

Research Article

Burden of *Helicobacter pylori* Isolated from Gastric Biopsies in Al-Jumhuri Hospital, Iraq

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A B S T R A C T

Introduction: *Helicobacter pylori* infection is one of the most common communicable gastrointestinal infections worldwide and is strongly associated with chronic gastritis, peptic ulcer disease, and gastric malignancy. The burden of infection remains high in developing countries due to poor sanitation and overcrowding. Accurate detection of *H. pylori* among symptomatic patients is essential for understanding disease burden and guiding effective management strategies.

Objective: The present study aimed to assess the burden of *H. pylori* infection among symptomatic patients undergoing upper gastrointestinal endoscopy using invasive detection methods.

Materials and Methods: This hospital-based cross-sectional study was conducted in the Endoscopy Unit of Al-Jamhuri Hospital, Nineveh Governorate, from December 2023 to February 2024. A total of 50 gastric biopsy samples were collected from symptomatic patients of gastritis undergoing endoscopy. Biopsy specimens obtained from the antrum and body of the stomach were subjected to culture on Columbia blood urea agar, rapid urease test (RUT), and polymerase chain reaction (PCR) targeting the 16S rRNA gene of *H. pylori*. PCR was considered the reference method for comparison.

Results: PCR detected *H. pylori* in 47 out of 50 samples, yielding a positivity rate of 94%. The rapid urease test was positive in 42 samples (84%), while culture yielded positive results in 20 samples (40%). When compared with PCR, RUT showed a sensitivity of 89.4% and specificity of 100%, whereas culture demonstrated a sensitivity of 42.6% and specificity of 100%. ROC curve analysis revealed excellent performance of RUT (AUC = 0.95) and moderate performance of culture (AUC = 0.71).

Conclusion: The study revealed a high burden of *Helicobacter pylori* infection among symptomatic patients of gastritis. PCR demonstrated the highest detection rate, while rapid urease testing provided a reliable and practical alternative for routine clinical use in endemic settings.

Keywords: *Helicobacter pylori*, Gastric biopsy, PCR, Rapid urease test, Communicable diseases

Introduction

Helicobacter pylori infection remains one of the most common chronic bacterial infections affecting humans worldwide and continues to pose a major public health challenge, particularly in developing countries. It is a gram-negative, spiral-shaped, microaerophilic bacterium that selectively colonizes the gastric mucosa and is implicated in a wide spectrum of gastroduodenal diseases, including chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma.¹⁻³ Owing to its strong association with gastric malignancy, the World Health Organization has classified *H. pylori* as a Group I carcinogen.⁴

The global prevalence of *H. pylori* infection is estimated to exceed 50% of the adult population, with disproportionately higher rates reported from low- and middle-income countries.^{5,7} High population density, poor sanitation, overcrowding, and low socioeconomic status have been consistently identified as key determinants facilitating transmission of this organism.^{8,9} Transmission is believed to occur predominantly via fecal–oral and oral–oral routes, although contaminated food and water sources also play a significant role, particularly in endemic regions.^{8,9} Despite widespread infection, many individuals remain asymptomatic, while others develop significant gastrointestinal symptoms such as epigastric pain, nausea, vomiting, dyspepsia, and gastrointestinal bleeding.¹⁰

Accurate detection of *H. pylori* infection is essential not only for individual patient management but also for understanding disease burden and guiding infection control strategies. A variety of invasive and non-invasive methods are available for detecting *H. pylori*, including rapid urease test (RUT), histopathological examination, culture, serology, urea breath test, stool antigen test, and molecular techniques such as polymerase chain reaction (PCR).^{6,11} Among these, invasive methods using gastric biopsy specimens obtained during endoscopy remain highly relevant in symptomatic patients, as they allow direct identification of the organism and enable confirmation through multiple complementary techniques.

Culture of *H. pylori*, although technically demanding due to its fastidious nature and strict growth requirements, is traditionally regarded as the reference method for confirmation of infection.¹¹ However, its sensitivity is often limited by factors such as biopsy quality, transport conditions, and prior antibiotic or proton-pump inhibitor use. Rapid urease testing offers a simple, rapid, and cost-effective alternative with good performance in routine clinical practice.¹⁷ Molecular detection using PCR targeting conserved regions such as the 16S rRNA gene has emerged as a highly sensitive method for detecting *H. pylori* directly

from gastric tissue, particularly in cases with low bacterial load or culture-negative results.¹⁸⁻²²

In regions with a high prevalence of *H. pylori* infection, limited laboratory infrastructure and cost constraints often influence the choice of detection methods. Therefore, evaluating the burden of *H. pylori* infection among symptomatic patients and assessing the utility of commonly employed invasive detection techniques is essential for improving diagnostic strategies and informing public health interventions. The present study was undertaken to determine the burden of *Helicobacter pylori* infection among symptomatic patients undergoing upper gastrointestinal endoscopy, using gastric biopsy-based detection methods.

