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Molecular and Serological Detection of *Helicobacter Pylori* Bacteria in Patients with Gastrointestinal Tract Infections

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***Abstract*—Helicobacter pylori is closely associated with gastrointestinal diseases, which are among the most common health problems worldwide. This study aimed to detect H. pylori using serological and molecular diagnostic methods to enhance the accuracy and reliability of testing. Samples were collected from the Digestive System Center/Imam Hussein Teaching Hospital in Thi Qar between October 2024 and March 2025. These included 115 patients of both sexes and ages with gastrointestinal diseases. Serological and molecular tests were performed to detect H. pylori. 45 stool samples were collected; antigen testing was performed, and 20 samples (44.4%) were positive. These samples were then molecularly diagnosed by polymerase chain reaction (PCR), with the following results: H. pylori infection was diagnosed using PCR targeting 16sRNA in 3 out of 20 stool samples (15%). The diagnosis was also confirmed by detecting the virulence factors VacAs1/s2 and CagA, with VacAs1/s2 detected in 3(100%) and CagA in 2(66.66%) patients with gastrointestinal disease. Seventy blood samples were collected, and the H. pylori detection rate using antibody testing was 31(44.28%). The results of this study demonstrated that combining serological and molecular diagnostic methods improves the accuracy of H. pylori detection in patients.**

***Keywords*—Helicobacter pylori, PCR, *VacA*, *CagA*, Serological tests, Gastrointestinal tract infection.**

I. INTRODUCTION

Helicobacter pylori is a curved, microaerobic, Gram-negative bacterium that is extremely picky and requires sophisticated growth conditions, such as blood or serum, as an additional source of nutrition [1]. *H. Pylori* is a motile, oxidase and catalase-positive bacterium that also produces a protease and a potent urease that alters stomach mucus and decreases the ability of acid to cross the mucous membrane [2]. *Helicobacter pylori* colonisation in the human stomach seems to have a key role in the development of a number of gastroduodenal malignancies, including gastric cancer (GC), low-grade B-cell mucosa-associated lymphoid tissue (MALT) gastric lymphoma, and non-cardia gastric carcinoma [3].

H. pylori infection has also been linked to conditions that are not related to the gastrointestinal tract. Shared risk factors like poverty and inadequate nutrition are likely the cause of an observed connection with coronary artery disease. Iron-deficiency anemia that cannot be explained [4]. For many gastroduodenal disorders to be effectively managed, an accurate diagnosis of *Helicobacter pylori* (*H. pylori*) infection is required. These infections can currently be detected using a variety of diagnostic techniques; the clinical condition, cost-effectiveness, accessibility, benefits, and drawbacks should all be considered when selecting a procedure [5].

Diagnostic tests are typically classified as either noninvasive or invasive. Molecular techniques, fast urease testing, histology, endoscopic imaging, and culture are examples of invasive diagnostic procedures. The urea breath test, stool antigen test, molecular analysis, and serological testing are among the non-invasive diagnostic procedures [6]. Serum *Helicobacter pylori* (*H. pylori*) IgG antibody tests and the stool antigen test (SAT) are both very useful for clinically diagnosing *H. pylori* infections and differentiating between acute and chronic infections [7]. One of the main drawbacks of the very cheap serology for *H. pylori* IgG detection is that it cannot distinguish between an ongoing infection and one that has already been resolved [8]. The stool antigen test (SAT) is another noninvasive technique for diagnosing *H. pylori* infection, with a specificity of 94% and 97%, respectively, according to a global meta-analysis [9]. PCR detection of virulence factors provides additional information to comprehend the clinical differences between patients infected with different strains of *H. pylori*. It aids in assessing the genetic variation within virulence factors of *H. pylori*. Numerous studies have demonstrated that the presence of virulence factors, such as the *VacA* gene and *CagA*, is linked to a higher incidence of gastric cancer and peptic ulcer disease as well as more severe stomach inflammation [10,11]. PCR is a quick and accurate method for detecting *H. pylori* in feces, and it is particularly appealing to kids as a noninvasive test. Moreover, stool PCR has the benefit of detecting particular genotypes and antibiotic resistance in the microbe [12-13].

This study aimed to detect *H. pylori* using serological and molecular diagnostic methods to enhance the accuracy and reliability of testing.

