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# Diagnostic performance between histopathological and molecular methods in the detection of *Helicobacter pylori*

## *Desempenho diagnóstico entre os métodos histopatológico e molecular na detecção de* Helicobacter pylori

Lucas Luiz L. Silva[1]; Igor M. C. Calassa[1]; Ana Karoline S. Oliveira[1]; Amanda F. P. L. Ramos[1]; Jaqueline C. Pontes[1]; Daniela M. M. Cardoso[2]; Lucas T. Rasmussem[3]; Lilian Carla Carneiro[1]; Monica S. Barbosa[1]

1. Universidade Federal de Goiás, Goiânia, Goiás, Brazil. 2. Hospital das Clínicas, Goiânia, Goiás, Brasil. 3. Faculdade de Medicina de Marília, Marília, São Paulo, Brazil.

## ABSTRACT

**Introduction:** *Helicobacter pylori* (*H. pylori*) is a Gram negative bacterium considered to be the etiologic agent of various gastric diseases. The prevalence of bacterial infection varies according to age, geographic location, ethnicity and socioeconomic status. The chronic infection caused by microorganism can favor the development of severe pathologies such as gastric adenocarcinoma. In this sense, early diagnosis is essential for a better prognosis and therapeutic success. Several diagnostic methods performed using invasive and non-invasive techniques, with different sensitivity and specificity, have been used in the detection of *H. pylori*. **Objective:** To compare the performance of the molecular and histopathological technique used in the diagnosis of *H. pylori* infection. **Methods:** 76 gastric tissue samples were collected from dyspeptic patients who underwent molecular and histopathological diagnosis. Molecular detection was performed using the ribosomal gene (16S rRNA) using the polymerase chain reaction (PCR) technique. **Results:** The PCR-based molecular diagnostic method detected the bacterium in 63.1% of the samples, while the histopathological test identified the microorganism in only 38.1% of gastric biopsies. The data demonstrated that the PCR technique was about 1.6 times more sensitive than the histopathological technique. **Conclusion:** The PCR technique was the most efficient diagnostic method for detecting *H. pylori* and can be implemented in the laboratory routine as a complementary test for the early detection of *H. pylori*.

**Key words:** bacteria; molecular biology; histology; pathology.

## RESUMO

***Introdução:*** Helicobacter pylori *(*H. pylori*) é uma bactéria Gram negativa considerada o agente etiológico de várias doenças gástricas. A prevalência da infecção bacteriana varia de acordo com idade, localização geográfica, etnia e* status *socioeconômico. A infecção crônica ocasionada por esse microrganismo pode favorecer o desenvolvimento de patologias severas, como o adenocarcinoma gástrico. Nesse sentido, o diagnóstico precoce é essencial para um melhor prognóstico e o sucesso terapêutico. Vários métodos de diagnóstico realizados com técnicas invasivas e não invasivas, com diferentes sensibilidade e especificidade, têm sido utilizados na detecção de* H. pylori*.* ***Objetivo:*** *Comparar o desempenho das técnicas molecular e histopatológica utilizadas no diagnóstico da infecção por* H. pylori*.* ***Métodos:*** *Setenta e seis amostras de tecido gástrico foram coletadas de pacientes dispépticos e submetidas ao diagnóstico molecular e histopatológico. A detecção molecular foi realizada utilizando o gene ribossomal (rRNA 16S) por meio da técnica de reação em cadeia da polimerase (PCR).* ***Resultados:*** *O método molecular de diagnóstico com base na PCR detectou a bactéria em 63,1% das amostras, enquanto o teste histopatológico identificou o microrganismo em apenas 38,1% das biópsias gástricas. Os dados demonstram que a técnica de PCR apresentou cerca de 1,6 vezes mais sensibilidade que a técnica histopatológica.* ***Conclusão:*** *A técnica de PCR foi o método de diagnóstico mais eficiente para detecção de* H. pylori *e pode ser implementada na rotina laboratorial como teste complementar para detecção precoce de* H. pylori*.*

