Detection of *Helicobacter pylori* in various oral lesions by nested polymerase chain reaction (PCR)

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Nested PCR was used for the detection of *Helicobacter pylori* DNA in specimens collected from seven different topographic sites in the oral cavity. Out of 161 patients, only 21 (13.04%) were positive. There was no correlation between *H. pylori* status and patient diagnosis and age. No preferential site for bacterial colonization was found in the oral cavity, nor was an association established between a bacterial presence and ulcerated versus non-ulcerated lesions. The results indicate that the oral mucosa does not appear to represent a preferred site of colonization for *H. pylori*. Furthermore, the evidence presented in this paper suggests that *H. pylori* is not pathogenic in the oral cavity, nor is it associated with common oral pathologic processes.

*Helicobacter pylori* (*H. pylori*) is a microaerophilic, S-shaped, gram-negative rod with a particular affinity for colonizing gastric mucosa. Therefore, it has strong implications as an etiological agent for acute and chronic gastritis and peptic ulcer diseases (1). The incidence of *H. pylori* is increased in association with gastric carcinoma (2), thus prompting it to be recently classified as a carcinogen (3). The development of gastrointestinal diseases after infection with this pathogen is determined by a complex interplay between bacterial (4), host (5) and environmental factors (6).

Recently, *H. pylori* has been detected in dental plaque, saliva (reviewed in 7,8), the subgingival region (9) and oral mucosal ulcers (10). This suggests that the oral cavity may be an important reservoir for this organism, and responsible for reinoculation of gastric mucosa after antibiotic treatment. Since there are difficulties in eradicating *H. pylori* from the gastrointestinal tract (11), it is important to determine whether it is a transient pathogen or a regular colonizer in the mouth. Because it is well known that gastrointestinal diseases are related to many oral diseases, it is therefore reasonable to examine the presence of *H. pylori* in oral lesions. This can provide additional explanations for the pathogenicity of *H. pylori* in the mouth. It would also be of interest to determine whether there are any oral sites or lesions infected more commonly by *H. pylori*. We employed the highly sensitive and specific nested polymerase chain reaction (PCR) method to address these issues.

**Material and methods**

**Patients and sample handling**

One hundred and sixty-one consecutive patients participated in this study. Twenty healthy volunteers (mean age 24 yr, equally distributed between the sexes, samples taken from lingual and buccal mucosa) served as controls. Samples were obtained from seven different oral cavity locations (defined by the World Health Organization) (for positive patients recorded in Table 2). In 99 out of 140 *H. pylori* DNA-negative patients, samples were collected from only one location. In the rest of the negative patients, samples were collected from two different topographic oral cavity sites, so that a total of 181 specimens, taken from different oral cavity locations, were negative for *H. pylori* DNA (Table 1).

The samples were collected from the lesion or intact mucosa by the cytobrush technique. The brushes were immediately immersed into an Eppendorf microtube containing 1.5 ml of sterile digestion buffer (50 mM Tris-HCl pH 8.5, 1 mM EDTA, 1% Tween 20), and kept frozen until tested. The cells were then removed from the brush by vigorous vortexing, and proteinase K was added in a final concentration of 100

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>Oral cavity location</th>
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<tbody>
<tr>
<td>106</td>
<td>Lingual mucosa</td>
</tr>
<tr>
<td>24</td>
<td>Buccal mucosa</td>
</tr>
<tr>
<td>7</td>
<td>Labial mucosa</td>
</tr>
<tr>
<td>9</td>
<td>Hard palate</td>
</tr>
<tr>
<td>7</td>
<td>Soft palate</td>
</tr>
<tr>
<td>23</td>
<td>Oral vestibule</td>
</tr>
<tr>
<td>5</td>
<td>Gingiva</td>
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</table>
Two to four microliters of each DNA solution were subjected to PCR using two primer pairs from the urease A gene of the *Helicobacter pylori* genome (12). The outer primer pair was 5' GCCA ATG GTA AAT TAG TTC C 3' and 5' CTC CCT AAT TGT TTT TAC AT 3'. PCR was carried out in a 25 μl mixture containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.75 U of recombinant Taq polymerase (Perkin Elmer), 200 μM dNTPs and 400 nM of each primer. In short, 35 cycles at 96°C for 30 s, 56°C for 30 s and 72°C for 30 s were performed in a Perkin Elmer ThermoCycler 2400. After PCR, one microliter of the final product was transferred to the second round reaction mixture and reamplified with the inner pair of primers under the conditions described above: 5' AGT TCC TGG TGA GTT GTT CT 3' and 5' AGC GCC ATG AAA ACC ACG CT 3'. Six microliters were taken to visualize the specific product in 2% agarose gel stained with ethidium bromide. The expected PCR product was 361 bp long. The gels were photographed with Polaroid 667 film. The reagents without DNA were used as negative controls in the PCR assay. In addition to PCR for *H. pylori* DNA, each sample was amplified for nm23-H2 repetitive sequence and for p53 exon 4 and intron 6 sequences. In all samples, both genes were amplified successfully.

### Results

Table 2 presents the results obtained with the nested PCR used for the detection of *Helicobacter pylori* infection in the oral cavity. The specimens were collected from 161 consecutive patients and seven different topographic sites (indicated in Tables 1 and 2). Twenty-one patients (13.04%) were positive for specific *H. pylori* sequence when nested PCR amplification products were tested on agarose gel (Fig. 1). None of the samples exhibited a visible DNA band after the first round of amplification with the outer primer pair, thus indicating that a low number of *H. pylori* DNA copies were present. All of the samples from the healthy volunteers were negative. Regardless of whether the samples were positive or negative for *H. pylori* DNA, each sample was successfully amplified for nm23-H2 repetitive sequence and for gene p53 sequences, indicating that the isolated DNA was representative for PCR and that *H. pylori* DNA negative samples were really negative. The percentage of positive patients with ulcer and non-ulcer diseases was almost the same (Table 3).

### Discussion

Many laboratories have detected *H. pylori* in the oral cavity (7,13,14,15). However, since data are dependent on the...
method of investigation and sample source, they cannot be generalized. Most of the studies used PCR assay on dental plaque and saliva specimens and yielded a 0–86% incidence of positive samples. In our opinion, an incidence of 86% (15) for the patients without H. pylori in their stomach is quite high. On the other hand, it is unusual that no positive dental plaque sample was found among the 29 samples obtained from gastric ulcer patients (13), even though PCR assay with primers for the 16S rRNA gene was applied. We believe that some degree of positivity would have been obtained if the authors had used DNA isolation in combination with nested PCR. We consistently used nested PCR in our study because one step PCR is not sufficiently sensitive.

Moreover, no correlation was found between H. pylori status and patient diagnosis and age.

In conclusion, our results with nested PCR show that H. pylori is rarely present in ulcerous and non-ulcerous oral cavity lesions. H. pylori in the oral cavity may be considered a transient pathogen. Therefore, we believe that the mouth is not a natural reservoir for this microorganism.

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Helicobacter pylori in oral lesions