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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 [^{13}C]urea breath test (^{13}C-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 absorbent 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 $\times$ *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 ^{13}C-UBT, 40 were also positive for the *ureA* gene by the nested PCR. In the 7 ^{13}C-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 ^{13}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 *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 (^{13}C-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 *ure*A 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

vacA genotype	No. of samples:		
	cagA positive	*cagA* negative	Total
vacAs1 vacAm1	14	3	17
vacAs1 vacAm2	3	2	5
vacAs2 vacAm2	0	6	6
s1[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 ^{13}C-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* virulennce 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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REFERENCES

1. **Marshall BJ.** 1994. *Helicobacter pylori.* Am. J. Gastroenterol. **89**(Suppl): S116–S126.
2. **Velapatiño B, Balqui J, Gilman RH, Bussalleu A, Quino W, Finger SA, Santivañez L, Herrera P, Piscoya A, Valdivia J, Cok J, Berg DE.** 2006. Validation of string test for diagnosis of *Helicobacter pylori* infections. J. Clin. Microbiol. **44**:976–980.
3. **Cavalcante MQ, Silva CI, Braga-Neto MB, Fialho AB, Nunes Fialho A, Barbosa AM, Cruz FW, Rocha GA, Queiroz DM, Braga LL.** 2012. *Helicobacter pylori vac*A and *cag*A genotypes in patients from northeastern Brazil with upper gastrointestinal diseases. Mem. Inst. Oswaldo Cruz **107**: 561–563.
4. **Rodrigues MN, Queiroz DM, Bezerra Filho JG, Pontes LK, Rodrigues RT, Braga LL.** 2004. Prevalence of *Helicobacter pylori* infection in children from an urban community in north-east Brazil and risk factors for infection. Eur. J. Gastroenterol. Hepatol. **16**:201–205.
5. **Queiroz DM, Carneiro JG, Braga-Neto MB, Fialho AB, Fialho AM, Goncalves MH, Rocha GA, Rocha AM, Braga LL.** 2012. Natural history of *Helicobacter pylori* infection in childhood: eight-year follow-up cohort study in an urban community in Northeast of Brazil. Helicobacter **17**:23–29.
6. **Cardinali LC, Rocha GA, Rocha AM, de Moura SB, de Figueiredo Soares T, Esteves AM, Nogueira AM, Cabral MM, de Carvalho AS, Bitencourt P, Ferreira A, Queiroz DM.** 2003. Evaluation of [^{13}C]urea breath test and *Helicobacter pylori* stool antigen test for diagnosis of *H. pylori* infection in children from a developing country. J. Clin. Microbiol. **41**:3334–3335.
7. **Samuels AL, Windsor H, Ho GY, Goodwin LD, Marshall BJ.** 2000. Culture of *Helicobacter pylori* from a gastric string may be an alternative to endoscopic biopsy. J. Clin. Microbiol. **38**:2438–2439.
8. **Wang JT, Lin LCW, Sheu JC, Yang JC, Chen DS, Wang TH.** 1993. Detection of *Helicobacter pylori* in gastric biopsy tissue by polymerase chain reaction. Eur. J. Clin. Microbiol. Infect. Dis. **12**:367–371.
9. **Atherton JC, Cao P, Peek RM, Jr, Tummuru MK, Blaser MJ, Cover TL.** 1995. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori.* Association of specific *vac*A types with cytotoxin production and peptic ulceration. J. Biol. Chem. **270**:17771–17777.
10. **Ashour AA, Magalhães PP, Mendes EN, Collares GB, de Gusmão VR, Queiroz DM, Nogueira AM, Rocha GA, de Oliveira CA.** 2002. Genotypes of *vacA* strains of *Helicobacter pylori* isolated from Brazilian adult with gastritis, duodenal ulcer or gastric carcinoma. FEMS Immunol. Med. Microbiol. **1412**:1–6.
11. **Queiroz DMM, Bitencourt P, Guerra JB, Rocha AM, Rocha GA, Carvalho AS.** 2005. *IL1RN* polymorphism and *cagA*-positive *Helicobacter pylori* strains increase the risk of duodenal ulcer in children. Pediatr. Res. **58**:892–896.
12. **Yoshida H, Hirota K, Shiratori Y, Nihei T, Amano S, Yoshida A, Kawamata O, Omata M.** 1998. Use of gastric juice-based PCR assay to detect *Helicobacter pylori* infection in culture-negative patients. J. Clin. Microbiol. **36**:317–320.
13. **Domínguez-Bello MG, Cienfuentes C, Romero R, García P, Gómez I, Mago V, Reyes N, Gueneau de Novoa P.** 2001. PCR detection of *Helicobacter pylori* in string-absorbed gastric juice. FEMS Microbiol. Lett. **198**: 15–16.
14. **Roth DE, Velapatino B, Gilman RH, Su WW, Berg DE, Cabrera L, Garcia E.** 2001. A comparison of a string test-PCR assay and a stool antigen immunoassay (HpSA) for *Helicobacter pylori* screening in Peru. Trans. R. Soc. Trop. Med. Hyg. **95**:398–399.
15. **Wang SW, Yu FJ, Lo YC, Yang YC, Wu MT, Wu IC, Lee YC, Jan CM, Wang WM, Wu DC.** 2003. The clinical utility of string-PCR test in diagnosing Helicobacter pylori infection. Hepatogastroenterology **50**: 1208–1213.
16. **Torres J, Camorlinga M, Pérez-Pérez G, González G, Muñoz O.** 2001. Validation of the string test for the recovery of *Helicobacter pylori* from gastric secretions and correlation of its results with urea breath test results, serology, and gastric pH levels. J. Clin. Microbiol. **39**:1650–1651.
17. **Mégraud F, European Paediatric Task Force on *Helicobacter pylori*.** 2005. Comparison of non-invasive tests to detect *Helicobacter pylori* infection in children and adolescents: results of a multicenter study. J. Pediatr. **146**:198–203.
18. **De Gusmão VR, Mendes EN, Queiroz DMM, Rocha GA, Rocha AMC, Ashour AAR, Carvalho AST.** 2000. *vacA* genotypes in *Helicobacter pylori* strains isolated from children with and without duodenal ulcer in Brazil. J. Clin. Microbiol. **38**:2853–2857.
19. **Morales-Espinosa, R, Castillo-Rojas G, Gonzalez-Valencia G, Ponce de Leon S, Cravioto A, Atherton JC, Lopez-Vidal Y.** 1999. Colonization of Mexican patients by multiple *Helicobacter pylori* strains with different *vacA* and *cagA* genotypes. J. Clin. Microbiol. **37**:3001–3004.