RESEARCH PAPER

Comprehensive multi-gene profiling of virulence and OMP gene clusters *in Helicobacter pylori* associated with gastroduodenal disorder

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Abstract
Helicobacter pylori, a common chronic bacterial infection that affects over half of the human population. *H. pylori* infection can result in a wide range of gastrointestinal illnesses, including gastritis and peptic ulcer and it can develop into stomach cancer if left untreated. Treating *H. pylori* infection in an early stage is crucial for mitigating health risks and improving digestive health. In the present study 200 biopsy samples were collected through endoscopy from patients exhibiting symptoms of dyspepsia, epigastric pain, Anaemia. The samples were subjected to bacterial culture (Columbia agar with Skirrow supplement) and genomic characterization using molecular methods. DNA extraction was carried out followed by polymerase chain reaction (PCR) using specific set of primer, 16srRNA designed specific for *H. pylori* bacteria. Further PCR tests were conducted targeting genes linked to bacterial survival and pathogenicity, such as the cell wall synthesis gene *glmM*, urease genes *ureA* and *ureB*, outer membrane protein genes *oip A*, *hof C*, *hopQ* and *homA*, virulence genes *cagA, cagE*, and *vacA m2*, as well as the adhesion-related gene *iceA1*.For molecular validation, Sanger sequencing was conducted on the amplified universal *16S rRNA* gene obtained from bacterial DNA derived from cultures, and the resulting sequence was deposited in the NCBI database. Additionally, fifteen biopsy DNA samples, randomly selected and positive for the *ureB* gene, underwent further confirmation via Sanger sequencing. The sensitivity, specificity, and accuracy of the rapid urease test (RUT), culture methods were evaluated comparatively to determine the reliability of each diagnostic approach. The combined application of PCR and sequencing enabled precise and dependable confirmation of *H. pylori* infection from both clinical biopsy specimens and culture-derived isolates. This study underscores the importance of integrating traditional and molecular diagnostic techniques to achieve accurate detection and genetic characterization of *H. pylori* in symptomatic patients.

Key words
H. pylori, PCR, Culture, Rapid urease test, virulence gene, Outer membrane protein

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1. INTRODUCTION

*Helicobacter pylor*i is an ubiquitous, gram-negative, microaerobic, spiral-shaped bacterium that primarily colonizes epithelial cells covering the gastric lining or it thrives ~300 micron thick overlying mucous membranes of the stomach [1,2]. *H. pylori* exhibits both chemotactic and swimming abilities to escape the acidic gastric juice, it also possesses the cytoplasmic enzyme urease. This flagellated bacterium induces chronic inflammation of the underlying gastric mucosa with humoral and tissue immune reactions causing gastritis and variety of gastroduodenal diseases, including gastric ulcer , peptic ulcer disease, lymphoma, and gastric adenocarcinomas[3,4] Approximately 50% of the world's population is chronically infected with this bacterium, with the incidence of infection in developing countries reaching 70-90% and about 25-50% of people in industrially developed countries [5](Figure1). The prevalence of *H. pylori* reported by ray et al was found to be 53% in Pondicherry[6]. A lack of proper sanitation

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and safe drinking water, improper hygienic conditions and low socioeconomic conditions are contributing to *H. pylori* infection and its prevalence in south India [7].This bacterial infection has the strongest risk factor for developing gastric cancer, accounting for 89% of all non-cardia gastric cancers[8] As a result of its ability to cause stomach cancer, *H. pylori* was classified as a human carcinogen by the International Agency for Research on Cancer of the World Health Organization[9]. There are several virulence factors and genes in *H. pylori* that are responsible for motility ,adhesion , urease enzyme for escaping from the acidic environment and cytotoxin production which are essential for infecting host tissues and causing gastrointestinal disorders, including cytotoxin-associated gene A protein (*CagA*), which interferes with the signaling pathways in gastric epithelial cells, leading to the production of cytokines that result in chronic gastritis and promote cancer development, making *CagA* the first identified bacterial oncoprotein whereas Vacuolating cytotoxin A (*VacA*) has the ability to disrupt endocytic trafficking, release organic anions and bicarbonate, increase immune tolerance, and facilitate chronic infections by suppressing immune cells, activating protein kinases, and modulating autophagy. [10,11]. The clinical outcome of the infection depends on the imbalance of the parasite-host relationship *H. pylori* presents high genetic heterogeneity, and the different virulence factors are determinants for the development of severe diseases during the infection period. Helicobacter pylori's outer membrane protein (OMP) family has a huge number of genes, including *homA, hopQ, hofC, and frpB*[12]. Many of these genes play important roles in adhesion, colonization, and immune evasion in Helicobacter pylori. *OipA* encodes an outer membrane protein that acts as an adhesin, by phosphorylating some signaling pathways which can induce inflammation along with those mediated by the cag pathogenic island (cag-PAI)[13]. *H. pylori* density has been associated with virulence factors (*cagA, CagE, vacA*), gastric inflammation, and duodenal ulceration, suggesting a central role in pathogenesis[14].

Recent developments in the diagnosis of *H. pylori* highlight the significance of both invasive and non-invasive approaches which is mentioned in Figure 2. Non-invasive techniques encompasses serology test, faecal antigen testing and urea breath testing, whereas invasive methods are characterized by endoscopic biopsies accompanied by immunohistochemical staining, Rapid urease test[15](Figure2). The gold standard for diagnosing *H. pylori* infection is Urea breath test (UBT)[16]. Nonetheless, proton pump inhibitors and antibiotics used to treat *H. pylori* can lead to inaccurate negative results[17]. The rapid urease test (RUT) detects *H. pylori* indirectly by measuring the presence of urease on the gastric mucosa but positive RUT requires around 10^5 *H. pylori* in the biopsy sample to change colour[18]. Molecular techniques, including Polymerase chain reaction, provide reliable alternatives for the identification of *H. pylori* and the detection of drug resistance. The selection of a diagnostic approach is influenced by various factors, including the age of the patient and the prevalence of *H. pylori* within the population[17]. PCR-based techniques are generally cost-effective and yield quicker results compared to bacterial culture, which can be time-consuming and often unfeasible for H. pylori detection[19]. Conventional PCR detection techniques provide key benefits such as speed, high sensitivity, and suitability for routine diagnostic use[20]. Nevertheless, to ensure maximum specificity and reduce the risk of false-positive or false-negative outcomes especially when there are sequence variations at the primer binding regions it is crucial to design primers carefully and choose target sequences thoughtfully.

