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Validation of String Test for Diagnosis of *Helicobacter pylori* Infections

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The method of recovering *Helicobacter pylori* DNA or viable cells absorbed on a string that a person has swallowed and that is retrieved an hour later (string test) should be a useful alternative to traditional analysis of cells or DNA obtained by endoscopy, which is invasive, uncomfortable, relatively costly, and ill-suited for community-based and pediatric studies. Here we assayed the sensitivity and validity of the string test versus conventional endoscopic biopsy for detecting and analyzing *H. pylori* infection. Forty-four people with gastric complaints were studied using both *H. pylori* culture and urease gene (*ureB*) PCR. *H. pylori* organisms cultured from strings and biopsy specimens from the same patients were fingerprinted by the randomly amplified polymorphic DNA (RAPD) method. Biopsy sections were also hematoxylin and eosin and silver stained for *H. pylori* detection. *H. pylori* was cultured from 80% of strings and detected by PCR from 91% of strings from participants whose biopsies had been *H. pylori* positive by culture, PCR, and/or histology. Strains recovered from strings and biopsy specimens yielded identical or closely related RAPD profiles in each of the 24 cases tested. We conclude that the string test is a useful method for *H. pylori* recovery and analysis when relatively noninvasive procedures are needed.

Helicobacter pylori is a gram-negative bacterium that chronically infects the gastric mucosa of billions of people worldwide and that constitutes a major cause of peptic ulcers and an early risk factor for gastric cancer (5). Poor sanitation and crowding are considered risk factors for *H. pylori* transmission (13, 19, 23, 27). For example, among Peruvian adults, some 90% of Lima shantytown residents but only about half of more prosperous people are infected with *H. pylori* (19, 20, 25). Also, infection with *H. pylori* is associated with chronic atrophic gastritis and hyperchlorhydria; the latter is a risk factor for diarrheal disease (6, 7). Definitive diagnosis of *H. pylori* infection has been based primarily on endoscopy and then bacterial culture and/or histologic analysis of gastric biopsy sections (28, 30). Each of these methods is highly sensitive and specific, but the requirement for endoscopy, which is costly, invasive, and discomfiting, makes it inappropriate for large-scale community-based studies or routine screening of children, the group in which most infections start.

The string test (or Entero-test) is designed as a capsule for easy swallowing with a protruding absorbent string whose end is held outside the mouth. This allows the ingested string to be retrieved and gastrointestinal microbes absorbed to the string to be recovered and studied. It has often been used to detect gastroduodenal parasites, such as *Giardia lamblia*, by microscopy (3, 11, 15) and *Salmonella enterica* serovar Typhi and *Mycobacterium tuberculosis* by bacterial culture (8, 9, 29). Recent studies also tested its potential for *H. pylori* culture (14, 18) but produced highly variable results: there were reported sensitivities of nearly 100% and 75% in Australia and Mexico,

respectively, relative to a standard urea breath test (24, 27) and 38% and 81% in China and Germany, respectively, relative to histologic examination or biopsy culture (16, 17). We have found that *H. pylori* can be detected efficiently by PCR of string test samples from Amazon rain forest residents (22). Left untested in these studies, however, was an assumption that *H. pylori* organisms obtained by string test were broadly representative of resident gastric *H. pylori* populations, as sampled by culture from biopsy samples. Here, we report efficient culturing of *H. pylori* from string test samples and DNA fingerprint evidence that strains cultured from strings are usually closely related to those from gastric biopsies from the same individual.

