2004

Multiplex PCR assay for rapid detection and genotyping of Helicobacter pylori directly from biopsy specimens

Santanu Chattopadhyay
National Institute for Cholera and Enteric Diseases

Rajashree Patra
National Institute for Cholera and Enteric Diseases

T. Ramamurthy
National Institute for Cholera and Enteric Diseases

Abhijit Chowdhury
Department of Gastroenterology, Institute of Post Graduate Medical Education and Research, Calcutta, India

Amal Santra
Department of Gastroenterology, Institute of Post Graduate Medical Education and Research, Calcutta, India

See next page for additional authors

Follow this and additional works at: https://digitalcommons.wustl.edu/open_access_pubs

Recommended Citation
Chattopadhyay, Santanu; Patra, Rajashree; Ramamurthy, T.; Chowdhury, Abhijit; Santra, Amal; Dhali, G. K.; Bhattacharya, S. K.; Berg, Douglas E.; Nair, G. Balakrish; and Mukhopadhyay, Asish K., "Multiplex PCR assay for rapid detection and genotyping of Helicobacter pylori directly from biopsy specimens." Journal of Clinical Microbiology.42.6. 2821-2824. (2004). https://digitalcommons.wustl.edu/open_access_pubs/2594

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact engeszer@wustl.edu.
Authors

This open access publication is available at Digital Commons@Becker: https://digitalcommons.wustl.edu/open_access_pubs/2594
Multiplex PCR Assay for Rapid Detection and Genotyping of *Helicobacter pylori* Directly from Biopsy Specimens


Updated information and services can be found at: http://jcm.asm.org/content/42/6/2821

These include:

**REFERENCES**

This article cites 13 articles, 9 of which can be accessed free at: http://jcm.asm.org/content/42/6/2821#ref-list-1

**CONTENT ALERTS**

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: http://journals.asm.org/site/misc/reprints.xhtml

To subscribe to another ASM Journal go to: http://journals.asm.org/site/subscriptions/
Multiplex PCR Assay for Rapid Detection and Genotyping of *Helicobacter pylori* Directly from Biopsy Specimens

Santanu Chattopadhyay,1 Rajashree Patra,1 T. Ramamurthy,1 Abhijit Chowdhury,2 Amal Santra,2 G. K. Dhali,2 S. K. Bhattacharya,1 Douglas E. Berg,3 G. Balakrish Nair,4 and Asish K. Mukhopadhyay1*

National Institute of Cholera and Enteric Diseases1 and Department of Gastroenterology, Institute of Post Graduate Medical Education and Research,2 Calcutta, India; Department of Molecular Microbiology, Washington University Medical School, St. Louis, Missouri 631103; and International Centre for Diarrhoeal Diseases Research, Dhaka, Bangladesh4

Received 1 October 2003/Returned for modification 4 December 2003/Accepted 2 February 2004

We developed and evaluated a simple, novel multiplex PCR assay for rapid detection of *Helicobacter pylori* infection and for the determination of *vacA* and *cagA* genotypes directly from gastric biopsy specimens. This assay did not require culturing of strains or extraction of DNA from biopsy samples. This multiplex PCR assay would be of particularly great value for laboratories in developing countries.

The gastric pathogen *Helicobacter pylori* causes gastritis and peptic ulcer disease and is an early risk factor for development of gastric adenocarcinoma (15). Because it is fastidious and microaerophilic, it cannot be cultured in many clinical or research laboratories in resource-poor developing countries. None of the methods to detect *H. pylori* infection, including the recently developed *H. pylori* stool antigen test (4) and PCR restriction analysis using a RNA polymerase gene (*rpoB*) (10), can give a clue to the virulence genes of *H. pylori* strains harbored by the host (6, 8, 9). Although Park et al. (13) developed a method for obtaining *H. pylori* genotype data directly from biopsy samples, it involves extraction of genomic DNA and requires several PCRs.

We report the development of a multiplex PCR assay by which *vacA* signal sequence (s1 and s2) and mid-region (m1 and m2) alleles and the presence or absence of a *cagA* gene can be detected in a single reaction, directly from gastric biopsy samples within 4 h after endoscopy, without a need for culture or genomic DNA extraction. This can save several weeks’ time and expensive reagents (e.g., antibiotic, serum, or blood- and growth-supplemented media and reagents for DNA extraction) and instrumentation (e.g., double gas incubator). The other *H. pylori* virulence gene multiplex PCR reported earlier was not standardized for biopsy samples, required expensive Ready-To-Go PCR beads, and does not type *vacA* alleles (11).

A total of 79 *H. pylori* strains isolated from India (65 strains), Japan (6), Spain (5), Australia (strain SS1; mouse colonized), and the United Kingdom and United States (strains 26695 and J99; genomes fully sequenced) were used to develop the multiplex PCR assay. Fresh biopsies (total, 90) were collected randomly from patients with duodenal ulcer (41 biopsies), gastrict ulcer (9), nonulcer dyspepsia (20), duodenitis (6), gastritis (4), and adenocarcinoma (7) and from three healthy volunteers for further evaluation of this method. Each person’s infection status inferred from multiplex PCR was further assayed by rapid urease testing (RUT), and culture and genotype data obtained from biopsy multiplex PCR were evaluated by PCR using DNA extracted from cultured strains isolated from the respective patients, as described earlier (5). Nucleotide sequences of primers used are given in Table 1.

