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REVIEW

Diagnostic methods for *Helicobacter pylori* infection: ideals, options, and limitations

Parisa Sabbagh[1] · Mousa Mohammadnia-Afrouzi[1,2] · Mostafa Javanian[1] · Arefeh Babazadeh[1] · Veerendra Koppolu[3] · VeneelaKrishna Rekha Vasigala[4] · Hamid Reza Nouri[2] · Soheil Ebrahimpour[1]

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Abstract

Helicobacter pylori (*H. pylori*) resides in the stomach, colonizes gastric epithelium, and causes several digestive system diseases. Several diagnostic methods utilizing invasive or non-invasive techniques with varying levels of sensitivity and specificity are developed to detect *H. pylori* infection. Selection of one or more diagnostic tests will depend on the clinical conditions, the experience of the clinician, cost, sensitivity, and specificity. Invasive methods require endoscopy with biopsies of gastric tissues for the histology, culture, and rapid urease test. Among non-invasive tests, urea breath test and fecal antigen tests are a quick diagnostic procedure with comparable accuracy to biopsy-based techniques and are methods of choice in the test and treatment setting. Other techniques such as serological methods to detect immunoglobulin G antibodies to *H. pylori* can show high accuracy as other non-invasive and invasive biopsies, but do not differentiate between current or past *H. pylori* infections. Polymerase chain reaction (PCR) is an emerging option that can be categorized as invasive and non-invasive tests. PCR method is beneficial to detect *H. pylori* from gastric biopsies without the need for the cultures. There is no other chronic gastrointestinal infection such as *H. pylori* with a set of comparable diagnostic methodologies. Despite the availability of multiple diagnostic methods, it remains unclear on the choice of any one method as the gold standard for detecting *H. pylori* infection, especially in epidemiological studies. In this work, we review the principal diagnostic methods used to detect *H. pylori* infection and their advantages and disadvantages, and applications in clinical practice.

Keywords *Helicobacter pylori* · Characteristics of infection · Diagnosis · Invasive tests · Non-invasive tests

Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that colonizes in gastric epithelium [1–5]. First, this bacterium was misrecognized as *Pseudomonas* spp. even though [6], during a clinical research project, Barry Marshall and Robin Warren discovered [7] it as *Campylobacter pyloridis*, which was later changed to *H. pylori* [8, 9].

H. pylori represent one of the most common bacterial infection in humans, which infected about half of the world's population [10, 11]. Often, *H. pylori* infection occurs in childhood and continue throughout life when proper treatment is not provided [11]. In this regard, some studies suggested that the infected mothers are the major source of this infection of their kids, through contact with the contaminated stomach juice from the mother's mouth [12].

Research indicates that *H. pylori* spread from East Africa about 58,000 years ago and subsequently developed into many strains with varying degrees of pathogenicity [13]. Generally, the prevalence of the bacterium infection varies according to age, region, race, and socioeconomic statuses. The prevalence of *H. pylori* infection in the developing countries is 50.8%, whereas the prevalence is 34.7% in the developed countries [14]. Many documents show that humans are

✉ Soheil Ebrahimpour
drsoheil1503@yahoo.com

[1] Infectious Diseases and Tropical Medicine Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Islamic Republic of Iran

[2] Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Islamic Republic of Iran

[3] Scientist Biopharmaceutical Development Medimmune, Gaithersburg, MD 20878, USA

[4] Rangaraya Medical College, NTR University of Health Sciences, Kakinada, India

the primary reservoir of *H. pylori*. The bacterium can also survive in dental plaques and saliva of a human [15]. The bacterial transmission can be through oral-oral, feco-oral, and gastro-oral routes [9, 16]. This bacterium has been found in water, as it is proven that the infection is transmitted through the water [17]. It is also reported that these bacteria can survive in the stomach of animals such as sheep and cats and milk of some others [18].

Effective treatment of the *H. pylori* infection is possible through antimicrobial therapy, prescribed to the susceptible patients. In order to treat the disease appropriately, suitable diagnostic procedures are necessary. Therefore, in this review, we aim to study the invasive and non-invasive diagnostic tests for *H. pylori* infection.

Pathogenicity of *H. pylori*

H. pylori is a gram-negative, helical bacillus, flagellated, slow-growing, microaerophilic, and fastidious bacterium [9, 19–21]. The bacterium isolated from the gastric mucosa is often seen as a spiral and curved in the culture medium [22].

