International Journal of Research in Medical Sciences
Paul S et al. Int J Res Med Sci. 2026 Nov;14(11):5023-5028
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20264158

Original Research Article

Molecular detection of *Helicobacter pylori* in rapid urease test-negative gastric biopsy samples

Swarna Paul[1]*, Muhammad Moazzem Hossain[2], Afreen Sultana[1], Banani Barua[3], Afroza Sultana[4], Masuma Nasrin[5], Sifat Saima[6], Rahella Begum[7], Abdul Quddus[8], Maraz Hossain Chayan[9]

[1]Department of Microbiology, Chittagong Medical College, Chattogram, Bangladesh
[2]Department of Virology, Chittagong Medical College, Chattogram, Bangladesh
[3]Department of Biochemistry, Chittagong Medical College, Chattogram, Bangladesh
[4]Alif Hospital and Diagnostic Center, Chattogram, Bangladesh
[5]Department of Neurology, Chittagong Medical College Hospital, Chattogram, Bangladesh
[6]Department of Dermatology, Chittagong Medical College Hospital, Chattogram, Bangladesh
[7]Department of Surgery, Chittagong Medical College Hospital, Chattogram, Bangladesh
[8]Department of ENT and Head-Neck Surgery, Parshuram Upazila Health Complex, Feni, Bangladesh
[9]Department of Endocrinology, Chittagong Medical College Hospital, Chattogram, Bangladesh

Received: 10 August 2026
Accepted: 11 September 2026

***Correspondence:**
Dr. Swarna Paul,
E-mail: dr.swarnapaul@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Accurate detection of *Helicobacter pylori* remains challenging in dyspeptic patients because urease-based assays may be negative in low-density or patchy infection. The study aims to evaluate biopsy-based Nested PCR targeting the ureA gene for detection of *H. pylori*, with emphasis on molecular detection among rapid urease test (RUT)-negative gastric biopsies.
Methods: This cross-sectional descriptive study enrolled 130 dyspeptic adults undergoing upper gastrointestinal endoscopy at the Department of Microbiology, Chittagong Medical College, Chattogram, in collaboration with the Department of Gastroenterology, Chittagong Medical College Hospital, over a one-year period from January 2023 to December 2023. Two antral biopsies were collected per patient, one for RUT and one for Nested PCR targeting ureA.
Results: The cohort included 76 males (58.46%) and 54 females (41.54%), with the largest age stratum 20-40 years. Endoscopy was normal in 52 (40.0%) and abnormal in 78 (60.0%), most commonly antral gastritis (31.5%). Nested PCR was positive in 97/130 (74.6%), RUT was positive in 87/130 (66.9%). Among RUT-negative cases (n=43), nested PCR detected *H. pylori* DNA in 12 (27.9%); among RUT-positive cases (n=87), PCR was positive in 85 (97.7%). RUT and PCR were strongly associated (χ^2=74.18, p<0.0001). Using RUT as comparator, PCR Sensitivity was 97.70%, Specificity 72.09%, positive predictive value (PPV) 87.63%, negative predictive value (NPV) 93.94%, accuracy 89.23%.
Conclusions: Nested ureA PCR demonstrated high detection and identification even with infections among RUT-negative biopsies, supporting its adjunct value in tertiary-care workflows.

Keywords: *Helicobacter pylori*, Nested PCR, UreA gene, Rapid urease test, Gastric biopsy

INTRODUCTION

Helicobacter pylori remain one of the most prevalent chronic bacterial infections worldwide and is the principal etiologic agent responsible for chronic gastritis, peptic ulcer disease, and gastric malignancy. Despite extensive global control efforts, it is estimated that more than half of the world's population is colonized by *H. pylori*, with

infection often acquired in early childhood and persisting throughout life if untreated.[1] The infection is of particular concern in low- and middle-income countries where hygiene conditions, population density, and healthcare access facilitate transmission. In these regions, high infection rates translate into a correspondingly elevated burden of dyspeptic symptoms and acid-peptic disorders, forming a major indication for endoscopic evaluation and antimicrobial therapy.