To find a suitable conserved part we aligned 20 full gene sequences of *cagA* of *H. pylori* deposited in the GenBank from different parts of the world and *cagA* sequences of the two reference (genome-sequenced) strains (strains 26695 and J99). Primers cag5c-F and cag3c-R were designed from conserved regions to amplify 350 bp (positions 109 to 459 of *cagA* of strain 26695) (Table 1).

For multiplex PCR from biopsies, specimens collected in microcentrifuge tubes containing 120 μl of sterile phosphate-buffered saline were vortexed vigorously for 2 min. The tubes were then boiled in a water bath for 15 min, cooled in ice, and centrifuged at 13,000 × g for 1 min. The supernatant was transferred to another tube, and 3 μl was added in 25-μl volumes containing 2.5 pmol of primers VAG-F and VAG-R, 25 pmol of primers VA1-F and VA1-R, 10 pmol of primers cag5c-F and cag3c-R, 0.25 mM of each deoxynucleoside triphosphate (Takara, Shuzo, Japan), and 1.5 mM of MgCl₂ in standard PCR buffer (Takara). Products were amplified under the following conditions: 3 min at 94°C for initial denaturation followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, with a final round of 10 min at 72°C, in a Perkin-Elmer 9700 thermal cycler. The sensitivity assay for multiplex PCR and nucleotide sequencing was carried out as described elsewhere (12, 14). Using this multiplex PCR, we characterized 65 previously isolated Indian strains. Of the strains, 44 carried an s1m1

---

* Corresponding author. Mailing address: National Institute of Cholera and Enteric Diseases, P-33, CIT Rd., Scheme XM, Beliaghata, Calcutta 700010, India. Phone: 91 33 2350 1176. Fax: 91 33 2350 5066. E-mail: asish_mukhopadhyay@yahoo.com.
cagA allelic combination, 16 carried s1m2 cagA+, and 5 carried s2m2 and were cagA-negative strains (Fig. 1a). These genotypes were confirmed in each case by PCR using single primer pairs. We also accurately typed the vacA and cagA status of six strains from Japan, five strains from Spain, one mouse-colonizing strain (strain SS1) from Australia, and the two reference strains 26695 and J99 from the United Kingdom and United States, respectively, by multiplex PCR (Fig. 1b). Each of the six strains (five from India and one from Spain) that gave no cagA amplicon yielded the 550-bp amplicon expected of the cag empty site (no cag pathogenicity island) in PCR with primers Lunil and R5280 (12) (Fig. 1c).

Assays with the addition of boiled cultures of bacterial strains after serial dilution showed this multiplex PCR to be

**TABLE 1. Primers used for the amplification of vacA alleles and cagA**

<table>
<thead>
<tr>
<th>DNA region(s) amplified</th>
<th>Primer name</th>
<th>Primer sequence</th>
<th>Amplicon Size(s) (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vacA s1/vacA s2</td>
<td>VAI-F</td>
<td>5’-ATGGAATACAAACAAACACAC-3’</td>
<td>259/286</td>
</tr>
<tr>
<td></td>
<td>VAI-R</td>
<td>5’-CTGCTTGAATGCGCAAAAC-3’</td>
<td></td>
</tr>
<tr>
<td>vacA m1/vacA m2</td>
<td>VAG-F</td>
<td>5’-CAATCTGTCATCAAGCGAG-3’</td>
<td>567/642</td>
</tr>
<tr>
<td></td>
<td>VAG-R</td>
<td>5’-GGCTCAAAATAATTCCAGG-3’</td>
<td></td>
</tr>
<tr>
<td>cagA</td>
<td>cag5c-F</td>
<td>5’-GTTGATAAAGCTGTTGCTTTC-3’</td>
<td>350</td>
</tr>
<tr>
<td></td>
<td>cag3c-R</td>
<td>5’-GGGTGTTATGTATATTTCCATAA-3’</td>
<td></td>
</tr>
</tbody>
</table>

**FIG. 1. Amplification of vacA s1, vacA s2, vacA m1, and vacA m2 alleles and the cagA gene of H. pylori by multiplex PCR assay.** (a) Lane M, 100-bp marker (New England Biolab); lanes 1 to 5, H. pylori strains isolated from India. Lanes 1 to 3, s1m1 cagA+ genotype; lane 2, s1m2 cagA+ genotype; lane 5, genotype of s2m2 cagA-negative strain. (b) Lane M, 100-bp marker (New England Biolab); lanes 1 to 5, H. pylori strains isolated from different part of the world. Lane 1, strain HUP 77 (s1m1 cagA+) from Spain; lane 2, strain TN2 (s1m1 cagA+) from Japan; lane 3, strain SS1(s2m2 cagA+) from Australia; lane 4, strain J99 (s1m1 cagA+) from the United States; lane 5, strain 26695 (s1m1 cagA+) from the United Kingdom. (c) Amplification of 550-bp fragment of cag-pathogenicity island empty site for cagA-negative strains discerned by multiplex PCR assay. Lanes 1 to 5, cagA-negative strains isolated from India; lane 6, HUP67, a cagA-negative strain isolated from Spain.

