastric mucosa. The percentage of *H. pylori*-infected children with chronic gastritis was 95.5% (43 patients), while 4.4% of the children (2 patients) had normal mucosa (*P* < 0.001).

*vacA* was detected in all 45 *H. pylori*-positive gastric biopsy specimens. The *vacA* genotypes *s1m1*, *s2m2*, and *s1m2 or s2m1* were found in 57.7, 33.3, and 4.4% of the specimens, respectively. The *iceA1* allele was detected in 9 (20%) and the *iceA2* allele in 31 (68.9%) of the samples. Of the 45 *H. pylori*-positive biopsy specimens, 28 (62%) were *cagA* positive and 38 (84.4%) were *babA2* positive. Correlation of histopathology results with *vacA*, *cagA*, and *iceA* genotypes showed that *vacA s1m1*, *cagA*, and *iceA2* positive strains were more frequently found in patients with moderate and marked gastritis (77%) in comparison to patients with mild gastritis (23%) (*P* < 0.001). Interestingly, in Slovenian children, *vacA s1* and *cagA* were also shown to be associated with more pronounced chronic gastritis (13). In contrast, in Korean children, although *vacA s1m1*, *cagA*, *iceA1* was the predominant genotype, no association with gastritis severity was observed (15).

In conclusion, we found a high incidence of clarithromycin-resistant *H. pylori* (27%) in Brazilian children. Furthermore we found an association between clarithromycin resistance and either *vacA s1m1* (*P* = 0.007) or *iceA2* (*P* = 0.038) genotypes. The high level of clarithromycin resistance among strains from children compared to adults (16, 19) suggests the importance of susceptibility testing, especially in Brazilian children. Altogether, these data stress the relevance of susceptibility testing and genotyping for establishing antibiotic treatment in pediatric *H. pylori* infection.

In our study, PCR-RFLP proved to be a rapid and accurate method for the detection of clarithromycin-resistance gene mutation directly in gastric biopsies.
Only a few groups have studied mutations involved in clarithromycin resistance in strains obtained from children, and their results are similar to those obtained in our study (6, 14, 29).

Our data also demonstrate an association between H. pylori infection and gastritis in Brazilian children. In addition, we confirmed the reported association of infection with vacAs1m1, cagA, iceA2-positive H. pylori strains and gastritis severity (7, 12, 24). Furthermore, a high frequency of babA2 was found among H. pylori isolates. Previous studies of adults in Brazil reported a high prevalence of babA2-positive strains from patients with different upper gastrointestinal diseases (9). The high incidence of babA2 in H. pylori Brazilian isolates suggests that this gene could be a useful marker for predicting the high-risk patients of H. pylori infection in Brazil.

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FIGURE 1. Distribution of MICs for the 45 H. pylori strains.