***Unitermos:*** *bactérias; biologia molecular; histologia; patologia.*

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## RESUMEN

***Introducción:*** Helicobacter pylori *(*H. pylori*) es una bacteria Gram negativa considerada agente etiológico de diversas enfermedades gástricas. La prevalencia de la infección bacteriana varía según la edad, la ubicación geográfica, la etnia y el nivel socioeconómico. La infección crónica provocada por esos microorganismos puede favorecer el desarrollo de patologías graves como el adenocarcinoma gástrico. Por esa razón, el diagnóstico precoz es fundamental para un mejor pronóstico y éxito terapéutico. En la detección de* H. pylori *se han utilizado varios métodos de diagnóstico realizados mediante técnicas invasivas y no invasivas, con diferentes sensibilidad y especificidad.* ***Objetivo:*** *Comparar el desempeño de las técnicas moleculares e histopatológicas utilizadas en el diagnóstico de la infección por* H. pylori*.* ***Métodos:*** *Se recolectaron 76 muestras de tejido gástrico de pacientes dispépticos y se las sometieron a diagnóstico molecular e histopatológico. La detección molecular se realizó mediante el gen ribosómico (ARNr 16S) mediante la técnica de reacción en cadena de la polimerasa (PCR).* ***Resultados:*** *El método molecular de diagnóstico basado en PCR detectó la bacteria en el 63,1% de las muestras, mientras que la prueba histopatológica identificó el microorganismo en solo el 38,1% de las biopsias gástricas. Los datos demostraron que la técnica de PCR era aproximadamente 1,6 veces más sensible que la técnica histopatológica.* ***Conclusión:*** *La técnica de PCR fue el método diagnóstico más eficaz para la detección de* H. pylori *y puede implementarse en la rutina del laboratorio como prueba complementaria para la detección precoz de* H. pylori*.*


***Palabras clave:*** *bacteria; biología molecular; histología; patología.*

## INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is a gram-negative, ubiquitous, spiral, pleomorphic, flagellated and microaerophilic bacteria. The bacterium was discovered in 1983 by Marshall and Warren, who established a relationship between microorganism infection to gastrointestinal disorders[1]. These findings led to the Nobel Prize for Medicine and Physiology awarded by researchers in 2005[2].

*H. pylori* infection has a cosmopolitan distribution and affects about 50% of the world population[3]. The prevalence rate of infection is higher in emerging countries, ranging from 20% to 90% depending on the geographical region. In Brazil, the prevalence varies from 50% to 80%, similar to Africa, where the prevalence is 70% to 90%[4].

Parasite-host imbalance relationship is required for occuring the *H. pylori* infection. The main accepted routes of transmission are oral-oral, gastric-oral, fecal-oral and iatrogenic routes[3-6]. However, other routes of transmission such as zoonotic and sexual have been proposed[7].

*H. pylori* is considered the etiological agent of several gastric pathologies such as gastritis, ulcer, atrophy, metaplasia, lymphoma and gastric adenocarcinoma. Due its high carcinogenic potential the bacterium was classified as a type I carcinogenic by the International Agency for Research on Cancer (IARC), subsided by World Health Organization (WHO)[8]. In addition, bacterial infection is associated with extra-gastric disorders such as immune thrombocytopenic purpura, anemia by iron and vitamin B12 deficiencies[9].

The methods used for screening *H. pylori* infection can be segregated into invasive and noninvasive. Invasive methods are those that involve the examination of upper digestive endoscopy to obtain the gastric tissue fragment. Among the invasive, there are: culture, urease test, polymerase chain reaction (PCR) and histopathological test, which is considered the gold standard according to the IV Brazilian Consensus Conference on *H. pylori* Infection. PCR can be used in several biological samples and several constitutive genes such as *ureA*, *glmM*, *ureC* and 16S ribosonal ribonucleic acid (rRNA) (*hpx*) have been targets for the detection of the bacterium. Noninvasive methods are not dependent on gastric biopsy and involve tests such as marked urea, serology and fecal antigen[10].

The use of appropriate techniques with high sensitivity and specificity is of utmost importance for a reliable infection diagnosis. An early diagnosis is associated with a better patient's quality of life, since the presence of the bacteria in the gastrointestinal tract may induce the several pathologies, including adenocarcinoma[11-13]. In this sense, the aim of this study was to compare the performance of the molecular and histopathological technique used in the diagnosis of *H. pylori* infection.

