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Prevalence and Molecular Characterization of Helicobacter pylori in Patients with Chronic Diarrhea in Thi-Qar Province

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Abstract. **General Background:** *Helicobacter pylori* is a globally prevalent bacterial pathogen increasingly linked to gastrointestinal and extra-gastric disorders. **Specific Background:** Its role in chronic diarrhea remains insufficiently characterized in Iraq, particularly in Thi-Qar Province, where epidemiological data are inconsistent. **Knowledge Gap:** Limited molecular evidence exists on local *H. pylori* strains and the performance of diagnostic methods in patients with chronic diarrhea. **Aims:** This study aimed to determine the prevalence of *H. pylori* and characterize circulating strains among chronic diarrhea patients using stool antigen testing and PCR targeting the *cagA* gene. **Results:** A high prevalence was detected by antigen testing, while PCR revealed lower positivity, and two local *cagA*-positive isolates were genetically characterized and registered in GenBank. **Novelty:** The study reports the first molecular registration of *H. pylori* strains from chronic diarrhea cases in Thi-Qar Province. **Implications:** These findings underscore diagnostic variability and highlight the relevance of local molecular data for epidemiological surveillance and future clinical research.

Keywords: Helicobacter pylori, chronic diarrhea, stool antigen test, PCR detection, molecular characterization

Highlights:

1. Rapid stool antigen testing revealed high Helicobacter pylori detection among chronic diarrhea patients.
2. PCR targeting cagA gene showed substantially lower positivity, indicating diagnostic sensitivity variation.
3. Genetic sequencing identified distinct local strains registered in international genomic databases.

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Introduction

One such bacteria that has colonized humans is Helicobacter pylori, which is spiral-shaped, Gram-negative, and microaerophilic. H. pylori have been acknowledged as one of the most common chronic bacterial infections in humans since its effective discovery and culture by Barry Marshall and Robin Warren in the early 1980s. It is estimated that half of the world's population is affected by this infection [1, 2]. Factors including overcrowding and insufficient sanitation contribute to its disproportionately high incidence in underdeveloped nations [3]. Pathogenicity of Helicobacter pylori is mostly due to its extraordinary capacity to live in the very acidic stomach environment. The bacteria do this mainly by producing an abundance of the enzyme urease, which neutralizes the microclimate around it by hydrolyzing urea to ammonia and carbon dioxide [4]. Because of its spiral structure and many flagella, this bacterium has a high motility, which allows it to stick to the stomach epithelial cells and penetrate the thick gastric mucus layer [5]. Although most infected people do not experience symptoms, colonization may create a chronic active gastritis. This gastritis can then lead to significant gastroduodenal disorders in certain cases [6].

H. pylori is a major clinical concern. Based on research showing its presence in more than 90% of duodenal ulcers and 70-80% of gastric ulcers, it is the main culprit behind chronic gastritis and plays a role in the development of peptic ulcer disease [7]. In addition, the World Health Organization has categorized H. pylori infection as a Group I carcinogen because of the substantial causative connection between the infection and stomach adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [8]. In addition to these well-established functions, new research points to a link between H. pylori infection and non-gastric issues, such as children's development retardation and persistent diarrhea, which may be caused by changes in the immune system's reaction to the infection [9].

A big clinical worry is H. pylori. Studies have shown that it is present in 70-80% of stomach ulcers and over 90% of duodenal ulcers, making it the primary cause of chronic gastritis and a risk factor for peptic ulcer disease [7]. Furthermore, due to the strong causal relationship between H. pylori infection and gastric mucosa-associated lymphoid tissue (MALT) lymphoma, the infection has been classified as a Group I carcinogen by the World Health Organization [8]. Along with these well-established roles, recent studies have linked H. pylori infection to non-gastric problems, such growth retardation in children and chronic diarrhea. This might be due to alterations in the immune system's response to the infection [9].

