Original Article
DOI: https://doi.org/10.70749/ijbr.v4i2.2911

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://ijbr.com.pk
ISSN: 2960-2793/ 2960-2807

Diagnostic Accuracy of Stool Antigen and Serological Tests for *Helicobacter pylori* Infection in Symptomatic Patients at a Tertiary Care Hospital in Islamabad

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ARTICLE INFO

Keywords: *Helicobacter pylori*, Immunochromatographic Test (ICT), Blood Antibody Test, Stool Antigen Test, Gastrointestinal Infections, Diagnostics Accuracy, Resource-Limited Settings.

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Declaration
Authors' Contribution
All authors equally contributed to the study and approved the final manuscript.
*Detail is given at the end.

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History
Received: 03-12-2025 Revised: 09-02-2026
Accepted: 21-02-2026 Published: 28-02-2026

ABSTRACT

Introduction: *Helicobacter pylori* is a gram-negative bacterium linked to chronic gastritis, peptic ulcer disease, and gastric cancer, with a high burden in developing countries. In Pakistan, up to 70% of the population is affected due to poor sanitation, contaminated water, overcrowding, and low socioeconomic conditions. Both invasive and non-invasive methods are used to detect *H. pylori*, but invasive tests are costly and impractical, making non-invasive tests preferable for routine diagnosis. Blood antibody tests are easy to perform but cannot distinguish past from active infection, limiting their reliability. Stool antigen testing is a reliable non-invasive method for detecting active *H. pylori* infection and is suitable for resource-limited settings. **Objectives:** This study aimed to compare the diagnostic effectiveness of blood antibody testing and stool antigen testing for the detection of *Helicobacter pylori* infection among patients presenting with gastrointestinal symptoms., to determine the prevalence of *Helicobacter pylori* infection in relation to age and gender among the study population and to evaluate the suitability of stool antigen testing as a reliable method for diagnosing active *Helicobacter pylori* infection in routine clinical practice. **Methodology:** A comparative prospective study was conducted among 80 patients presenting gastrointestinal symptoms at Al-Nafees Medical College and Hospital, Islamabad, with the Serology Department of Nayab Labs & Diagnostic Center - Blue Area, Islamabad from September 2025 to December 2025. Participants were selected using non-probability convenience sampling based on the inclusion and exclusion criteria of this study. Blood and stool samples were collected and tested using commercially available ICT kits. Data were analyzed using SPSS version 27.0 and the McNemar test was applied to compare diagnostic test results of both blood and stool tests with a statistical significance set at $p < 0.05$. The study adhered to the ethical standards and informed consent was obtained from all participants. **Results:** The stool antigen test was positive in 68 patients (85%), and the blood antibody test was positive in 59 cases (73.75%) with a statistical significance of $p < 0.03$. The greater positivity rate on stool antigen testing points to the fact that the test has a better capability to identify active infection. It was found that the prevalence of infection was higher among the male participants than it was among the female participants and the age group of between 26-50 years reported the highest number of positive cases. A statistically significant difference was noted between the two diagnostic methods, favoring stool antigen testing. **Conclusion:** In conclusion, stool antigen testing was more effective than blood antibody testing for identifying of active *H. pylori* infection. According to our results, stool antigen testing should be preferred for routine diagnosis and as a clinical decision-making tool in symptomatic patients, especially in resource-limited settings.

INTRODUCTION

Helicobacter pylori is a gram-negative, spiral-shaped bacterium that resides in the mucus layer of the human stomach. It uses urease to protect itself from the acidic environment, neutralizes local acids, and breaks down urea into ammonia.[1] The bacterium adheres to gastric epithelial cells and uses several virulence factors, most notably the *cytotoxin-associated gene A* (*CagA*) and the *vacuolating cytotoxin* (*VacA*), to damage the mucosa and modify the host immune response.[2] These interactions produce chronic inflammation that can last for years if untreated. Basic laboratory detection methods include invasive tests (gastric biopsy with histology, rapid urease test, culture) and non-invasive tests (urea breath test,

stool antigen, serology), each with advantages and limits depending on clinical context.[3]