## METHODS

### Ethical considerations

This study was approved by the Research Ethics Committee of an University Hospital (CAAE: 83422017.7.0000.5078), published

under the number 2.519.032. All patients enrolled in this study have read and signed the Consent Form.

### Patients and samples

Patients using antibiotics, proton pump inhibitors (PPIs), immunosuppressants, participants under 18 years of age, pregnant, lactating, individuals diagnosed with gastrointestinal bleeding and a history of gastrectomy were excluded from this study. Patients who could not access the histopathological diagnosis result were also excluded.

Sample collection was performed at a University Hospital of Goiânia, Goiás, Brazil, between November 2017 and September 2018. The procedure for obtaining gastric samples followed the recommendations of the Sociedade Brasileira de Endoscopia Digestiva (Brazilian Society of Digestive Endoscopy). Two fragments of the antrum and two of the gastric body were obtained from each patient and were sent to the hospital's own clinical pathology laboratory for histopathological analysis and to the Núcleo de Estudo da *Helicobacter pylori* (NEHP) for molecular analysis.

### Histopathological exam

Histological evaluation of the specimens was performed to verify the microorganism presence and histological alteration. Histological sections of the body and gastric antrum were about $0.6 \times 0.3 \times 0.2$ cm in diameter and the fixation of samples were performed in Giemsa stained 10% buffered formalin. Sidney Classification System[14] was used for the analysis of inflammatory infiltrate. The slides were analyzed by an experienced pathologist who had no access to molecular data.

### Molecular diagnosis

Deoxyribonucleic acid (DNA) extraction and molecular analyzes were performed by NEHP. DNA extraction from gastric tissue samples followed the QIAamp® Kit protocol (Qiagen, Valencia, CA, United States). A 10 µl aliquot was reserved for DNA quantitation on NanoDrop® (ND-1000 UV-Vis).

Genomic DNA was amplified by the PCR method as described by Nevoa *et al.* (2017)[15]. Molecular detection was performed using the 16S rRNA ribosomal gene, which was amplified using the *hpx/hpx1* primers. Oligonucleotides sequences, amplification conditions, and fragment size are described in **Table 1**.

Amplification was performed in an Amplitherm® TX96 thermocycler and each reaction consisted of: 33.5 µl Milli-Q water, 5 µl PCR buffer 10×, containing MgCl2 (1.5 mM), 2 µl dNTP (2.5 mM), 2 µl of each oligonucleotide pair (10 pmol each), 5 µl of DNA sample (50 ng) and 0.5 µl of Taq DNA polymerase (2.5 units), totaling a volume of 50 µl per reaction. A negative and positive control was used for each reaction.

For the evaluation of gene amplification PCR products were submitted to 2% agarose gel electrophoresis using BlueGreen nucleic acid dye (Lab Biotechnology) and a 100-base pair molecular DNA marker (Cellco). The visualization occurred in a transluminator under ultraviolet (UV) light and samples that amplified a 150 bp fragment (base pairs) were considered positive.

### Data analysis

For data analysis, the GraphPad Prism 8 software was used, applying the Chi-square test, considering $p < 0.05$ as statistically significant.

## RESULTS

117 samples of gastric tissue were collected from dyspeptic patients and 41 were excluded because they did not answer the inclusion criteria. The analyzed samples were 60/76 (78.94%) to females and 16/76 (21.05%) to males and participants' ages ranged from 18 to 83, with an average of 46.6 years.

Samples were analyzed using PCR and histopathological technique for detection of *H. pylori*. They were considered positive when the bacterium was detected in at least one of the tests and negative when the microorganism was absent in both tests. Positive samples submitted to 16S rRNA gene amplification generated a 150 bp fragment (**Figure 1**). Results showed that 55/76 (72.3%) were positive and 21/76 (27.7%) negative for *H. pylori.* Among the positive samples, 43/55 (78.2%) were female and 12/55 (21.8%) male, and most were aged 18 to 40 years (45.5%) (**Table 2**).