Past research has shown conflicting prevalence percentages of Helicobacter pylori in Iraq, and more especially in the Thi-Qar area. One research indicated a prevalence of 62.86% in individuals with stomach ulcers [12], whereas another study identified a rate of 38.9% [13] using real-time PCR. Molecular characterisation of the local strains in circulation, however, has to be more thorough. Thus, the purpose of this research is to make a contribution to regional H. pylori knowledge by examining:

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- Examining the frequency of Helicobacter pylori in Thi-Qar province's chronic diarrhoea patients by means of a stool antigen test and polymerase chain reaction.
- Using DNA sequencing and cagA gene targeting to characterize local H. pylori isolates.

Materials and Method

Study Design and Setting

A cross-sectional study was carried out between January and May 2024. Stool samples were gathered from individuals experiencing chronic diarrhea, characterized by loose stools persisting for over 4 weeks, who visited prominent public hospitals in Thi-Qar province, Iraq, such as Al-Hussain Teaching Hospital, Bent Al-Huda Hospital, and Al-Musawi Hospital.

Study Population

There was a total of 500 participants that were recruited in the trial who suffered from chronic diarrhea. One month to 58 years old was the age range of those who took part in the study. During the process of collecting samples, a questionnaire was used to record demographic information, such as the respondent's age, gender, and habitat (whether it was urban or rural).

Sample Collection and Microscopic Examination

Sterile containers were used to collect fresh stool samples. Routine parasitological inspection was conducted on a part of each sample right away using the formalin-ether concentration procedure, direct wet mount with normal saline (0.9%) and Lugol's iodine. These procedures were conducted as part of the first patient screening; however, their primary focus is intestinal parasites. We also saw consistency in the stool.

Detection of H. pylori by Stool Antigen Test

Following the instructions provided by the manufacturer, a fast immunochromatographic test kit (CTM, UK) was used to detect the presence of H. pylori antigens in all stool samples. To summarize, using the supplied sample diluent, a little amount of feces was emulsified. To the well of the test cassette, four drops of the diluted sample were then inserted. When the test and control lines appeared after 4 minutes, it meant the result was positive; when just the control line appeared, it meant the result was negative.

Molecular Detection of H. pylori by PCR

In order to further investigate the presence of H. pylori, a subset of 96 samples that had been microscopically confirmed to be positive for intestinal parasites underwent polymerase chain reaction (PCR) testing.

DNA Extraction

The PrestoTM feces DNA Extraction Kit (Geneaid, Taiwan) was used to extract genomic DNA from 210 milligrams of feces, and the procedure that was described by the manufacturer was followed. An instrument called a Nanodrop spectrophotometer (THERMO, USA) was used in order to determine the concentration and purity of the DNA that was extracted.

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PCR Amplification:

The cagA gene, also known as the cytotoxin-associated gene A, was the focus of the amplification. The Taq DNA polymerase, dNTPs, MgCl2, and reaction buffer were included in the AccuPower® PCR PreMix Kit (Bioneer, Korea) that was used in the preparation of the PCR master mix. There were 5 microliters of DNA template and particular primers (Bioneer, Korea) included in the reaction mixture. The primers had the following sequences:

- Forward: 5'-ATAATGCTAAATTAGACAACTTGAGCGA-3'
- Reverse: 5'-TTAGAATAATCAACAAACATCACGCCAT-3'

The cagA gene, which is another name for cytotoxin-associated gene A, was amplified. The PCR master mix was prepared using the AccuPower® PCR PreMix Kit (Bioneer, Korea), which includes the following ingredients: Taq DNA polymerase, deoxyribonucleotide triphosphates (dNTPs), magnesium chloride (MgCl2), and reaction buffer. The reaction mixture included specific primers (Bioneer, Korea) and 5 microliters of DNA template. The primers' sequences were as follows.

Gel Electrophoresis

Electrophoresis on a 1% agarose gel stained with ethidium bromide was used to evaluate the PCR results. A 100 bp DNA ladder was used to compare the amplified fragments, which were seen using a UV transilluminator.