A broad-spectrum molecular method was developed to sensitively and specifically detect *H. pylori* by targeting thirteen genes that encode species-specific markers, cell wall biosynthesis, outer membrane adhesins or virulence factors. Species identification was verified by *ureB* gene and molecular profiling of *16S rRNA* conserved housekeeping gene and *glmM* gene's that are equally important for survival of bacteria, urease activity and peptidoglycan biosynthesis.

In order to get a more comprehensive view of bacterial adherence and colonization, we also choose OMP genes (*oipA, hopQ, homA, frpB, hof C*), which encode adhesins that are implicated in both attachment onto the host epithelium and persistence within the gastric mucosa. Furthermore, virulence-related-genes *cagA, cagE, vacAm2* were also examined to assess the pathogenicity of the strains. These genes are involved in the crucial processes of host cell signalling interference, toxin synthesis and inflammation stimulation. Considering the genetic diversity of *H. pylori* and its impact on clinical outcomes, analysing multiple genes offers a thorough method to understand strain differences and their potential to cause disease. In this study we compared different detection methods, including rapid urease test (RUT), *H. pylori* culture and PCR to evaluate the sensitivity and specificity of each method and validated using sanger sequencing. This combined approach seeks to improve the understanding of H. pylori genetic variation and evaluate the accuracy of traditional diagnostic methods against molecular techniques, ultimately aiding in the development of better diagnostic strategies and patient care.

2. MATERIALS AND METHOD

Cross sectional study which was conducted after getting Institutional Ethical Committee clearance (IHEC No: AV/IHEC/2023/013) in Aarupadai Veedu Medical College and Hospital from March 2023 to November 2025 two hundred patients undergoing upper GIT endoscopy were included after clinical examination based on purposive sampling technique, with informed and written consent.

Subjects 18-75 years of age of both sexes with symptomatic UGI disorders such as dyspepsia,

abdominal pain, and loss of appetite, dysphagia were included.

Individuals who provide informed consent for Tissue sample collection and data usage

Patients already on antibiotics, anticoagulants, NSAIDS in the past 4 weeks, previous gastric surgery, active GI bleeding, pregnant and lactating women, patients with severe cardiac diseases were excluded. Informed and written consent was obtained from each patient. Among the 200 selected subjects, male (121) and female (79) were unequally distributed with age varying from 18 to 75.

2.1 Sample size Calculation

Two hundred gastric tissue biopsies from the study subjects were enrolled. Estimation of sample size was done based on the following formula:

where N = sample size

p = sensitivity (70%),

q = 100-p,

d = absolute error which is 10% N= (2) 2 x 30 x70 = 171N

z-1.96-2 0.01

N = 200

Error = 10%, sensitivity = 70%, 95% confidence interval

2.2 Sample preparation

Patients scheduled for endoscopy the next day were instructed to fast overnight. Following which endoscopy was done in medical gastroenterology (MGE) unit by physician using fibre optic endoscopy apparatus. The endoscope and biopsy forceps were thoroughly cleaned in water and sterilized by soaking it in 2% glutaraldehyde over a period of 20 min and rinsed again with sterile normal saline just before the procedure and specimen collection. Patients were sprayed with lidocaine spray 10% local anaesthesia in the pharyngeal region. Gastric biopsy samples were taken from antrum of stomach which is 2 cm from the pylorus of stomach Gastric biopsy samples were collected using biopsy needle which was guided by the camera. Following 3 tissue bites were collected one was inoculated in RUT card (Rapid urease test) and another one was collected in a Brain heart infusion agar for culturing and another tissue was collected in Phosphate buffer saline for DNA extraction for further downstream process (Figure3).

2.3 Sample processing

2.3.1 Rapid urease test

RUT was carried out at the time of endoscopy by adding few drops of sterile water followed by the biopsy specimens to the RUT card. The test was reported positive if colour turned from yellow to pink within two hours. If it remains yellow after 2h, then it was noted as a negative for *H. pylori*

2.3.2 Culture of *H. pylori* strain

Biopsy samples were manually grinded until homogenization and then streaked on columbia agar (Hi-media) and was supplemented with 2% agar and 5% sheep blood supplemented with Skirrow selective supplement to avoid other bacterial contamination. The plates were then incubated at 37°C for 1 or 2 days in an anaerobic jar to maintain microaerophilic environment. Characteristic features indicative of small (<2 cm), circular, translucent, grey colonies were corroborated by positive results obtained from standard diagnostic tests (Figure 4a). Gram's stain showed gram negative spiral shaped bacilli (Figure4a). Biochemical tests like urease, catalase, oxidase, tests were performed to confirm the bacterial species (Figure 4b). Additionally hanging drop method was done to identify motility of the bacteria. These tests were performed according to the manufacturer's instruction.

2.3.3 DNA extraction

Bacterial DNA was extracted from gastric mucosa samples isolation was done using Tissue kit (QIAGEN). The tissue samples were homogenised and lysed with a help of ATL tissue lysis buffer and proteinase K solution the tubes were placed in a water bath for 3 -5 hours at 60° C and immediately chilled on ice for 10 min and precipitated by using ethanol and transferred into spin column tube and excess derbies were washed using wash buffer and finally DNA was eluted using 200 µl of elution buffer and Genomic DNA extracts were stored at -20° C until used.

