

THE INFLUENCE OF ENDOSCOPIC PROCEDURES UPON THE CONTAMINATION OF *Helicobacter pylori* CULTURES

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ABSTRACT - Background - Among the various diagnostic methods for the detection of *Helicobacter pylori* infection, histological examination and microbiological processing of gastric biopsy samples are assumed to be the gold standard techniques. **Aims** - Since *H. pylori* culture can be affected by the presence of non-*H. pylori* bacteria, we evaluated the efficacy of endoscope disinfection and the influence of endoscopic procedures on culture contamination. **Patients and Methods** - The procedures used during the first two routine endoscopies were evaluated during 28 consecutive days. Endoscopy room, forceps and endoscopic channel were analyzed before and after the beginning of normal procedures. After disinfection, a biopsy simulation was performed to verify the gastric bacteria. **Results** - Endoscope disinfection removed all organisms from forceps and endoscopic channel with 100% efficacy. The most frequent non-*H. pylori* bacteria detected were *Streptococcus bovis*, *Enterobacter hormaechei*, and *Staphylococcus aureus*. The sensibility of the *H. pylori* culture was affected by the presence of non-*H. pylori* bacteria. **Conclusion** - The risk of transmission of microorganisms was not detectable when sterilized biopsy forceps and stringent disinfection standards were employed. Whilst *S. bovis* and *E. hormaechei* may be common in gastric microbial flora, the presence of *P. aeruginosa* and *S. aureus* indicated that the manipulation of biopsies could be responsible for culture contamination with these bacteria.

HEADINGS - *Helicobacter* infections. *Helicobacter pylori*. Endoscopes, gastrointestinal. Equipment contamination. Disinfection, methods.

INTRODUCTION

Helicobacter pylori is a gram-negative, microaerophilic, curved bacterium that persistently colonizes the human stomach, establishing a long-term infection of the gastric mucosa⁽⁴⁾. The bacterium has a worldwide distribution, with a prevalence ranging from 25% in developed countries to more than 90% in developing areas, however not all infected individuals develop clinically relevant disease⁽⁴⁾. *H. pylori* occupies a narrow ecological niche due to its tropism for gastric mucosal surface cells⁽⁷⁾.

Among the various methods for the detection of *H. pylori* infection, histological examination and microbiological processing of gastric biopsy samples are assumed to be gold standard techniques⁽⁶⁾. In clinical practice, however, the identification of *H. pylori* in gastric mucosal samples relies mostly on the detection of urease activity by commercial urease tests, since culturing the bacterium is time consuming and the sensitivity rate is affected by the number of biopsies, the transport medium, the duration and the temperature during

the transport period, the culture media, the microaerophilic conditions, as well as the presence of culture contaminants leading to false-negative results^(6, 9).

Since the endoscope comes into close contact with the mucous membrane or non-intact skin, these instruments are routinely contaminated during their use. Despite the use of rigorous disinfection procedures, there is considerable evidence documenting the presence of pathogens and of a persistent biofilm or encrusted patient material within endoscope channels^(8, 13, 18). Endoscopic transmission of infectious agents may occur via three primary routes: a) from a previous patient, b) during cleaning and disinfection, or c) from the manipulation of a previously infected system⁽¹⁾.

Several reports have described endoscope-transmitted infections, the most commonly-identified organisms being *Salmonella* spp, *Staphylococcus epidermidis*, alpha-hemolytic *Streptococcus*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Candida* spp, and *Pseudomonas aeruginosa*. These infections were mostly related

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to improper cleaning and disinfection procedures^(10,17). The occurrence of bacteremia during endoscopic therapy, indicating the possibility of bacterial displacement, has also been reported⁽⁵⁾.

The presence of contamination in *H. pylori* cultures, derived from gastric biopsies, can reduce the sensitivity of this method. The sources of contamination, however, have not been well characterized. Our previous results have suggested that bacterial culture and susceptibility tests were necessary to define the resistance patterns of *H. pylori* in a particular geographical area before the general use of an eradication schedule⁽¹²⁾. Therefore, we evaluated the efficiency of endoscope disinfection, the influence of endoscopic procedures upon *H. pylori* culture contamination and the most frequent non-*H. pylori* bacteria detected in *H. pylori* culture.