Materials And Methods

Study Design and Setting

This hospital-based cross-sectional study was conducted in the Endoscopy Unit of Al-Jamhuri Hospital, Nineveh Governorate, Iraq, during the period from December 2023 to February 2024.

Study Population

A total of 50 symptomatic patients undergoing upper gastrointestinal endoscopy for complaints suggestive of gastritis were included in the study. Patients of both sexes, aged between 7 and 85 years, were enrolled. Individuals who had received antibiotics, proton-pump inhibitors, or bismuth compounds within two weeks prior to endoscopy were excluded.

Sample Collection

During endoscopy, gastric biopsy specimens were obtained from the antrum and body of the stomach under aseptic conditions. Each biopsy sample was placed in 2 mL of sterile normal saline and transported to the laboratory in a refrigerated container within two hours of collection for further processing.

Culture of *Helicobacter pylori*

Preparation of Culture Medium

Columbia blood urea agar was prepared by dissolving 42 g of the medium in distilled water, followed by autoclaving at 121°C for 20 minutes. After cooling to 50°C, 7% sheep blood, urea (20 g), phenol red (0.0012 g), and selective antibiotics (vancomycin 5 mg/L, trimethoprim 10 mg/L, and amphotericin B 2.5 mg/L) were added aseptically. The medium was poured into sterile Petri dishes and stored at 4°C until use.

Inoculation and Incubation

Biopsy specimens were homogenized, and 0.5 mL of the homogenate was inoculated onto the prepared culture

medium. The plates were incubated at 37°C for 3–5 days under microaerophilic conditions using a gas-generating system providing 5% O₂, 10% CO₂, and 85% N₂. Plates were examined daily for characteristic *H. pylori* growth.

Rapid Urease Test (RUT)

Preparation of RUT Solution

The rapid urease test solution was prepared by dissolving 10 g of urea and 0.2 g of phenol red in 100 mL of distilled water.

Test Procedure

Fresh gastric biopsy specimens were placed directly into 200 µL of the prepared RUT solution. A color change from yellow to pink or red within 24 hours was interpreted as a positive result, indicating the presence of urease-producing *H. pylori*.

DNA Extraction from Biopsy Samples

Biopsy samples were incubated in 2 mL of brain heart infusion broth supplemented with antibiotics for one hour with gentle shaking. Samples were then centrifuged at 10,000 rpm for 10 minutes. Genomic DNA was extracted using a commercial DNA purification kit (Geneaid), according to the manufacturer's instructions. DNA concentration and purity were assessed using a NanoDrop spectrophotometer.

PCR Detection of *Helicobacter pylori*

Target Gene and Primers

PCR amplification targeted the species-specific 16S rRNA gene of *H. pylori* using forward primer 27F (AGAGTTTGATCMTGGCTCAG) and reverse primer 1522R (AAGGAGGTGATCCARCCGCA).

PCR Amplification Conditions

PCR was performed in a thermal cycler with an initial denaturation at 95°C for 6 minutes, followed by 35 cycles of denaturation at 95°C for 45 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. A final extension step was carried out at 72°C for 5 minutes.

Gel Electrophoresis

PCR products were analyzed by electrophoresis on a 2.5% agarose gel stained with an appropriate DNA stain. Amplified products of approximately 1500 bp were considered positive for *H. pylori*.

Ethical Considerations

The study protocol was approved by the Ethics and Research Committees of the Nineveh Department of Health. Written informed consent was obtained from all participants or their guardians prior to enrollment.

Results

A total of 50 gastric biopsy specimens obtained from symptomatic patients undergoing upper gastrointestinal endoscopy were evaluated for the presence of *Helicobacter pylori* using culture, rapid urease test (RUT), and polymerase chain reaction (PCR). The results of the three invasive detection methods are summarized below.