DNA Sequencing and Phylogenetic Analysis

In order to undergo Sanger sequencing in both directions using the same primers, PCR products that tested positive for the cagA gene were purified and delivered to Macrogen Inc. in South Korea. To verify their identification and find similarities with known sequences, the acquired nucleotide sequences were modified and examined using the Basic Local Alignment Search Tool (BLAST) on the NCBI website. The MEGA6 program was used to create phylogenetic trees and to align multiple sequences. Accesssion numbers were obtained by submitting newly discovered sequences to the NCBI GenBank database.

Statistical Analysis

An analysis of the data was carried out using SPSS version 22, which is a statistical package for the social sciences. The prevalence rates were determined using descriptive statistics. Variables including gender, environment, and age groups were examined for their relationships with H. pylori infection using the Chi-square (χ^2) test. Statistical significance was determined by a p-value of less than or equal to 0.05 [14].

Results

Prevalence of H. pylori Infection

A total of 109 stool samples (or 70.3% of the total) were positive for Helicobacter pylori antigens when tested using the fast antigen test. This was in addition to the 155 samples that tested positive for intestinal parasites under the microscope. Figure 1 shows that 46 samples, or 29.7 percent, were negative for H. pylori.

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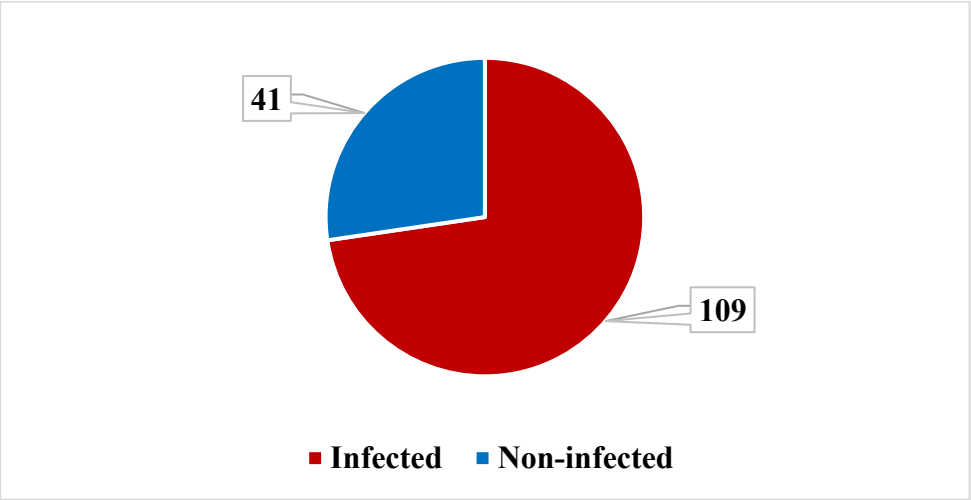

Figure 1: *H. pylori* percentage in stool samples by using Ag. rapid test**.**

A decreased prevalence was detected when PCR targeting the cagA gene was used to evaluate a subset of 96 samples that tested positive for parasites under a microscope. The bulk of the samples (78 out of 112) came back negative for H. pylori, while just 18 out of 112 (or 18.75%) tested positive (Figure 2).

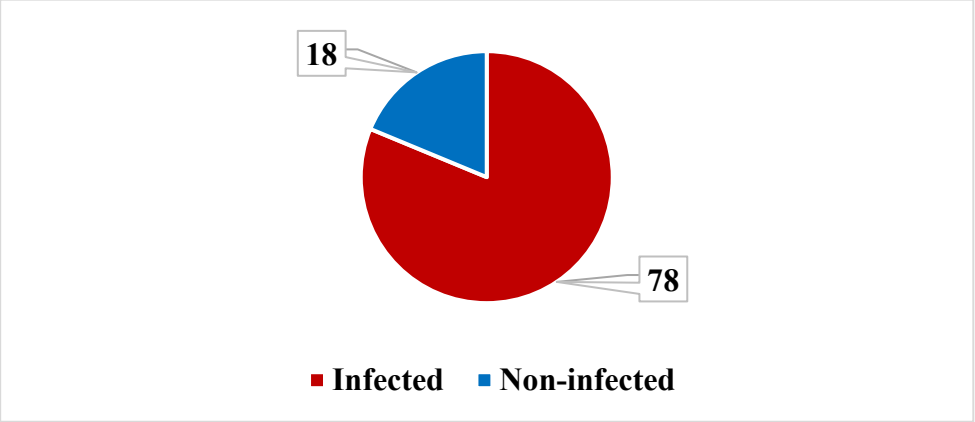

Figure 2: *H. pylori* percentage in stool samples by using PCR technique.