In Bangladesh and similar South Asian settings, dyspepsia constitutes one of the most common reasons for upper gastrointestinal endoscopy, with *H. pylori* infection being a frequent underlying cause. However, laboratory confirmation of infection remains inconsistent, leading to widespread empirical use of acid-suppressive or eradication regimens without bacteriologic verification. Such practices may contribute to underdiagnosis, antimicrobial resistance, and treatment failure.[2] Therefore, accurate and reliable diagnostic methods are essential to improve patient outcomes and guide evidence-based therapy. Multiple invasive and non-invasive tests are available for *H. pylori* detection, including histopathology, RUT, culture, urea breath test, and stool antigen assays. However, no single test is universally ideal. Diagnostic accuracy varies depending on bacterial density, biopsy site, recent use of antibiotics or proton-pump inhibitors, and laboratory infrastructure.[1,2] Invasive biopsy-based methods such as histology, culture, and RUT are frequently applied in tertiary hospitals, but each has intrinsic limitations. Culture, though specific, requires viable organisms, stringent microaerophilic conditions, and prolonged incubation. Histology provides visual confirmation of mucosal pathology but depends on experienced interpretation and may yield false negatives when bacterial load is low. Similarly, the RUT, though rapid and inexpensive, shows reduced sensitivity in patients recently exposed to PPIs or antibiotics, reflecting the fragility of these conventional diagnostic systems in routine clinical settings.[3] To overcome these challenges, molecular diagnostic methods, particularly PCR, have gained increasing importance in *H. pylori* detection. PCR is capable of identifying low copy numbers of bacterial DNA directly from gastric biopsy specimens, making it less dependent on bacterial viability or culture success. It provides higher sensitivity and specificity, with the ability to detect infection even when bacterial growth is suppressed by prior therapy or suboptimal sampling.[4] Comparative studies have demonstrated that PCR can outperform traditional methods and maintain high diagnostic accuracy across different specimen types and clinical contexts.[3,5] Moreover, PCR assays can be adapted to detect virulence genes and antimicrobial resistance markers, offering valuable epidemiologic and therapeutic insights.[4] The performance of PCR largely depends on the choice of target gene. Ideal targets are conserved, species-specific, and biologically essential, ensuring both sensitivity and specificity. Housekeeping genes such as glmM (also referred to as ureC) and 16S rRNA, as well as urease-associated genes (ureA and ureB), have been extensively validated in this regard.[5,6] Among these, ureA encodes one subunit of the urease enzyme complex, a critical determinant of *H. pylori* survival in the acidic gastric environment. Because of its functional indispensability and genetic conservation, ureA has emerged as a preferred target in PCR assays.[7] Molecular investigations have confirmed that ureA is a highly stable marker and that its amplification correlates strongly with clinical infection, supporting its diagnostic utility.[8] Recent open-access molecular studies further emphasize that PCR targeting conserved ureA or glmM genes can achieve detection rates exceeding 90%, even in biopsy samples where culture or RUT results are negative.[4,7] The use of Nested PCR further enhances sensitivity by allowing amplification of minimal DNA templates without compromising specificity. Such methodological advances hold particular relevance for countries like Bangladesh, where laboratory resources are variable and empirical management remains common. Given this background, the present study was designed to evaluate the diagnostic performance of Nested PCR targeting the ureA gene for detection of *H. pylori* in gastric biopsy specimens from dyspeptic patients attending a tertiary-care hospital in Bangladesh. By focusing on a robust molecular assay applicable within a resource-constrained clinical setting, this work aims to contribute locally generated evidence on the practical utility of ureA-based PCR as a reliable diagnostic tool for *H. pylori* infection.