H. pylori can survive in the gastric tissue, due to the presence of urease, its mobility, and ability to connect to the gastric epithelium [23, 24]. Some of the factors that provide an advantage for the successful bacterial colonization in the gastric epithelium include the shape of this bacterium, polar-sheathed flagella, mobility, chemotaxis, adherence, and persistence. Pathogenic factors such as cytotoxin-associated gene A (CagA), vacuolating cytotoxin A (VacA), outer inflammatory protein A (OipA), duodenal ulcer promoting gene a (dupA), sialic acid–binding adhesin (SabA), and blood group antigen–binding adhesin (BabA) are associated with increased virulence of *H pylori* [25–28]. This bacterium, with the help of the urease enzyme, breaks down urea to carbon dioxide and ammonia [29], through which it can neutralize the gastric acid, penetrate, and colonize in the gastric epithelium [30].

Immunopathogenesis of *H. pylori*–induced infection in gastric mucosa

The immune response towards pathogenic agents can be divided into innate and adaptive responses. *H. pylori* is an activator of both the innate and adaptive immune responses. The colonization of *H. pylori* in gastric mucosa triggers innate host defense mechanisms, including NOD1, TLR2, TLR4, TLR5, and TLR9, thus stimulating the expression of pro-inflammatory and antimicrobial peptides including defensins and cathelicidins by gastric epithelial cells as well as dendritic cells (DCs), neutrophils, and macrophages [31]. DCs and macrophages are activated and produce cytokines, including IL-6, IL-10, IL-12, IL-18, and IL-8 in inflamed mucosa of *H. pylori*–infected individuals [32].

The Th1/Th2 cell paradigm is an important concept to understand mucosal adaptive immunity and inflammation induced by *H. pylori*. Cytokine profiles indicate a Th1-predominant host immune response in the gastric mucosa, illustrated by IFN-γ production, which is associated with IL-12, IL-18, and TNF-α pro-inflammatory cytokines expression by DCs and macrophages [33]. Many studies support the involvement of Th17 cells in *H. pylori* infection by production of IL-17. IL-17 induces expression of IL-8, as a chemokine with the strong neutrophil chemoattractive property [34]. *H. pylori* can also elicit a strong specific systemic and mucosal IgG and IgA antibody responses. However, the humoral immune response is not protective in this infection. Many reports show that *H. pylori* induces regulatory T cell (Treg) responses to avoid both innate and adaptive immune defenses and maintain prolonged colonization of the gastric mucosa (Fig. 1).

Diseases and clinical manifestation

H. pylori is the cause of some gastric disorders (peptic ulcer disease (PUD), gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [7, 35, 36]), which are the results of an interaction between bacterial virulence factors, host, and environmental factors. Several extra-gastric manifestations have been reported to be linked to *H. pylori* infection such as neurological, dermatological, hematologic, ocular, cardiovascular, metabolic, and allergic diseases [19]. Most of *H. pylori* infections usually are without clinical manifestation [37]. However, signs and symptoms associated with the disease are primarily due to gastric or peptic ulcer illness or duodenal inflammation. Furthermore, other symptoms such as abdominal pain, nausea, and vomiting may be attributed to other gastrointestinal diseases [38].

Peptic ulcer disease

Peptic ulcers are usually found in the stomach or proximal duodenum but can also be found in the esophagus or Meckel's diverticulum. Peptic ulcer refers to the acid peptic injury of the digestive tract, resulting in mucosal break reaching the submucosa [39]. The lifetime prevalence of this disease in the general population has been estimated to be about 5–10% and incidence 0.1–0.3% per year. Over the past two centuries, PUD has been a major threat to the world's population [39]. Some studies showed that PUD is at least fourfold higher in *H. pylori*–infected individuals than in non-infected individuals [40]. *H. pylori* along with non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin is the main cause of gastric and duodenal ulcers (imbalance of