Culture Findings

Culture of gastric biopsy specimens on Columbia blood urea agar yielded *H. pylori* growth in 20 out of 50 samples, giving a culture positivity rate of 40%. Positive cultures showed small, translucent colonies with a characteristic change in the color of the medium from yellow to red, indicating urease activity. These isolates were further confirmed by positive catalase, oxidase, and urease biochemical tests. No growth was observed in the remaining 30 samples even after 5 days of incubation under microaerophilic conditions, and these samples were considered culture negative.

Rapid Urease Test Results

The rapid urease test demonstrated positive results in 42 of the 50 gastric biopsy samples, corresponding to an overall positivity rate of 84%. In positive cases, a clear color change from yellow to pink or red was observed within 24 hours of incubation. The remaining 8 samples (16%) showed no change in color and were interpreted as negative. The high proportion of RUT-positive samples indicated a substantial burden of *H. pylori* infection among the symptomatic patients included in the study.

PCR Detection of *Helicobacter pylori*

PCR analysis targeting the species-specific 16S rRNA gene identified *H. pylori* DNA in 47 of the 50 gastric biopsy specimens, yielding the highest positivity rate of 94%. Agarose gel electrophoresis revealed amplified DNA fragments of approximately 1500 bp in PCR-positive samples, confirming the presence of *H. pylori*. Three samples (6%) did not show amplification and were considered PCR negative. PCR detected *H. pylori* in several samples that were negative by culture, suggesting the presence of low bacterial load or non-viable organisms.

Comparison of Invasive Detection Methods

When the three invasive methods were compared, PCR demonstrated the highest detection rate, followed by the rapid urease test and culture. Culture showed the lowest positivity rate, reflecting the fastidious nature of *H. pylori* and the technical challenges associated with its isolation. In contrast, RUT and PCR identified a significantly higher proportion of infected individuals. Overall, these findings

highlighted a high burden of *Helicobacter pylori* infection among symptomatic patients undergoing endoscopy, with molecular detection providing the greatest yield, while rapid urease testing offered a reliable and practical method for routine clinical assessment.

from a biopsy specimen of a chronic gastritis patient on Columbia Urea Agar medium. A red zone is formed around the bacterial colony due to the change in pH of the medium from acidic to alkaline by hydrolysis of urea by the enzyme

urease. The plate was incubated for 65–70 h at 37°C and 5% CO₂. (B) *H. pylori* positive colonies from the samples were applied to the medium designated for the bacteria and the color of the medium changed from yellow to red. The plate was incubated for 96 h at 37°C and 5% CO₂.

Figure 3 : Agarose gel (2.5%) electrophoresis of 16S rRNA of *H. pylori*. Species-specific 16s rRNA of *H. pylori* was amplified and 1500 bp amplified DNA is shown Lane 1–24: 16S rRNA amplified from *H. pylori* of biopsy samples of chronic gastritis patients.