METHODS

This cross-sectional descriptive study was conducted in the Department of Microbiology, Chittagong Medical College, Chattogram, in collaboration with the Department of Gastroenterology, Chittagong Medical College Hospital, over a one-year period from January 2023 to December 2023. The study population comprised adult patients with dyspeptic symptoms suggestive of peptic ulcer disease (PUD) attending the outpatient department or admitted to the Department of Gastroenterology, CMCH, who underwent upper gastrointestinal endoscopy as part of routine clinical evaluation. A total of 130 symptomatic patients were enrolled by purposive sampling after being informed about the objectives and procedures of the study, and written informed consent was obtained from each participant. Patients were included if they had clinical features consistent with PUD, such as nausea, vomiting, burning epigastric pain, abdominal fullness, or related upper gastrointestinal complaints, along with endoscopically proven gastritis, ulcer, or erosion. Patients were excluded if they had received proton pump inhibitors or bismuth compounds within the preceding two weeks, antibiotics within the preceding one month, or prior *H. pylori* eradication therapy within the last six months, and also if they had peptic ulcer disease complicated by active bleeding or perforation, were regular users of NSAIDs or systemic steroids, had significant co-morbidities including cardiac arrhythmia or congestive cardiac failure, or declined consent for biopsy. During endoscopy, two gastric antral biopsy specimens were collected aseptically

from each participant, one specimen was used for the RUT and the other specimen was used for Nested PCR targeting the ureA gene of *H. pylori*. Urease test is the basic test for the diagnosis of *H. pylori* infection from endoscopy. The test is based on the potent urease activity of the bacteria. This enzyme hydrolyses available urea to release ammonia and carbon-di-oxide. The free ammonia increases the pH of the media and the pH indicator phenol red that changes its colour from yellow to red. For RUT, the biopsy material was placed directly into the a commercially manufactured RUT kit-PYLO DRYTM kit (Halifax Research Laboratory, India), consist of a dry filter paper containing urea, phenol (a pH indicator), buffers and bacteriostatic agent in a sealed plastic slide. The biopsy tissue was placed directly on the commercial rapid urease agar gel kit and results are monitored at 5, 10, 15 and 30 minutes, 1 hour and 24 hours according to the kit manual. No change of colour of yellow gel is considered as negative. The colour change from yellow to red or pink or magenta are considered as positive results. The intensity of the colour depends on bacterial load.[9]



Figure 1: Photography shows positive RUT of *H. pylori*.

The biopsy specimen intended for molecular testing was transported promptly to the Microbiology laboratory, and DNA was extracted using a commercially available extraction kit (QIAGEN DNA Mini Kit) following the manufacturer's protocol and extracted DNA was stored at −20°C until amplification. PCR amplification was performed in a total reaction volume of 20 µl with 2 µl template DNA (20 µg/ml) and Taq Polymerase (Biolab 2X master mix) according to the supplier's instructions. PCR amplification was performed in a thermal cycler (Applied Biosystem) with the following cycling parameters: Initial denaturation at 95°C for 4 min, followed by 30 cycles at 95°C for 30 s, 55°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 7 min. In the first steps, PCR for detection of H. pylori was performed using primers ureA-aF (5'-ATG AAA CTC ACC CCA AAA GA-3') and ureA-bR (5'-CCG AAA GTT TTT TCT CTG TCA AAG TCT A-3'). It was done by amplifying 2 µl template genomic DNA extracted from gastric biopsy samples in reaction of 20 µl total volume. In the second steps, PCR was performed using primers ureA-bF (5'- AAA CGC AAA GAA AAA GGC ATT AA -3') and ureA-aR (5'- TTC ACT TCA AAG AAA TGG AAG TGT GA-3'). It was done by amplifying 1 µl of the first PCR product used as a template in reactions of 20 µl total volume. Both the PCR cycles of first and second steps were performed as the single PCR method using the Taq polymerase enzyme (Biolab 2X master mix).[10]

PCR run included positive controls (*H. pylori* ATCC 43504 DNA), negative controls (nuclease free water) to monitor assay performance and minimize contamination risk. Amplified products were analyzed by agarose gel electrophoresis, stained with an intercalating dye, visualized under ultraviolet illumination, and interpreted as positive when a band of the expected amplicon size (383 bp) was observed. Clinical and laboratory data were recorded in a structured format and summarized using frequencies and percentages, and diagnostic performance indices were calculated using standard formulas that were computed bidirectionally by treating Nested PCR and RUT in turn as the comparator test in order to describe operational performance and agreement in this setting. Ethical approval was obtained from the Institutional Ethical Review Committee of Chittagong Medical College, and confidentiality and voluntary participation were ensured throughout the study.

