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Detection of *Helicobacter pylori* by Using the Polymerase Chain Reaction

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A 1.9-kb cloned fragment of chromosomal DNA randomly selected from a *Helicobacter pylori* cloned library was evaluated as a potential probe. The probe detected 19 of 19 *H. pylori* strains and yielded a specificity of 98.7% when tested against 306 other bacterial strains representing 32 different species. False-positive results with non-*H. pylori* strains were due to the presence of contaminating vector sequences. A polymerase chain reaction (PCR) assay was developed by using 20-base oligonucleotide primers homologous to a portion of the 1.9-kb fragment. The PCR assay amplified a 203-nucleotide-pair product which was analyzed by agarose gel electrophoresis and Southern hybridization by using a third 20-base ^{32}P-labeled oligonucleotide complementary to a region of DNA between the primers. The PCR assay was 100% sensitive, detecting all 35 *H. pylori* strains tested, and did not amplify sequences in several closely related species. The assay was sensitive for as little as one copy of the cloned plasmid DNA or 100 *H. pylori* bacterial cells. To evaluate the PCR assay for clinical samples, gastric biopsy and aspirate specimens were tested by PCR, and the results were compared with those of microbiologic culture and histologic examination. In fresh biopsy specimens, *H. pylori* sequences were detected by PCR in 13 of 14 (93%) positive tissues and 0 of 19 negative tissues. In gastric aspirate specimens, 11 of 13 (85%) positive tissues were positive by PCR. *H. pylori* DNA was detected in 1 of 14 aspirate specimens negative by culture, histology, and PCR of the accompanying biopsy tissue. PCR is a rapid, accurate, and sensitive method for the detection of *H. pylori*.

Helicobacter pylori (formerly *Campylobacter pylori*) is a microaerophilic spiral gram-negative rod implicated in peptic ulcer disease and active chronic gastritis. While numerous clinical studies have confirmed the association of *H. pylori* with gastritis and peptic ulcer disease, many questions remain unanswered about the organism's mode of transmission, the natural history of the disease, and possible environmental reservoirs. In response to these questions, several assays have been developed to diagnose the presence of *H. pylori*, including microbiologic culture (7), histologic examination (5, 10, 23, 26, 30, 31, 34), rapid gastric biopsy urease tests (17), urea breath tests (9, 16, 22), serologic tests (10, 21), and more recently, the detection of *H. pylori* by DNA and RNA probe technology (4, 19, 28, 32).

Because of the fastidious growth requirements and the lengthy 3- to 7-day incubation period necessary to isolate *H. pylori*, DNA probe technology offers a more rapid and less cumbersome approach to the detection of this organism in clinical samples. Several nucleic acid techniques have been developed for the detection of *H. pylori*. These hybridization techniques have used ^{32}P-labeled and nonradioactive genomic DNA probes (32), a molecularly cloned DNA probe (4), in situ hybridization (28), and a synthetic oligonucleotide probe to 16S rRNA (19).

In this study, a 1.9-kb chromosomal DNA probe with high sensitivity and specificity for the detection of *H. pylori* was identified. Nucleic acid sequences for a portion of this fragment were determined, and primers were synthesized for use in a polymerase chain reaction (PCR) assay to maximize the sensitivity of detection. The sensitivity and specificity of PCR for the detection of *H. pylori* was established, and the detection of *H. pylori* in gastric biopsy and aspirate specimens was evaluated.

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MATERIALS AND METHODS

Bacterial strains. The *H. pylori* strains used in this study included 4 American Type Culture Collection (ATCC) reference strains (ATCC 43504, ATCC 43526, ATCC 43579, ATCC 43629); 7 strains from primates (81269, 83163, 84038, 84042, F82200, F83121, J88162) kindly provided by Melinda Bronsdon, Regional Primate Research Center, University of Washington, Seattle (3); and 24 clinical strains. The 24 clinical strains were recovered by inoculation of Skirrow selective medium with gastric biopsy material and incubation for 3 to 7 days at 37°C under microaerobic conditions (7). The isolates were identified by Gram stain and colonial morphology; positive oxidase, catalase, and urease reactions; and cellular fatty acid analysis (8). The *H. pylori* strains were suspended in brain heart infusion broth with 0.25% yeast extract–20% glycerol and were frozen in liquid nitrogen until testing.