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II. Materials and Methods

A. Ethical Considerations

Consent was obtained from patients participating in this study at Imam Al-Hussein Teaching Hospital, No. 17443, Date 20/10/2024

B. Samples Collection

Samples were collected from the Digestive System Center/Imam Al-Hussein Teaching Hospital in Thi Qar during the period from October 2024 to March 2025, about 115 patients with gastrointestinal diseases. Stool samples were placed in a sterile container and stored in a cool box. Antigen testing was then performed in the Bacteria Unit Laboratory/ Imam Al-Hussein Teaching Hospital. 45 stool samples were collected after antigen testing and 20 samples were positive for the test. 20 stool samples were stored in phosphate buffer solution and frozen until diagnosis by polymerase chain reaction (PCR), 70 blood samples were collected and stored in gel tubes, and then an antibody test was performed.

c. Stool Antigen Test

A non-invasive diagnostic method for the qualitative detection of *Helicobacter pylori*. After collecting stool samples, a small amount of stool is taken from multiple locations within the sample using a wooden stick. The sample is placed in a container containing a buffer solution, and the components are mixed. A specific amount is then drawn up using a pipette and added to the cassette opening until it is full. The results are then waited ten minutes. If a single line appears, the result is negative, while if two lines appear, the result is positive. According to the manufacturer's instructions OnSite Ag test kit, Hannover /Germany.

d. Detection of H. pylori by Molecular Methods

DNA extraction: DNA was extracted using the MagicPure Stool and Soil Genomic DNA Kit. Extraction was performed according to the manufacturer's instructions. DNA quantity and quality were measured by a NanoDrop device (TransGen, China) [14].

e. Amplification Reaction

The PCR master mix reaction was prepared following the manufacturer's instructions provided by TransGen Biotech Inc. (China). The total volume of the reaction was 50 µL. The primer was designed based on the references in Table 1.

Table (1): Prime sets used in this study with their references.

Gene name	Primer sequence (5'–3)'	Annealing temperature (°C)	Length of an amplified segment (bp)	Reference
16SrRNA H. pylori gene	F CTGGAGAGACTAAGCCCTCC R ATTACTGACGCTGATTGTGC	57°C	110 bp	[15]
cagA gene	F ACCGCTCGAGAACCCTAGTCGGT AATGGG R CAGGTACCGCGGCCGCTTAAGAT TTTTGGAAACCAC	65°C	981 bp	[16]
vacAs1/S2 gene	F ATGGAAATACAACAAACACAC R CTGCTTGAATGCGCCAAAC	56°C	259/286bp	[16]

f. Electrophoresis

The PCR products from various genes were analyzed using agarose gel electrophoresis. The procedure involved the following steps:

Preparation of a 1% agarose gel: Agarose was dissolved in 1X TBE buffer, heated in a water bath at 100°C for 15 minutes, and then cooled to 50–60°C [17]. Addition of ethidium bromide dye: 2µL of ethidium bromide dye was added to the agarose gel solution.

Gel casting: The agarose gel solution was poured into a casting tray, ensuring proper comb placement, and left to solidify at room temperature for fifteen minutes. Subsequently, each well received 10 µL of PCR product and 2 µL of a 1500 bp ladder after carefully removing the comb.

Electrophoresis: The gel tray was placed in an electrophoresis chamber filled with 1X TBE buffer. Electrophoresis was conducted at 110 volts for the first 30 minutes and then at 75 volts for an additional 60 minutes. Through the use of a UV transilluminator, the PCR products were detected.

g. PCR Master Mix Reaction Preparation

The PCR master mix reaction was prepared following the manufacturer's instructions provided by TransGen Biotech Inc., China.

Table (2) PCR master mix contents

Component	Quantities
EasyTaq® DNA Polymerase	500 U×1
10×*EasyTaq*® Buffer and Mgcl2	1.2 ml×1
2.5 mM dNTPs	800 µl×1
6×DNA Loading Buffer	1 ml×1

h . PCR Thermocycling Conditions

PCR thermocycler conditions were set using a conventional PCR system, detailed in the accompanying table. The reaction components are listed in the table below.