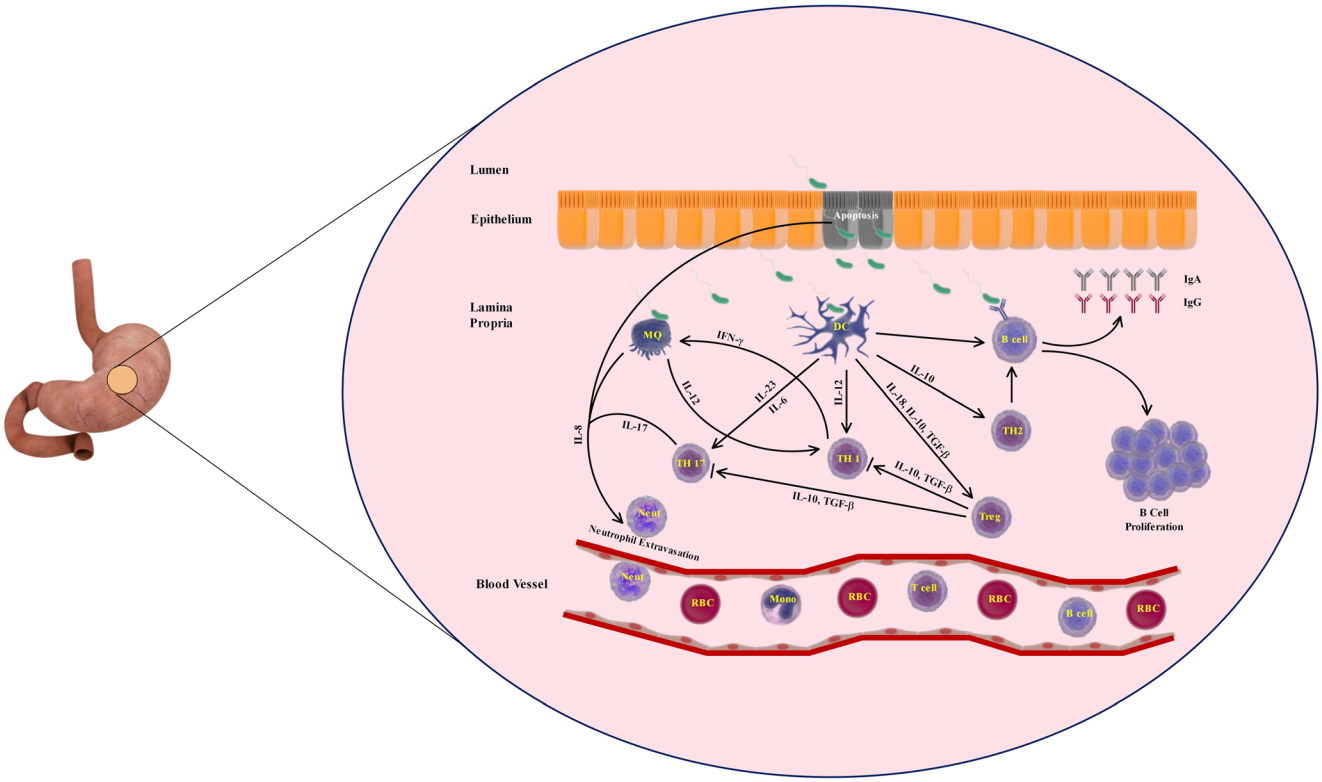

Fig. 1 Immunopathogenesis of *H. pylori* in gastric mucosa. *H. pylori* colonizes in gastric epithelium using urease. Binding and entering of *H. pylori* to epithelial cells results in the production of IL-8 and activation of the innate and adaptive immune systems as well as apoptosis of epithelial cells. Dendritic cells capture, process, and present bacterial antigens to the T cells in immunogenic or tolerogenic forms. Immunogenic DCs induce Th1/Th17 differentiation by producing IL-12, IL-6, and IL-23. IL-17 produced by Th17 cells targets innate immune cells and epithelial cells, to produce IL-8 (CXCL8), which results in neutrophil production and recruitment. Tolerogenic DCs provide a high level of IL-18 as well as TGF- /IL-10 for Treg differentiation. Macrophages are important activators of immune response to *H. pylori*, along with immunogenic DCs, by producing IL-12. IL-12 stimulates Th1 cells, resulting in production of IFN-γ, a key cytokine for activation of macrophages. *H. pylori* induces enhanced expression of indoleamine 2,3-dioxygenase (IDO) which results in diminished IFN- production by Th1 cells and differentiation of Th2 cells. Colonization of *H. pylori* elicits the production of IgA and IgG antibodies, but it seems that antibodies are not essential for protection

aggressive gastric luminescence and pepsin and protective mucosal barrier function) [41].