Bacterial species that are closely related to *H. pylori* and that were used in specificity studies included *Helicobacter mustalae* ATCC 43772, *Wolinella succinogenes* ATCC 29543, *Wolinella recta* ATCC 33238, *Succinomonas amylolytica* ATCC 19206, and six clinical *Campylobacter* isolates, including *C. coli* (two isolates) and *C. jejuni* (six isolates). Additional bacterial strains representing 32 different species, including members of the family *Enterobacteriaceae* and members of the genera *Pseudomonas*, *Mobiluncus*, *Vibrio*, *Flavobacterium*, *Achromobacter*, *Acinetobacter*, *Staphylococcus*, and *Streptococcus* (Table 1), were also used to assess the specificity of nucleic acid hybridization techniques for *H. pylori*. The strains were

TABLE 1. Bacteria tested by hybridization with the ^{32}P-labeled pCYP202 probe[a]

Organism	No. of isolates tested	Organism	No. of isolates tested
Achromobacter xylosoxidans	2	*Proteus mirabilis*	18
Acinetobacter anitratum	11	*Providencia rettgeri*	1
Aeromonas hydrophila	1	*Providencia stuartii*	1
Bordetella bronchosepticum	1	*Pseudomonas aeruginosa*	64
Citrobacter freundii	10	*Pseudomonas cepacia*	1
Enterobacter aerogenes	4	*Pseudomonas maltophilia*	5
Enterobacter cloacae	18	*Pseudomonas paucimobilis*	2
Enterobacter spp.	9	*Salmonella enteritidis*	3
Escherichia coli	66	*Serratia marcescens*	13
Flavobacterium indologenes	1	*Shigella* sp.	1
Flavobacterium meningosepticum	1	*Staphylococcus aureus*	7
Hafnia alvei	1	*Staphylococcus* spp., coagulase negative	9
Klebsiella oxytoca	4	*Streptococcus viridans*	3
Klebsiella pneumoniae	34	*Vibrio parahaemolyticus*	1
Mobiluncus spp.	7	*Wolinella succinogenes*	1
Morganella morganii	4	*Yersinia enterocolitica*	2

[a] Nineteen *H. pylori* and 306 non-*H. pylori* strains were tested.

obtained from the Johns Hopkins Hospital Clinical Microbiology Laboratory and were isolated and identified by standard microbiologic techniques.

Biopsy material. Gastric biopsy specimens were obtained from 33 patients undergoing endoscopic examinations at the Johns Hopkins Hospital. Gastric aspirate specimens were collected from 27 of the 33 patients undergoing endoscopy. All biopsy specimens were cultured as described above and examined histologically, and an aliquot was frozen at −70°C. Histologic examination and Giemsa staining for *H. pylori* in the biopsy tissues were performed by the Pathology Department of the Johns Hopkins Hospital. The gastric aspirates were divided into aliquots and frozen at −70°C.

Specimen processing. Bacterial genomic DNA from *H. pylori* and closely related strains was extracted as described previously (33). Briefly, bacterial cells grown for 48 h on Skirrow medium were added to a 1.5-ml microcentrifuge tube, rinsed with phosphate-buffered saline, and pelleted by centrifugation. The pellet was resuspended in TE buffer (10 mM Tris hydrochloride, 1 mM EDTA [pH 8.0]) and digested with 100 μg of proteinase K (Boehringer Mannheim, Indianapolis, Ind.) per ml in 0.5% sodium dodecyl sulfate (SDS) for 1 h at 37°C. The solution was then treated with 5 M NaCl and 10% hexadecyltrimethylammonium bromide (H-5882; Sigma, St. Louis, Mo.) in 0.7 M NaCl for 10 min at 65°C to precipitate cellular debris. The DNA was further purified by phenol-chloroform extraction, ethanol precipitation, and resuspension in 100 μl of TE buffer. From 0.5 to 2 μg of DNA was obtained from each sample. For PCR studies, *H. pylori* strains were digested in 50 μl of tissue extraction buffer (50 mM Tris hydrochloride–1 mM EDTA containing 0.45% Tween 20, 0.45% Nonidet P-40, and 100 μg of proteinase K per ml) and incubated at 55°C for 2 h; this was followed by phenol-chloroform extraction. The resulting DNA pellet was resuspended in 50 μl of TE.