2.6 Polymerase chain reaction

In this research, the molecular identification of *H. pylori* in biopsy samples was achieved by amplifying the *16srRNA* specific for *H. pylori* and urease gene using specific primers (*ureA & ureB*). Samples that tested positive for the urease gene were further analysed for pathogenic markers such as *cagA, cagE, vacAm2, oip A, and iceA1, glm* gene responsible for cell wall synthesis in *H. pylori* bacteria. Outer-membrane proteins such as *hopQ, hof C, hom A, frp B* using a specific set of primers. These oligonucleotide primers were synthesized by IDT in Singapore. The sequences and lengths of the targeted gene fragments are listed in (table1). PCR gels were visualized in UV gel doc system. Conventional PCR was performed using the thirteen different primers designed for identification purposes.

2.7 Statistical analysis

All statistical analyses were conducted using IBM SPSS software (Version 23.0). Sensitivity and specificity of various detection methods were determined using the case definition of *H. pylori*-positive cases as the reference standard. Associations between variables were examined using the Chi-square test. A P value below 0.05 was regarded as statistically significant for all tests.

3. RESULT

A total of 200 patients were recruited from the endoscopy unit, with ages ranging from 14 to 80 years (mean age ± standard deviation). A certain percentage of the participants were Male, and another percentage were female resided in rural areas. None of the patients had

received nonsteroidal anti-inflammatory drugs prior to the study. Table 2 Shows the overall prevalence of *H. pylori* (52.5%) in Puducherry population. Gender wise distribution shows more prevalence among males (66.6%) compared to Females (33.3%). Maximum positivity of *H. pylori* was seen among the age group 41-50. The most prevalent symptom reported was recurrent epigastric pain, followed by loss of appetite, dyspepsia, Anaemia or weight loss and hematemesis with respective frequencies of 60%, 14.3%, 12.4% ,5.7% and 5.7% which is shown in table 3. Endoscopic examination revealed that gastritis was the most frequently observed finding, followed by Gastric ulcer, gastroesophageal reflux disease (GERD), Oesophageal varices and PHT gastropathy, Duodenitis and gastric cancer with occurrences of 50%, 100%, and 35.7%, 53.8%, 27.3% ,71.2% 100% respectively which is mentioned in (table 4). Additionally, among gastritis, duodenitis, gastric ulcer, and gastric ulcer and gastritis exhibited a statistically significant correlation with PCR results ($p < 0.001$). Even the patients who were diagnosed with normal gastric mucosa has *H. pylori* infection which accounts for 44.8 %. Normal gastric mucosa was identified in only 16 patients (7%). Among the 200 patients studied, 53.5 (%) were tested positive for the rapid urease test (RUT) and It has generated 33 false positive and 31 false negative results. All patients diagnosed with gastric ulcer and those with combined gastritis and gastric ulcers were RUT positive. The chi-square test showed a statistically significant association between RUT positivity and gastric ulcer ($\chi^2 = 11.582$, $p = 0.0007$). In contrast no significant association was observed between gastritis (p=1.000), duodenitis (p=0.850), GERD/LAX LES(p=1.000), PHTG(p=0.138), Oesophageal varices(P=0.794), gastric cancer with p-value 0.944 respectively. Polymerase chain reaction (PCR) analysis was conducted on all 200 samples, revealing that105 samples (52.5%) were positive for the *H. pylori ureB* gene and 32% of patients were positive for virulent associated genes like *cagA* and *vacAm2* refer table5. Among the patients who tested positive for virulent *cagA*, gastritis was the most common endoscopic finding, followed by gastric ulcer, duodenal ulcer and GERD, with respective frequencies of 22.6%, 50%, 60%and 60% which is represented in table 6. Out of 200 cases, H. pylori infection rates were notably high in individuals who consumed alcohol [39 out of 45 (86.6%)] and those who smoked [29 out of 34 (85.2%)]. Conversely, the infection rate was low among vegetarians [2 out of 15 (13.3%)] and higher among non-vegetarians [102 out of 185 (55.1%)] shown in table7. The *16S rRNA* gene was amplified in 73 isolates (69.5%), and the glm gene was amplified in 56 isolates (53.3%). Regarding outer membrane protein (OMP) genes, *frp B* was found in 73 isolates (69.5%), *hof C* in 62 isolates (59.0%), *oipA* in 45 isolates (42.9%), *hom A* in 20 isolates (19.0%), and *hopQ* in 10 isolates (9.5%) (Table 8) (Figure5). The presence of OMP genes was analysed based on cag A status and clinical symptoms. Additionally, The DNA obtained by the bacterial culture of *H. pylori 16srRNA* gene was sequenced using sanger sequencer and the obtained sequences were submitted to NCBI database Accession no: PQ394642.1. Furthermore, 15 randomly selected *ureB* positive samples and one *vacAm2* positive sample were sanger sequenced to confirm the presence of *H. pylori* species.