**TABLE 1 – Amplication conditions and sequence primeers used for *H. pylori* detection**

| Gene | Primer | Sequence (5' → 3') | Amplification conditions | pb | Reference |
|---|---|---|---|---|---|
| 16S rRNA | *hpx* | CTGGAGARACTAAGYCCTCC | 94°C 5', 40 ciclos 94°C 1' | 150 | Luscenti and Gatti (2008)[16] |
| | *hpx1* | GAGGAATACTCATTGCGAAGGCGA | 59°C 1'/72°C 1' e 72°C 7' | | |

*rRNA: ribosonal ribonucleic acid.*

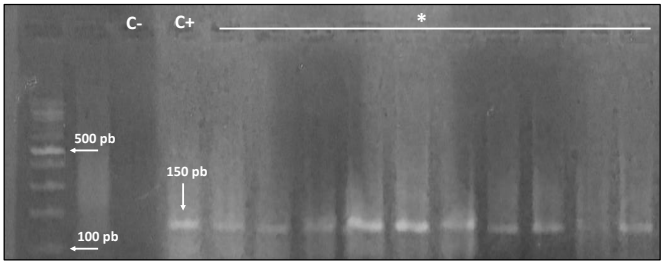


**FIGURE 1** – *Agarose gel electrophoresis of the PCR amplified products of the 16S rRNA ribosomal gene (*hpx/hpx1*)*
*M: 100 bp molecular DNA marker; C-: negative control; C+: positive control; *DNA from gastric tissue samples from dyspeptic patients; PCR: polymerase chain reaction; DNA: deoxyribonucleic acid.*

**TABLE 2** – Profile of dyspeptic patientes according to sex and age group

| Variables | Total ($n$ = 76) | | *H. pylori*-positive ($n$ = 55) | | *H. pylori*-negative ($n$ = 21) | |
|---|---|---|---|---|---|---|
| | $n$ | % | $n$ | % | $n$ | % |
| Sex | | | | | | |
| Female | 60 | 78.9 | 43 | 78.2 | 17 | 80.9 |
| Male | 16 | 21.1 | 12 | 21.8 | 4 | 19.1 |
| Age range | | | | | | |
| 18-40 | 27 | 35.5 | 25 | 45.4 | 2 | 9.5 |
| 41-60 | 35 | 46.1 | 21 | 38.2 | 14 | 66.7 |
| > 60 | 14 | 18.4 | 9 | 16.4 | 5 | 23.8 |

The samples submitted to histopathological examination were positive when showed the presence of *H. pylori* by Giemsa staining (**Figure 2**).

The results showed that 26 (34.2%) samples were positive for the PCR technique and negative for the histopathological, while only seven (9.2%) samples were positive for histopathological and negative for PCR. Moreover, 22 (28.9%) samples were positive and 21 (27.6%) negative for both tests (**Table 3**).

The PCR technique was about 1.6 times more sensitive than the histopathological diagnosis. The results showed that the molecular tool detected 48/76 (63.1%) positive samples, while the histological technique only 28/76 (38.1%). Statistical analyzes showed a significant difference between the methods ($p < 0.05$) (**Figure 3**).

Gastric pathologies were classified according to the endoscopic and histopathological diagnostics. More than one clinical outcome could be found in the same infected patient. Results showed that 1/79 (1.2%) patients had gastric adenocarcinoma, 4/79 (5.0%) atrophy, 11/79 (15.1%) duodenitis, 8/79 (10.1%) esophagitis, 43/79 (55.6%) gastritis, 2/79 (2.5%) ulcer, 2/79 (2.5%) metaplasia and 7/79 (7.5%) presented normal endoscopic diagnosis (**Table 4**).