Fresh frozen gastric biopsy specimens were digested in 100 μl of the tissue extraction buffer and incubated at 55°C for 2 h; this was followed by phenol-chloroform extraction and reconstitution in 50 μl of TE. For gastric aspirate samples, 20 μl of 1 N NaOH was added to a 250-μl aliquot of specimen to eliminate acidity. The aspirates were then digested with 250 μl of tissue extraction buffer (50 mM Tris hydrochloride–1 mM EDTA containing 1% Laureth-12 and 100 μg of proteinase K per ml) and incubated at 55°C for 2 h; this was followed by phenol-chloroform extraction. The resulting DNA pellet was resuspended in 50 μl of TE.

Hybridization with the molecularly cloned probe. A cosmid clone, pCPY101, from an *H. pylori* chromosomal DNA gene bank constructed in *Escherichia coli* (18) was selected at random from among 2,000 clones. Plasmid DNA was isolated, and the majority of insert DNA was deleted by digestion with *Cla*I followed by religation and transformation of *E. coli* HB101. The resultant 8-kb plasmid, designated pCPY202, was used as a source of a 1.9-kb fragment of chromosomal DNA for use as a potential gene probe. The *H. pylori* insert was separated from vector sequences by digestion with *Cla*I and *Pst*I, electrophoresed on a 1% agarose gel, and electroeluted.

To evaluate the ability of this probe to detect *H. pylori*, chromosomal DNA from *H. pylori* strains was digested with *Hin*dIII at 37°C for 18 h. The DNA fragments were electrophoresed on 0.7% agarose gels and transferred to nylon filters (Nytran; Schleicher & Schuell, Inc., Keene, N.H.) for Southern blot analysis (25). The filters were probed with ^{32}P-labeled *H. pylori* DNA, which was labeled by nick translation to a specific activity of 3×10^7 to 2×10^8 cpm/μg. The filters were prehybridized at 42°C for 3 h in 10 ml of 10× Denhardt solution (10× Denhardt solution is 0.2% bovine serum albumin, 0.2% polyvinylpyrrolidone, and 0.2% Ficoll), 0.5% SDS, 6× SSC (20× SSC is 3 M NaCl plus 0.3 M sodium citrate [pH 7.0]), 43% formamide, and 100 μg of tRNA per ml. The hybridization solution consisted of 10× Denhardt solution, 0.5% SDS, 4× SSPE (20× SSPE is 3 M NaCl, 200 mM NaH_2PO_4 [pH 7.4], 20 mM EDTA [pH 7.4]), 43% formamide, 100 μg of tRNA per ml, and a sufficient quantity of labeled probe to obtain 10^7 cpm per filter. The filters were hybridized overnight at 42°C; washed in 2× SSC containing 0.1% SDS at room temperature; and then washed three times at 68°C for 10, 20, and 30 min, respectively. The filters were exposed to X-Omat XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) with intensifying screens for 4 to 18 h.

To evaluate the specificity of the 1.9-kb fragment for *H. pylori*, non-*H. pylori* strains were grown on Mueller-Hinton agar and tested by colony lift hybridization on nylon filters. *H. pylori* ATCC 43504 and *Mobiluncus* strains were spotted onto the filters to serve as positive and negative controls, respectively. The cells were lysed, and the DNA was fixed by the method of Maniatis et al. (15). The filters were

hybridized with the molecularly cloned probe under the conditions described above.

Selection of PCR primers and oligonucleotide probe. Sequence data on a 288-bp fragment of the 1.9-kb *H. pylori* DNA was used to select two 20-base oligonucleotide primers designated CAM-2 (5′-CATCTTGTTAGAGGGATTGG-3′) and CAM-4 (5′-TAACAAACCGATAATGGCGC-3′). The expected product of amplification of the target sequence with these primers was 203 nucleotide pairs (np) in length. A third oligonucleotide designated CAM-3 (5′-CGCTCTTTA GTTTTGGAGCG-3′), which was complementary to a region of DNA between the two primers, was synthesized for use as a specific probe to identify the amplified *H. pylori* DNA in the reaction products.

