Helicobacter pylori virulence genes detected by string PCR in children from an urban community in Northeastern Brazil

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Running title: “String test for H. pylori virulence gene detection”

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The accuracy of a nested PCR in gastric DNA obtained by string test for the diagnosis of 
*H. pylori* infection in asymptomatic children was 94.0%. The *cagA*-positive toxigenic *vacA* 
s1m1 strains were the most prevalent indicating that this population is early colonized by the 
strains associated with gastric cancer.

*H. pylori* infection significantly increases the risk of developing peptic ulcer disease, 
distal gastric carcinoma and gastric lymphoma (1). Infection of the general population with 
virulent strains, especially those carrying the *cag* gene and *vacA* s1 genotype, is a predictor of 
increase risk for developing the severe *H. pylori*-associated diseases. However, the majority of 
the methods used for genotyping *H. pylori* strains requires 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 non- 
endoscopic 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 *vacA* s1 allele in dyspeptic adult patients 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 \textit{H. pylori} circulate in asymptomatic children from

the population, by obtaining \textit{H. pylori} DNA in gastric juice/mucus by string test. We also aimed
to evaluate the accuracy of an \textit{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 Federal University of
Ceará. All children and their parents signed the informed consent. Individuals who had
participated in previous \textit{H. pylori} epidemiological studies in Parque Universitário, a poor income
urban community in Fortaleza, Brazil, were invited to participate (5). Children who had taken
antibiotics potentially active against \textit{H. pylori} were not included. Fifty children (24 females and
26 males) 8 to 18 years old, mean age of 14.3 years were evaluated. After six hour fast the
children were submitted to the $^{13}$C-UBT (6) and, immediately after, to the string test. We
used a homemade string test with a small size capsule, which increased the adherence of the
participants, following the protocol previously described with minor modifications (7). Gelatin
capsule containing a 90 cm long absorbent cotton string was swallowed with up to 200 ml of
water. Twenty 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
were discarded. The distal gastric mucus/juice-impregnated string was flushed with 5 ml of
saline to reduce contamination by oropharyngeal organisms, 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 \textit{H. pylori} DNA detection a nested PCR specific for \textit{H.
pylori} ureA was employed (8). PCR amplification of the \textit{vacA} signal sequence and mid region
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 \( \chi^2 \) or Fisher test. All individuals swallowed the capsule without inconveniences. Among 43 \emph{H. pylori}-positive children by \( ^{13}\text{C-UBT} \), 40 were also positive for \emph{ureA} gene by the nested PCR. In the 7 \( ^{13}\text{C-UBT} \)-negative children, the \emph{ureA} nested PCR was also negative. String \emph{ureA} nested PCR had a sensitivity of 93.0% and a specificity of 100% when compared with \( ^{13}\text{C-UBT} \). 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 \emph{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 non invasive test (\( ^{13}\text{C-UBT} \)) that has high sensitivity and specificity for the diagnosis of \emph{H. pylori} infection in children older than 6 year of age (17).

\emph{vacA} gene was detected by conventional PCR in 82.5% of the \emph{ureA} nested-positive samples. Among them, the most virulent \emph{vacA} s1 genotype was the most prevalent, being observed in 27 (81.8%) samples, higher frequency than we demonstrated in symptomatic children from the southeast region of Brazil (18). Otherwise, \emph{vacA} s2 non-toxigenic genotype was observed in 6 (18.2%) samples. All 27 \emph{vacA} s1 strains were s1b. \emph{cagA} was detected in 22 (66.7%) of 33 \emph{vacA}-positive strains. Neither \emph{vacA} nor \emph{cagA} was amplified in the samples from \( ^{13}\text{C-UBT} \) -negative children. \emph{vacA} m1 and m2 alleles were detected in 17 (60.7%) and 11 (39.3%) samples, respectively. The \emph{vacA} s1m1 genotype, considered the higher cytotoxin producer, was the most frequent \emph{vacA} allelic combination and was associated with \emph{cagA}-
positivity (p=0.005) (Table 1). cagA-positive status and vacA s1 genotype neither associated with the age (p≥0.55) nor with the gender (p≥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 intra-host 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 by nested and conventional simple, accurate and inexpensive PCR the detection of H. pylori virulent 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.

REFERENCES


Table 1 - Distribution of vacA genotypes according to the cagA status of the H. pylori strains

<table>
<thead>
<tr>
<th>vacA genotype</th>
<th>cagA-positive</th>
<th>cagA-negative</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>s1m1</td>
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<td>3</td>
<td>17</td>
</tr>
<tr>
<td>s1m2</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
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<td>6</td>
</tr>
<tr>
<td>s1*</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

* Only s region was detected; N, number