Distribution of H. pylori Infection by Demographic Factors

As shown by the fast antigen test, the prevalence of H. pylori infection differed throughout demographic categories. More men (71, or 65.1% of the total) than women (38, or 34.9% of the total) contracted the illness. It was more common among those living in rural regions [73, or 67.0%] than in urban areas [36, or 33.0%] according to the data analyzed. See Figure 3.

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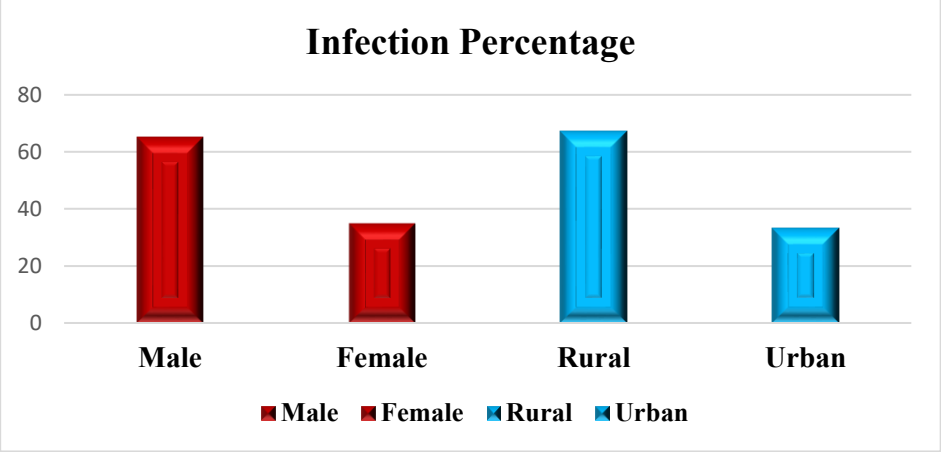

Figure 3: Percentage of *H. pylori* according to sex and residency by rapid test

The youngest cohort (less than 1-10 years), which accounted for 49 (45.0%) of the positive cases, had the greatest prevalence of H. pylori infection, according to the age group analysis. The age range of 31–40 years had the lowest infection incidence, with just 6 cases (5.5%) reported (Figure 4).