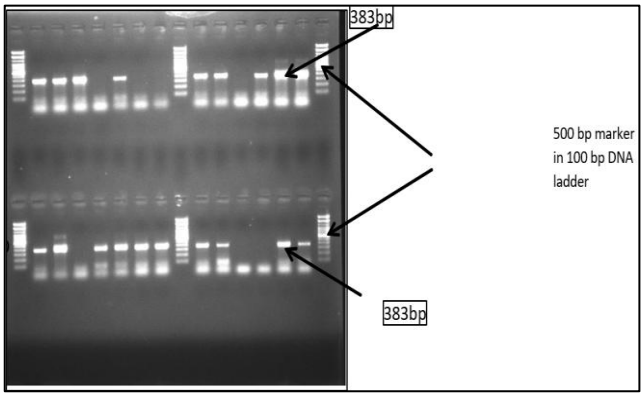


Figure 2: Photography shows gel electrophoresis visualizing ureA nested PCR amplicons.

RESULTS

Among the 130 participants included in the study, male patients constituted the majority, accounting for 76 individuals (58.46%), while 54 participants (41.54%) were female, indicating a modest male predominance in the dyspeptic patient population undergoing upper gastrointestinal endoscopy (Figure 1).

Across the study population (n=130), the largest subgroup comprised patients aged 20-40 years, including 43 males (33.1%) and 28 females (21.5%), followed by those aged 40-60 years with 21 males (16.2%) and 20 females (15.4%); participants aged more than 60 years represented the smallest proportion, consisting of 12 males (9.2%) and 6 females (4.6%), indicating that the study cohort was predominantly composed of younger and middle-aged

adults with a higher male representation across all age strata (Table 1).

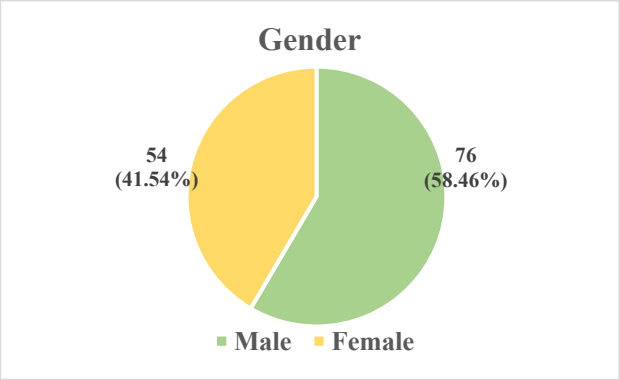


Figure 3: Distribution of participants by gender, (n=130).

Table 1: Distribution of age and sex among the study subjects, (n=130).

Age groups (in years)	Male, n (%)	Female, n (%)
20-40	43 (33.1)	28 (21.5)
40-60	21 (16.2)	20 (15.4)
>60	12 (9.2)	6 (4.6)

Table 2: Distribution of endoscopic findings among the study subjects, (n=130).

Endoscopic finding	N	Percentage (%)
Normal	52	40.0
Antral gastritis	41	31.5
Duodenal ulcer	12	9.2
Gastric ulcer	11	8.5
Antral erosion	11	8.5
Reflux oesophagitis	3	2.3

Endoscopic evaluation of the 130 dyspeptic patients showed that 40.0% (n=52) had a normal upper GI endoscopy, while abnormal findings were present in 60.0% (n=78). The most frequent abnormality was antral gastritis observed in 31.5% (n=41), followed by duodenal ulcer in 9.2% (n=12). Gastric ulcer and antral erosion were each identified in 8.5% (n=11) of cases, and reflux oesophagitis was the least common finding, occurring in 2.3% (n=3) of participants (Table 2).

Table 3: Result of RUT for detection of *H. pylori*, (n=130).

RUT	N	Percentage (%)
Positive	87	66.9
Negative	43	33.1

RUT of *H. pylori* was positive in 87 (66.9%) out of 130 gastric biopsy specimens, while 43 samples (33.1%) tested negative, indicating a high prevalence of *H. pylori* infection among the dyspeptic patients evaluated in this study (Table 3).