Gastric adenocarcinoma

Worldwide, gastric cancer (GC) is the fifth most commonly diagnosed malignancy and the fourth leading cause of cancer-related deaths per year [41, 42]. On the other hand *H. pylori* has been implicated as the strongest risk factor in the pathogenesis of gastric adenocarcinoma [40, 43]; thus, it has been classified as a class I carcinogen by the World Health Organization (WHO) [44]. GC is triggered by a multifactorial process, beginning with *H. pylori*–induced chronic gastritis, which results in atrophic gastritis, intestinal metaplasia, dysplasia, and eventually gastric cancer [45, 46].

Mucosa-associated lymphoid tissue lymphoma

The molecular pathogenesis of MALT lymphoma is incompletely understood, but it seems to include strain-specific *H. pylori* factors as well as host genetic factors, such as polymorphisms in inflammatory cytokine promoters such as TNF and IL-1β [47]. The mentioned lymphoma is an indolent extranodal marginal zone B cell lymphoma, originating in acquiring MALT that is induced in mucosal barriers as part of a normal adaptive immune response to a chronic immunoinflammatory stimulus, most notably chronic infections by *H. pylori* [48]. In addition to the disease caused by this bacterium, it should also be noted that the treatment of *H. pylori* infection has improved considerably since the early experiments in 1987. Currently, three or four drug regimens used for 7 to 14 days lead to the cure of the infection in 85 to 95% of the patients [49].

Evidence shows that the eradication of *H. pylori* or vaccination (vaccines were composed of different antigens and adjuvants applied by different routes and delivery systems) [50] may reduce the risk of ulcers and gastric cancer. Of course, in the case of vaccines, it should be reminded that some immunization strategies were tested in humans, but they almost never reached sterilizing immunity [51]. It should be noted

Table 1 Evaluation of advantages and disadvantages regarding to diagnostic tests of *Helicobacter pylori*

Test	Advantages	Disadvantages
Urea breath test	Simple; non-invasive; safely; high sensitivity, specificity and accuracy; detection of eradication of infection	False-negative findings, as a result of bleeding, use of antibiotics and proton-pump inhibitors; having a low accuracy in atrophic gastritis, intestinal metaplasia, gastric cancer cases
Stool antigen test	Fast, simple, and inexpensive	False-negative results occur in the low bacterial load, recent use of the antibiotics, bismuth, and proton-pump inhibitors; the unwillingness of patients and doctors to do this test; problems of keeping and carrying samples
Serology	Cost-effectiveness cheapest; widely available; applicable to diagnosis for patients treated with antibiotics and PPIs	Failure to distinguish between acute infection and previous contact; unusable in confirming cure after therapy
Endoscopy	Acquisition of gastric biopsies and leads to a definitive diagnosis of infection	Time-consuming, and requires so much skill
Rapid urease test	Fast, inexpensive, and simple	Sensitivity and specificity are lower in gastric ulcer bleeding and intestinal metaplasia
Histology	The gold standard in the direct diagnosis of *H. pylori* in mucosa and investigation of the eradication condition	Observer dependency; time-consuming; needing a lot of skill, and high cost
Culture	Determination of patterns of antimicrobial resistance and sensitivity	Expensive, complicated, and time-consuming test
Polymerase chain reaction	High sensitivity, specificity; no need for specific transportation	Expensive; need special skills; false-positive results due to detect DNA pieces of dead bacteria

that scientists argue that the co-evolution of *H. pylori* with the human population might have positive effects and protect children from diarrhea and asthma [52].

Diagnostic methods

Diagnostic methods have also been expanded with the evolution of *H. pylori* infection treatments. In spite of this, the standard methods applicable, especially in the population at risk, is still missing [11].

These methods should fulfill the common standards of clinical diagnostics like accuracy, sensitivity, and specificity. The methods should also be applicable in developing areas where hygiene standards and medical supports are poor [11].

The diagnostic tests are separated into two divisions: invasive tests (endoscopy, histology, culture, and molecular methods) and non-invasive (urea breath test, fecal antigens, serological and molecular tests) for *H. pylori* infection (Table 1).

Non-invasive methods

These methods are based on the presence of bacterial enzymes, antigens, antibodies, or DNA sequences [53]. They include ^{13}C or ^{14}C urea breath test, stool antigen test (SAT), serology, and molecular methods [54].