PATIENTS AND METHODS

Endoscopic procedures

The procedures used during the first two routine endoscopies performed at the Clinical Pharmacology and Gastroenterology Unit at the São Francisco University, Bragança Paulista, SP, Brazil, were evaluated in a total of 56 biopsies. The clinical protocol was approved by the São Francisco University Ethics Committee, and the study was conducted in accordance with the Declaration of Helsinki.

Initially, before the first two endoscopic procedures of each day, a biopsy forceps was inserted into a collection tube (Brucella broth (Difco, Detroit, MI, USA)). Subsequently, endoscope disinfection was performed using a sequential three-step procedure: a) initial manual brushing and cleaning of debris with detergent and tap water immediately after use, b) a 20 min immersion in 2% glutaraldehyde (Glutacide, Johnson & Johnson, Brazil) followed by a tap water rinse, and 3) manual flushing with 70% isopropyl alcohol and forced air drying. After this procedure, the biopsy forceps were passed through the accessory channel cap into the accessory port and through the endoscope channels, and inserted into collection tubes. During the endoscopic procedure, a biopsy simulation was performed by inserting the biopsy forceps through the accessory channel of the endoscope until they reached the gastric lumen, the forceps were then removed and immersed in collection tubes. Following collection, the biopsy sample was inserted in collection tubes and immediately transported to the microbiology laboratory. *Helicobacter pylori* infection was determined by histological evaluation (hematoxylin-eosin (H&E) and Giemsa staining) and rapid urease test (Probac, São Paulo, Brazil).

Bacterial culture

One hundred microliters taken from the collection tubes were inoculated in BHI-agar (Difco, Detroit, MI, USA) with 10% sheep blood (BBV, Campinas, SP, Brazil) in aerobic and microaerophilic (8-10% CO₂, 5-6% O₂, 80-85% NO₂, at 98% humidity) conditions. The biopsies were carefully homogenized and 100 µL were inoculated in BHM-YE plates⁽¹²⁾ [Brain Heart Infusion (Difco, Detroit, MI, USA) supplemented with 2.5 g L⁻¹ yeast extract (Difco), 10% lysed sheep blood 6 mg L⁻¹, vancomycin (Sigma Aldrich Chemie, St. Louis, MO, USA), 20 mg L⁻¹ nalidixic acid (Sigma Aldrich Chemie), 2 mg L⁻¹ amphotericin B (Sigma Aldrich Chemie) and 40 mg L⁻¹ 2,3,5, triphenyl-tetrazolium chloride (Sigma Aldrich Chemie)] and incubated under aerobic and microaerophilic conditions. The cultures were examined after 48-72 h of incubation, and bacteria were Gram stained and tested for production of catalase, urease and oxidase.

All bacteria observed were isolated and identified in the Clinical Pathology Laboratory at São Francisco University.

RESULTS

In the endoscope disinfection procedures, the manual cleaning, followed by 20 minutes of glutaraldehyde exposure and ETOH drying, removed all organisms from forceps and endoscope channel, providing a 100% rate of disinfection. Forceps contamination was observed on four occasions before the first disinfection procedure. These bacteria were identified as *Staphylococcus aureus* (75%) and *Pseudomonas aeruginosa* (25%).

In the biopsy simulation, a non-*H. pylori* bacterial growth was obtained in 61% samples, and bacteria identified were *Streptococcus bovis* (77%), *Enterobacter hormaechei* (62%) and *Staphylococcus aureus* (6%). A mixed bacterial population was observed in 44% of these cultures (Table 1).

Biopsy analysis, by means of rapid urease test and histology, revealed the presence of *H. pylori* in 45 patients (80%), and non-*H. pylori* bacteria were detected among 31%. The time-consuming method for *H. pylori* isolation from mixed culture was possible in 20% of these cases. In the remaining 11%, the sensibility of culture was clearly affected by the presence of non-*H. pylori* bacteria (Table 1).

DISCUSSION

Helicobacter pylori occupies a narrow ecological niche, requiring gastric type epithelium. The bacterium has an advantage over potential competing bacteria in that it can adhere to gastric mucosa and survive the hostile gastric acidic environment⁽¹⁴⁾. Differences in the diversity of the gastric microbial population have yielded a high rate of non-*H. pylori* contamination, primarily involving alpha-hemolytic streptococci, micrococci, staphylococci, and various Enterobacteriaceae⁽¹⁷⁾.