Table 4: Result of nested PCR for detection of *H. pylori*, (n=130).

PCR results	N	Percentage (%)
Positive	97	74.6
Negative	33	25.4

Nested PCR targeting the ureA gene of *H. pylori* was positive in 97 out of 130 gastric biopsy specimens, yielding an overall positivity rate of 74.6%, while 33 (25.4%) samples were tested negative, indicating a high prevalence of *H. pylori* infection among the dyspeptic patients evaluated in this study (Table 4).

Cross-tabulation of ureA nested PCR results against RUT showed that among RUT-positive patients (n=87), 85 (97.7%) were PCR positive and 2 (2.3%) were PCR negative. Among RUT-negative patients (n=43), 12 (27.9%) were PCR positive while 31 (72.1%) were PCR negative, indicating that over one-quarter of RUT-negative cases demonstrated molecular evidence of *H. pylori* by nested PCR. Overall, RUT was positive in 87 of 130 patients (66.9%) and negative in 43 (33.1%), while ureA nested PCR was positive in 97 (74.6%) and negative in 33 (25.4%). The association between RUT status and nested PCR results was statistically significant (χ^2=74.18, df=1, p<0.0001) (Table 5).

Table 5: Cross-tabulation used for diagnostic performance estimation, (n=130).

RUT	PCR positive	PCR negative	Total	
RUT positive	85 (97.7)	2 (2.3)	87	χ^2=74.18 p≤0.0001
RUT negative	12 (27.9)	31 (72.1)	43	
Total	97	33	130	

Table 6: Sensitivity, specificity, PPV, NPV, and accuracy of ureA nested PCR, (n=130).

Statistics	Value
Sensitivity	97.70%
Specificity	72.09%
PPV	87.63%
NPV	93.94%
Accuracy	89.23%

Using RUT as the comparator method, ureA-targeted nested PCR demonstrated a sensitivity of 97.70% and a specificity of 72.09%. The positive predictive value was 87.63%, while the negative predictive value was 93.94%. Overall, nested PCR showed an accuracy of 89.23% for detection of *H. pylori* infection in gastric biopsy specimens (Table 6).