4. DISCUSSION

The discovery of *H. pylori* 40 years ago set a milestone in modern medicine. The discovery led to the rejection of the dogma that the stomach is a sterile organ and the need to rewrite the chapters on gastric pathophysiology and gastroduodenal diseases[21]. *H. pylori* infections affect 80 to 90% of the Indian population, with the pathogen's prevalence being influenced by factors like race, age, and socioeconomic status[22]. The inflammation of the gastric epithelium, along with various factors such as cyclooxygenase activity, prostaglandin production, and the disruption of cadherin–catenin interactions, has been implicated in the pathogenesis of gastric adenocarcinoma[23]. The endoscopy image of normal gastric mucosa and gastric ulcer are shown in figure 6 The epidemiological aspects of *H. pylori* infection remains an interesting area of research, particularly regarding its colonization and varying consequences. In one of the studies conducted by Sharma et al, the prevalence of *H. pylori* was found to be 40.85%.[24] which was lower as compared to our current study, in which *H. pylori* were detected in 105 biopsy samples from 200 symptomatic subjects by PCR (52.5%). The gender-based prevalence of *H. pylori* in a patient with epigastric pain and dyspepsia showed notable variation, with 33.3 % of women and 66.6% of men testing positive. Although our study observed a marginally higher prevalence in men, this difference was not statistically significant. These findings are consistent with those reported by Louw et al, who documented infection rates of 64% in male patients compared to 61% in female patients[25]. Almashhadany *et al* study shows high incidence in females which was contrary to our study [26] In the analysis of age group-based prevalence of *H. pylori* , it was found to be most common in 41-50 years of age group with a prevalence of 25%. The mean age in the infected group was found to be 47 years, and in the noninfected group, it was found out to be 50 years. Indicating that the distribution of *H. pylori* is likely affected by geographical, cultural, and behavioural factors In one of the studies conducted, it was found out to be 44 years in the infected group, and 53 years in the non-infected group, which was almost the same as compared to our study[25].It is likely that *H. pylori* infection varies by region, race, age, gender, and ethnicity. On the other hand, specific gastrointestinal symptoms were significantly associated with infection. Abdominal pain ($p = 0.55$) and Loss of appetite ($p = 0.766$) were notably more frequent in infected patients. These results corroborate the pathogenic role of *H. pylori* and align with existing literature that recognizes the bacterium as a significant factor in the contribution of upper gastrointestinal symptoms. However, the lack

of association with symptoms like bloating and heartburn aligns with findings by Ieva Renata Jonaityte who reported mixed symptom associations[27]. In our study, in patients with findings on esophagogastroduodenoscopy, majority of patients had been diagnosed with gastritis. Gastritis was found in 107 patients (60.9%), of which in 53 patients (49.5%), were positive for *H. pylori*. Majority of patients had been diagnosed with gastritis out of 107gastritis cases 53 patients were tested positive for *H. pylori* and shows no statistical significance. patients diagnosed with gastric ulcer, where out of 18 patients 18 were tested positive (100%) and it shows statistical significance of P = 0.0001, gastric cancer was found in one patient 100% followed by Duodenitis 71.2%, osophageal varices 53.8% , even those who are diagnosed as normal had *H. pylori* infection in which 13 out of 29 patients were positive for *H. pylori* , GERD/LAX LES was found in 5 patients out of 14 (35.7%) and PHT gastropathy was found to be 27.3% respectively . *H. pylori* cag A is a strongly immunogenic protein which is linked to cellular damage and more serious clinical conditions, such as duodenal ulcers and gastric adenocarcinoma [11]. *H. pylori* infection is associated not only with gastric diseases, but also with extra-gastric comorbidities such as diabetes, hypertension, and liver disease. Kountouras et al. showed that *H. pylori* infection leads to increased insulin resistance and contributes to the development of metabolic syndrome and obesity[28]. In our study majority of patients who were positive for *H. pylori* has comorbidity such as diabetes and liver diseases. In the current study, the *ureB* gene was chosen as the target detection to compare with other methods due to its highly conserved region and the absence of cross-homology other closely related bacteria or Helicobacter subspecies. The vacA protein disrupts the tight junctions of epithelial cells, modifies the host's inflammatory response, and inhibits T cell activation[29]. In our study, *cagA and vacAm2* prevalence was 32.5%, with maximum positivity among 100%, 66.6%, 60%, and 50% ,20%, 16.6% of gastric ulcer, GERD/LAX LES Duodenitis, gastritis and normal respectively. Another study in Algeria identified the *cagA* gene in 58% patients[30] , and the percentages of positive cases in Pakistan was 24.2% [31].

Culture have been considered the gold standard due to their high sensitivity of 80.30%(above 68.68%-89.07%) and specificity100% (between 89.72%-100%) in a study conducted by priadko et [32].which was in contrary to our study where culture shows Sensitivity of 15.24 and specificity 100% (Table9) . It is challenging to rely on it solely as a routine diagnostic tool. Despite being used for a long time, culturing remains difficult because the bacterium is fastidious and requires specific environmental and atmospheric conditions to grow[33]. Another study conducted by Hussein et al showed 22.6% false negative for culture method[34]. This could be because *H. pylori* can take on two different forms: an actively reproducing spiral shape and a coccoid shape. The coccoid form is often referred to as "viable but non-culturable[33].

Based on our findings RUT test had the sensitivity of 70.4 %, but the specificity was lower at 65.2%(Table7). In this study, there were 33 false positives and 31 false negatives generated by RUT. The study conducted by Nga et al showed the sensitivity and specificity of 67.1% and 64%, respectively, for RUT, which is in line with our study [35]. RUT results are influenced by many factors including the biopsy condition, disease type, and the size and density of the bacteria in the sample[36]. The high prevalence of *frpB* (69.5%) and *hofC* (59.0%) genes suggests that outer membrane protein genes are extensively distributed among clinical isolates. The increased occurrence of *oipA, hofC*, and *frpB* genes in *cagA*-positive strains may indicate a coordinated regulation of adhesion-related factors in conjunction with components of the cag pathogenicity island. This genetic clustering potentially facilitates enhanced epithelial adherence and augments the inflammatory response. The OMP burden is positively associated with the virulence burden, suggesting a coordinated pathogenic structure instead of random gene presence. This study showed that PCR assays are sensitive and specific for detecting *H. pylori* in clinical specimens, since almost all of the infected subjects were able to detect *H. pylori* in tissue sample with increased in sensitivity and specificity compared to other conventional detection method.