H. pylori affects a large part of the global population, with 40–45% prevalence in adults, especially in low-income countries. It is often acquired in childhood and can lead to peptic ulcers and gastric cancer.[4] The incidence of pylori in Pakistan and its neighboring countries is significantly greater compared to developed countries, with some groups having estimates that exceed 50%.[5]

Helicobacter pylori causes illness by surviving in the acidic stomach environment. It uses urease to neutralize acid, adheres to the stomach lining, and employs toxins *CagA* and *VacA* to disrupt cell function and trigger inflammation.[6] It can also affect the duodenum. The bacteria's location influences disease severity, with antral colonization linked to duodenal ulcers and corpus dominance associated with an increased risk of gastric cancer.[7] If untreated, *H. pylori* infection can lead to serious issues like peptic ulcers, chronic gastritis, stomach atrophy, intestinal metaplasia, and stomach cancer. It may also be linked to lymphoma and other health problems.[6]

H. pylori often causes chronic gastritis, but most infected people have no symptoms. When symptoms occur, they may include nausea, heartburn, abdominal pain, bloating, early fullness, or bleeding.[8] Around 10–15% of people may develop an ulcer in their lifetime. If an infection lasts for a long time, it can raise the chances of serious problems, such as stomach cancer and lymphoma. Getting help quickly is key, and finding the problem early is also crucial.[9]

Antibiotic resistance, which is the bacteria's waning response to the medications used to kill them, is one of the main obstacles in treating *H. pylori*. Antibiotic resistance makes it hard to treat *H. pylori*. It leads to the need for stronger medications and raises treatment failure risks.[10]Recent research indicates that resistance is growing all over. For instance, one worldwide review revealed that in several nations, resistance to metronidazole and clarithromycin is exceptionally high, often exceeding the limits where conventional triple therapy becomes ineffective.[11]An investigation in China found *H. pylori* resistance rates of almost 38% to clarithromycin (CLR), 44% to levofloxacin (LVX), and 13.7% to furazolidone (FR) and smaller (~8%) for amoxicillin (AMX) in Asia.[12] Among patients who had failed earlier treatment, yet another Malaysian study found very high secondary resistance: roughly 82% of isolates resistant to metronidazole, ~72.5% to clarithromycin, ~52.9% to levofloxacin.[13]A recent investigation of cultured isolates from Pakistan revealed remarkably high resistance to metronidazole (approximately 97.8%), moderate resistance to ofloxacin (roughly 30.1%), levofloxacin (approximately 16.2%), and low resistance to clarithromycin (approximately 5.4%), tetracycline (about 4.3%), and amoxicillin (around 2.2%). It also claimed that around 40% of isolates were resistant to several antibiotics.[14]

Treatment of *H. pylori* entails taking into account cost, patient adherence, and local antibiotic resistance. Recent research indicates that usual triple therapy is less effective due to increasing resistance. One-week vonoprazan treatment yielded comparable results but better patient tolerance in a Pakistan trial.[15] Areas with great clarithromycin resistance also suggest bismuth-containing quadruple therapy, adding bismuth and a third antibiotic.[16] High-dose dual antibiotic regimens, rifabutin based regimens, or therapies guided by antimicrobial sensitivity testing comprise rescue treatments (for patients who fail first-line therapy).[17]

Who contracts *H. pylori* and how the illness manifests depend on age, gender, and immune status. The infection is usually contained by a strong mucosal immune response, but *H. Pylori* overcomes the body's natural defenses (TLRs, macrophages, dendritic cells), enabling prolonged colonization in certain hosts.[18] Age is important since infection is frequently acquired during childhood and builds with age, resulting in a higher lifetime prevalence in older groups and a lower prevalence in youngsters and adolescents. The trend continues to have high rates in areas with high transmission.[19] Gender differences are inconsistent; some large studies report higher rates in men when young but greater prevalence or recurrence in older women, suggesting hormonal or behavioral influences.[20]