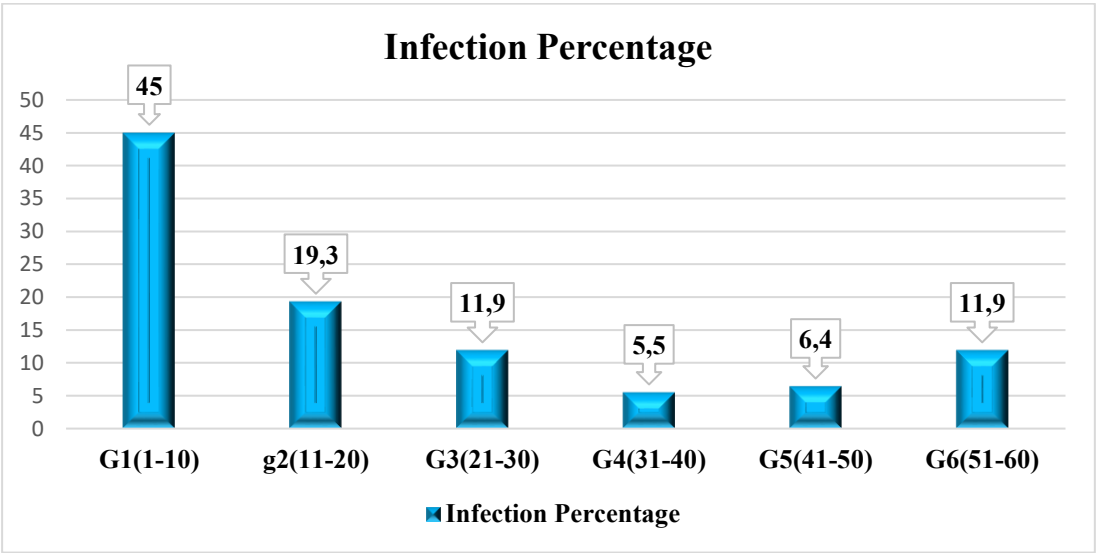

Figure 4: Percentage of *H. pylori* according to age group by rapid test.

Within the PCR-positive group, a similar pattern of demographic distribution was seen. A higher proportion of men (14, 77.8%) than women (4, 22.2%) and a higher proportion of rural (15, 83.3% of the population) than urban (3, 16.7% of the population) residents were infected. Furthermore, among children less than 10 years old, the incidence of infection verified by PCR was greatest (9 cases, or 50.0%). Figures 5 and 6.

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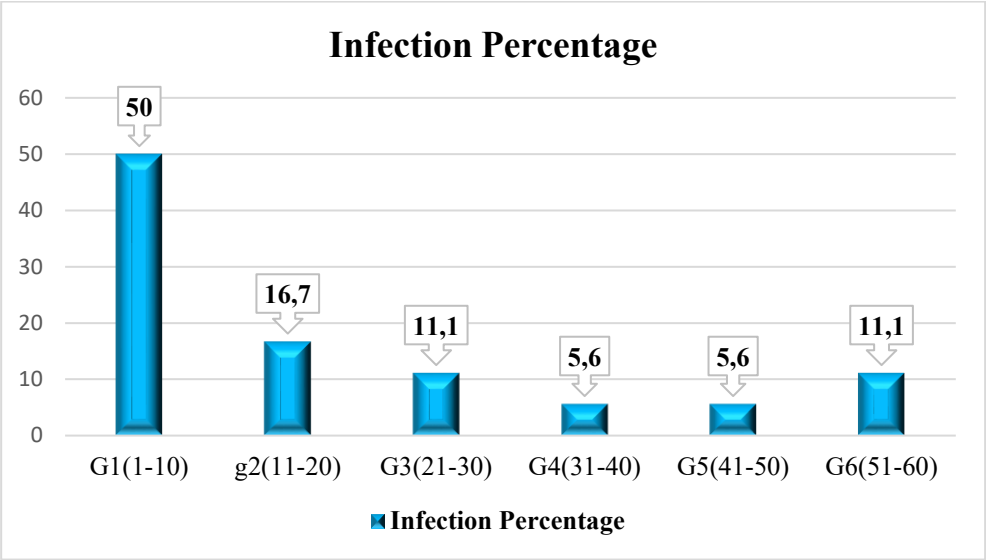

Figure 5: Percentage of *H. pylori* according to age group by PCR technique.