Urea breath test

Urea breath test (UBT) is regarded as a gold standard non-invasive method for *H. pylori* diagnosis [55, 56]. This non-invasive test has high sensitivity, specificity, and accuracy [57, 58]. UBT has been used for about 30 years and is still the most popular, accurate, and common non-invasive test for the diagnosis of *H. pylori* infection [10].

This test can detect the infection indirectly by measuring the activity of bacterial urease produced by *H. pylori* in the stomach [11]. The test exploits the hydrolysis of orally administered urea by the *H. pylori*. An isotopically (^{13}C or ^{14}C) labeled urea is hydrolyzed into ammonia and carbon dioxide, which diffuses directly into the blood and excreted out through the lungs. The released carbon dioxide can be measured [10, 59, 60]. A ^{13}C-labeled urea is preferred over ^{14}C since ^{13}C is stable and nonradioactive. An isotype ratio mass spectrometer is typically used to measure the release of ^{13}C-enriched carbon dioxide from breath samples. However, this technique is expensive and requires a lot of skill. Recently, other less expensive methods like infrared spectroscopy and laser-assisted ratio analysis are developed as valid alternatives. The increase of ^{13}C-labeled carbon dioxide in breath samples (taken before and about 30 min after drinking the test solution) indicates the bacterial urease activity. The intensity of the ^{13}C signal in breath indicates the density of the microorganism colonization [54].

The ^{13}C UBT has shown a variable level of accuracy in the pediatric population. Some meta-analyses confirmed that the

^{13}C UBT is less accurate for the diagnosis of *H. pylori* infection in young children [60].

Several factors including the patient's condition, bacterium, and the test itself can affect the results of the UBT [61]. Nonetheless, the urea breath test is widely available because breath samples are easy to collect for rapid testing [62]. UBT is useful for epidemiological studies and for assessing the effectiveness of eradication therapy [63]. This method has advantages such as non-invasive, safe, accurate, and with a sensitivity of 95.9% and a specificity of 95.7% [60, 64].

Factors can cause the false-negative test results; the patients have been received proton-pump inhibitors (PPIs) 2 weeks and antibiotic 4 weeks before this exam [65]. The bleeding also affects the diagnostic accuracy of UBT, and thus, UBT should be performed after recovery from bleeding [66]. Corpus-predominant gastritis can cause false-negative UBT results [67]. It should also be noted that although it is rarely true, the urease production of other pathogens in the stomach (such as *H. heilmannii*) which might lead to false-positive results [49].

It has been shown that UBT can detect an ongoing from past infections; hence, it can identify the eradication progress after treatment [68]. Also, according to various existing protocols, the accuracy of UBT test results depends on the amount of urea applied, sampling time, and the set point of the cutoff value [69].

Stool antigen test

In infected individuals, *H. pylori* sticks to the gastric epithelial wall and is excreted in the feces. This test is a direct test of initial infection that results in the superiority of serologic tests [70]. The test is based on the detection of *H. pylori* antigens in the stool. There are two types of SATs used for *H. pylori* detection: enzyme immunoassay (EIA)– and immunochromatography assay (ICA)–based methods, using either polyclonal antibodies or monoclonal antibodies [30, 49]. Monoclonal antibody–based tests show better results compared to polyclonal-based tests mainly because of the difficulty in obtaining polyclonal antibodies of consistent quality every time. EIA-based tests provide more accurate and reliable results than ICA-based tests although both tests can be performed with monoclonal antibodies [30, 71].

The systematic review and meta-analysis conducted by Leal et al. [72] established that stool enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies is an efficient non-invasive test for the diagnosis *of H. pylori* infection in children [62, 72, 73]. The sensitivity and specificity of this method are 94% and 97%, respectively [74]. Stool samples need to be refrigerated when stored before analysis; otherwise [75], the sensitivity of this test will be critically reduced [76].

The accuracy of this test can be influenced by some gastrointestinal problems, PPIs, antibiotics, and *N*-acetylcysteine (NAC) treatments, and bleeding ulcers [49].

Similar to the urea breath test, false-negative results occur when the bacterial load is relatively low and due to the use of the antibiotics, bismuth, and proton-pump inhibitors [77]. However, SAT may not require fasting and recently monoclonal antibodies unaffected by PPI are also developed. These advantages make SAT a better test compared to UBT. Many studies showed that SATs could distinguish actively infected from treated patients as well as to assess the effectiveness of *H. pylori* eradication [78]. However, to confirm the definitive eradication, it is advisable to wait until about 3 months after the end of the treatment [70]. SAT is a fast, simple, and inexpensive test [70, 79] and is also a useful tool for epidemiological studies and screening programs [80, 81].