DISCUSSION

This study examined molecular detection of *H. pylori* in gastric biopsy specimens from dyspeptic patients undergoing upper gastrointestinal endoscopy in a tertiary hospital in Bangladesh, with a particular focus on infections that may be missed by rapid urease testing. In our cohort of 130 patients, males constituted 58.46% of participants, and most patients were within the younger and middle-aged groups, particularly the 20–40-year age category. Similar demographic trends have been reported in Bangladesh-based studies. For example, Aftab et al investigated diagnostic tests for *H. pylori* infection in symptomatic Bangladeshi patients and reported that the majority of patients presenting for endoscopic evaluation were young to middle-aged adults, reflecting a similar epidemiological pattern of dyspepsia and *H. pylori* infection in the region.[9] Endoscopic evaluation in the present study revealed abnormal findings in 60.0% of patients, while 40.0% had a normal endoscopy. Among abnormal findings, antral gastritis was the most frequent lesion (31.5%), followed by duodenal ulcer, gastric ulcer, and antral erosion. These findings are consistent with previous observations that gastritis is commonly the predominant endoscopic manifestation associated with *H. pylori*. Patel et al reviewed diagnostic approaches for *H. pylori* and highlighted that gastritis is the most frequent endoscopic presentation in infected patients, although a considerable proportion of dyspeptic individuals may show no visible abnormalities during endoscopy.[11] This emphasizes the importance of laboratory-based diagnostic methods rather than relying solely on endoscopic appearance to determine infection status. Using *ureA*-targeted nested PCR, *H. pylori* DNA was detected in 74.6% of gastric biopsy specimens in this study, indicating a substantial molecular detection rate among symptomatic patients. Previous molecular studies have also demonstrated high PCR detection rates in biopsy samples. Lu et al evaluated five PCR assays targeting different genetic loci in gastric biopsy specimens.[5] In their analysis of 50 biopsy samples, PCR targeting *ureA*-targeted PCR detected 75% (18/24) of the biopsy specimens. Their results demonstrated that PCR detection rates can vary depending on the gene target but generally provide high sensitivity when optimized assays are used. Similarly, Elnosh et al examined PCR detection of *H. pylori* using multiple gene targets including *ureA* and reported high diagnostic sensitivity of PCR-based methods in biopsy specimens, supporting the usefulness of molecular detection in clinical diagnosis.[7] The most important finding of the present study was the identification of *H. pylori* DNA in a notable proportion of RUT-negative biopsy samples. Among 43 patients who tested negative by RUT, Nested PCR detected *H. pylori* DNA in 12 cases (27.9%), demonstrating additional molecular detection among individuals who would otherwise have been classified as negative using urease-based testing alone. Similar discrepancies between PCR and conventional diagnostic methods have been reported in earlier studies. Vinette et al compared nested PCR with histology, rapid urease testing, and serological assays in 101 pediatric patients undergoing biopsy evaluation.[12] Their Nested PCR assay demonstrated 100% sensitivity and 94.6% specificity, outperforming serological methods and showing strong concordance with biopsy-based tests. The authors concluded that nested PCR can detect infections that may be missed by other diagnostic approaches, particularly when bacterial distribution is patchy within the gastric mucosa. Additional evidence for such discordance has also been reported in comparative diagnostic studies. Kismat et al, who analyzed gastric biopsy specimens using histology, culture, RUT, and PCR, found that PCR sensitivity was 89.4% and specificity was 100%, while RUT sensitivity was 91.5% with specificity 77.0%.[13] Their study demonstrated that PCR can detect infections in cases where other methods show negative results, highlighting the complementary role of molecular techniques in invasive diagnosis. In the present study, concordance between RUT and PCR was very high among RUT-positive patients. Among the 87 RUT-positive cases, 85 (97.7%) were also PCR positive, indicating that urease activity detected by RUT usually corresponds with molecular evidence of infection. The statistical association between the two diagnostic methods was highly significant (χ^2=74.18, p<0.0001), confirming that both tests generally detect the same underlying infection, although discordant cases can occur. Similar strong associations between PCR and urease-based tests have been reported in comparative diagnostic studies where both methods are applied to biopsy specimens.[5,13] When diagnostic performance was evaluated using RUT as the comparator method, *ureA* nested PCR showed 97.70% sensitivity, 72.09% specificity, 87.63% positive predictive value, 93.94% negative predictive value, and 89.23% overall accuracy. These findings indicate that PCR has excellent ability to detect infection when present, while the lower specificity may reflect limitations of the comparator method rather than true PCR false positivity. Patel et al noted that no single diagnostic test can serve as a perfect gold standard for *H. pylori*, and discrepancies between methods are expected because each test detects different biological characteristics such as urease activity, bacterial DNA, or histological presence.[11] Therefore, PCR-positive but RUT-negative results may represent true infections that are not detected by urease testing due to low bacterial density or uneven mucosal distribution. Taken together, the findings of this study demonstrate that *ureA* Nested PCR is a highly sensitive diagnostic method for detecting *H. pylori* in gastric biopsy specimens and can identify additional infections among patients with negative urease tests. Incorporating molecular detection alongside conventional invasive methods may therefore improve diagnostic accuracy in tertiary-care endoscopy settings, particularly in regions with high prevalence of *H. pylori* infection.

Limitations

The study was single-center with purposive sampling and antral-only biopsies, these factors may limit generalizability and may not fully capture patchy gastric colonization patterns.

CONCLUSION

In this tertiary-care Bangladeshi dyspeptic cohort (n=130), *H. pylori* DNA was detected by ureA nested PCR in 74.6% of gastric antral biopsies, while RUT was positive in 66.9%. A key operational finding was that 27.9% of RUT-negative biopsies were PCR positive, indicating that reliance on urease-based testing alone may miss a clinically meaningful subset of infections. Using RUT as the comparator, ureA nested PCR demonstrated high sensitivity (97.70%) and accuracy (89.23%), supporting its utility as an adjunct biopsy-based diagnostic tool, particularly when RUT is negative despite ongoing clinical suspicion.