MATERIALS AND METHODS

Patients and the string test. Adults (18 to 70 years of age) presenting with abdominal pain and referred for endoscopy at the Gastroenterology Endoscopy Service of the Hospital Cayetano Heredia (Lima, Peru) were recruited into this study after informed consent. Excluded were patients with active bleeding, prior gastric surgery, known gastric cancer, human immunodeficiency virus, or cirrhosis or who within 7 days of endoscopy had received antibiotics or a proton pump inhibitor. Subjects came to the outpatient clinic after an overnight fast, gave their medical history, and filled out a socioeconomic questionnaire. Consenting subjects swallowed a gelatin capsule that dissolves in the stomach and that contains a metal weight and an absorbent cotton string (the “string test”), along with 100 ml of water. One end of the string protrudes from the capsule via a small hole and was attached to the patient’s cheek with tape before the capsule was swallowed (16, 17, 24, 27). The string was retrieved from the patient after 1 h, and 20 to 30 cm of its distal portion was put in 500 μ l of transport medium, which contains brain heart infusion (BHI) broth (Difco), 20% glycerol (Sigma), and 1% Skirrow supplement (Oxoid). Each sample was held at 4°C and processed for culture and PCR within 4 h of endoscopy. During the endoscopy procedure, three antral biopsy specimens were collected from each patient: one each for urease and PCR assays, for *H. pylori* culture, and for histopathology. This study was approved by the Human Research Committees of Cayetano Heredia University (UPCH), AB PRISMA, and the Johns Hopkins University School of Public Health.

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TABLE 1. Comparison of gastric biopsy to string test using culture, Warthin-Starry stain, and PCR

Gastric biopsy specimen (<i>n</i>)	No. of culture assay-positive strains/total no. of strains (% sensitivity) ^a		No. of PCR assay-positive strains/total no. of strains (% sensitivity) ^a	
	String	Gastric biopsy	String	Gastric biopsy
Histologic stain and/or culture-positive group (35)	28/35 (80%)	35/35 ^a (100%)	31/34 ^b (91.1%)	34/35 (97.4%)
Histologic stain or culture-negative group (9)	0/9	0/9	4/9	3/9

^a One gastric biopsy was positive only by stain.

^b One string did not have sufficient material present for PCR.

^c There was no significant difference between PCR and culture of the string nor between PCR of the string and PCR of the biopsy. Culture of the biopsy sample was significantly more sensitive in detecting *Helicobacter pylori* than was the string culture ($P = 0.021$).

String culture and biopsy. Each string sample in transport medium was vortexed vigorously for 3 min, and 150- μ l aliquots were spread in duplicate on BHI agar containing Skirrow supplement (Oxoid) and on Columbia colistin-nalidixic acid agar with Dent supplement (Oxoid). Gastric biopsy samples were homogenized, and 100 μ l was spread on the BHI agar with Skirrow supplement (Oxoid). Plates were incubated under microaerobic conditions (5% O₂, 10% CO₂, 85% N₂) at 37°C for 4 to 7 days. *H. pylori* colonies were identified by characteristic morphology, positive urease test, and appearance of cells recovered from them after Gram staining (25). *H. pylori* cells in biopsy samples were cultured similarly. For each *H. pylori*-positive culture (whether string or biopsy), five representative colonies were picked, and remaining colonies were pooled. Each of the resulting samples (generally 12 per patient) was processed separately for DNA extraction and randomly amplified polymorphic DNA (RAPD) fingerprinting.

Urease test. One biopsy sample was placed on urea agar, which contains 1.5% Bacto agar (Difco) and 1.5% urea agar base concentrate 10 \times (BBL). The test is considered positive if the color changes from yellow to pink within 1 h at room temperature. The biopsy sample used for this urease test was also used for DNA preparation and PCR.

PCR. To prepare DNA from the string, remaining liquid from the vial containing the string was collected and centrifuged at 13,000 $\times g$ for 10 min. DNA was extracted from resulting pellets and similarly from gastric biopsy specimens using a QIAamp DNA Mini kit (QIAGEN) (25). *H. pylori* was detected by PCR under standard conditions (1, 25) with primers specific for a 463-bp segment of the *ureB* gene (urease) (UreB-F, 5'-CGTCCGGCAATAGCTGCCATAGT; UreB-R, 5'-GTAGGCTCTGCTACTGAAGCCTTA) (10, 22).