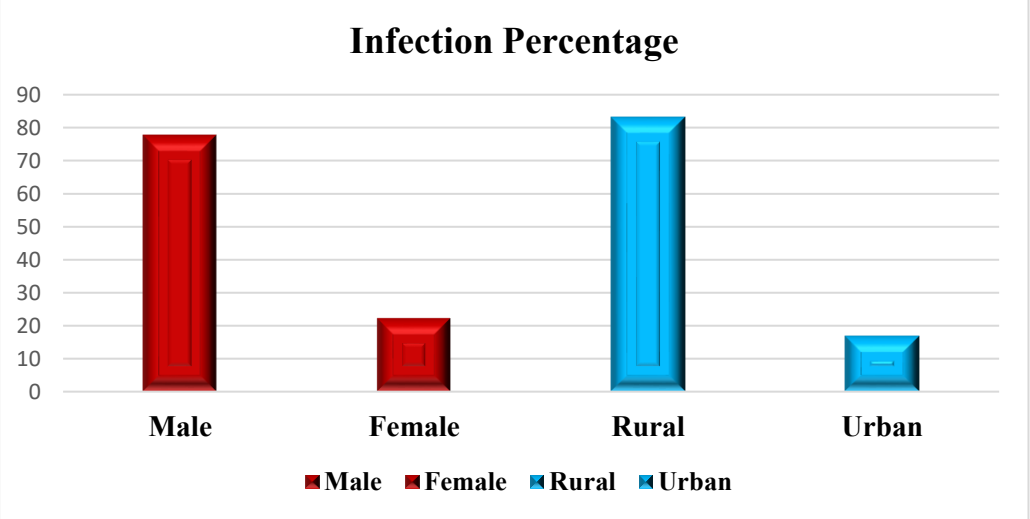

Figure 6: Percentage of *H. pylori* according to sex and residency by PCR technique

H. pylori Co-infection with Intestinal Parasites

Results from fast antigen tests for Helicobacter pylori and polymerase chain reaction (PCR) tests for intestinal parasites showed that co-infections occurred often. There was a significant prevalence of co-infection with Cryptosporidium parvum; in fact, 87.5% of the samples that tested positive for this parasite also tested positive for Helicobacter pylori. After that, Entamoeba histolytica and Giardia lamblia were found co-infected in 44 out of 59 samples (74.6%) and 26 out of 38 samples (68.4%), respectively. (Referring to table 1).

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Table 1: Co-infection of G. lamblia, E. histolytica and C. parvum with H. pylori, by PCR of INPs and Ag. rapid test Results for H. pylori.

Parasites	Total	Only		With *H. pylori*	
		NO.	%	NO.	%
E. histolytica	59.0	15.0	25.4	44.0	74.6
G. lamblia	38.0	12.0	31.6	26.0	68.4
C. parvum	24.0	3.00	12.5	21.0	87.5

Molecular Characterization and Genetic Analysis

We successfully sequenced the cagA gene from two local H. pylori strains. A significant level of similarity with known sequences in the NCBI database was shown by BLAST analysis, which also validated their identification. There was a 99.64% identity between the South Korean isolate (Accession No. KX673192.1) and the isolate labeled IQ No.1 (Subject sequence). Comparing IQ No.2 to the reference sequence revealed a level of 99.26% identity. Figure 7 shows that there are modest genetic changes between the two Iraqi isolates, despite a 98.89% similarity between them.

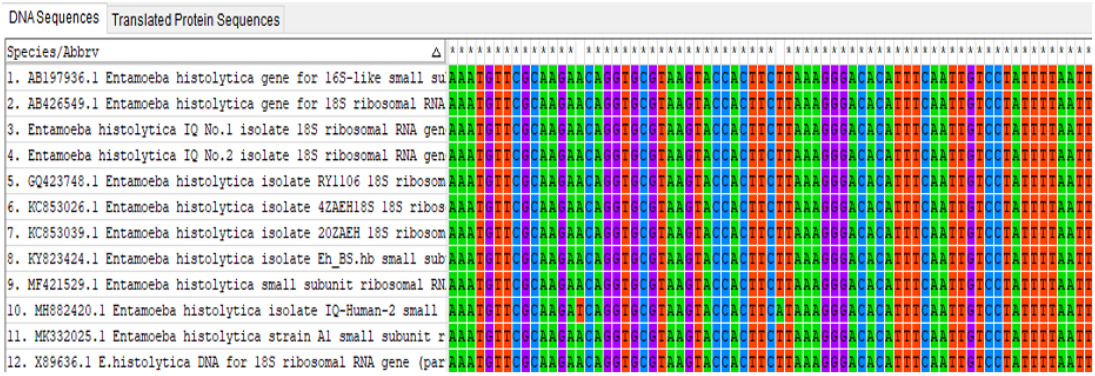

Figure 7: Multiple sequence alignment analysis of 18S ribosomal RNA gene in local E. histolytica isolates and NCBI-Genbank E. histolytica isolate No.1 and No.2.