PCR amplification. Amplification was performed in 50-μl reaction volumes containing 0.5 μM (each) oligonucleotide primer (CAM-2 and CAM-4); 200 μM (each) dATP, dCTP, dTTP, and dGTP; 1× reaction buffer (50 mM KCl, 10 mM Tris hydrochloride [pH 8.3], 1.5 mM MgCl, 0.01% [wt/vol] gelatin); 1.25 U of *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, Conn.); and a 10-μl volume of sample DNA. Prior to the addition of sample DNA, the tubes were overlaid with mineral oil. Forty cycles of amplification were performed in a DNA thermal cycler (Perkin-Elmer Cetus). Each cycle consisted of a 1-min denaturation step at 95°C, a 30-s annealing step at 42°C, and a 1-min extension step at 72°C.

The PCR-amplified products were analyzed by agarose gel electrophoresis and Southern hybridization. A 10-μl sample of PCR-amplified products was electrophoresed on a 4% gel (3% NuSieve, FMC Bioproducts, Rockland, Maine; 1% BRL agarose, BRL Products, Bethesda, Md.). The gel, which was stained with ethidium bromide, was examined under UV light for the presence of the 203-np fragment of amplified DNA. Southern transfer was performed on Nytran filters as described above. The filters were exposed to UV light for 2 min and allowed to air dry. Hybridizations were performed by using the oligonucleotide CAM-3 that was end-labeled with [^{32}P]ATP and T4 polynucleotide kinase (U.S. Biochemical Corp., Cleveland, Ohio) to a specific activity of 10^8 to 10^9 cpm/μg. The filters were prehybridized for 1 h at 42°C in 3× SSPE–5× Denhardt solution–0.5% SDS–0.25 mg of tRNA per ml. The CAM-3 probe was added to the hybridization tube (final concentration, 5 ng/ml), and the filters were reincubated at 42°C for 1 h. The filters were washed at room temperature in 3× SSPE (once) and 1× SSPE containing 0.1% SDS (three times) for 5-min periods. A final wash was performed for 10 min at 50°C in 5× SSPE–0.1% SDS. The filters were exposed to X-Omat XAR-5 film (Eastman Kodak Co.) with intensifying screens for 1 to 4 h.

RESULTS

Hybridization studies with the molecularly cloned probe. To evaluate the sensitivity of the full-length, 1.9-kb molecularly cloned probe, *Hin*dIII digests of chromosomal DNA from 19 *H. pylori* strains were electrophoresed on 0.7% agarose gels and transferred to filters for Southern blot analysis. The probe detected all 19 *H. pylori* strains, including 18 clinical isolates and *H. pylori* ATCC 43504. Figure 1 shows the results of Southern hybridization of seven representative *H. pylori* clinical isolates that were digested with *Hin*dIII. *H. pylori* ATCC 43504 and *W. succinogenes* ATCC 29543 were included as positive and negative controls, respectively. Genomic variation was reflected in the heterogeneity of band sizes seen on the Southern hybridizations; however, a majority of the *H. pylori* clinical isolates had similar patterns with two to five bands. The predominant patterns consisted of two major bands, usually a smaller fragment of 1.2 to 1.4 kb and a larger fragment ranging from 3.2 to 5.8 kb. Following hybridization, the probe was removed from the complementary sequences on the filters by washing the filters for 15 min (twice) at 90°C in 0.1× SSC–0.1% SDS. No hybridization was observed when the same filters were retested with the pHC79 vector (nick translated with [^{32}P]ATP), demonstrating that signals seen with the molecularly cloned *H. pylori* probe were due to hybridization of the homologous sequences of *H. pylori* and not to homology with vector sequences.

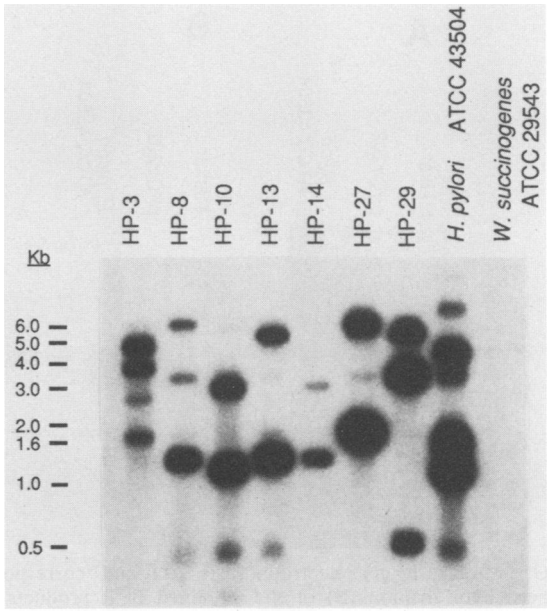

FIG. 1. Southern blot analysis of *Hin*dIII digests of chromosomal DNA from seven *H. pylori* clinical isolates, *H. pylori* ATCC 43504, and the negative control strain *W. succinogenes* ATCC 29543. Some heterogeneity was observed among the *H. pylori* isolates; however, a majority of the clinical strains had patterns similar to that of either HP-10 or HP-13 shown on this hybridization.