Laboratory diagnosis of *H. pylori* infection relies on both invasive and non-invasive approaches. Endoscopic biopsy followed by histopathology, culture, or rapid urease testing is still considered the gold standard, but it is expensive and less practical for large populations.[21] The urea breath test, serology, and stool antigen testing are all examples of less intrusive procedures that are more useful.[22] Stool antigen tests are widely used in routine labs due to their high accuracy, low cost, and ability to detect active infection, while serological assays are limited by their inability to distinguish past from current infection.[23]

Though highly common in Pakistan and directly related to gastrointestinal disorders, uncertainty still exists about the most appropriate diagnostic technique in local clinical practice. Despite being costly, uncomfortable, and not easily available, invasive methods continue to be the standard of reference.[24] While non-invasive methods are more affordable and easier on patients, their accuracy can vary based on the diagnostic tools used and the surrounding conditions.[25] Healthcare professionals struggle to identify appropriate assessments for routine application due to a lack of clear consensus on the most economical diagnostic methods.[26] This knowledge gap supports a comparative assessment of stool antigen tests with blood (serological) for the detection of *H. pylori* among patients with gastrointestinal symptoms in Pakistan.[27]

This study will examine how accurate blood-based serological tests and stool antigen tests are for detecting *H. pylori* infection. Reliable local data will aid doctors in selecting suitable, non-invasive tests for patients.[28]

H. pylori is frequently found in Pakistan and is linked to a rise in peptic ulcers, gastritis, and stomach cancer.[29] Timely detection through cost-effective testing is essential. This research aims to enhance medical resources and guidelines for identifying and addressing *H. pylori* infections.[30]

Identifying *H. pylori* early improves patient outcomes. Non-invasive assessments lessen the necessity for invasive interventions, resulting in improved adherence.

Timely identification enables swift therapy, minimizing symptoms and the likelihood of cancer.[31]

The purpose of this research stems from the demand for precise, effective, and cost-efficient diagnostic methods for *H. pylori*, particularly in areas with limited resources. Although invasive techniques are dependable, they are impractical for everyday screening; meanwhile, non-invasive assessments demonstrate varying levels of precision based on the specific population and laboratory circumstances. Serological tests using blood frequently mistake previous infections for ongoing diseases, whereas tests that analyze stool samples are more effective at identifying active infections but need local verification. Through the analysis of blood and stool specimens, this research seeks to determine the best diagnostic strategy for individuals presenting gastrointestinal symptoms in our area.

There is a limited local evidence regarding the effectiveness of blood antibody and stool antigen tests for diagnosing Helicobacter pylori infection in patients with symptoms. Blood tests are common but cannot tell if the infection is current or past, and stool tests need more validation. Data on H. pylori infection distribution by age and gender in Islamabad is lacking.

The objective of this study was to compare blood antibody and stool antigen tests for the detection of *H. pylori* infection among patients with gastrointestinal symptoms and to determine and compare the positivity rates and diagnostic performance of blood antibody and stool antigen tests for *Helicobacter pylori* infection, and to assess its distribution by age and gender, while evaluating the suitability of stool antigen testing for diagnosing active infection

MATERIALS AND METHODS

This study was designed as a comparative prospective study aimed at comparing the Blood Antibody Test (BAbT) and Stool Antigen Test (SAgT) in patients presenting gastrointestinal symptoms. The research was conducted over a four-month period, from September 2025 to December 2025 at Al-Nafees Medical College and Hospital, Islamabad, with the Serology Department of Nayab Labs & Diagnostic Center - Blue Area, Islamabad.

Patients of all genders and ages, presenting with gastrointestinal symptoms such as dyspepsia, abdominal pain, bloating, nausea, or heartburn, patients who were willing to provide both their blood and stool samples and patients who provided informed consent to participate in the study were considered eligible. Patients currently on antibiotics, proton pump inhibitors, or bismuth compounds within the last 4 weeks (as these may affect *H. pylori* detection), with a history of gastric surgery and severe systemic illness (liver disease, renal failure, malignancy), and patients unwilling or unable to provide stool or blood samples were excluded from the study.

A sample size of 80 symptomatic patients was collected using non-probability convenience sampling. Both blood and stool samples were collected from each patient for antibody testing and antigen testing and then the positivity rate of *H. pylori* was determined. The diagnostic positivity of *H. pylori* by age and gender was also determined to identify significant patterns across subgroups.