The persistent contamination of endoscopes has been related to the presence of a bacterial biofilm, which is either resistant or inaccessible to current disinfection procedures⁽³⁾. Although current disinfection procedures for endoscopes are still imperfect^(3,19), we have demonstrated that the biopsy procedure itself does not introduce contamination, as also previously described⁽¹⁰⁾. Similarly, individual patients were unlikely to be a source of contamination.

In several reports, endoscopic procedures seem to be a source of contamination by *S. aureus* and *P. aeruginosa*^(10,17). NOGUERAS et al.⁽¹⁵⁾, evaluating the influence of hand washing on microorganism transportation, detected the presence of *P. aeruginosa* and *S. aureus* among health care workers before and after physical examination of the patients. The presence of these bacteria, in a few cases, pointed to hand contamination as a possible transmission source of infectious agents.

The presence of *S. bovis* in the gastric flora has been associated with colorectal cancer by several authors^(2,11). Additionally, the presence of another enteric pathogen – *E. hormaechei* – seems to be related to the infection of vulnerable patients through patient-to-patient transmission^(16,20). Thus, our data confirm that these bacteria are commonly observed in the gastric microbial flora.

The composition of the microbial flora in gastric biopsies may vary in different geographic regions. OSATO et al.⁽¹⁷⁾, comparing the

TABLE 1 - Presence of bacterial contaminants before and after endoscopy

| Day | Endoscopic channel | Disinfection | Endoscopic channel | First patient | H. pylori infection | Biopsy Culture | Disinfection | Endoscopic channel | Second patient | H. pylori infection | Biopsy Culture |
|-----|--------------------|--------------|--------------------|---------------|---------------------|---------------------|--------------|--------------------|----------------|---------------------|---------------------|
| 1 | no growth | | no growth | S. b., E. h. | + | S. b., H. p. | | no growth | no growth | + | H. p. |
| 2 | S. a. | | no growth | no growth | - | no growth | | no growth | no growth | + | H. p. |
| 3 | no growth | | no growth | S. b., E. h. | + | S. b., E. h. | | no growth | S. b., E. h. | + | E. h., H. p. |
| 4 | no growth | | no growth | S. a. | + | H. p. | | no growth | no growth | + | H. p. |
| 5 | no growth | | no growth | E. h. | + | H. p. | | no growth | S. a. | - | S. a. |
| 6 | no growth | | no growth | no growth | + | H. p. | | no growth | S. b., E. h. | + | S. b., E. h., H. p. |
| 7 | no growth | | no growth | E. h. | + | E. h., H. p. | | no growth | E. h. | + | H. p. |
| 8 | no growth | | no growth | S. b., E. h. | - | no growth | | no growth | E. h. | - | E. h. |
| 9 | no growth | | no growth | S. b., E. h. | + | S. b., E. h., H. p. | | no growth | no growth | + | H. p. |
| 10 | P. a. | | no growth | no growth | + | H. p. | | no growth | no growth | + | H. p. |
| 11 | no growth | | no growth | E. h. | + | E. h. | | no growth | no growth | + | H. p. |
| 12 | no growth | | no growth | no growth | + | H. p. | | no growth | S. b. | - | S. b. |
| 13 | S. a. | | no growth | S. b., E. h. | + | H. p. | | no growth | S. b. | + | H. p. |
| 14 | no growth | | no growth | S. b., E. h. | + | H. p. | | no growth | S. b., E. h. | + | S. b., H. p. |
| 15 | no growth | | no growth | S. b., E. h. | + | E. h., H. p. | | no growth | S. b., E. h. | + | S. b., E. h. |
| 16 | no growth | | no growth | S. b., E. h. | + | S. b., E. h. | | no growth | no growth | + | H. p. |
| 17 | S. a. | | no growth | S. b. | + | H. p. | | no growth | no growth | - | S. b. |
| 18 | no growth | | no growth | no growth | + | H. p. | | no growth | no growth | + | H. p. |
| 19 | no growth | | no growth | no growth | - | S. b. | | no growth | S. b. | - | no growth |
| 20 | no growth | | no growth | S. b., E. h. | + | H. p. | | no growth | S. b., E. h. | + | E. h., H. p. |
| 21 | no growth | | no growth | no growth | + | H. p. | | no growth | S. b., E. h. | + | H. p. |
| 22 | no growth | | no growth | no growth | + | H. p. | | no growth | S. b. | - | S. b. |
| 23 | no growth | | no growth | S. b. | + | S. b., H. p. | | no growth | S. b. | + | S. b. |
| 24 | no growth | | no growth | S. b. | + | H. p. | | no growth | no growth | + | H. p. |
| 25 | no growth | | no growth | S. b. | + | H. p. | | no growth | no growth | + | H. p. |
| 26 | no growth | | no growth | S. b. | - | no growth | | no growth | no growth | - | no growth |
| 27 | no growth | | no growth | no growth | + | H. p. | | no growth | no growth | + | H. p. |
| 28 | no growth | | no growth | S. b. | + | H. p. | | no growth | E. h. | + | H. p. |