1. H. pylori isolate IQ No.1: MN065500
2. H. pylori isolate IQ No.2 (co-infection with intestinal parasites): MN065501

Using several sequence alignments, we were able to see that there were SNPs between the two Iraqi isolates, the reference strain, and the local isolates (Figures 8,9,10).

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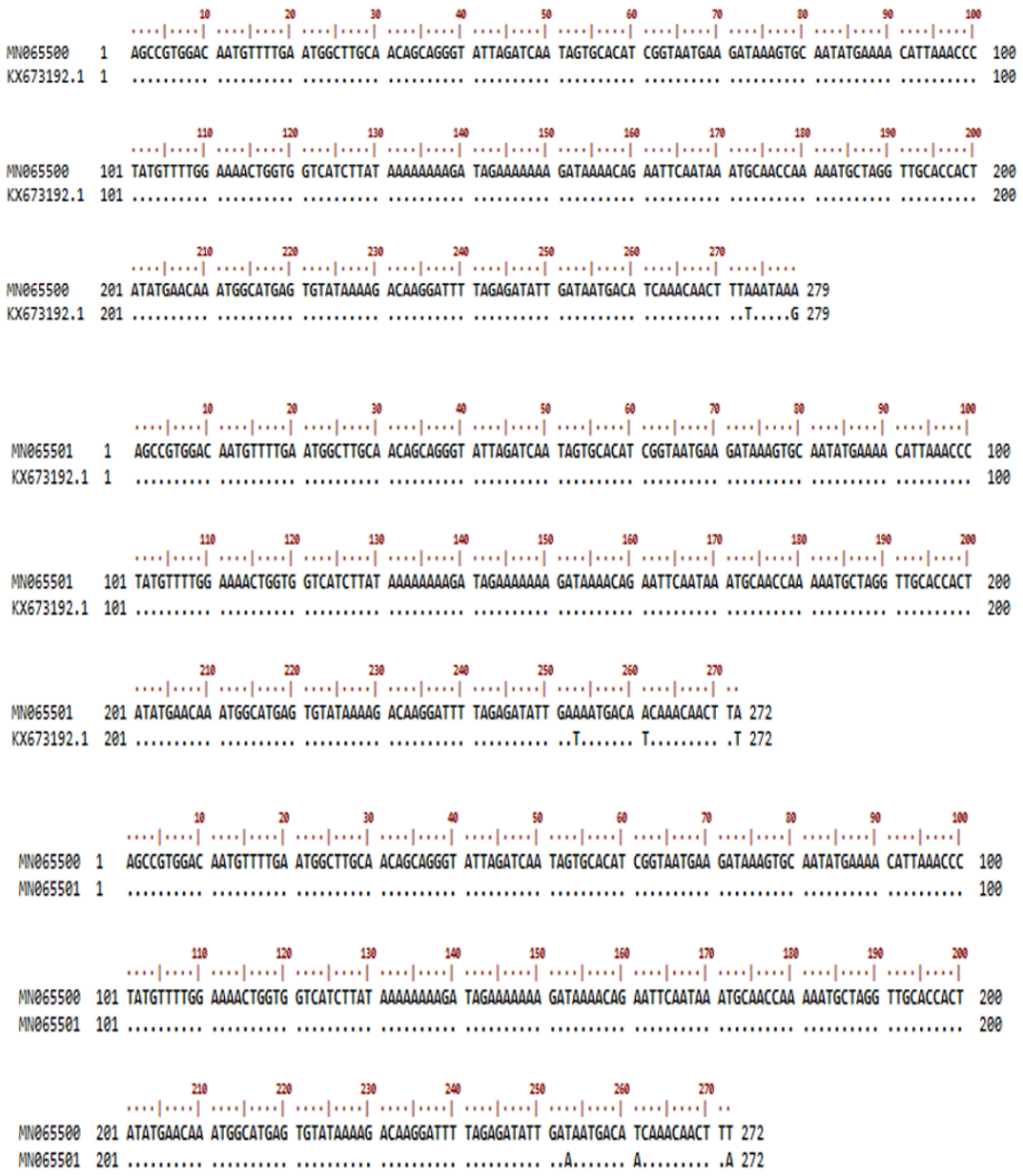

Figure 8: Sequencing analysis of H. pylori isolates in NCB alignment.

