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Culture of *Helicobacter pylori* from a Gastric String May Be an Alternative to Endoscopic Biopsy

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Helicobacter pylori **was isolated from a swallowed string from 32 of 33 adult subjects (97%) with selective culture media. With this method, antibiotic susceptibility testing and molecular epidemiology studies of *H. pylori* can be carried out without the need for the collection of specimens by endoscopic biopsy.**

Eighteen years after the original isolation of *Helicobacter pylori* (8, 9), most investigators still use essentially the same techniques as those devised in 1982. These involve culture of a freshly collected gastric biopsy specimen taken during fiberoptic endoscopic examination of the stomach (4, 5).

To avoid the necessity for endoscopy, gastric mucus samples can be collected with a swallowed gastric string. Perez-Trallero and colleagues first reported this methodology in 1995 (11), when they achieved a 50% success rate with the Entero-Test Hp (HDC Corporation, San Jose, Calif.), followed by bacterial culture in patients previously determined to be *H. pylori* positive by endoscopic biopsy. In other recent reports (2, 7, 10), successful culture at a rate between 37 and 84% has been achieved using a similar string device. When we reviewed the literature carefully, it was clear that the methodology for isolation of *H. pylori* from a swallowed string was poorly described and that most investigators had ultimately resorted to showing the presence of the organisms with a rapid urease test or to detecting *H. pylori* DNA with PCR rather than carrying out the steps necessary to culture it (3, 12, 13). Our interest in the possibility of noninvasive collection of gastric *H. pylori* samples was reactivated by the use of the rapid, 1-μCi capsule [^{14}C] urea breath test (UBT) to diagnose a number of patients who had failed *H. pylori* therapy. As many patients did not want to undergo further endoscopy to obtain cultures for *H. pylori*, we saw a need for noninvasive monitoring of antibiotic resistance in these individuals.

The study protocol was approved by our Institutional Ethics Committee (Sir Charles Gairdner Hospital, Perth, Western Australia), and 40 volunteer subjects were enrolled in this study after having given informed consent. Subjects were initially defined as *H. pylori* positive if they had a positive UBT or a positive bacterial culture from a biopsy collected during endoscopy. Subjects were negative for *H. pylori* if the UBT was negative. When the string test was negative in a predefined *H. pylori*-positive subject, endoscopic biopsy was performed, where possible, to confirm the results of the noninvasive test.

The string test chosen for our study was the Entero-Test Hp, which consists of a 90-cm length of nylon fiber enclosed in a 2.5-cm-long weighted gelatin capsule. Two types of nylon string are used within the capsule—30 cm of nonabsorbent thread and 60 cm of absorbent fiber. The patients fasted overnight, and the test was performed first thing in the morning. It was determined that the device was easiest to swallow if the first 30 cm of nonabsorbent string was pulled out and coiled on the back of the tongue so that the capsule could be swallowed as rapidly as possible by the patient. Patients were given 100 ml of water to drink until they felt that the capsule had passed through the esophagus. The string was then taped to the cheek and the patient sat quietly for 1 h. After an initial few minutes of discomfort, most patients tolerated the string very well. Patients were not asked to expectorate but to swallow their saliva normally during the test. When the string was retrieved, we found that it was best to pull it out rather quickly in one swift motion, since pulling it out slowly caused greater discomfort and more often an urge to gag.

The proximal 30 cm of the retrieved string, which had been in contact with the oral and nasopharangeal flora for 1 h, was discarded. A small 2-cm central section of the remaining 60 cm of string was placed in a rapid urease test (CLOtest) and left at room temperature for 24 h before being checked for any color change from yellow to red. The remaining string was divided into four equal sections, and two pieces of string were placed together in 3 ml of sterile saline (to eliminate adhering contaminants), while the other two sections were immediately placed in 3 ml of brain heart infusion broth (BHIB) (Acumedia, Baltimore, Md.) for transport to the laboratory. After 10 min, the string was removed from the saline wash and placed into a second volume of 3 ml of BHIB in the laboratory. Two aliquots from each BHIB mixture were plated onto three different selective medium plates. The broth suspensions were concentrated 15-fold by centrifugation at 20,000 × *g*, and two aliquots of each resuspended pellet were also plated onto the selective media.