S. b. - *Streptococcus bovis*; E. h. - *Enterobacter hormaechei*;
 S. a. - *Staphylococcus aureus*; P. a. - *Pseudomonas aeruginosa*;
 H. p. - *Helicobacter pylori*

presence of non-*H. pylori* organisms in patients from Korea, Colombia and USA, found the highest level of biopsy contamination in Colombia. The high level of non-*H. pylori* organisms detected in our study is in agreement with this observation, and may be related to the socioeconomic conditions, as well as to the high microbial diversity found in these tropical underdeveloped countries. Additionally, the presence of non-*H. pylori* bacteria in biopsy samples may be a problem for *H. pylori* culturing, since in 11% of *H. pylori*-positive patients we were unable to culture bacteria.

CONCLUSIONS

Despite the limitations of the present study, we conclude that stringent disinfection standards can reduce the transmission of bacterial pathogens

to nearly zero. *S. bovis* and *E. hormaechei* are normally present in the gastric microbial flora, whereas the presence of *P. aeruginosa* and *S. aureus* probably results from contamination through manipulation. The presence of non-*H. pylori* bacteria in biopsies may be a problem in *H. pylori* routine culture. Since the experience of laboratory staff and the use of different selective medium is necessary to increase the sensibility of isolation methods of *H. pylori*.

ACKNOWLEDGEMENT

Dr. Anita P.O. Godoy is supported by a fellowship from the Fundação Clara de Assis - São Francisco University. The authors gratefully acknowledge the invaluable assistance of the endoscopy staff: Maristela Deguer and Marco A. M. Marchioretto.

Ribeiro ML, Godoy APO, Benvenço YHB, Ecclissato CC, Mendonça S, Pedrazzoli Jr J. A influência dos procedimentos endoscópicos na contaminação de culturas de *Helicobacter pylori*. Arq Gastroenterol 2004;41(2): 100-3.

RESUMO - Racional e Objetivos - Dentre os vários métodos diagnósticos empregados na detecção da infecção por *Helicobacter pylori*, o diagnóstico histológico e a análise microbiológica de biópsia gástrica são consideradas as técnicas mais sensíveis. Entretanto, a sensibilidade da cultura de *H. pylori* pode ser reduzida pela presença de outras bactérias. Desse modo, avaliou-se a eficácia da desinfecção do endoscópio e a influência dos procedimentos endoscópicos na contaminação da cultura bacteriana. Para tal, as duas primeiras endoscopias durante 28 dias consecutivos foram estudadas. A sala de endoscopia, o fórceps e o canal do endoscópio foram analisados antes e depois do início dos procedimentos endoscópicos rotineiros. Depois da desinfecção, uma simulação de coleta de biópsia foi realizada para verificar a presença das bactérias gástricas. **Resultados** - A desinfecção do endoscópio foi capaz de remover todos os organismos do fórceps e do canal do endoscópio. As bactérias não-*H. pylori* mais frequentemente detectadas foram *Streptococcus bovis*, *Enterobacter hormaechei* e *Staphylococcus aureus*. Em alguns casos a sensibilidade da cultura de *H. pylori* foi reduzida pela presença de bactérias contaminantes. **Conclusão** - Não houve risco de transmissão de microorganismos quando fórceps esterilizados e desinfecção adequada foram empregadas. A presença de *S. bovis* e *E. hormaechei* parece ser comum na microflora gástrica; por outro lado, a detecção de *P. aeruginosa* e *S. aureus* indica que a manipulação de biópsias pode ser responsável pela contaminação da cultura por essas bactérias.

DESCRIPTORIOS – Infecções por *Helicobacter*. *Helicobacter pylori*. Endoscópios gastrointestinais. Contaminação de equipamentos. Desinfecção, métodos.

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Recebido em 17/6/2003.
Aprovado em 19/1/2004.