5. CONCLUSION

This study found that *H. pylori* strains have a consistent genetic foundation related to colonization, along with diverse virulence factors. The simultaneous presence of outer membrane protein (OMP) and virulence genes indicates that the bacteria's pathogenicity is influenced by interactions among multiple genes rather than by single genes alone. Therefore, analysing multiple genes involved in adhesion and virulence could be an effective approach to gain deeper insights into H. pylori's disease-causing mechanisms and the diversity among its strains. PCR-based detection is a sensitive and dependable technique for identifying *H. pylori* in gastric biopsy samples compared to traditional diagnostic methods like the rapid urease test (RUT) and culture. The comparison of sensitivity, specificity, and accuracy among RUT, culture, and PCR highlights the value of molecular approaches in confirming *H. pylori* infection. The amplification of *16S rRNA* and other key pathogenicity-related genes, along with Sanger sequencing verification of *16S rRNA* amplicons from culture derived DNA and also sanger sequenced randomly chosen 15 samples of *ureB* gene from biopsies, reinforces the reliability of the findings. Additionally, submitted the *16S rRNA* gene sequence to NCBI further validates the molecular characterization of the isolate. Future research combining gene expression analysis, protein validation, and clinical outcomes in patients may provide a clearer understanding of the functional importance of these

adhesion–virulence markers in the development of H. pylori-related disease.

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Institutional Review Board Statement: This study was reviewed and approved by institutional ethics committee Board of Aarupadai veedu medical college Puducherry under approval number IHEC No: AV/IHEC/2023/013). The study was conducted in accordance with ethical standard of the institutional research committee

Informed Consent Statement Written informed consent was obtained from all participants prior to inclusion in the study. Patients were informed about the purpose of the study, the procedures involved, and the use of their gastric biopsy samples for research related to *Helicobacter pylori* detection.

Availability of Data and Materials The data supporting the findings of this study are available upon reasonable request from the corresponding author.

Authors contribution IK have done the wet lab work and wrote the manuscript. SS contributed to the collection of gastric tissue samples and provided clinical support for the study. KR and TS critically evaluated and gave suggestions for the revised manuscript. SR gave approval for the final version to be published.

Conflict of Interest The authors have no conflicts of interest to declare.

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igure 1

The image depicts the worldwide distribution and spreads of a bacterial pathogen, probably Helicobacter pylori, and its association with cancer. A global map displays the considerable differences in infection rates among various continents, with certain areas experiencing a much higher prevalence. The diagram in the upper right corner outlines the main transmission pathways, such as contaminated food and water, along with person-to-person transmission through oral-oral and faecal-oral contact. The bottom section offers a microscopic perspective of the bacteria interacting with human cells, demonstrating their capacity to cause cellular damage and eventually trigger the development of cancer cells, thus directly linking the infection to disease progression

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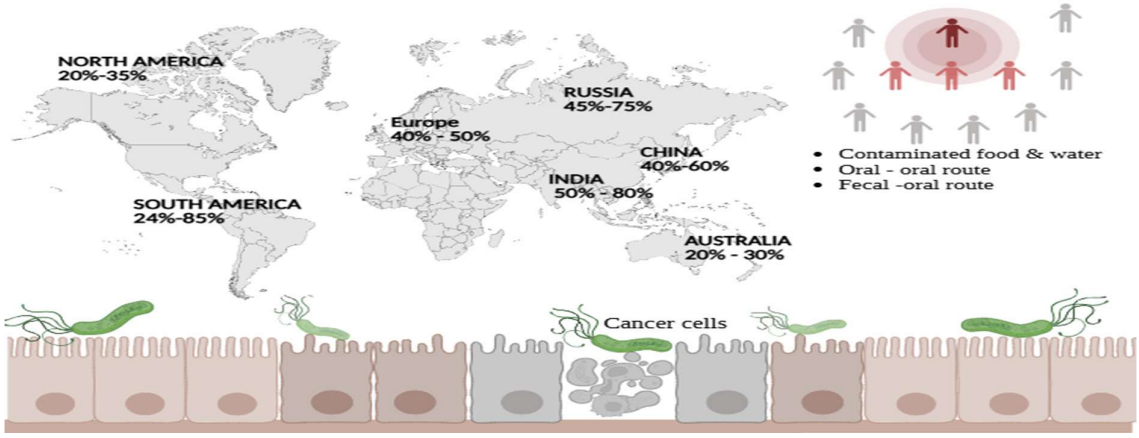


Figure2
There are many diagnostic tests today, but each has its own advantages and disadvantages, followed by limitations. The use of one or another test depends on the accessibility of those tests, equipment from laboratories, and the clinical conditions of patients. Screening and laboratory diagnoses are based on non-invasive methods and invasive methods. Non-invasive methods include respiratory tests, stool antigens, and serology. Invasive methods include endoscopy, histological examination, rapid urea test, culture, and PCR test