Aseptic techniques were used to draw 5 mL of venous blood from a participant with a sterile syringe. The blood was put in a plain Vacutainer tube to allow natural clotting and stored at room temperature for 20–30 minutes. After centrifugation for 10 minutes at 1500–2000 × g, the clear serum was pipetted into sterile cryovials for analysis. Two drops of serum were placed in the ICT cassette sample well, followed by one drop of buffer. Results were read after 15 minutes. A positive result showed two lines, a negative result showed one line, and no control line meant the test was invalid and needed to be repeated. A stool sample was collected from each participant and stool suspension was prepared. Then two to three drops of the suspension were dropped into the ICT cassette's sample well and started a timer. After 15 minutes, the results were checked. A positive result showed two colored lines, a negative result showed one line, and no lines meant an invalid result, requiring a new test with a different cassette.

The collected data were entered and analyzed using SPSS version 27.0 and Microsoft Excel. Categorical variables were summarized using frequencies and percentages. The McNemar test was used to compare the qualitative results of the blood antibody test and stool antigen test because blood and stool samples were obtained from the same patients, making the data paired in nature. Then test positivity with demographic variables such as age and gender was evaluated. A p-value of less than 0.05 was considered statistically significant. No missing data were observed in this study. Sensitivity and specificity were not calculated due to the lack of a gold standard reference method. The non-invasive nature and simplicity of the diagnostic tests used in this study allowed for convenient analysis and made them suitable for use in settings with limited resources.

RESULTS

The analysis of Helicobacter pylori infection used two methods: the Stool Antigen Test (SAgT) and the Blood Antibody Test (BAbT). Both tests were evaluated to compare their effectiveness in identifying *H. pylori* infections among symptomatic patients. In the Blood Antibody Test, 59 out of 80 patients (73.75%) tested positive for *H. pylori* antibodies, suggesting a high rate of past exposure. However, this test does not confirm an active infection, as it can also reflect old or resolved infection instead of ongoing disease. On the other hand, the Stool Antigen Test showed that 68 out of 80 patients (85%) were positive for *H. pylori*, indicating an ongoing infection since it detects bacterial antigens in stool. This test was found to be more effective in identifying active cases of *H. pylori* infection among patients with gastrointestinal symptoms. A comparative analysis of both tests highlighted significant differences, with SAgT identifying more infections than BAbT. Statistical testing revealed a significant difference between the two methods ($p = 0.003$). The results are shown in Table1 and Graph1.

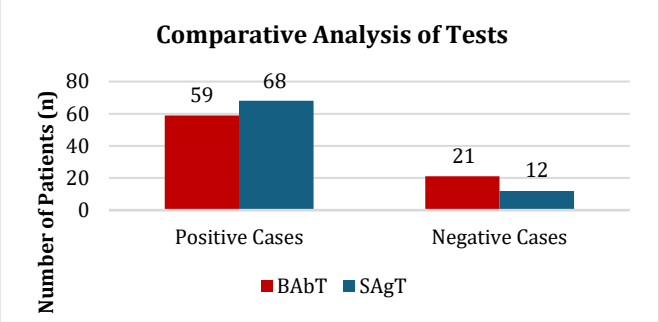
Table 1

Comparison of Diagnostic Positivity between Blood Antibody Test (BAbT) and Stool Antigen Test (SAgT) for the detection of Helicobacter pylori.

Tests	Positive Cases (n=80)	Negative Cases (n=80)
BAbT	59 (73.75%)	21 (26.25%)
SAgT	68 (85%)	12 (15%)

Graph 1

Comparison of Diagnostic Positivity Between Stool Antigen Test (SAgT) and Blood Antibody Test (BAbT) for detection of Helicobacter pylori.



The study involved 80 patients with gastrointestinal complaints, consisting of 49 males (61.25%) and 31 females (38.75%), showing a higher occurrence of these complaints in males. Among those testing positive for *H. pylori*, 39 males and 20 females were identified by the Blood Antibody Test, while the Stool Antigen Test showed 43 males and 25 females positive. Overall, the results indicated that *H. pylori* infection is prevalent in both genders, although more males tested positive in both tests, as detailed in Table 2 and Graph 2.