The disadvantage of the SAT is the lack of enthusiasm for patients in the stool sample preparation. In addition, storage and handling of stool samples can also impact the assay results. For example, the stool should be frozen to keep the antigen intact when samples are not tested in a short period of time. The storage may become a problem in areas where freezing is not available. Selection of a cutoff point is an important factor for specificity and sensitivity of detection and may vary among the different population. Thus, a local validation of the test at a particular location is needed for better results [54].

Serology

In this method, antibodies against *H. pylori* are detected by ELISA, immunoblotting, and enzyme immunoassays (EIA) [30]. Although more tests for IgA, IgG, and IgM antibodies are performed, only the IgG antibody test is reliable. These tests involve the use of serum, saliva, or urine; however, the use of whole blood is still a controversial topic [82]. This method has a sensitivity and specificity of 76–84% and 79–90%, respectively [30].

Based on findings from many studies, such tests have a high negative predictive value (NPV). The ability of this test to detect active infections depends on the patient's age, clinical conditions of infection, the choice of the antigen used for antibody preparation in ELISA kit, and the prevalence of infection [54, 83]. In patients treated with colloidal bismuth, antibiotics, and PPIs, if it is not possible to discontinue the mentioned medications, the IgG serologic tests may be beneficial since serological methods are less likely confounded by suppression of *H. pylori* by these treatments [83]. Therefore, in particular, in clinical scenarios such as gastrointestinal bleeding, gastric carcinoma, MALT lymphoma, and atrophic gastritis, the serological method is the efficient diagnostic method. Serological methods offer other advantages such as cost-effectiveness, wider availability, simplicity, and thus are

commonly used in most studies of *H. pylori* epidemiological studies [84, 85]. Besides, a serological test for the evaluation of *H. pylori* infection in children is also found to be very helpful [85]. Another benefit of the serology test is that the accuracy of them is not affected by ulcer bleeding and gastric atrophy, which cause false-negative results in other invasive or non-invasive experiments [10]. However, if UBT and SAT are available, it will not be used for initial diagnosis because it only represents the previous exposure [54].

A urine-based ELISA is also found to be inexpensive, convenient serological method to detect anti-*H. pylori* antibodies in adults. Serological test from urine samples is much easier than serum samples because it does not require skills in sample collection and does not need preparation steps like centrifugation. However, the urine-based ELISA method is found to be unacceptable for children due to its low specificity (76.4%). The low specificity may be due to low concentrations of anti-*H. pylori* antibodies in the urine.

The major disadvantage of the serological approach is its inability to distinguish between the current infection and the previous exposure leading to misinterpretation. The IgG antibodies can be found even for the months after treatment and thus provide a positive result, even after the bacterial clearance [83, 84]. Therefore, it is usually not useful in confirming cure after antimicrobial therapy, but it is useful for epidemiological examinations [86]. False negatives can also result during early infection since the antibody levels are not sufficiently elevated during the early infection.

Invasive methods

These methods include endoscopy and gastric biopsy followed by either rapid urease test (RUT) or histology or culture, or molecular methods on biopsy samples. Each individual invasive test offers a specific clinical advantage. Rapid urease test (RUT) is the quickest test the provides an opportunity to start the treatment immediately. The histological examination provides a comprehensive assessment of gastric mucosa and thus help in short-term and long-term management strategies. Cultures have the highest specificity and are particularly useful in antibiotic susceptibility testing prior to choosing an appropriate eradication therapy. PCR is an emerging option to detect the bacterium without needing for cultures. The specific details and methodology of each technique are described below.

Endoscopy

A typical endoscopy exam is performed to detect *H. pylori*–related diseases. Endoscopy is the method for obtaining biopsies from the gastric mucosa that can be used in further studies on other invasive methods [87, 88]. Also, the endoscopy offers the precise and clear image of gastric mucosa, but it may not have better results than other diagnostic tests [89]. The major disadvantages of this procedure include the time-consuming process and require a lot of skill and experience [83].