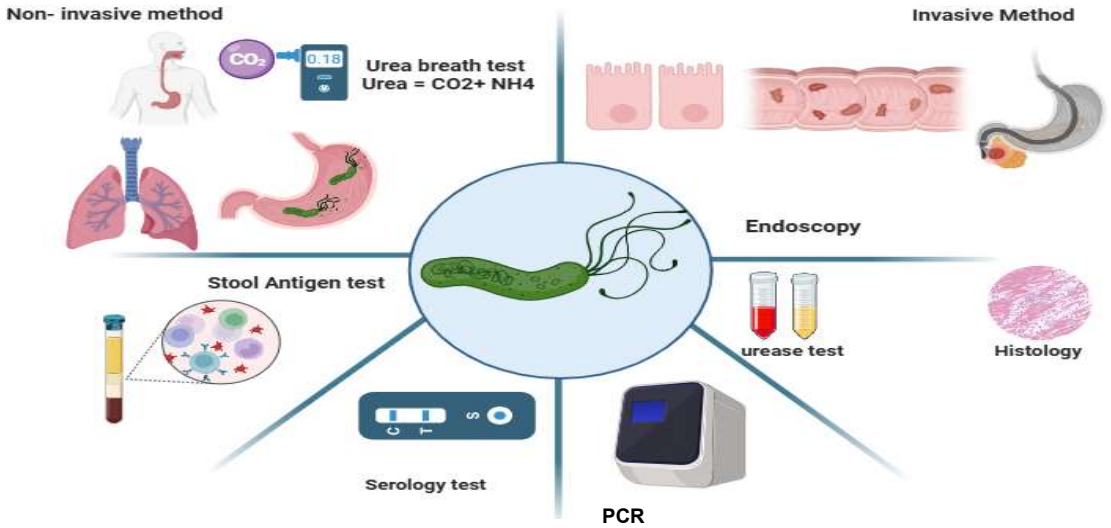


Figure 3
Gastric biopsy specimens were collected via endoscopy from patients suspected of *H. pylori* infection. The biopsy samples were split into three portions: one was kept in BHI broth for culture and one was inoculated in rapid urease test card (RUT), while the other was stored in PBS for molecular analysis. DNA was extracted from the samples and stored at –20 °C for PCR amplification of specific target genes. The amplified DNA products were then analysed using Sanger sequencing to confirm results and enable further genetic characterization.

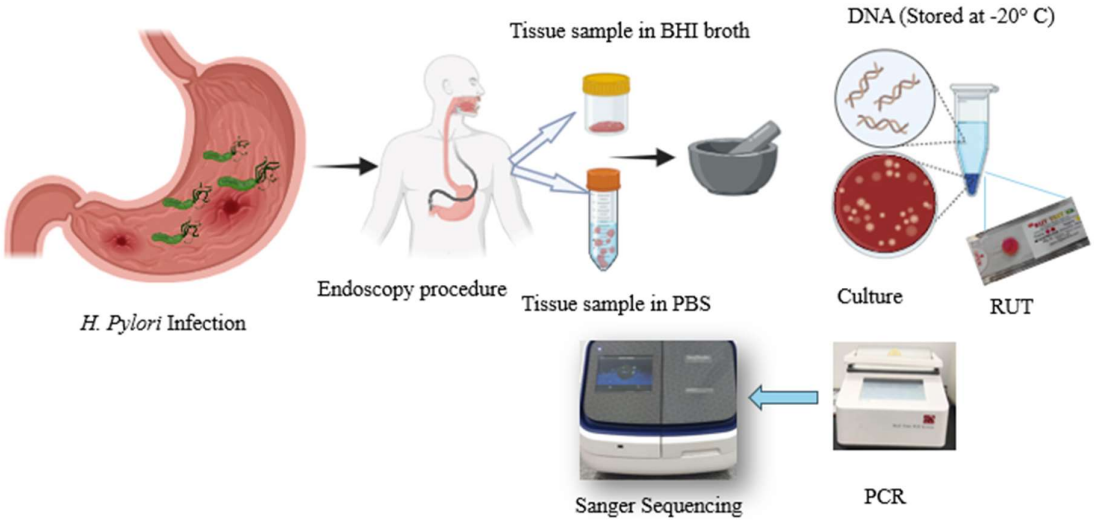
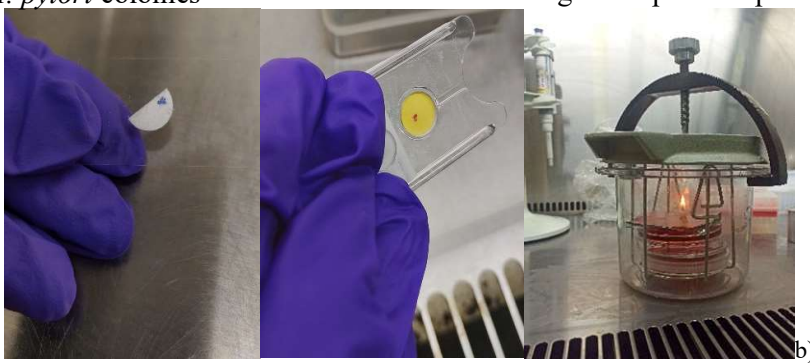


Figure 4
a) Representative images display *H. pylori* colonies cultured on growth medium (Columbia agar supplemented with Skirrow selective supplement), Gram staining revealing Gram-negative spiral-shaped bacteria.
b) Images shows an important biochemical traits such as growth in microaerophilic conditions within an anaerobic jar, along with positive results for urease, catalase, and oxidase tests.

a)

H. pylori colonies

Gram negative-spiral shaped bacteria



b)

Anaerobic jar Urease – positive

Catalase - positive Oxidase- Positive

Figure 5
Agarose gel electrophoresis showing PCR amplification of *H. pylori 16srRNA*, virulence genes (*cagE, vacAm2, iceA1*) and OMP genes (*oipA, hofC, hop, frpB, glm*). Lane M -100bp ladder. Bands correspond to the expected amplicon sizes in bp