Comparison of Diagnostic Techniques

Results from the fast antigen test and the PCR method for identifying H. pylori were noticeably different. On the subgroup of samples analyzed by both procedures, the antigen test yielded a much greater number of positive cases (70.3%) than PCR (18.8%) (Table 2).

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Table 2: Comparison between PCR-technique and Microscope and Ag rapid test according to infectious Samples

Microorganisms	Microscopic examination for INPs & Ag. test for *H. pylori*		PCR technique Examination For INPs & *H. pylori*	
	NO:	%	NO:	%
E. histolytica	136/155	87.7	59.0/84	70.2
G. lamblia	30.0/155	19.4	38.0/84	36.9
C. parvum	19.0/155	12.3	24.0/84	28.6
H. pylori	109/155	70.3	18.0/96	18.8

Discussion

Important insights into the diagnostic and molecular features of Helicobacter pylori infection were provided by this study's results, which show that it is very prevalent among patients with chronic diarrhea in Thi-Qar region, Iraq. The fast antigen test found a much greater frequency of H. pylori (70.3% vs. rates reported in several other countries). For example, Cardenas et al. [15] found a frequency of 38.2% in a US-Mexico border community while Chang et al. [16] found a prevalence of 42.2% in the US. The exclusive inclusion of individuals with chronic diarrhea in our research most likely explains this higher-than-average prevalence. This might mean that the involvement of H. pylori in chronic diarrhea is more than previously thought, or that the people being studied are a subset of the population that is more likely to be exposed to the bacteria because of things like poor hygiene and sanitation [17]. There may be comparable epidemiological circumstances in Iraq, since the high prevalence is in line with aresearch from Erbil, Iraq, which found an infection rate of 55.8% Hussen et al. [18].

Using the PCR method yielded findings that were significantly different from the fast antigen test. When comparing the antigen test to PCR, the former yielded a much greater percentage of positive cases (70.3%). The underlying concepts of each test account for this variation. Bacterial feces may include many proteins (antigens), which the antigen test can identify. If the bacterial burden is low or DNA extraction is inadequate, PCR might provide false-negative findings because it identifies bacterial DNA, which could be present in lesser amounts or more easily degraded by stool inhibitors Shahadoust et al. [19]. In addition, antigen tests may stay positive for a while after the active infection has disappeared, while PCR can only identify living or complete bacteria due to its great specificity. The significance of taking into account the clinical setting and the limits of each diagnostic procedure is emphasized by this.

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This study's molecular characterisation and registration of local H. pylori strains is a significant contribution. Despite a high degree of similarity (99.26%-99.64%) to a South Korean strain (KX673192.1), single-nucleotide polymorphisms (SNPs) were detected in the two Iraqi isolates (GenBank Accession Nos. MN065500 and MN065501). Scientists all across the world will have access to this invaluable resource once these sequences are registered in the NCBI GenBank. In the future, comparative genomic studies may use this information to study the transmission patterns, local strain development, and illness consequences in Iraqi populations linked to particular genetic markers (such as cagA variants) [20].

Key trends emerged from the analysis of H. pylori infection rates by demographic category. Previous research from Iraq and underdeveloped nations has shown similar trends, including greater infection rates in men and rural populations Al-mashhadany, [21] and Yousif et al., [22]). Socioeconomic status, food choices, and environmental cleanliness are often mentioned as causes of these differences. The fact that the virus is most often acquired in children under the age of 10 in underdeveloped nations is supported by the fact that this age group has the greatest prevalence overall. [23].

Lastly, we must note that this research has several limitations. Patients with persistent diarrhea were the only ones included in the trial, which is the main restriction. We cannot, however, extrapolate the stated prevalence of H. pylori to the larger, asymptomatic population of Thi-Qar province. To determine the actual prevalence in the community, more research with larger populations will be necessary. This caveat aside, the research does a good job of demonstrating the heavy load that H. pylori.

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