Rapid urease test

Rapid urease test (RUT) is the popular invasive and cost-effective test for the detection of *H. pylori* infection [90]. If the biopsy is done, then the rapid urease test from gastric biopsies is the first choice [91]. This test is based on the production of urease enzyme by *H. pylori* bacteria and the presence of this enzyme in the gastric mucosa [92]. After the biopsy, the specimen is transferred to the solution comprising urea and a pH indicator [91, 93]. If *H. pylori* exists, urease will convert the urea into ammonia and CO_2, which leads to change in color of the indicator due to an increase in pH [30]. In order to get the best results, biopsy specimens are taken from the gastric antrum and corpus [92].

The RUT is a very fast, inexpensive, reliable, and simple technique that provides the results in a few hours. An accurate detection would depend on the bacterial density in the biopsy samples [92, 94, 95]. It is worth noting that the sensitivity and specificity of this method are more than 90% [30, 91].

False-positive results of this test are possible in certain conditions [92, 96]. Several organisms such as *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus mirabilis*, *Enterobacter cloacae*, and *Citrobacter freundii*, isolated from the oral cavity and/ or stomach, also present urease activity and give false-positive results. Treatment with proton-pump inhibitors, antibiotics, and bismuth compounds [93] may cause false-negative results. As these agents can prevent the production of urease by *H. pylori* [92]. Also, we find similar findings in the cases with achlorhydria [95]. Besides, the sensitivity and specificity of the test are significantly lower in the cases of gastric ulcer bleeding and also in patients with intestinal metaplasia [54, 92, 96]. Therefore, in these cases where the RUT result is negative, a confirmation is needed by using appropriate alternate tests.

Histology

Histology is reviewed and considered as the gold standard in the direct diagnosis of *H. pylori* in mucus [83, 88]. This method is faster and, of course, more expensive than RUT and give essential data about the different types of gastritis (especially chronic form), atrophy, dysplasia, metaplasia, and malignant neoplasms [30]. The accuracy of the histopathological diagnosis of *H. pylori* is dependent on the number and location of

the collected biopsy materials [97]. The diagnosis of *H. pylori* by this method can be made with only one biopsy sample taken from a suitable location, but multiple biopsies are recommended for high diagnostic accuracy and sensitivity [98, 99]. Due to the various distributions of these bacteria in the mucus layers, tissue samples should be taken from several areas of the stomach [100]. For this reason, the biopsy is better to be collected from both antrum and corpus [90, 101, 102]. Usually, two different stain methods are used for tissue samples from biopsy; hematoxylin and eosin (H&E) for assessment of inflammatory cells, and Giemsa for discernment of pathogen. Giemsa stain is commonly used in medical diagnostic laboratories [30, 54]. But when the results are unclear, other diagnostic techniques like toluidine blue, acridine orange, genta, the Romanowski, and the McMullen are beneficial [103, 104]. However, many of these methods are purely research-related. Some studies showed that histology had the higher sensitivity and specificity than the UBT and the RUT for the *H. pylori* diagnosis [105] so that the mentioned sensitivity and specificity were 80–95% and 99–100%, respectively [30].

Many factors affect the diagnostic accuracy of histological examination, like pathologist and gastroenterologist potentials and experiences, respectively, and in sampling and observation of biopsy specimens, staining techniques; the used medications are PPIs, antibiotics, and peptic ulcers bleeding [54, 83]. Also, the presence of other bacterial species, but with structural similarity to *Helicobacter*, can have adverse and dramatic effects on the results of this test [97, 98].

Based on the abovementioned, the several biggest weaknesses of this test are the observer dependency, the relative-to-the-long-time-to-get results, the need for specialized skills for relatively high performance, and the high cost [106].

Culture

The culture of *H. pylori* is performed on the gastric biopsy samples to confirm the *H. pylori* infection and is performed only in specialized laboratories [107]. Culturing of gastric biopsy samples to detect *H. pylori* is not a routine method for detecting *H. pylori* [108, 109]. Bacterial culture is carried out mainly for scientific research and when the prior treatments have failed to detect an appropriate bacterium. It is recommended that the mentioned test should be performed before the next treatment line to determine the microbial susceptibility [110, 111].

Although the culture is an expensive, complicated, and time-consuming test for *H. pylori* detection, an antibiotic susceptibility test of *H. pylori* by culture is a useful clinical practice for accurate detection. Besides, culture allows the isolation of *H. pylori* for phenotypic and genotypic studies [10, 84].