**TABLE 2. Sensitivity and specificity of biopsy multiplex PCR assay for the detection of H. pylori virulence genes in comparison with tissue rapid urease test (RUT) and culture**

<table>
<thead>
<tr>
<th>Test and result</th>
<th>No. of specimens with multiplex PCR result that was:</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>RUT*</td>
<td>41</td>
<td>2</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>43</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Culture*</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>45</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>45</td>
</tr>
</tbody>
</table>

* Sensitivity, 95.3%; specificity, 91.5%; positive predictive value, 91.1%.

* Sensitivity, 100%; specificity, 91.8%; positive predictive value, 91.1%.
highly sensitive, allowing detection of few cells per reaction. This motivated us to standardize the assay with boiled biopsy specimens.

Positive PCR was obtained with biopsies from 41 of 43 patients that had given positive results in RUT, and no PCR amplicon was obtained with biopsies from 43 of 47 patients that had given negative results in RUT (Table 2). Moreover, the method also provided preliminary H. pylori virulence genes by multiplex PCR from biopsy specimens. As shown in lane 2, biopsy 23 gave a shorter amplicon for the vacA middle region. (b) Lane M, 100-bp marker (New England Biolab); lanes 1 to 5, multiplex PCR results obtained from DNA extracted from strains isolated from the respective patients. As shown in lane 2, strain PCR 23 gave a shorter amplicon similar to that obtained with biopsy 23.

FIG. 2. Genotype of H. pylori obtained by multiplex PCR format. (a) Lane M, 100-bp marker (New England Biolab); lanes 1 to 5, multiplex PCR results obtained from biopsy specimens. As shown in lane 2, biopsy 23 gave a shorter amplicon for the vacA middle region. (b) Lane M, 100-bp marker (New England Biolab); lanes 1 to 5, multiplex PCR results obtained from DNA extracted from strains isolated from the respective patients. As shown in lane 2, strain PCR 23 gave a shorter amplicon similar to that obtained with biopsy 23.

Another advantage of this multiplex PCR is that it was able to detect a deletion in vacA, since an amplicon for a vacA middle region, which is shorter than both vacA m1 and vacA m2, was obtained by biopsy multiplex PCR from one individual (Fig. 2a). The strain isolated from the same individual also showed the same vacA mid-region profile when subjected to multiplex PCR (Fig. 2b). Subsequent PCR using primer pairs VAm-F3–VAm-R3 and VA4-F–VA4-R (12) proved this particular vacA allele to be m1 (Fig. 4a and b), though the reason that a shorter amplicon was obtained in our newly designed multiplex PCR system could not be determined. Further amplification of the vacA middle region of this strain by PCR using primers VAG-F and VAG-R and sequencing showed a 120-bp deletion (from the second base of the codon) in this vacA allele when it was aligned with an m1 allele carrying the vacA gene sequence of strain 26695. This deletion resulted in a frameshift, which should give rise to a premature termination in peptide synthesis (Fig. 4c).

In summary, we believe the multiplex PCR developed here could be of great value in clinical microbiology and H. pylori population studies, especially for rapid screening of many sam-

FIG. 3. Multiplex PCR assay for simulated mixed infection of strains 26695 (s1m1 cagA⁺) and I-80 (s2m2 cagA-negative strain). Lane M, 100-bp marker (New England Biolab); lanes 1 to 6, strains 26695 and I-80 at ratios of 1:1 to 1:32; lane 7, strain 26695; lane 8, strain I-80.
ples to detect of *H. pylori* infection, to determine *vacA* and *cagA* status, to identify multiple-infection hosts, and to detect variant alleles.

This work was supported by grants from the Department of Biotechnology, Government of India (BT/MB/VAP/3/2/98), Indian Council of Medical Research [5/8-1 (161)/H. pylori], Ministry of Health and Family Welfare [H. pylori], Government of India (BT/MB/VAP/3/2/98), Indian Council of Medical Research [5/8-1 (161)/H. pylori], and in part by National Institutes of Health grants (AI 38166, DK 53727, AI 38166). This work was supported by the Department of Biochemistry, Government of India (BT/MB/VAP/3/2/98), Indian Council of Medical Research [5/8-1 (161)/H. pylori], Ministry of Health and Family Welfare [H. pylori], Government of India (BT/MB/VAP/3/2/98), Indian Council of Medical Research [5/8-1 (161)/H. pylori], and in part by National Institutes of Health grants (AI 38166, DK 53727, AI 38166). This work was supported by grants from the Department of Biochemistry, Government of India (BT/MB/VAP/3/2/98), Indian Council of Medical Research [5/8-1 (161)/H. pylori], Ministry of Health and Family Welfare [H. pylori], Government of India (BT/MB/VAP/3/2/98), Indian Council of Medical Research [5/8-1 (161)/H. pylori], and in part by National Institutes of Health grants (AI 38166, DK 53727, AI 38166).

REFERENCES