The three selective media used were Wilkins-Chalgren agar plus Dent supplement (Oxoid), colistin-nalidixic acid agar plus Dent supplement, and Skirrow's agar. Dent supplement contains 10 mg of vancomycin per liter, 5 mg of trimethoprim per liter, 5 mg of cefsulodin per liter, and 5 mg of amphotericin B per liter and was specifically developed for the isolation of *H. pylori* (1). Skirrow's agar contains 10 mg of vancomycin per liter, 5 mg of trimethoprim per liter, and 2,500 IU of polymyxin B per liter and is usually used to isolate *Campylobacter* and *Helicobacter* species. Inoculated plates were incubated at 37°C in an atmosphere of 10% CO_2 and a relative humidity of 95 to 100% for 7 to 10 days. Bacterial colonies were identified as *H. pylori* on the basis of colonial morphology, positive tests for urease, catalase, and oxidase, and a positive Gram stain.

There were 16 male and 24 female patients in the study,

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ranging from ages 22 to 70, the average age being 46. Prior to the string test, 35 of 40 subjects were determined to be *H. pylori* positive. In 32 of these 35 subjects, *H. pylori* was isolated by the string test. In three *H. pylori*-positive subjects in whom the string test failed to detect *H. pylori*, we performed further studies. Of two subjects who underwent endoscopy, *H. pylori* was detected by culture in one. In the other subject, both culture and histology were negative, indicating that the UBT result had been a false positive. The third patient was only able to repeat the UBT, and a negative result was obtained. Thus in the final analysis, culture from the string test was positive in 32 of 33 patients, giving a sensitivity of culture from gastric string of 97%. (confidence interval = 84 to 99%). Naturally, as with all diagnostic culture of *H. pylori*, the test was 100% specific.

Adhesion of *H. pylori* to the string was confirmed by PCR for the amplification of a 314-bp segment of the urease A gene of *H. pylori*, as described by Kawamata et al. (6). The organisms in the resuspended pellets were lysed by being boiled for 10 min, and the DNA was separated from the cellular debris by the addition of a volume of chloroform and isoamyl alcohol (24:1). Amplification of this DNA mixture showed that *H. pylori* DNA was present on the strings of 34 of 40 patients. Of these, 32 patients were determined to be *H. pylori* positive by culture from the string and, as mentioned above, one patient was later determined to be *H. pylori* positive by subsequent culture from an antral biopsy. PCR results were also positive for one patient who was ultimately determined to be *H. pylori* negative by repeat UBT.

Previous researchers have used the string test in conjunction with either a CLOtest or other urease detection medium to diagnose *H. pylori* infection (3, 12). Our data shows that this would not be a reliable diagnostic method, as string sections from 21 patients gave positive urease results, but 2 of these patients had an *H. pylori*-negative status. Similarly, string samples from 19 patients gave negative urease reactions, but *H. pylori* was successfully cultured from the string of 16 of these patients.

Some weeks after the completion of the study, subjects were mailed a questionnaire to determine the level of discomfort they felt during the string test. When asked to compare the test with seven other common procedures and rank it in degree of difficulty, the string test was rated third after having a dental filling or an upper endoscopy; 73% of patients said they would choose a string test in preference to an endoscopy.

In our study, simple methodology and the use of selective bacterial culture media enabled nearly all patients with *H. pylori* (97%) to be noninvasively cultured with the string test. Since the string test is somewhat labor intensive and a little uncomfortable, patients should first receive follow-up testing with the UBT and should only have the string test when the UBT is positive. The string test can easily be performed by a trained nurse or technician, since it is apparently without risk, and it could be carried out in a general practice setting, provided that a microbiology laboratory is nearby. Our study is the first study in which high rates of isolation have been achieved with this test. Our methodology was similar to that of Perez-Trallero et al. (11), but different selective media were chosen, and the methodology of rinsing the string in saline is new. A comparison of the isolation rates obtained from the saline-washed and unwashed strings showed that there were fewer contaminants on the plates after the string had been washed. However, there were also fewer *H. pylori* colonies, which promoted the decision to pass the string through the saline wash more rapidly in future—for 1 min instead of 10 min. One drawback of this study was that we used a wide range of preculture manipulations and dilutions, as well as several selective media. We found that at least three selective media and at least two aliquots of the bacterial suspension must be plated out for each patient, as every patient had different bacterial loads of *H. pylori* and other bacterial flora. Some patients had strains that grew better on one of the plates, while others had strains that only grew on one selective medium. However, regardless of the preparation method used, a selective medium was always required. We propose that, according to our current results, if the whole length of string is washed in saline briefly for 1 min, rather than being split in two, and one 5-μl aliquot is plated onto each of the three selective media before and after centrifugation of the BHIB suspension, the number of plates is reduced to six per patient without affecting the isolation rate achieved previously.

The string test can be used for molecular epidemiological studies, as well as for the routine determination of antibiotic susceptibility prior to further therapy. If we are to control the rate of new infections in developing countries, accurate knowledge of the mode of transmission is essential.

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