

## Letter to the Editor

# Isolation of viable *Helicobacter pylori* in the tonsillar tissues of chronic tonsillitis patients

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*Helicobacter pylori* is a Gram-negative, spiral, microaerophilic bacterium that originally colonizes the human stomach. Chronic *H. pylori* infection has been associated with chronic gastritis, peptic ulcer disease, atrophic gastritis, mucosa associated lymphoid tissue (MALT) lymphoma, and gastric cancer [1]. *H. pylori* has been also detected in the mouth and the middle ear as well as in tonsillar and adenoid tissues [2-5]. However, the relationship of *H. pylori* with the pathogenesis of diseases that involve these organs is controversial.

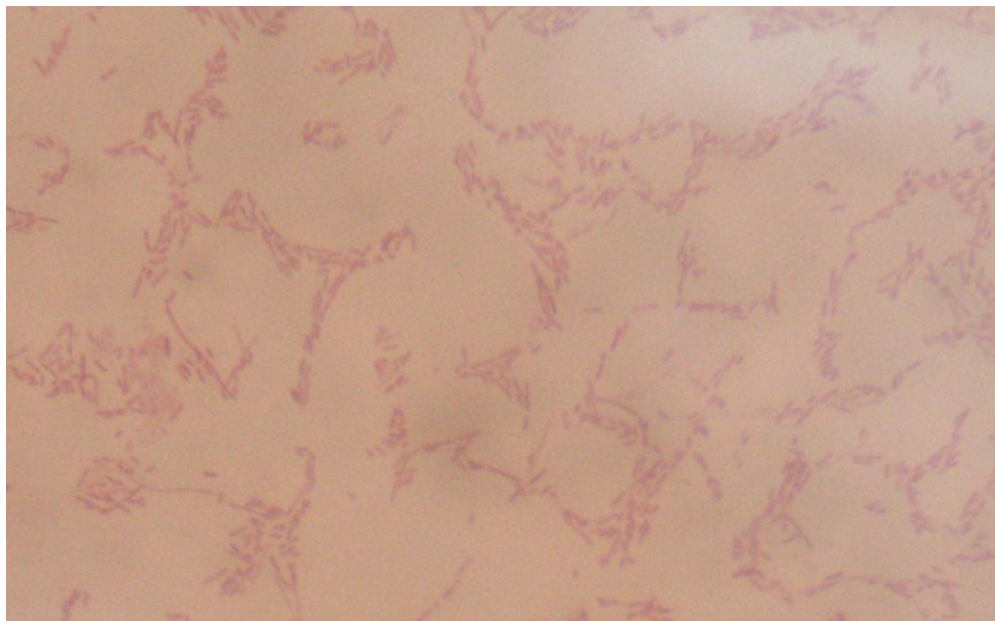
Accumulated reports show controversies regarding the role of *H. pylori* in oropharyngeal infection. The hypothesis that *H. pylori* can colonize tonsillar and adenoid tissue has not yet been well elucidated due to the difficulty in isolating viable *H. pylori* [3,6,7]. The results of this study indicate that viable *H. pylori* can be detected in the tonsillar tissues of chronic tonsillitis patients using a combination of conventional culture and histological examination employing modified Giemsa staining and immunohistochemistry.

The study included nineteen patients with chronic tonsillitis who were admitted for elective tonsillectomy. Patients were evaluated as having chronic tonsillitis based on Ballantyne and Groves criteria [8]. All patients underwent tonsillectomy under general anesthesia. The tonsillar tissues were divided in two parts and subsequently used for microbiological and histological analysis. Informed consent was obtained from all patients or their parents.