Recommendations

Nested ureA PCR should be considered as an adjunct test in tertiary endoscopy pathways for dyspeptic patients, particularly for RUT-negative biopsy specimens where missed infection may contribute to under-recognition and delayed targeted therapy. Where feasible, future diagnostic workflows should incorporate a composite reference standard, for example histopathology with special stains plus at least one additional test, to better characterize discordant results and refine specificity estimates. Multicentered studies with standardized multi-site gastric sampling would further strengthen external validity and clarify the incremental yield of nested PCR across endoscopic phenotypes.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Miftahussurur M, Yamaoka Y. Diagnostic Methods of *Helicobacter pylori* Infection for Epidemiological Studies: Critical Importance of Indirect Test Validation. BioMed Res Int. 2016;2016(1):4819423.
2. Costa LCMC, das Graças Carvalho M, La Guárdia Custódio Pereira AC, Teixeira Neto RG, Andrade Figueiredo LC, Barros-Pinheiro M. Diagnostic Methods for *Helicobacter pylori*. Med Princ Pract. 2024;33(3):173-84.
3. Bogiel T, Mikucka A, Szaflarska-Popławska A, Grzanka D. Usefulness of Molecular Methods for *Helicobacter pylori* Detection in Pediatric Patients and Their Correlation with Histopathological Sydney Classification. Int J Mol Sci. 2023;24(1):179.
4. Fernandez-Caso B, Miqueleiz A, Valdez VB, Alarcón T. Are molecular methods helpful for the diagnosis of *Helicobacter pylori* infection and for the prediction of its antimicrobial resistance? Front Microbiol. 2022;13:962063.
5. Bai W, Xia P. 'Diagnosis of Helicobacter pylori infection and recent advances', Diagnostics, 2021;11(8):1305.
6. Liu H, Rahman A, Semino-Mora C, Doi SQ, Dubois A. Specific and Sensitive Detection of *H. pylori* in Biological Specimens by Real-Time RT-PCR and In Situ Hybridization. PLOS one. 2008;3(7):e2689.
7. Elnosh M, Altayb H, Hamedelnil Y, Elshareef W, Abugrain A, Osman E, et al. Comparison of invasive histological and molecular methods in the diagnosis of *Helicobacter pylori* from gastric biopsies of Sudanese patients: a cross-sectional study. F1000Research. 2022;11:123.
8. Abdulrahman AT, Ismail Shna I, Hussain SS, Ahmed NJ, Hassan AN. Detection of *Helicobacter pylori's* Virulence Gene (UreA) and its Influence on the Result of Rapid Urease Test (RUT). Al-Mustansiriyah J Sci. 2022;33(4):42-8.
9. Aftab H, Yamaoka Y, Ahmed F, Khan AA, Subsomwong P, Miftahussurur M, et al. Validation of diagnostic tests and epidemiology of *Helicobacter pylori* infection in Bangladesh. J Infect Dev Ctries. 2018;12(5):305-12.
10. Szymczak A, Ferenc S, Majewska J, Miernikiewicz P, Gnus J, Witkiewicz W, et al. Application of 16S rRNA gene sequencing in Helicobacter pylori detection. PeerJ, 2020;8:e9099.
11. Patel SK, Pratap CB, Jain AK, Gulati AK, Nath G. Diagnosis of *Helicobacter pylori*: What should be the gold standard? World J Gastroenterol WJG. 2014;20(36):12847-59.
12. Vinette KM, Gibney KM, Proujansky R, Fawcett PT. Comparison of PCR and clinical laboratory tests for diagnosing *H. pylori* infection in pediatric patients. BMC Microbiol. 2004;4:5.
13. Kismat S, Tanni NN, Akhtar R, Roy CK, Rahman MM, Molla MdMA, et al. Diagnosis and Comparison of Three Invasive Detection Methods for *Helicobacter pylori* Infection. Microbiol Insights. 2022;15:11786361221133947.

Cite this article as: Paul S, Hossain MM, Sultana A, Barua B, Sultana A, Nasrin M, et al. Molecular detection of Helicobacter pylori in rapid urease test-negative gastric biopsy samples. Int J Res Med Sci 2026;14:5023-8.