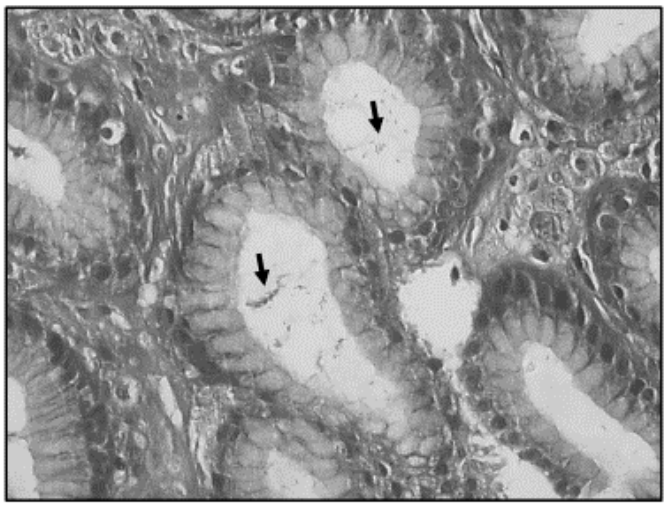

**FIGURE 2** – *Histological section image of the gastric antrum (400× magnification), stained by Giemsa's technique, showing the presence of* H. pylori
*The arrow indicates the presence of* H. pylori.

**TABLE 3** – Comparison of positive and negative results according to the molecular and histopathological tests

| PCR | Golden pattern | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 22 | 26 | 48 |
| Negative | 7 | 21 | 28 |

*PCR: polymerase chain reaction.*

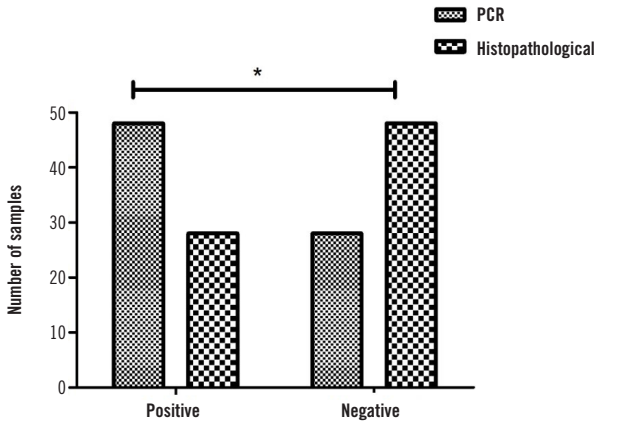


**FIGURE 3** – *Results comparison obtained by the PCR method and histopathological test*
*PCR: polymerase chain reaction*

**TABLE 4** – Clinical outcomes obtained from endoscopic diagnosis and histopathological analysis in *H. pylori* positive patients

| Clinical outcames ($n$ = 79) | $n$ | % |
|---|---|---|
| Gastric adenocarcinoma | 1 | 1.3 |
| Atrophy | 4 | 5.1 |
| Duodenitis | 12 | 15.2 |
| Esophagitis | 8 | 10.1 |
| Gastritis | 44 | 55.7 |
| Ulcer | 2 | 2.5 |
| Metaplasia | 2 | 2.5 |
| Normal | 6 | 7.6 |

## DISCUSSION

In the present study the prevalence of infection was 55/76 (72.3%), similar to other developing countries. Other study[15] conducted in central Brazil with similar sampling showed prevalence of infection of 77.6%, similar to the present study. Several diagnostic methods are used to screen for *H. pylori* infection and to choose the best test it is required to evaluate parameters of sensitivity, specificity, cost and clinical condition of the patient, therefore it is of utmost importance studies evaluating diagnostic efficacy[16-18]. In this study 78.1% patients were female, corroborating with study by Ortiz *et al.* (2011)[19], which used a similar sample and identified that 65.3% of the individuals were women. The study by Nevoa *et al.* (2017)[15], analyzed gastric biopsies of 85 patients and most were women (65.8%). The higher prevalence of infected women can be explained by the fact that they seek the public health care more often. The age of the participants in this study ranged from 18 to 83, with a mean of 46.6 years, similar to the study by Nevoa *et al.* (2017)[15], where the range was from 15 to 89 years with a mean of 40.77 years.

The histopathological method for decades is considered the most common test to identify the infection[10]. This diagnostic is considered the gold standard by the IV Consenso Brasileiro sobre infecção por *Helicobacter pylori* for detecting the bacterium presence and to evaluate the gastric mucosa conditions. Some studies showed that the histopathological test sensitivity is higher to several techniques used to detect the microorganism. In addition, the gold standard technique is inexpensive and simple to perform[20-23]. In contrast, previous studies have shown disadvantages of the technique, such as the professional's ability to analyze the slides and besides that false negative results occur when the collected fragment does not contain the bacteria. This is due to the irregular distribution of the microorganism in the gastric mucosa, requiring the removal of two fragments from the body and two from the stomach antrum to minimize this event[10, 20, 21].