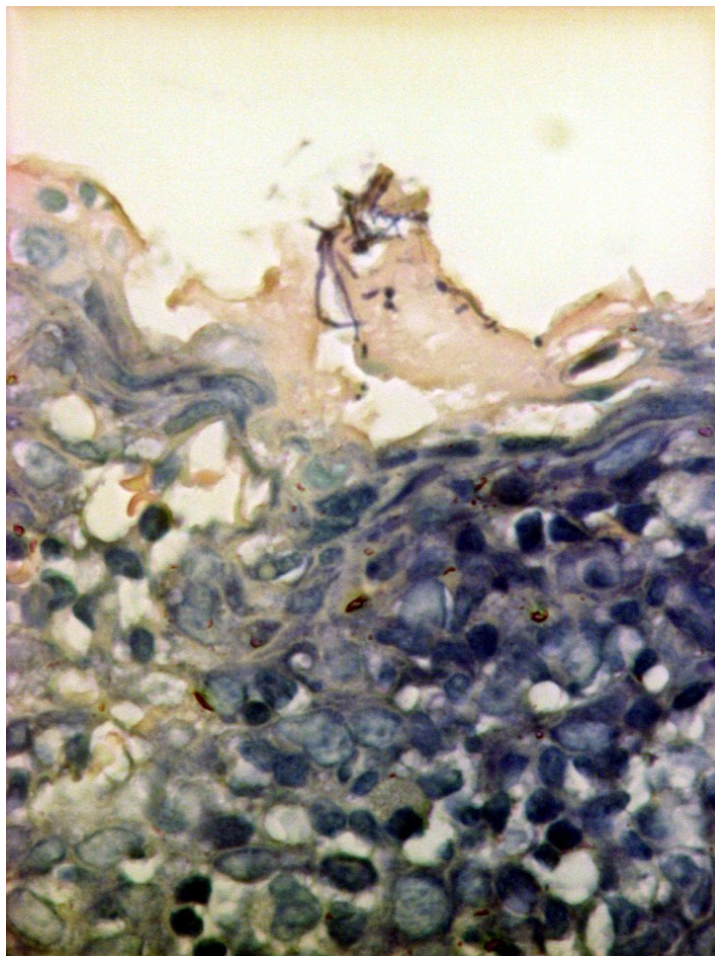
Specimens obtained from patients were directly inoculated into Stuart transport medium. Soon after arriving at the laboratory, specimens were inoculated directly on Trypton Soy Agar (TSA) medium (Oxoid Ltd, Cambridge, UK) supplemented with 5% defibrinated sheep blood and *H. pylori* selective Skirrow's medium supplemented with vancomycin, trimethoprim, polymyxin B, and amphotericin B (Sigma-Aldrich, St. Louis, USA). Plates were incubated micro-aerobically using the gas generating kit (Mitsubishi Gas Chemical Co Inc, Tokyo, Japan) at 37°C for 5 to 7 days. Colonies of suspected *H. pylori* were identified by Gram and Giemsa staining, and a positive reaction of the urease test.