RAPD PCR. Only 24 of 28 *H. pylori* culture-positive patients were tested by RAPD-PCR. RAPD fingerprinting was carried out as described previously (2). Briefly, genomic DNA was prepared using a QIAamp DNA Mini kit (QIAGEN) and quantitated by electrophoresis in 1% agarose gels. PCR was carried out in 25- μ l volumes containing about 9 ng of *H. pylori* DNA, 3 mM MgCl₂, 20 pmol of primer, 1.2 U of *Taq* DNA polymerase, and 250 μ M concentrations of each deoxynucleoside triphosphate in standard PCR buffer. The primers used were 1254 (5'-CCGCGAGCCAA), 1281 (5'-AACGCGCAAC), 1283 (5'-GCGATCCC CA), and 1290 (5'-GTGGATGCGA). The following cycling program was used: 45 cycles of 94°C for 1 min, 36°C for 1 min, and 72°C for 1 min. Each isolate was RAPD profiled separately with each of the four primers. After PCR, 10 μ l of product was mixed with 2 μ l of gel loading buffer (0.1% bromophenol blue and 50% glycerol), and samples were loaded on a 2% agarose gel containing 0.5 mg/ml ethidium bromide in 1 \times Tris-acetate-EDTA buffer. The 1-kb DNA ladder (Gibco BRL) was used as a size standard, and gels were photographed under UV light after electrophoresis.

If RAPD profiles of isolates were identical with each of the four primers, the isolates were classified as the same within the limits of resolution. When just 1 of the approximately 20 bands obtained (~5 bands per primer, on average) was different, the isolates were considered closely related but not identical. If two or more bands were different, then the sample pairs were provisionally designated as not closely related.

Histology. One antral biopsy specimen was fixed in 10% formalin. Paraffin sections were cut, Warthin-Starry silver and hematoxylin and eosin stained, and scored for *H. pylori* microscopically. Pathological criteria were as described previously (21). Biopsy samples positive either by culture or histologically were considered to be *H. pylori* positive and taken as the "gold standard" against which the sensitivity of the string test was compared. Statistics were calculated using the Proportion test (Stata version 8.0).

RESULTS

Forty-four of the 50 eligible patients (21 men and 23 women, each from a different household) who were invited to participate in this study provided informed consent. Their mean age was 41.6 years (standard deviation, 15.08), and their socioeconomic status was lower to lower middle class; 38 of 44 (86.3%) had permanent housing with piped potable water and sewage connections, and the others had temporary (reed or wood plank) housing. Endoscopic observation of these 44 patients indicated that 3 (6.8%) had duodenal ulcers, 2 (4.5%) had gastric ulcers, 1 (2.3%) had an early gastric cancer, 33 (75%) had only gastritis, and 5 (11.3%) had apparently normal gastric mucosa. However, histologically, none of the 42 biopsy samples that were informative were normal (2 biopsy samples were superficial and not well suited for histologic analysis): 10 of them (24%) had chronic superficial gastritis, 20 of them (47.6%) had chronic active superficial gastritis, 1 (2.4%) had chronic deep gastritis, 5 (11.9%) had chronic atrophic gastritis alone, and 6 (14.3%) had chronic atrophic gastritis and intestinal metaplasia.

H. pylori was found by both histology and culture in biopsy samples from 34 of the 44 patients (77.27%) and by histology but not culture in 1 other patient. Only 29 of the 35 (82.8%) patients that were positive by these two tests were also positive by biopsy urease assay (a typical result, probably due to patchy or low-density infection). All nine biopsies that seemed to be *H. pylori* negative by culture and histology were also negative by urease test. PCR of biopsy samples was positive for 34 of the 35 patients considered positive by culture or histology, whereas 1 patient was positive by histology (viewed separately by two observers) but negative by culture and PCR.

Most important for the present study, *H. pylori* was cultured from the strings of 28 of the 35 patients with apparently *H. pylori*-positive biopsy samples (80% sensitivity), whereas no organisms were cultured from strings from the 9 patients whose biopsy samples had scored negative (Table 1). This indicated a sensitivity of 80% and a specificity of 100%, with negative and positive predictive values of 56.2% and 100%, respectively (95% confidence interval, 0.63 to 0.92). We could not culture *H. pylori* from strings from 7 of the 35 patients who were found by other tests to be *H. pylori* infected. In three cases, there was heavy contamination with other bacterial species that would have overgrown any possible *H. pylori* colonies; the other four were not studied further.

TABLE 2. Comparison by RAPD PCR fingerprinting of *Helicobacter pylori* strains isolated from string and gastric biopsy specimens, pools, and five single colonies

RAPD comparison result	No. (%) of patients with indicated RAPD comparison result for:			
	Pooled vs single-colony biopsy isolates	Pooled vs single-colony string isolates	Pooled biopsy vs pooled string isolates	Single-colony biopsy vs single-colony string isolates
Same ^a	15 (62.5)	15 (62.5)	13 (54.1)	13 (54.1)
Similar ^b	9 (37.5)	9 (37.5)	11 (45.8)	11 (45.8)
Different ^c	0	0	0	0
Total	24 ^d	24	24	24

^a Identical profiles.