The specificity of the molecularly cloned probe was evaluated by testing 306 non-*H. pylori* clinical isolates by colony lift hybridization. The overall specificity was 98.7%. Of 306 bacterial strains tested (Table 1), all except 3 of 65 *E. coli* isolates and 1 of 64 *Pseudomonas aeruginosa* isolates were negative. These false-positive results were due to contaminating vector sequences which were not completely eliminated during the electrophoretic purification of the 1.9-kb fragment (data not shown). The *P. aeruginosa* isolate was not available for further testing, but the three *E. coli* isolates were subsequently shown to be nonreactive by PCR.

PCR assay. Since the studies with the molecularly cloned probe demonstrated that the *H. pylori* sequences in the 1.9-kb fragment of chromosomal DNA were specific and detected all *H. pylori* strains tested, a PCR assay was developed. Sequence data for a 288-base fragment of the 1.9-kb *H. pylori* DNA were used to select primers (CAM-2, CAM-4) and a probe (CAM-3) for the amplification and detection of a 203-np fragment (Fig. 2A). These primers successfully amplified a product of the expected size when either pCPY202 or *H. pylori* ATCC 43504 DNA was used as

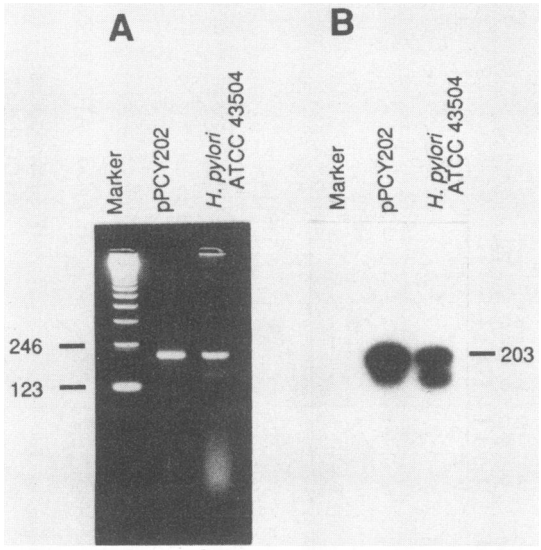

FIG. 2. Agarose gel electrophoresis (A) and corresponding Southern hybridization (B) of the amplified PCR products from pCPY202 plasmid DNA and *H. pylori* ATCC 43504 resulting in a 203-np product. The marker lane contains 123-bp ladder (Bethesda Research Laboratories, Rockville, Md.). The numbers next to the figures are in nucleotide pairs. The lower band seen on agarose gel electrophoresis and Southern blot hybridization represents specific single-stranded PCR product.

a template and the PCR products were analyzed by agarose gel electrophoresis (Fig. 2). The presence of the *H. pylori*-specific sequences in the 203-np product (Fig. 2A) was confirmed by the hybridization with the CAM-3 oligoprobe (Fig. 2B). The limit of detection of the *H. pylori* sequences by PCR was evaluated by preparing 10-fold dilutions of the plasmid DNA and bacterial suspensions. As little as one copy of the pCPY202 DNA per 10 μl of reaction mixture (prior to amplification) could be detected when a 10-μl volume of PCR product was tested by Southern hybridization with a 2-h autoradiographic exposure (Fig. 3). The sensitivity of PCR for detection of bacterial cells was determined by using *H. pylori* ATCC 43579 grown in brucella broth (Oxoid, Ltd., Basingstoke, England) and 5% fetal bovine serum. Cells were quantitated microscopically, 10-

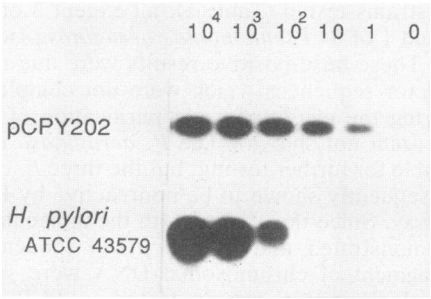

FIG. 3. PCR sensitivity of detection for pCPY202 plasmid DNA and *H. pylori* ATCC 43579. The numbers represent the number of plasmid copies in 10 μl of reaction mixture prior to amplification or the number of *H. pylori* bacterial cells per reaction tube. The products were analyzed by Southern hybridization with a 2-h autoradiographic exposure. The limit of detection was one copy of pCPY202 DNA or 100 whole bacterial cells.