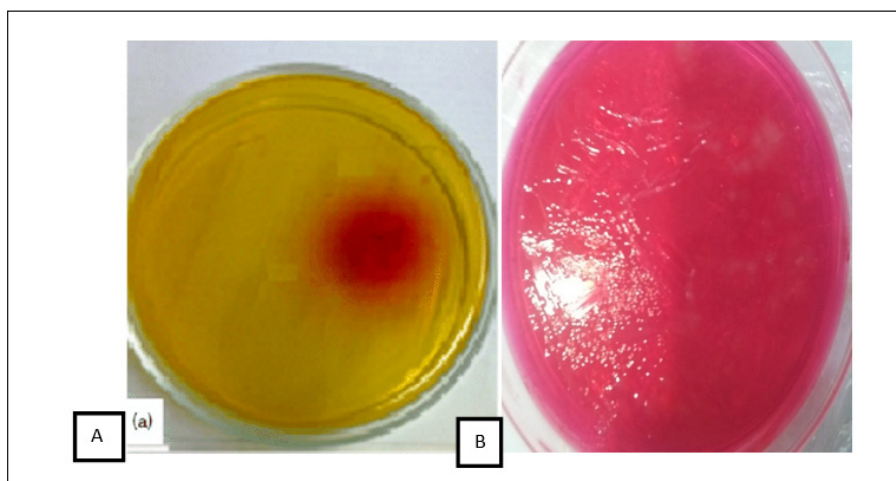


Figure 1./ *H. pylori* culture. (A) Growth of *H. pylori*

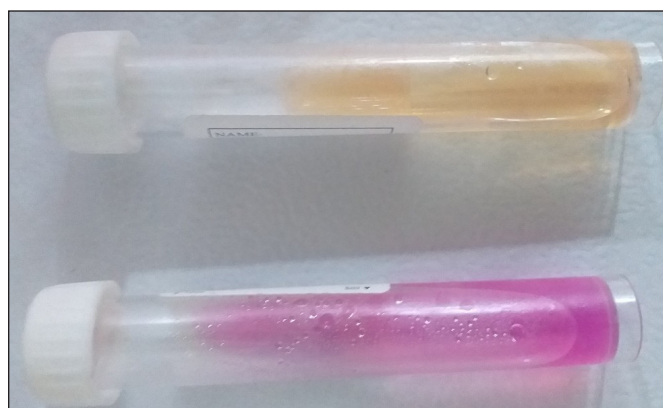


Figure 2.Result of RUT on Biopsy tissue A:Negative,B:Positive

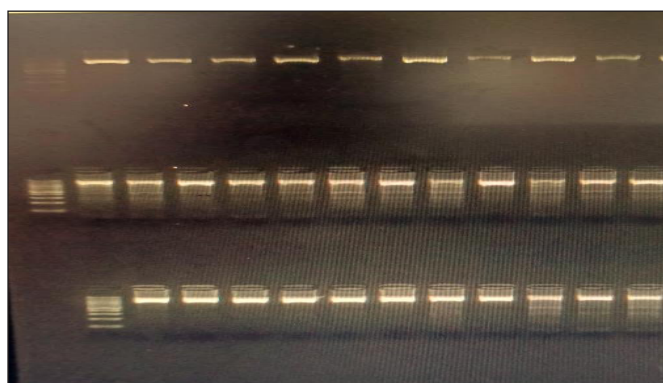


Figure 3.Agarose gel (2.5%) electrophoresis of 16S rRNA of *H. pylori*.

Table 1. Detection of *Helicobacter pylori* by Different Invasive Methods

(n = 50)

Detection Method	Positive (n)	Positive (%)	Negative (n)	Negative (%)
Culture	20	40.0	30	60.0
Rapid Urease Test	42	84.0	8	16.0
PCR (16S rRNA)	47	94.0	3	6.0

Table 2. Comparison of *Helicobacter pylori* Detection Rates Among Invasive Methods

Method	Detection Rate (%)	Rank of Detection Yield
PCR (16S rRNA)	94.0	Highest
Rapid Urease Test	84.0	Intermediate
Culture	40.0	Lowest

Table 3. Summary of Culture, RUT, and PCR Findings in Gastric Biopsy Samples

Parameter	Culture	Rapid Urease Test	PCR
Type of test	Invasive	Invasive	Invasive
Target	Viable bacteria	Urease enzyme	16S rRNA gene
Positive samples (n)	20	42	47
Positivity rate (%)	40.0	84.0	94.0

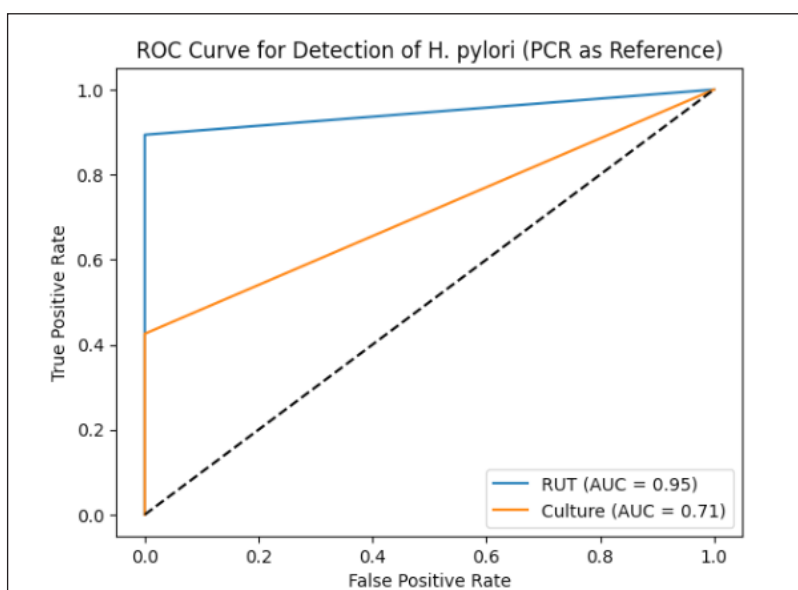


Figure 4. ROC Curve for Detection of *H. pylori* (PCR as Reference)