^b Similar profiles were ones in which just 1 band was different among the ~20 usually scored.

^c Different profiles were ones in which there were multiple band differences.

^d Only 24 of 28 *H. pylori* culture-positive patients were tested using RAPD PCR.

String test PCR of 34 patients judged to be *H. pylori* positive by histology and/or biopsy specimen culture indicated the presence of *H. pylori* in 31 cases and thus a sensitivity of 91% (1 of the 35 strings was not tested by PCR due to insufficient material). Similarly, *H. pylori* was detected by string PCR from 31 of 33 (93.9%) persons considered *H. pylori* positive by gastric biopsy PCR. The two PCR-negative strings still yielded *H. pylori* cultures; the lack of PCR product might be ascribed to sequence divergence in a primer-binding site.

The *H. pylori* organisms cultured from the biopsy sample and string were tested for similarity by comparing RAPD profiles of single colonies and also of pools of colonies from 24 patients (Table 2). With 13 of 24 (54%) patients, the RAPD profiles from each single colony and each pool were identical with each of four primers used (some four to six informative fragments per primer overall); with 11 of 24 patients (46%), all isolates cultured exhibited very similar profiles with a one- or two-band difference. In no patient were the strains from the string and

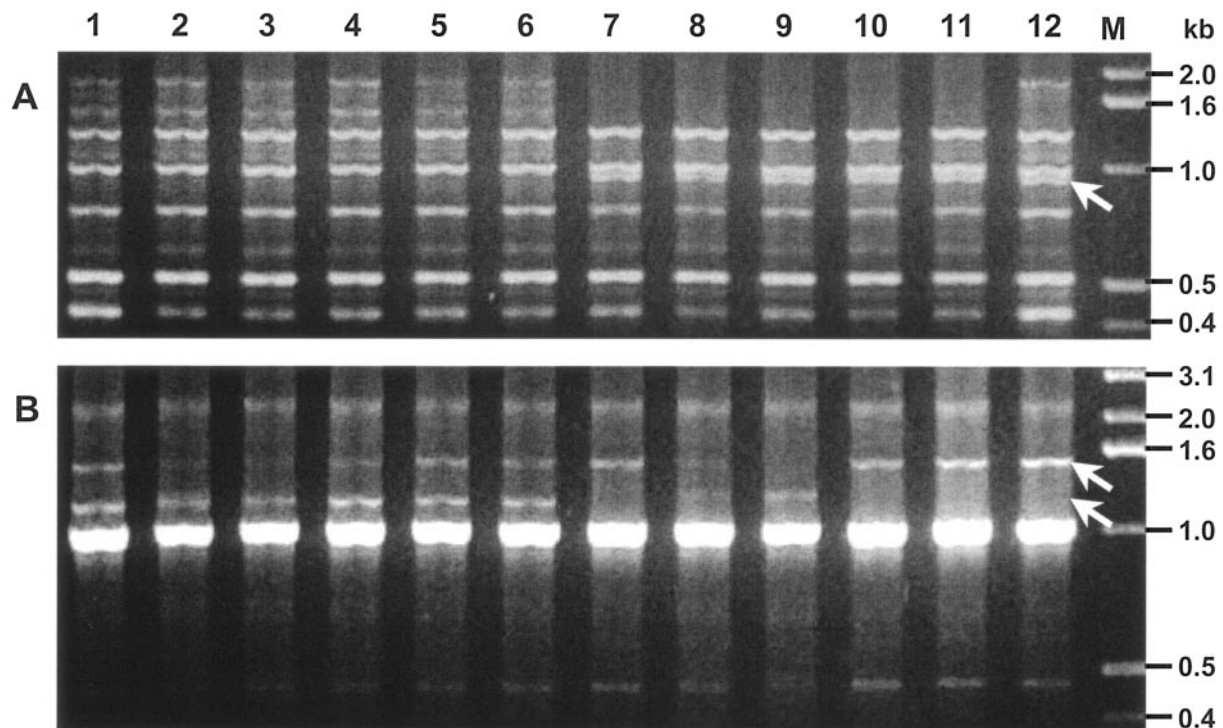


FIG. 1. Representative RAPD fingerprint patterns. The profiles shown here were generated with DNAs from five single colonies and a pool of colonies. Lane M, molecular size markers (sizes shown are 0.4, 0.5, 1.6, 2.0, and 3.1 kb); lanes 1 to 6, pool and five single colonies from biopsy samples; lanes 7 to 12, equivalent cultures from a string test of the same patients. The profile in panel A was generated with RAPD primer 1281 and was chosen to illustrate a case of subtle but real difference between predominant genotypes of strains cultured from biopsy specimen versus string test: the lower polymorphic band in the 0.9-kb doublet is identified by an arrow in lane 12. (The additional differences in yield of RAPD products ≥ 1.8 kb long may reflect differences in size of genomic DNAs used for RAPD analyses and not differences in strain genotype.) The profiles in panel B were generated with primer 1283, are from another subject, and were chosen to illustrate heterogeneity among isolates (in this case, presence/absence of 1.2-kb and 1.5-kb bands in isolates from string).

biopsy sample clearly different. Equivalent results were obtained when pools of colonies rather than individual colonies were used for comparison of biopsy sample- and string test-derived isolates. RAPD profiles of isolates cultured from a patient's biopsy sample versus a string test that were judged to be very similar but slightly different are illustrated in Fig. 1.

DISCUSSION