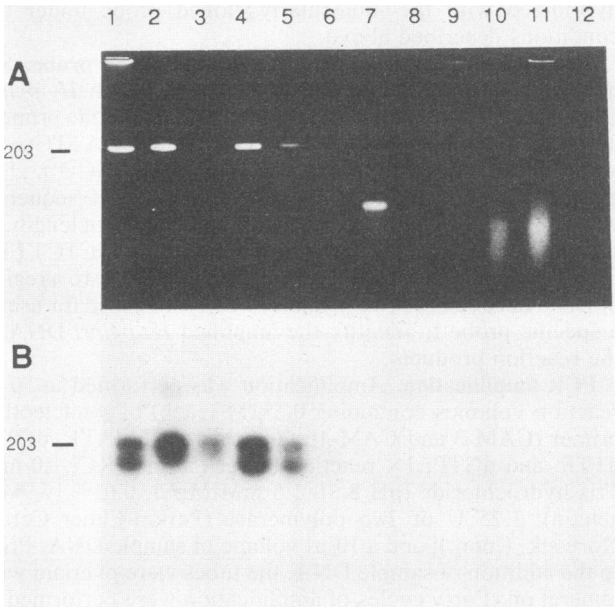

FIG. 4. Agarose gel electrophoresis (A) and the corresponding Southern hybridization (B) of amplified PCR products. The first four lanes represent 2 of 4 *H. pylori* ATCC reference strains (lane 1, ATCC 43504; lane 3, ATCC 43579), the plasmid pCPY202 DNA (lane 2), and 1 of the 24 *H. pylori* clinical isolates (lane 4), all of which demonstrate the presence of the target 203-np fragment. Lane 5, Results from a representative gastric biopsy tissue specimen. The 203-np band was present in the fresh tissue, as determined by agarose gel electrophoresis and Southern blot analysis. Lanes 7 to 12 demonstrate that the primers did not amplify sequences in several closely related strains, including *H. mustalae* ATCC 43772 (lane 7), two *Wolinella* ATCC strains (lane 8, *W. succinogenes* ATCC 29543; lane 9, *W. recta* ATCC 33238), two of six clinical isolates representing *C. coli* (lane 10) and *C. jejuni* (lane 11), and *S. amylolytica* ATCC 19206 (lane 12). Lane 6, Amplification products for a paraffin-embedded biopsy tissue (see text). The paraffin-embedded PCR product is barely visible on the agarose gel and is positive by Southern blot analysis.

fold dilutions were prepared, and the dilutions were treated with tissue extraction buffer and amplified by PCR. One hundred organisms were required for a positive hybridization signal.

Thirty-five *H. pylori* strains were tested by PCR, including 4 ATCC reference strains, 7 primate strains, and 24 clinical isolates from the Johns Hopkins Hospital. All 35 strains were positive when the products were examined by agarose gel electrophoresis and Southern hybridization. To confirm the specificity of the assay, purified chromosomal DNAs from several closely related bacterial species and clinical isolates were tested. These microorganisms included *H. mustalae* ATCC 43772, *W. succinogenes* ATCC 29543, *W. recta* ATCC 33238, *S. amylolytica* ATCC 19206, and clinical isolates of *C. jejuni* (four strains) and *C. coli* (two strains). PCR results for these organisms were uniformly negative (Fig. 4). Two clinical strains of *Haemophilus influenzae* and one *Haemophilus parainfluenzae* that were not previously screened with the molecularly cloned probe were also tested and shown to be negative by PCR.