The histopathological technique may also be affected by recent use of medications such as bismuth salts, PPIs and antibiotics, which lead to decreased bacterial density. After eradication treatment, this methodology presents a decrease in sensitivity that may be even smaller than the urease test, however the histopathological test is commonly used to monitor inflammatory lesions after treatment[10, 19]. In this study, patients who were undergoing treatment or using PPIs and antibiotics were excluded from the study; therefore, the histological technique may not have been influenced by these factors.

Over the past few decades, molecular detection has dramatically changed the clinical management of many infectious diseases[21]. PCR is one of the best molecular methods used in a wide range of clinical applications such as simplicity of execution, high sensitivity and specificity and allows genotyping different strains and identifying virulence and antibiotic resistance genes[24, 25]. The present study evaluated 76 samples which 48 were positives using PCR, in contrast, only 29 were detected using the histopathological diagnosis, showing the better detection of *H. pylori* using the molecular tool. These data corroborate the study by Ruparelia *et al.* (2013)[26] which evaluated several parameters of conventional and PCR tests, demonstrating a greater accuracy of molecular methodology. The research by Rasmussen *et al.* (2010)[27], also showed a better detection of *H. pylori* using PCR compared to the histological test, however, the effectiveness of PCR was lower compared to the Southern Blotting test.

A recent study by Nevoa *et al.* (2017)[15], compared the sensitivity of the rapid urease test between PCR technique using the *hpx1/hpx2* ribosomal gene and the constitutive urease *h5/h6* gene. The authors demonstrated that regardless of the *H. pylori* gene used for amplification, the PCR technique demonstrated better sensitivity compared to the urease test.

The study by Ortiz *et al.* (2011)[19] showed an increase in sensitivity from 80.3% using the histopathological test to 90.3% when using the molecular methodology. Furthermore, the authors showed that the molecular test combined with the histological test presented higher sensitivity than the rapid urease test combined with the PCR or the histological test[26]. Our results demonstrated that individually the histological technique presented a positivity of 38.1%, the PCR of 63.1% and when combined presented 72.3%. Besides that, it was observed that 26 (34.2%) samples were positive exclusively for the molecular technique and seven (9.2%) exclusively for the histopathological test. Despite the advantages of PCR, the technique may have some limitations, such as inadequate sample collection and the presence of reaction inhibitors. Additionally, PCR can detect DNA from dead bacteria present in the gastric mucosa of patients after treatment generating false positive results[23, 28, 29]. These data reinforce the importance of using the histological PCR technique to increase sensitivity, avoiding possible cases of false negative results.

*H. pylori* infection may manifest asymptomatically, in this situation it is not possible to determine the presence of the bacterium based on clinical symptoms or findings. In this sense, a tool with high sensitivity that allows the detection of bacteria in asymptomatic individuals is important. Infection clinical outcomes may be severe or non-severe and will be determined according to the parasite-host relationship. This study showed that gastritis was the main pathology associated with *H. pylori*

infection, followed by esophagitis and duodenitis. The study by Barbosa and Schinonni (2011)[30] showed that 95% of cases of chronic gastritis have *H. pylori* as etiological agent.

## CONCLUSION

*H. pylori* represent one of the most common bacterial infection in humans, that causes severe anomalies, including chronic gastritis and gastric cancer. An accurate diagnosis of *H. pylori* infection is a critical first step in the successful treatment. The PCR was more advantageous because showed sensitivity about 1.6 times higher than the histopathological. Currently, PCR is not used in the laboratory routine due to its high cost when compared to other techniques, making its use unfeasible, especially in the public health system. As regard, it is required to continue our efforts to achieve more appropriate and reliable diagnostic tests.

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**CORRESPONDING AUTHOR**

**Monica Santiago Barbosa** ID 0000-0001-6964-5219
e-mail: santiagosant@gmail.com