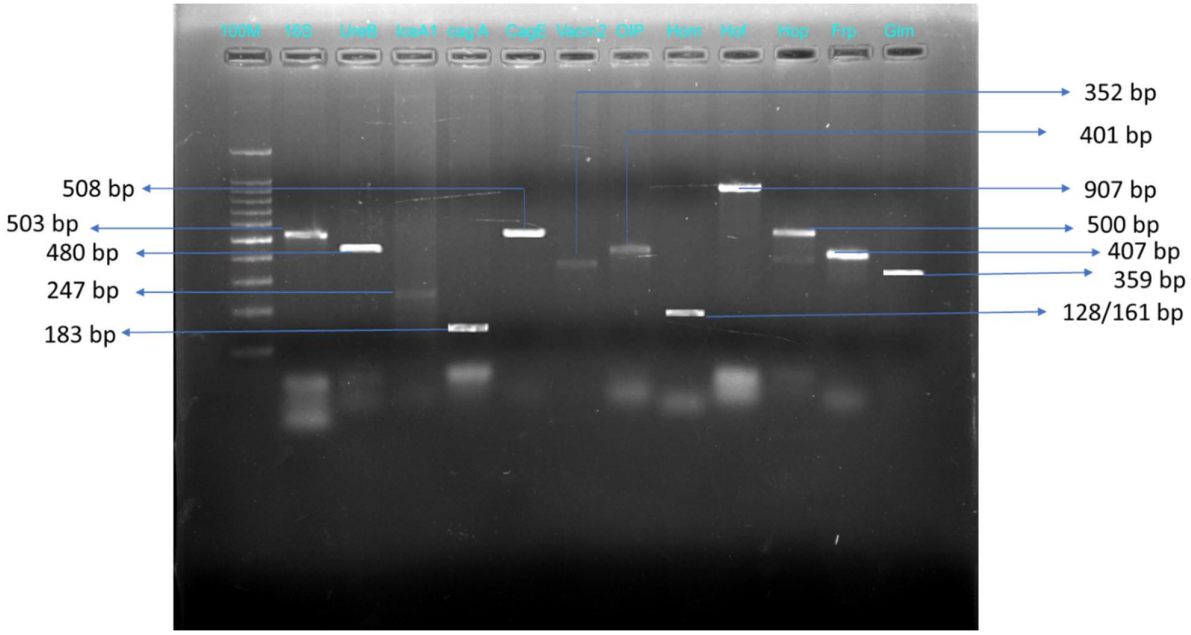


Figure 6
Endoscopic appearance of gastric antrum
(a) The normal gastric antrum exhibits a smooth mucosal surface with intact rugal folds, without any signs of erosion or ulceration.
(b) The gastric ulcer is characterized by well- defined mucosal defect accompanied by surrounding erythema and inflammatory alterations.

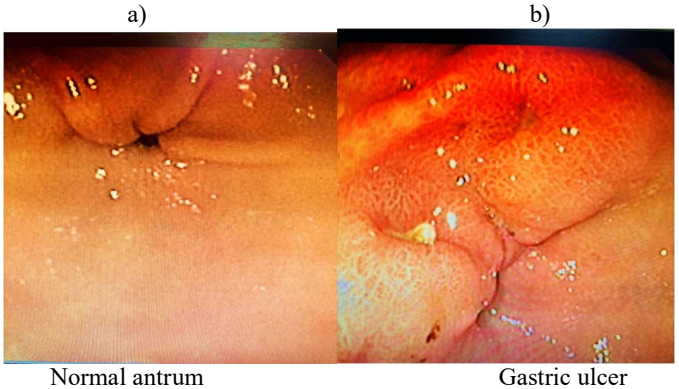


Table 1
Primer sequence used for PCR amplification of *H. pylori* genes and the corresponding amplicon size

Primer Name	Primer Sequence	PCR condition	Base pair	Reference

*16SrRNA*F *16SrRNA* R	5'-GCGCAATCAGCGTCAGGTAATG-3' 5'-GCTAAGAGAGCAGCCTATGTCC-3'	95 °C, 1 min; 55 °C, 1 min; 72 °C, 1 min (34 cycles)	503bp	[37]
27F *1492R* (Outer primer)	5'-AGAGTTTGATCCTGGCTCAG-3' 5'-TACGGYTACCTTGTTACGACTT-3'	95 °C, 1 min; 54 °C, 1 min; 72 °C, 1 min (34 cycles)	1465bp	[38]
799F *U1492R* (Inner primer)	5'-AACMGGATTAGATACCCKG-3' 5'-GGTTACCTTGTTACGACTT-3'	95 °C, 1 min; 56 °C, 1 min; 72 °C, 1 min (35 cycles)	750bp	[39]
ure B F *ure B* R	5'-CGTCCGGCAATAGCTGCCATAGT-3' 5'-GTAGGTCCTGCTACTGAAGCCTTA-3'	95 °C, 2 min; 62 °C, 30 sec; 72 °C, 1 min (35 cycles)	480 bp	[40]
ure A F *ure A* R	5'-CGTGGCAAGCATGATCCAT-3' 5'-GGGTATGCACGGTTACGAGTT T-3'	95 °C, 1 min; 62 °C, 1 min; 72 °C, 1 min (40 cycles)	77 bp	[41]
cag A F *cag A* R	5'-TTGACCAACAACCACAAACCGAAG-' 5'-CTTCCCTTAATTGCGAGATTCC-3'	95 °C, 1 min; 54 °C, 1 min; 72 °C, 2 min (40 cycles)	183 bp	[42]
cagE F *cagE* R	5'-TTGAAAACTTCAAGGATAGGATAGAGC-3' 5'-GCCTAGCGTAATATCACCATTACCC-3'	95 °C, 1 min; 56 °C1 min; 72 °C, 1 min (35 cycles)	508 bp	[43]
vac Am2 F *vac Am2* R	5'-CATAACTAGCGCCTTGCAC -3' 5'-GGAGCCCCAGGAAACATTG-3'	95 °C, 1 min; 57 °C, 1 min; 72 °C, 1 min (34 cycles)	352 bp	[44]
ice A1 F *iceA1* R	5'-GTGTTTTTAACCAAAGTATC-3' 5'-CTATAGCCASTYTCTTTGCA-3'	95 °C, 1 min; 55 °C, 1 min; 72 °C, 1 min (34 cycles)	247 bp	[45]
oip A F *oip A* R	5'-GTTTTTGATGCATGGGATTT-3' 5'-GTGCATCTCTTATGGCTTT-3'	94 °C, 1 min; 56 °C, 1 min; 72 °C, 1 min (34 cycles)	401bp	[46]
hom A F *hom A* R	5'-AGAGGGTGTTTGAAACGCTCAATA-3' 5'-GGTGAATTCTTCTGCGGTTTG-3'	95 °C, 1 min; 60 °C30 sec; 72 °C, 1 min (35 cycles)	A-128 bp B-161 bp	[47]
hopQ F *hopQ* R	5'CAACGATAATGGCACAAACT-3' 5'GTCGTATCAATAACAGAAGTTG-3'	94 °C, 1 min; 54 °C, 45 sec; 72 °C, 1 min (35 cycles)	500bp	[48]
Hof C F *Hof C* R	5'-GCTTGCCACTRTTGTTCACT-3' 5'-CGACCGTATTCAGCGTTATT-3'	95 °C, 1 min; 55 °C, 1 min; 72 °C, 1 min (34 cycles)	907bp	[49]
frpB F *frpB* R	5'-AGCCGTCTCTTAAGGGTAAC-3' 5'-TCGCTATTGCTTGGATCTTG-3'	94 °C, 1 min; 56 °C, 30 sec; 72 °C, 1 min (35 cycles)	407bp	[49]
Glm F *Glm* R	5'-CCATGCACGATATTCCCTAA-3' 5'-GATAGACGATGTGATAGGGC-3'	94 °C, 1 min; 55 °C, 30 sec; 72 °C, 1 min (35 cycles)	359bp	[50]