H. pylori needs selective media and microaerobic conditions (80–90% N_2, 5–12% CO_2, 5–10% O_2) for growth [112, 113] and requires an incubation for 5–7 days at 37 °C [30, 114]. Several types of media can be used for *H. pylori* culture, including selective and non-selective agars (*H. pylori* agar, the Wang media, the Wilkins-Chalgren, brain-heart infusion (BHI), trypticase agar bases, Columbia and blood agar) [113, 114]. Antibiotics are used in the culture media to prevent the growth of other types of bacteria [11].

H. pylori should be cultured quickly after the biopsy [112]. A biopsy can be preserved in the transport medium (*Portagerm pylori* or Stuart) for up to 24 h at 4 °C and also isolates of *H. pylori* can be stored frozen at − 80 °C [113, 114]. GESA transport medium is a new medium that can store gastric biopsy specimens at 4 °C for up to10 days and provide a quantifiable recovery rate of *H. pylori* [115]. This method has a sensitivity and specificity of 70–80% and 100%, respectively [30, 116].

The culture's results are affected by the skill and experience of the microbiologist, sample quality, exposure to the aerobic environment, and the use of the transport medium [113]. Also, other factors may affect the diagnostic accuracy of culture examination, like the decreased density of these pathogens in atrophic gastritis, alcohol drinking, bleeding, and the use of antibiotics, H2 receptor antagonists, and PPIs [117, 118].

Polymerase chain reaction

Over the past few decades, molecular detection has dramatically changed the clinical management of many infectious diseases [119]. Polymerase chain reaction (PCR) is one of the best molecular methods used in a wide range of clinical applications including broad-spectrum infection detection, evaluation of emerging infections, genotypic bacterial identification, antibiotic resistance, and in epidemiological studies [120, 121]. PCR-based detection of *H. pylori* could be classified as both invasive and non-invasive [119]. Samples are frequently used in this method including gastric juice and biopsy, saliva, and feces [120, 122, 123].

This method has high sensitivity and specificity (> 95%) [122, 124, 125]. PCR offers a simple, accurate, fast, automatic, and high efficiency of the *H. pylori* detection [120, 123]. In comparison to other common tests, PCR is more accurate to detect *H. pylori* in patients with bleeding [122, 125]. An accurate primer design and a proper gene selection are critical for a successful PCR reaction [11]. *H. pylori* genes such as vacA, cagA, UreA, GlmM, HSP60, 16SrRNA, 23SrRNA, and ureC can be used to amplify the *H. pylori* genome [30, 116]. Two or more target genes are amplified to increase the specificity of *H. pylori* diagnosis and to reduce the false-

positive rates (particularly in specimens other than gastric biopsy samples) [122, 123, 125].

Bacterial resistance to antibiotics has become an increasingly difficult challenge for the health care community [126]. This problem is considered an essential challenge in medicine and microbiology. Alarmingly, the World Health Organization (WHO) recently issued the following statement: "the world is heading towards a post-antibiotic era in which common infections will once again start to kill" [127]. Molecular techniques like PCR is an appropriate methodology for pathogen detection can detect antibiotic resistance mutations and would help us in choosing an appropriate treatment strategy [128].

PCR can also be used to identify *H. pylori* in environmental samples for epidemiological researches. The relatively high prevalence of this pathogen in drinking water has been confirmed by PCR [129]. Moreover, the higher detection rate of *H. pylori* in unwashed vegetables suggests that the hygienic consumption of vegetables and complete washing of such foods is beneficial in reducing the infection with *H. pylori* [130]. The major disadvantages of PCR are that the technique is expensive and requires a lot of skill and experience. Also, false-positive results can be found in PCR due to its detection of DNA fragments from the killed bacteria [120, 121, 131].

Conclusion

H. pylori is a common bacterial infection of stomach epithelial tissue that causes severe anomalies, including chronic gastritis and gastric cancer. An accurate diagnosis of *H. pylori* infection is a critical first step in the successful treatment of this infection. Several methodologies are developed for *H. pylori* diagnosis and the choice of a particular depends on several factors such as clinical situations, availability of the appropriate technology to run the method at a clinical setting, and the accuracy, sensitivity, and specificity of the test. In this regard, our view is that we must continue our efforts to achieve more appropriate and reliable diagnostic tests.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval This study received the approval from the Babol University of Medical Science, Ethical Committee.

Informed consent There is no informed consent for this review article.

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