Helicobacter pylori Virulence Genes Detected by String PCR in Children from an Urban Community in Northeastern Brazil

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The accuracy of a nested PCR in gastric DNA obtained by a string test for the diagnosis of *Helicobacter pylori* infection in asymptomatic children was 94.0%. The *cagA*-positive toxigenic *vacAs1m1* strains were the most prevalent strains, indicating that this population is colonized early by the strains associated with gastric cancer.

Helicobacter pylori infection significantly increases the risk of development of peptic ulcer disease, distal gastric carcinoma, and gastric lymphoma (1). Infection of the general population with virulent strains, especially those carrying the *cagA* gene and *vacAs1* genotype, is a predictor of increased risk for development of severe *H. pylori*-associated diseases. However, the majority of the methods used for genotyping *H. pylori* strains require an invasive procedure, endoscopy, for tissue sample collection and are not indicated in epidemiological studies evaluating asymptomatic individuals, especially children. The string test, a minimally invasive nonendoscopic procedure, seems to be an accurate method to obtain gastric specimens in order to investigate *H. pylori* virulence genes. It has been demonstrated that the genotypes of *H. pylori* strains in DNA from the gastric juice or tissue samples are identical (2).

Previously we have shown a high prevalence of infection by *H. pylori* strains carrying the *cagA* gene and the *vacAs1* allele in dyspeptic adult patients who underwent endoscopy in northeastern Brazil (3). Furthermore, we have demonstrated that in this population, the infection is acquired earlier in childhood (4), another predictor of gastric cancer. However, we are unaware of studies evaluating the profile of the circulating strains in children of the general population living in areas at increased risk of gastric cancer. Therefore, our aim was to investigate whether the most virulent strains of *H. pylori* circulate in asymptomatic children from the population, by obtaining *H. pylori* DNA in gastric juice or mucus by the string test. We also aimed to evaluate the accuracy of an *H. pylori*-specific nested PCR for the diagnosis of the infection in asymptomatic children.

The study was approved by the Ethics and Research Committee of the Federal University of Ceará. All children and their parents signed the informed consent. Individuals who had participated in previous *H. pylori* epidemiological studies in Parque Universitário, a low-income urban community in Fortaleza, Brazil, were invited to participate (5). Children who had taken antibiotics potentially active against *H. pylori* were not included. Fifty children (24 females and 26 males) 8 to 18 years old with a mean age of 14.3 years were evaluated. After a 6-h fast, the children were submitted to the [¹³C]urea breath test (¹³C-UBT) (6) and immediately after to the string test. We used a homemade string test with a small capsule, which increased the adherence of the participants, following the protocol previously described, with minor modifications (7). A gelatin capsule containing a 90-cm-long ab-

sorbent cotton string was swallowed with up to 200 ml of water. A 20-cm-long portion of the string was pulled out from the capsule and taped to the subject's cheek. After an hour, the string was retrieved orally. The proximal 30 cm of the string was discarded. The distal gastric mucus/juice-impregnated string was flushed with 5 ml of saline to reduce contamination by oropharyngeal organisms and then placed into a sterile bottle containing 3 ml of brain heart infusion broth and immediately sent for processing. The liquid from the vial containing the string was centrifuged at 13,000 × g for 10 min. The DNA was extracted from the pellet using the QIAamp (QIAGEN, Hilden, Germany) kit according to the manufacturer's recommendations. For *H. pylori* DNA detection, a nested PCR specific for *H. pylori ureA* was employed (8). PCR amplification of the *vacA* signal sequence and midregion was performed by using the primers described by Atherton and colleagues (9), and the *s1* genotype was further characterized into *s1a*, *s1b*, or *s1c* variants (10). The *cagA* gene was amplified as described previously (11). Negative and positive controls were included in all reactions. Data were analyzed by two-tailed χ^2 or Fisher test. All individuals swallowed the capsule without inconvenience. Among 43 children *H. pylori* positive by ¹³C-UBT, 40 were also positive for the *ureA* gene by the nested PCR. In the 7 ¹³C-UBT-negative children, the *ureA* nested PCR was also negative. The string *ureA* nested PCR had a sensitivity of 93.0% and a specificity of 100% compared with the ¹³C-UBT. The agreement between the two tests was of 94.0%, higher than that reported in the literature in different countries when conventional PCRs for detecting *H. pylori* specific genes were used (12–16). The accuracy of the string nested PCR was excellent, because we compared its results with those of a noninvasive test (¹³C-UBT) that has high sensitivity and specificity for the diagnosis of *H. pylori* infection in children older than 6 years (17).

The *vacA* gene was detected by conventional PCR in 82.5% of the *ureA* nested-PCR-positive samples. Among them, the most

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TABLE 1 Distribution of *vacA* genotypes according to the *cagA* status of the *H. pylori* strains in this study

<i>vacA</i> genotype	No. of samples:		Total
	<i>cagA</i> positive	<i>cagA</i> negative	
<i>vacAs1 vacAm1</i>	14	3	17
<i>vacAs1 vacAm2</i>	3	2	5
<i>vacAs2 vacAm2</i>	0	6	6
<i>s1</i> ^a	5	0	5

^a Only the *s* region was detected.

virulent *vacAs1* genotype was the most prevalent, being observed in 27 (81.8%) samples, a higher frequency than we demonstrated in symptomatic children from the southeast region of Brazil (18). Otherwise, the *vacAs2* nontoxigenic genotype was observed in 6 (18.2%) samples. All 27 *vacAs1* strains were *s1b*. *cagA* was detected in 22 (66.7%) of 33 *vacA*-positive strains. Neither *vacA* nor *cagA* was amplified in the samples from ¹³C-UBT-negative children. *vacAm1* and *-m2* alleles were detected in 17 (60.7%) and 11 (39.3%) samples, respectively. The *vacAs1 vacAm1* genotype, considered the higher cytotoxin producer, was the most frequent *vacA* allelic combination and was associated with *cagA* positivity ($P = 0.005$) (Table 1). *cagA*-positive status and *vacAs1* genotype were associated neither with the age ($P \geq 0.55$) nor with the gender ($P \geq 0.32$) of the children.

Infection by multiple *vacA* genotypes was not observed, in agreement with the knowledge that it occurs more frequently in adults with the *H. pylori*-associated severe diseases, probably due to the microevolution that may represent intrahost diversification during long-term colonization (19).

In conclusion, we found that the string test is a safe and simple method to obtain gastric DNA in children, which allowed using nested and conventional simple, accurate, and inexpensive PCR the detection of *H. pylori* virulence genes. This approach may be of particular value in *H. pylori* molecular epidemiological studies. Of note, asymptomatic children from the community we studied are frequently colonized by the most virulent *H. pylori* strains.

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The authors declare they have no conflict of interests.

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