Our study shows that the string test, which is minimally invasive, inexpensive, and not dependent on sophisticated or costly equipment or radioactivity, allows culture of *H. pylori* from infected persons about 80% as efficiently as endoscopic gastric biopsies. Contamination with upper gastrointestinal tract bacteria as the string passes the esophagus and pharynx may be most responsible for the slightly lower efficiency of string than biopsy sample culture; changes in culture medium or inclusion of different antimicrobial supplements might further improve efficiency. PCR on DNA extracted from strings exhibited a sensitivity of 91.2%, nearly as high as biopsy DNA PCR (97.4%). PCR is rapid, highly specific, and not affected by bacterial contamination but is not well suited for phenotype analysis or extensive genetic analysis.

Our previous comparison of isolates from antrum and corpus biopsy samples revealed mixed infections in 15% of Peruvian patients, although often one strain tended to predominate (4). In addition, experimental mouse infection has shown that certain strains can differ in gastric tropism, i.e., that not all *H. pylori* strains are antrum specific (1). Even though infections can be mixed, in all cases tested here, the strains recovered from the string were closely matched to those from the antrum biopsy sample by DNA fingerprinting. Therefore, *H. pylori* organisms cultured from strings are likely to be representative of those predominating in the gastric mucosa and not a special, atypical subset. Indeed, given that *H. pylori* infection can be patchy and mixed, the strain that predominates stomach-wide may be sampled more effectively from a string (that collects from throughout the stomach) than from any single biopsy.

Small but real differences were also often seen, however. Most of these may have been generated by recombination after transiently mixed infection with an unrelated strain, which is probably quite common in this population (25), potentially coupled with selection for recombinants in at least certain gastric regions. Population genetic analysis has revealed a rich history of gene exchange among *H. pylori* lineages (26), and a case of extensive recombination during mixed infection has been documented (12).

We suggest that the *H. pylori* string test assays will be of increasing importance in a public health context. The much-used serologic tests cannot distinguish active from past infection. Stool antigen and breath tests can detect active infection but are not highly accurate after antimicrobial treatment (25) and do not give genetic, antibiotic susceptibility, or other phenotype information. Endoscopy is invasive and uncomfortable, which makes it unsuitable for children unless anesthesia is used. In contrast, the string test is minimally invasive and can be highly sensitive and specific. It should be of particular value for children under 3 years of age, the most critical group for studies of how infections begin and types of initial bacteria-host interactions that may shape infection outcomes many

years later. Pediatric string capsules can be used in children over 1 year of age (P. Herrera and R. H. Gilman, unpublished data), which ensures that the string test could be valuable for community-based and pediatric studies of the timing, specificity, and duration of active *H. pylori* infections.

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