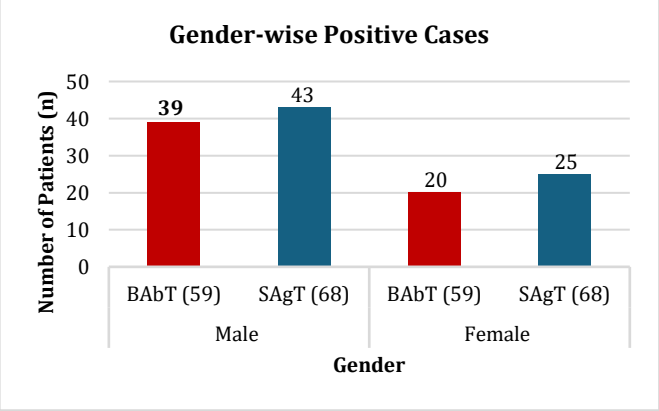
Table 2

Gender-wise Positive Cases among Participants.

Gender	Tests (positive cases)	Positive Cases
Male	BAbT (59)	39 (66.1%)
	SAgT (68)	43 (63.2%)
Female	BAbT (59)	20 (33.8%)
	SAgT (68)	25 (36.7%)

Graph 2

Gender-wise Positive Cases among Participants.



The age distribution of positive cases for *H. pylori* infection showed that under 25 years 16 individuals were positive by BAbT and 19 by SAgT. In the 26-50 age group, there were 32 positive cases by BAbT and 36 by SAgT. For those over 50 years, 11 were positive by BAbT and 13 by SAgT. The highest cases were in the 26-50 age group, indicating that middle-aged adults are more affected as shown in |Table 3 and Graph 3.

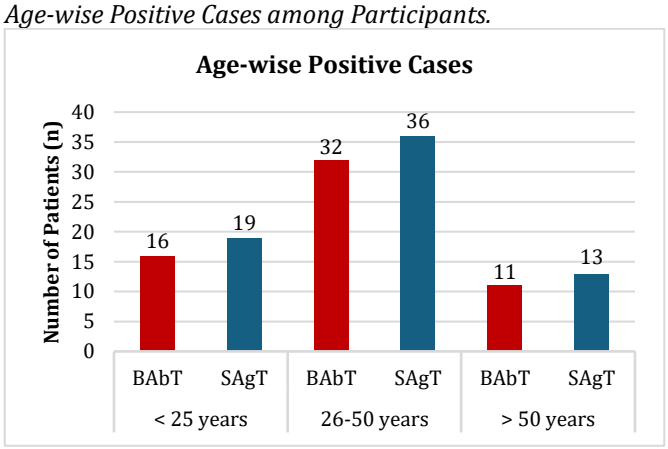
Table 3

Age-wise Positive Cases among Participants.

Age Group	Test type (positive cases)	Positive Cases (n)
< 25 years	BAbT (59)	16 (27.1%)
	SAgT (68)	19 (28%)
26-50 years	BAbT (59)	32 (54.2%)
	SAgT (68)	36 (53%)
> 50 years	BAbT (59)	11 (18.6%)
	SAgT (68)	13 (19.1%)

Graph 3

Age-wise Positive Cases among Participants.



Overall, the findings suggest that stool antigen testing is a more reliable method for diagnosing active *H. pylori* infections, supporting its use in clinical settings, particularly for symptomatic patients.

DISCUSSION

This study was conducted to compare the diagnostic performance of the blood antibody test (*BAbT*) and the stool antigen test (*SAgT*) for detecting *Helicobacter pylori* infection among patients presenting with gastrointestinal symptoms. We were interested in testing the relative performance of two diagnostic procedures in the process of diagnosing *H. pylori*.