Table (3): Reaction components for PCR reaction

Component	50 µL reaction
10 µM Forward Primer	1 µL
10 µM Reverse Primer	1 µL
Template DNA	Variable
Easy Taq 2X Master Mix with Standard Buffer	25 µL
Nuclease-free water	Variable
Total	50 µL

Table (4): PCR amplification program for PCR

Step	**Temperature(˚C)**	**Time**	**No. of cycles cycle**
Initial denaturation	94 ˚**C**	3 min	1
Denaturation	94 ˚**C**	30**sec**	35
Annealing	Variables according to (primer's TM)	30**sec**	
Extension Step	72 ˚**C**	1 min	
Final extension	72 ˚**C**	5 min	1
Hold temperature	4 ˚**C**	∞	-

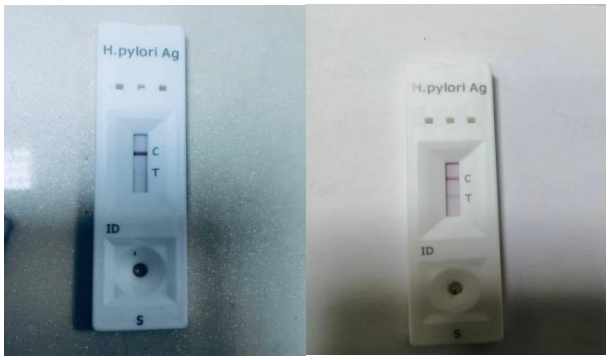
i. Blood Antibody Test

H. pylori Ab Rapid Test is a sandwich lateral flow chromatographic immunoassay for the qualitative detection of antibodies. After collecting blood samples (3-5 mL) and placing them in a gel tube, we expose them to centrifugation to obtain the serum that is added to the antibody test, which is a strip with a hole in which the serum is placed. If this serum contains IgG antibodies formed against bacteria, it reacts with the bacterial antigen molecules that ignore the surface of the hole. If the sample contains antibodies to bacteria, a colored test line will appear, indicating a positive result. However, if the red line is absent in this area, it indicates a negative result, according to the manufacturer's instructions OnSite Ab test kit,Hannover /Germany.

III. RESULTS

A. *Identification of H. Pylori in Stool by Antigen Test*

Antigen test was performed on stool samples. H. pylori was primarily diagnosed using an antigen test (Ag) before molecular diagnosis. About 20/45 (44.44%) as a positive test was revealed for antigen test as in figure 1.



(B)Negative results (A)Positive results

Fig. (1): The results of antibody tests in the serum of patients infected with *H. pylori*

B. *Identification of H. pylori by molecular assay*

The positive samples for the *H. pylori* antigen test were confirmed by PCR as DNA was extracted and amplified from stool samples. Diagnosis was made using 16sRNA, with the following results: 3 out of 20 stool samples were diagnosed as Helicobacter pylori, representing a 15% infection rate. Figure (2)

- Identification of *H. Pylori* Virulence Factors

  The positive samples diagnosed using 16sRNA were used to detect the virulence factors *VacAs1*/*s2* with the following results: 3 out of 20 stool samples were diagnosed as *Helicobacter pylori*, representing a 100% in patients with gastrointestinal tract infections. Figure (2)

  As for the *CagA* gene, the results: 2 out of 20 stool samples were diagnosed as *Helicobacter pylori*, representing a 66.66%. Figure (2)

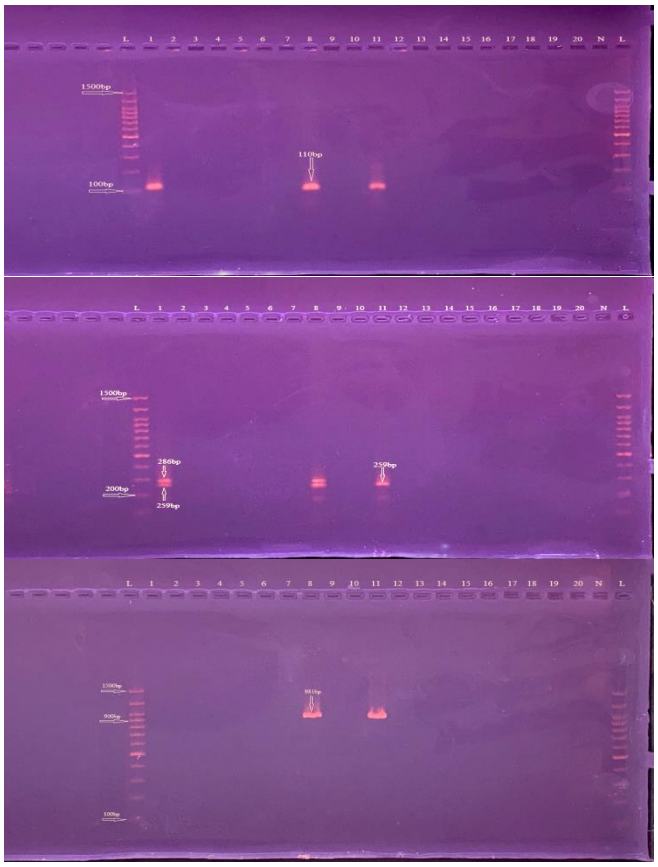
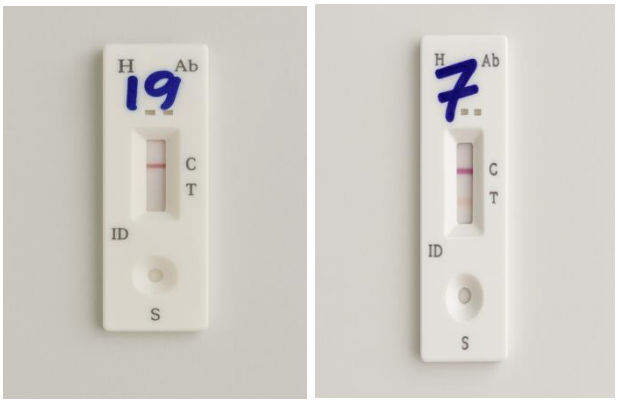