PCR assay of gastric tissue. Gastric tissue biopsy specimens obtained from 33 patients were tested by PCR, and the results were compared with those of the Giemsa stain and culture methods. Fourteen specimens were positive by ei-

TABLE 2. Detection of *H. pylori* in gastric biopsies and gastric aspirates from 33 patients

Culture or histology[a]	PCR			
	Biopsy		Aspirate	
	No. tested	No. (%) positive	No. tested[b]	No. (%) positive
Positive	14	13 (93)	13	11 (85)
Negative	19	0 (0)	14	1[c] (7)

[a] Analysis of biopsy specimen.
[b] Aspirates were not available for six patients.
[c] The patient had active chronic gastritis, but no organisms were seen on histologic examination; culture was negative.

ther the culture or Giemsa stain method, the reference standard. Twelve specimens were detected by histology, and 11 specimens were detected by culture. One specimen was not examined histologically but was positive by culture, another specimen was negative by histology but was positive by culture, and two tissues were negative by culture but were positive by histologic examination. One of these negative cultures was likely due to a 24-h delay in transport; the other negative culture was accidentally transported in Formalin rather than saline prior to culturing. *H. pylori* sequences were detected by PCR in 13 of 14 fresh specimens that were positive by the reference method (Table 2).

Two biopsy tissue specimens from the only patient that was positive by the reference method and negative by PCR were sent to the pathology laboratory; the specimens were collected during a single endoscopic examination. No organisms were seen in one biopsy specimen, and only very few *H. pylori* organisms were seen in the other specimen; however, both culture and PCR results were negative, suggesting that a sampling error may have been responsible for the negative PCR result (2, 20). The specificity of PCR was 100%, because all gastric tissue specimens negative by the reference method were also negative by PCR (Table 2).

PCR assay of gastric aspirates. Gastric aspirates were collected from 27 of the patients and tested by PCR. The assay detected *H. pylori* sequences in 11 of 13 aspirates (85%) for which the corresponding biopsy was positive by Giemsa stain, the culture method, or both. PCR failed to detect *H. pylori* in one aspirate from a patient whose gastric biopsy specimen was positive by culture, histologic staining, and PCR assay. This negative PCR result may reflect the lower number of organisms found in the gastric aspirate compared with that found in the gastric tissue. The second aspirate that was negative by the PCR assay was from a patient whose biopsy specimen was PCR and culture negative, although very few *H. pylori* organisms were seen in one of two tissue specimens sent to the pathology laboratory for analysis. Of 14 aspirates whose corresponding tissue biopsy specimens were negative by Giemsa stain or culture, 13 were also negative by the PCR assay. Only 1 of 14 negative aspirates gave positive results by PCR. This false-positive result may be a result of sample contamination or carry-over during the PCR assay; however, histologic examination of the corresponding tissue demonstrated slightly active chronic gastritis typical of *H. pylori* infections, although no organisms were seen and culture and PCR results of the original tissue were negative.

DISCUSSION

Since the discovery of *H. pylori*, several nucleic acid techniques have been applied to the characterization and detection of *H. pylori*. Initial work by Langenberg et al. (12) demonstrated the genomic variation of *H. pylori* using restriction endonuclease analysis with *Hin*dIII. While Langenberg et al. (12) suggested the usefulness of this technique for typing strains, Majewski and Goodwin (14) found this method unsuitable for typing strains because of the demonstrated variation in the profiles of consecutive isolates from the same patient. In our study, *Hin*dIII digests of *H. pylori* chromosomal DNA were similarly run on agarose gels and then transferred to nylon filters for Southern blot analysis with the 1.9-kb DNA probe to examine the resulting hybridization patterns. While some band size heterogeneity was observed on Southern blot analysis, the majority of the resulting *H. pylori* patterns fell into two predominant patterns. We did not evaluate multiple specimens or isolates from the same patient; therefore, we cannot assess the utility of the probe for detecting the consistency of patterns in sequential isolates from the same patient.

Several nucleic acid techniques have been developed for the detection of *H. pylori*. Wetherall et al. (32) used dot blot hybridization to evaluate three nonradioactive probes and a ^{32}P-labeled probe from whole genomic *H. pylori* DNA. Vandenberg et al. (28) developed an in situ hybridization assay using whole genomic DNA and a biotinylated probe. More recently, Clayton et al. (4) cloned *H. pylori* DNA into *E. coli* K-12 and developed a DNA probe with a 17-kb restriction fragment insert.