Our results showed that the Stool Antigen Test detected 85% positive cases, but the Blood Antibody Test detected 73.75% positive cases. This greater rate of detection using SAgT emphasizes its usefulness in detection of active *H. pylori* infection. Qui et al., 2021 stated that the stool Antigen Test had 91% sensitivity, 97% specificity and 95.7% accuracy which makes it a possible procedure to screen *H. pylori* infection massively.[32]

According to a 2025 study, Stool antigen tests show high levels of diagnostic accuracy. It comes as a non-invasive, less expensive alternative to urea breath tests and can be used with a wide variety of individuals, such as children and the elderly. Results may however be affected by factors like sample handling, antigen stability and bacterial load.[33]

However, unlike the stool antigen testing, the positivity rate of the blood antibody testing was lower in our study (73.75%) than the stool antigen test (85%). The difference can be attributed to the fact that serological testing has been documented to be limited in certain aspects as the IgG antibodies are identified as opposed to

the fact that active infection is present. Serological assays are not dependable indicators of current infection despite the possibility of the antibodies remaining for months or even years after exposure or effective eradication. As a result, the blood antibody test will not detect the active cases especially in patients who have been recently infected. We therefore indicate that blood antibody testing is not as sensitive to identify active infection in *H. pylori* as stool antigen testing making it appropriate to use stool antigen tests to identify *H. pylori* infection accurately in symptomatic patients.

Our results are also similar to the ones obtained by Zeeshan et al., 2024 who did a comparative analysis on *Helicobacter pylori* diagnostic tests within the tertiary care hospital environment in Mardan. The stool antigen test used in their study revealed a greater rate of active *H. pylori* infection detection than the serological test. Authors emphasized the fact that stool antigen assays proved to be more efficient in the diagnosis of persistent infection in symptomatic patients, whereas serological tests were not very effective in diagnosis because of the failure to distinguish between previous exposure and the current infection. The data obtained from these observations is quite similar to our findings, as stool antigen testing revealed a larger percentage of positive cases as compared to blood antibody testing, which supports the validity of stool-based diagnostics in the regular clinical setting.[34]

Likewise, the cross-sectional research of Ali et al. 2025 was conducted to determine the testing of antibody and antigen as a method of diagnosis of *H. pylori* infection in the symptomatic patients of District Mardan. Their results showed that stool antigen testing was more useful in the diagnosis of active infection, whereas blood antibody testing had lower diagnostic efficacy. The authors have observed that antibody-based tests could be misleading because of differences in immune response together with the presence of antibodies even after prior exposure or treatment. These findings are upheld by our study because we found a reduced rate of positivity with blood antibody testing than with stool antigen testing, and this finding indicates the inefficacy of serology in the accurate diagnosis of active *H. pylori* infection.[35]

Moreover, Khorsheed SA performed a comparison study to evaluate the diagnostic accuracy of serological and stool antigen tests to detect *H. pylori* infection and demonstrated similar results that matched ours. The research found that stool antigen testing had a greater potential in identifying active infection, compared to serological testing which had lower sensitivity and probability of misclassification because of antibody persistence. Khorsheed also stressed that pure serology may be the cause of underdiagnosis or overdiagnosis, based on existing exposure and immune response. Concurring with such findings, our findings confirm that the stool antigen test is more clinically accurate than the blood antibody test, which justifies its use as a method of choice as a non-invasive diagnostic tool to diagnose *H. pylori* infection in symptomatic patients.[36]

The analysis of demographic data revealed a predominance of male participants, with 61.25% being male and 38.75% female. Our study is in line with the findings of a study carried out at the Mayo Hospital in Lahore, which demonstrated the high prevalence of *H. pylori* infection in males (61.7%) compared to females (38.3%), as per the existing risk factors of this behavior and lifestyle within the Pakistani population. Males are more likely to eat out and consume street food that is cooked in poor conditions, exposing them to *H. pylori* infection due to contaminated water and utensils. The irritation of the gastric lining is also caused by increased prevalence of smoking, high levels of stress, irregularity in the schedule of meals, and frequent intake of spices and strong tea in men, which increases the ease with which the bacteria can colonize. Moreover, men will usually delay medical care even in the event of persistent gastrointestinal problems and this will lead to these infections becoming chronic and more evident upon testing.[37]