Fig.(2): PCR amplification of 16S rRNA, *VacA*, and *CagA* genes of *H. pylori* showing specific bands on agarose gel electrophoresis.

c. Identification of *H. Pylori* in Blood by Serological Tests

As for serological diagnosis, 70 blood samples were tested from patients with gastrointestinal diseases, and 31 samples were positive for the test, at a rate of 44.28%, as shown in Figure (3)



(A) Negative results (A) Positive results

Fig. (3): The results of antibody tests in the serum of patients infected with *H. pylori*

IV. Discussion

Antigen testing was performed on stool samples prior to diagnosis by PCR, with 20 samples testing Positive, at a rate of 44.44% and the number of negative samples was 25, at a rate of 55.55%. Our results differ from a study conducted by [18]. where the results of positive samples were 26.33%, which is lower than the result we obtained. In a similar study conducted by researchers [19].on patients suffering from gastrointestinal diseases, their results were higher than the results we obtained at a rate of 67%. The samples were then diagnosed with *H. pylori*. After DNA extraction and amplification from stool samples, diagnosis was performed using 16S rRNA, with the following results: 3 of the 20 stool samples were diagnosed as *H. pylori*, at a rate of 15%. In a study by [20]. *H. pylori* was diagnosed based on 16S rRNA (39.2%), a higher rate than the diagnosis in this study. In a similar study conducted by researchers [21], on stool samples, no positive sample was obtained at a rate of 0%, which is a lower rate than the diagnosis in our study. Regarding the *CagA* gene, the study showed a positive result (66.66%), while in the study by [20], the diagnosis rate was 40%, a lower rate than the diagnosis in the current study. Serological diagnosis: 70 blood samples from patients with gastrointestinal disease were tested, and 31 samples tested positive, representing a rate of 44.28%. In a study by [22], 55.6% of patients tested positive, which is close to our results. A study by [23], showed 80% positive test results for gastrointestinal disease. This result disagrees with the current study. The reason for the difference in the diagnosis of *H. Pylori* bacteria may be due to the difference in the immune response from one person to another. One possible explanation for the disparity in the studies' findings is that each person's immune response is unique [24]. The discrepancy in our results compared to previous studies could be attributed to several factors. First, differences in the study population—including immune status, age, and regional exposure—can significantly impact detection rates. Second, variations in diagnostic tools, such as differences in antigen test kits or polymerase chain reaction (PCR) primer sensitivity, affect detection accuracy. In addition, sample quality and timing of collection can affect detection accuracy. The genetic diversity of *H. pylori*, particularly in virulence genes such as *CagA*, may also contribute to the variability of results across regions. Furthermore, host immune responses vary from person to person, especially in immunocompromised individuals, which may explain the variability in serological results. These combined factors help explain the discrepancy between our results and previous studies. One of the advantages of serological testing is that its accuracy is not affected by ulcer bleeding or the use of antibiotics. However, serological testing is not a reliable test for evaluating eradication therapy because antibody levels can persist in the blood for long periods [25].

V. CONCLUSION

Based on the results, we recommend combining serological and molecular diagnostic methods to improve the accuracy of *H. pylori* detection in patients with gastrointestinal tract infections. While serological diagnosis is used for the initial detection of *H. pylori* infection, molecular diagnosis is more sensitive and accurate for confirming active infection.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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