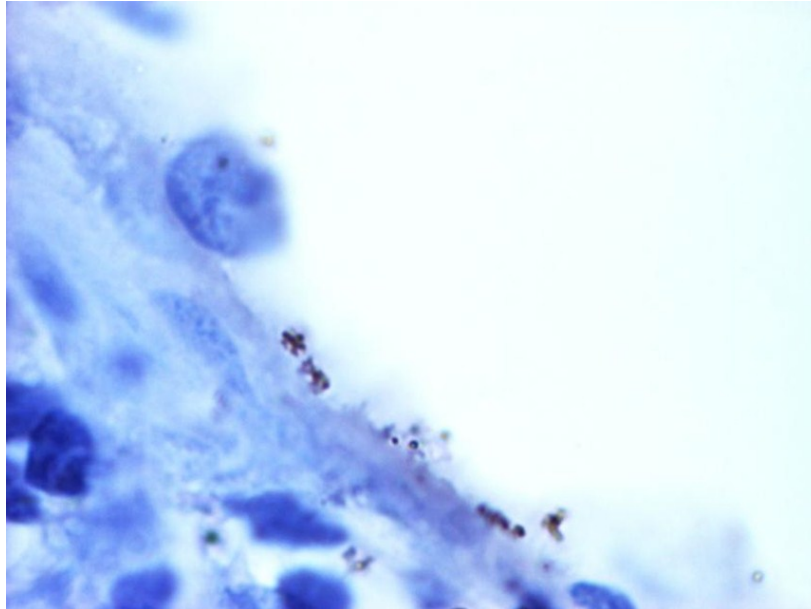
Tonsillar tissue samples were fixed in 10% (v/v) formalin, then embedded in paraffin, and subsequently used for modified Giemsa staining and immunohistochemistry. Modified Giemsa staining was performed according to the standard procedure described elsewhere [9]. Immunohistochemistry detection was performed using a specific *H. pylori* antibody (Dako, Glostrup, Denmark). Briefly, tissue sections were immersed in 10 mmol/L sodium citrate buffer, pH 6.0, and then autoclaved at 120°C for 10 minutes. The sections were then treated with 3% H<sub>2</sub>O<sub>2</sub> for 10 minutes at room temperature to inactivate endogenous peroxidase, and then they were blocked with 10% goat serum for 20 minutes at room temperature. The sections were incubated with a-*H. pylori* Ab (Dako, Glostrup, Denmark) overnight at 4°C. The sections were washed with PBS and incubated for 20 minutes with biotinylated goat anti-rabbit IgG (Dako, Glostrup, Denmark). The sections

**Figure 1a.** Giemsa staining of *H. pylori* colonies cultured on selective medium



**Figure 1b.** Modified Giemsa staining of tonsillar tissue taken from patients with chronic tonsillitis (1000 x magnification)



**Figure 1c.** Detection of *H. pylori* using immunohistochemistry

were washed again with PBS and incubated in a solution of avidin-conjugated horseradish peroxidase (Dako, Glostrup, Denmark) for 20 minutes, and then washed with PBS for 5 minutes. Peroxidase activity was detected with H<sub>2</sub>O<sub>2</sub>/diaminobenzidine substrate solution. The sections were counterstained with hematoxylin before dehydration and mounted.

Viable *H. pylori* was detected by conventional culture in three male patients out of 19 patients (15.7%) and confirmed by histology using modified Giemsa staining and immunohistochemistry (Figure 1). Culture results were in 100% agreement with modified Giemsa staining and immunohistochemistry results.

Culture is the gold standard method for the determination of *H. pylori*. It indicates the presence of viable bacteria, though several factors may lower its sensitivity, such as antibiotic administration, local disinfection, and anesthetic application. Modified Giemsa staining and immunohistochemistry staining were used to confirm culture results due to their superior and reproducible capability to detect *H. pylori* in tissue samples.

Some studies have suggested that *H. pylori* may have an association with oropharyngeal infection [3,4,7]. However, this notion was not supported by the work of several groups which were not able to detect the colonization of *H. pylori* in tonsillar tissue [6,10]. These contradictory results may be due to

the specific characteristics of each study population or to the variety of methods employed in the studies. Yilmaz *et al.* [5] were the first who isolated *H. pylori* from tonsillar tissue cultures from patients suffering from otitis media with effusion. Our study appears to be the first reporting isolation of viable *H. pylori* from the tonsillar tissues of patients with chronic tonsillitis. Eyigor *et al.* [11] reported that *H. pylori* can be detected in tonsillar tissue of 5.5% of chronic tonsillitis patient by rapid urease test (RUT) but not with PCR. Vayisoglu *et al.* [12] detected *H. pylori* using RUT in only 2.2% of adenoidectomy specimens and in none of the tonsillectomy specimens. A positive result was not obtained in any tonsillectomy specimen using immunohistochemistry. Our results showed *H. pylori* colonization in the tonsillar tissue of 15.7% of patients suffering from chronic tonsillitis. The different findings may be related to distinct population characteristics. The combination of culture and histological examination may be useful in detecting *H. pylori* colonization of tonsillar tissue in chronic tonsillitis cases.

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**Conflict of interests:** No conflict of interests is declared.