The prevalence of males (BAbT:66%, SAgT:63%) in our study population was higher, which is supported by the research conducted by Qiao et al. (2024), who also indicated that there is a significant gender difference in *Helicobacter pylori* prevalence and the risk factors. Their research indicated that men were more likely to be infected with *H. pylori* because of the following factors, lifestyle habits, occupational exposure, and behavioral differences, namely, smoking and dieting habits. These findings give a potential reason behind why the male gender has been more prevalent in our study and show the need to look into gender related factors when explaining *H. pylori* infection trends.[38]

Besides this, the prevalence of *H. pylori* infection in the sample of symptomatic adults is similar to the regional data in the study by Shrestha et al. (2023). Their results indicated that the *H. pylori* infection is still very widespread in the region with adults presenting with gastrointestinal symptoms, which highlights the persistence of the infection on public health. This regional consistency promotes the applicability of our results and the importance of proper diagnostic methods, including stool antigen testing, to detect *H. pylori* infection and manage it in symptomatic groups with appropriate diagnostic methods and observations.[39]

The distribution in the study according to age revealed that gastrointestinal complaints were more likely to be reported by middle-aged people, those aged between 25 and 50 years more specifically. In the study of Ali and colleagues (2025), the highest cases of *H. pylori* infection were observed in people who were between the ages of 26-50 (Blood test: 32, Stool test: 36) compared with other younger and older groups. This may be attributed to increased exposure to risk factors during the middle-aged life such as contaminated water and food, and lifestyle changes. Besides, the lack of immunity to infection may be inhibited by stress and an unbalanced diet. The youngsters are less exposed, and the elderly may have developed immunity hence the corresponding change in patients in the middle age in Pakistan.[35]

Immunochromatographic tests (ICTs) are helpful in low-resource settings because they are quick and inexpensive. However, they have some limitations. These tests usually have lower accuracy than methods like the urea breath test (UBT) or polymerase chain reaction (PCR).[40] The study lacked a gold standard reference test,

making it hard to measure sensitivity and specificity. Small sample size and convenience sampling may limit the findings' relevance to other groups. Diagnostic errors could occur, especially in patients with similar symptoms.

The study presents strengths despite its limitations, including a direct comparison of two non-invasive diagnostic tests in symptomatic patients. It maintains internal consistency through standardized procedures and simultaneous sample collection. The findings provide valuable local data for clinical decision-making and health policies in resource-limited settings.

The study had a small sample size, limiting the results' applicability to the larger population. It only evaluated two diagnostic tests and was conducted in two laboratories in one city, potentially missing regional variations. Factors like diet and cleanliness were not examined.

Future recommendations include studying larger and more diverse samples, using additional diagnostic techniques, assessing the impact of recent medication on false negatives, and investigating patient lifestyle and dietary patterns for preventable risks.

CONCLUSION

Our study shows that the Stool Antigen Test is more effective than the Blood Antibody Test in detecting active *Helicobacter pylori* infection among patients presenting gastrointestinal symptoms, as it identified a higher number of positive cases. Although the Blood Antibody Test was useful in indicating exposure to *H. pylori*, its ability to differentiate between past and current infections limits its reliability for diagnosing active disease. Based on our results, we conclude that stool antigen testing should be preferred in routine clinical practice for accurate diagnosis of active *H. pylori* infection, particularly in symptomatic patients.

Author Contributions

Rumeela Iqbal conceptualized the study, collected samples, performed the laboratory work, analyzed the data, and wrote the original manuscript. *Muhammad Ahmad* was involved in gathering samples, performing lab tests, entering data, and reviewing related literature. *Muhammad Bilal* helped with manuscript revision. *Dr. Shahzeera Begum* supervised the study and provided a critical evaluation of the manuscript. Every author reviewed and consented to the final version of the manuscript.

Acknowledgment

The writers wish to express their gratitude to the Department of Medical Laboratory Technology at Al-Nafees Medical College and Hospital, ISRA University Islamabad Campus, for its institutional support. We also extend our heartfelt thanks to Nayab Labs & Diagnostic Center Blue Area, Islamabad, for their support with laboratory resources, technical help, and cooperation throughout the laboratory testing process. The authors are grateful to all participants who voluntarily participated in this research.

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