Table 2
Sociodemographic factors with *H. pylori* Infection

Variables	Category	Total number of cases (200)	Number of positive cases (105)	Percentage of positive cases
Gender	Male	123	70	66.66
	Female	77	35	33.33
Age (Years)	14-20	7	2	1.9
	21-30	22	17	16.1
	31-40	38	21	20.1
	41-50	54	28	26.6
	51-60	46	21	20.1
	61-70	26	12	11.4
	71-80	7	4	3.8

Table 3
Symptoms distribution among *H. pylori* positive patients

Symptoms	Present	*H. pylori* Positive	*H. pylori* negative	Percentage	P value
Epigastric pain	118	64	54	60.5	0.555
Dyspepsia	30	13	17	12.4	0.275
Loss of appetite	30	15	15	14.3	0.766
Weight-loss/ Anaemia	11	6	5	5.7	0.889
Hematemesis/ melena	10	6	4	5.7	0.626

Table 4
Various endoscopy findings among the study participants with *H. pylori*

Endoscopic findings	Total no of cases	*H. pylori* Positive	*H. pylori* negative	Percentage	P value
Gastric ulcer	18	18	0	100	0.001
Gastritis	107	53	54	49.5	0.367
Duodenitis	7	5	2	71.2	0.307
GERD/LAX LES	14	5	9	35.7	1.192
PHT gastropathy	11	3	8	27.2	0.0
Oesophageal varices	13	7	6	53.8	0.920
Gastric cancer	1	1	0	100	0.340
Normal	29	13	16	44.8	0.340

Table 5
Prevalence of *cagA* virulence gene among *H. pylori* positive samples identified by PCR.

PCR	*cag A*	
H. pylori	Positive	Negative
105(52.5%)	32(30.5%)	73(69.5%)

Table 6
Association between *Cag A* genotype and Endoscopic findings from *H. pylori* patients.

Endoscopic findings	PCR positive *H. pylori*	*cagA* positive	*cag A* negative	Percentage of *cagA* positive
Gastric ulcer	18	9	9	50
Gastritis	53	12	41	22.6

Duodenitis	5	3	2	60
PHT gastropathy	5	3	2	60
GERD/LAX LES	3	0	3	0
Oesophageal varices	7	1	6	14.3
Gastric cancer	1	1	0	100
Normal	13	3	10	23

Table 7
Distribution of *H. pylori* infection according to various factors

Variables	**Total number of cases (200)**	***H. pylori* Positive**	***H. pylori* negative**	**Percentage of positive cases**
Alcohol consumption	45	39	6	86.6
Smokers	34	29	5	85.2
Veg	15	2	13	13.3
Non-veg	185	102	83	55.1

Table 8
Distribution of *H. pylori* virulence and outer membrane protein (OMP) genes in relation to endoscopic findings among PCR (*ure B*) positive cases

Endoscopic findings	**PCR (*ureA*& *ureB*) positive *H. pylori***	***16srRNA***	***cagE***	***vacAm2***	***iceA1***	***glm***	***Oip A***	***hofC***	***frp B***	***hopQ***	***homA***
Gastric ulcer	18	16	10	7	2	10	7	14	15	0	6
Gastritis	55	35	17	14	5	31	23	31	35	6	10
Duodenitis	4	3	3	2	0	3	3	3	3	1	0
PHT gastropathy	5	2	0	0	0	1		1	2	1	0
GERD/LAX LES	3	4	1	1	0	2	4	3	4	1	1
Oesophageal varices	7	4	2	2	0	2	3	2	4	0	0
Gastric cancer	1	1	1	1	0	1	1	1	1	0	1
Normal	12	8	5	4	1	6	4	7	9	1	2

Table 9
Diagnostic performance of RUT and culture for the detection of *H. pylori*

Diagnostic test	**RUT**	**Culture**
Sensitivity	70.4%	15.2%
Specificity	65.2%	100%
Positive predictive value	69.1%	100%
Negative predictive value	66.6%	51.6%
Accuracy	68%	55.5%