While DNA probes with whole genomic DNA or large restriction fragments allow for a high level of radioactive incorporation, they have the disadvantage of requiring lengthy hybridization times (32). Improvements may be made to these methods by the use of oligoprobes, which have the advantage of rapid hybridization kinetics, allowing for much shorter hybridization periods. By synthesizing an oligoprobe homologous to 16S rRNA sequences, Morotomi et al. (19) developed a DNA-RNA hybridization assay with more rapid hybridization times. The use of 16S rRNA as the target molecule which is present within the cell in a high copy number increased the sensitivity (19).

A further improvement to the sensitivity of DNA probes is the amplification of the target DNA by PCR. PCR offers the advantages of maximum sensitivity, specificity, and rapidity when combined with an oligoprobe for detection of reaction products. The PCR assay can detect the DNA from as few as 100 bacterial cells, which represents a 100-fold increase in sensitivity over that of the 16S rRNA oligoprobe method (19) and a 4-log-unit increase in the sensitivity reported for the whole genomic DNA probes (32). This low level of detection may be well-suited for verifying eradication following therapy. The exquisite sensitivity of PCR is a potential disadvantage, since false-positive reactions can result from sample contamination or carry-over, a possible explanation for the patient whose gastric aspirate tested positive by PCR, despite negative histology and culture results for the corresponding tissue. This problem can be overcome with meticulous laboratory technique and careful attention to negative controls.

The PCR assay described here correctly identified all 35 *H. pylori* strains tested, including seven strains from monkeys isolated by Bronsdon and Schoenknecht (3). This finding supports previous data which suggest that these strains from primates may be identical to *H. pylori* from

humans. Although closely related to *H. pylori*, the strain from ferrets, *H. mustalae*, was nonreactive by PCR, which is in agreement with earlier studies showing that there are differences between these organisms (6). For detection of *H. pylori* in fresh gastric biopsy material, the PCR assay demonstrated a good correlation with microbiologic culture and histologic examination. The assay requires only the presence of intact DNA for diagnosis, as was seen for two patients in whom nonviable organisms were present.

The recovery of *H. pylori* by culture of gastric aspirates is poor, with reported sensitivities ranging from 38 to 56% (27, 29). This poor sensitivity of the culture method probably reflects low numbers of organisms or the loss of viability of organisms in acidic aspirate specimens. In this study, the sensitivity of the assay for the detection of *H. pylori* in gastric aspirates was 85% when compared with the results of culture and histologic examination of biopsy tissue. This represents a significant improvement over the results reported in previous studies that used the culture method to detect *H. pylori* in gastric aspirates (27, 29). Collection of gastric aspirate specimens and testing by PCR may be an attractive alternative to invasive endoscopic procedures.

Amplification of *H. pylori* sequences by PCR is a useful technique for diagnosing *H. pylori* infections. PCR has the requisite sensitivity and specificity, and compared with other hybridization techniques, it has a short turnaround time. The use of the oligoprobe to detect the correct fragment in the reaction products ensures the specificity of the test and permits shorter hybridization times to minimize the diagnosis time to 8 h if the products are analyzed by agarose gel electrophoresis or 12 h if the products are further analyzed by dot or slot blot hybridization. Use of a nonradioactive probe and detection system would make use of the PCR method more appealing for clinical laboratories without any significant loss of sensitivity.

The examination of paraffin-preserved tissue by PCR has also been used to diagnose infections (1, 24). The study of archival material for *H. pylori* would permit a retrospective research of this newly recognized pathogen. In preliminary studies of paraffin-embedded gastric biopsy specimens, *H. pylori* sequences were successfully amplified (Fig. 4). The specimens were processed as described by Shibata et al. (24). The level of detection of *H. pylori* in paraffin-embedded material, however, was poorer than when fresh tissue from the same biopsy specimen was tested. The decreased sensitivity of PCR when testing the paraffin-embedded samples may be due to decreased tissue volume, the inhibitory effects of the tissue fixative, or DNA loss during tissue extraction. Further optimization of sample preparation procedures will be required to make PCR a useful technique for the detection of *H. pylori* in paraffin-preserved material.

A future application of the PCR assay is to search for other vegetative and nonculturable coccoid forms of *H. pylori* which may be present in gingival scrapings, oral secretions, or feces (11, 13). Likewise, it could be applied to evaluate further a more noninvasive diagnostic method or to elucidate the mode of transmission of this organism. Finally, the assay could be used as an epidemiologic tool for the investigation of possible environmental or animal reservoirs of the organism.

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