Table 4. Sensitivity and Specificity of Invasive Tests for Detection of *Helicobacter pylori* (PCR as Reference Standard)

Test Method	Sensitivity (%)	Specificity (%)
Rapid Urease Test	89.4	100
Culture	42.6	100

Discussion

The present study demonstrated a high burden of *Helicobacter pylori* infection among symptomatic patients undergoing upper gastrointestinal endoscopy. Using PCR as the reference method, *H. pylori* was detected in 47 out of 50 patients, yielding an overall positivity rate of 94%. This finding indicates widespread infection in the study population and highlights the continuing public health relevance of *H. pylori* as a common communicable gastrointestinal pathogen in endemic settings.

These findings are comparable with earlier reports from developing countries, where prevalence rates ranging from 60% to over 90% have been documented among symptomatic individuals.^{5,7} Salem et al. reported a prevalence of 78.6% among dyspeptic patients in Egypt⁷, while Zsikla et al. detected *H. pylori* in 92% of biopsy samples from patients with chronic gastritis using PCR.¹² The high burden observed in the present study may be attributed to factors such as poor sanitation, overcrowding, and low socioeconomic conditions, which have been consistently implicated in facilitating transmission.^{8,9}

In the present study, PCR targeting the 16S rRNA gene demonstrated the highest detection rate (94%), followed by the rapid urease test (84%), while culture yielded the lowest positivity rate (40%). These findings underscore the variability in detection rates among invasive methods and emphasize the influence of test sensitivity on estimating infection burden.

Similar observations have been reported in previous studies. Jabbar and Al-Obaidi detected *H. pylori* in 90% of gastric biopsies using PCR, compared to 76% by rapid urease test and only 38% by culture.¹⁸ Likewise, Alrubaye et al. reported PCR positivity of 93.3%, RUT positivity of 86.7%, and culture positivity of 41.7% among gastritis patients in Iraq.²⁰ The close agreement between these findings and the present study supports the reliability of PCR and RUT in detecting *H. pylori* in endemic populations.

The rapid urease test showed a high positivity rate of 84% in the present study, with a sensitivity of 89.4% and specificity of 100% when compared with PCR. The ROC analysis further demonstrated excellent diagnostic performance of RUT, with an area under the curve (AUC) of 0.95.

Comparable results have been reported by Negash et al., who observed RUT sensitivity of 88.2% and specificity of 98.6% when evaluated against ELISA-based reference tests.¹¹ Zsikla et al. also reported RUT positivity of 85% in patients with chronic gastritis, closely aligning with the findings of the present study.¹² The high sensitivity and rapid turnaround time of RUT make it a practical and cost-effective method for routine detection of *H. pylori*, particularly in resource-limited settings. Culture positivity

in the present study was observed in only 40% of biopsy samples, with a sensitivity of 42.6% despite a specificity of 100%. This low yield reflects the fastidious nature of *H. pylori* and the strict requirements needed for its isolation and growth.

Several studies have reported similarly low culture yields. Jabbar and Al-Obaidi reported a culture positivity rate of 36.7%¹⁸, while Alrubaye et al. observed a rate of 41.7%.²⁰ Factors such as prior antibiotic exposure, low bacterial load, improper transport conditions, and conversion of bacillary forms into coccoid forms may significantly reduce culture sensitivity.^{1,11} Despite these limitations, culture remains valuable for antimicrobial susceptibility testing and epidemiological surveillance. PCR detection of *H. pylori* using the conserved 16S rRNA gene provided the highest detection rate in the present study and identified infections missed by both RUT and culture. The high sensitivity of PCR is particularly useful in cases with low bacterial density or non-viable organisms.

Zsikla et al. reported PCR detection of *H. pylori* in 92% of biopsy samples compared to 78% by histology and 65% by culture.¹² Ansari and Yamaoka emphasized that molecular methods significantly improve detection rates and provide a more accurate estimation of infection burden in endemic areas.²¹ The findings of the present study further support the utility of PCR in epidemiological assessment of *H. pylori* infection.

Conclusion

The present study demonstrated a high burden of *Helicobacter pylori* infection among symptomatic patients undergoing upper gastrointestinal endoscopy. PCR detected the highest proportion of infections, followed by the rapid urease test, while culture showed comparatively lower yield. These findings highlight the importance of using sensitive invasive methods to accurately assess the burden of *H. pylori* infection in endemic settings.

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