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

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Received 28 May 2010/Returned for modification 2 August 2010/Accepted 26 August 2010

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, 20, 24). 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 (25). However, antibiotic resistance is frequently associated with eradication failure (3, 16). Resistance to metronidazole and clarithromycin is population dependent, and several studies suggest that clarithromycin resistance is higher in strains isolated from children than in strains isolated from adults (10). In Brazil, the prevalence of clarithromycin-resistant strains in adults is reported to be from 7 to 10% (15, 18). However, little is known about the prevalence of clarithromycin-resistant H. pylori infection in Brazilian children.

The primary aims of this study were 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 was also studied. Furthermore, the study aimed to genotype the vacA and iceA genes and to detect the cagA gene in gastric biopsy specimens, since recent studies found a high frequency of cagA-positive and iceA2-positive strains as well as a strain with the vacA signal region genotype s1 and middle region sequence m1 among pediatric H. pylori isolates in Brazil (6, 7, 11, 23). This is also the first investigation of babA2 gene prevalence in Brazilian children.

A total of 217 consecutive child patients, aged 1 to 18 years (mean age, 10 years) (105 girls and 112 boys), who underwent upper gastrointestinal endoscopy for the evaluation of dyspeptic symptoms at the outpatient clinic of Pediatric Gastroenterology at the Instituto da Criança, 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 thiosulfate broth (Difco, Detroit, MI) in an ice bath and ground before submission to DNA extraction and PCR-restriction fragment length polymorphism (PCR-RFLP) analysis with primers specific to the H. pylori 23S rRNA gene (17). The QI Amp 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-RFLP using BsaI and MboII enzymes (27). The vacA, cagA, iceA, and babA2 genotypes were detected by PCR, as described elsewhere (1, 9, 21, 26, 28). In each experiment, H. pylori strain 26695 (ATCC 700392) was used as the positive control strain.

H. pylori strains were cultured on Belo Horizonte medium (22) 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 the disc diffusion method (Oxoid), and MICs were determined by the Etest 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/liter and to metronidazole or amoxicillin if the MIC was >4 mg/liter (19).

Data were analyzed by the two-tailed χ² test and Fisher exact test. P values of <0.05 were 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 suscep-

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† Published ahead of print on 8 September 2010.
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 vacA s1m1 cagA iceA2-positive H. pylori strains and gastritis severity (6, 11, 23). 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 (8). The high incidence of babA2 in H. pylori Brazilian isolates suggests that this gene could be a useful marker for identifying patients with a high risk of H. pylori infection in Brazil.

Gabriella T. Garcia and Katia R. S. Aranda contributed equally to this work.

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo a Pesquisa do Estado de São Paulo.

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

Clarithromycin Metronidazole Amoxicillin Tetracycline

TABLE 1. Distribution of isolates according to MICs for each antibiotic.

MIC (µg/mL)

Clarithromycin Metronidazole Amoxicillin Tetracycline

Number of isolates

0.016 0.031 0.063 0.125 0.250 0.500 1.000 2.000 4.000 8.000 16.000 32.000

Clarithromycin Metronidazole Amoxicillin Tetracycline

0 5 10 15

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