

Review Article

Diagnosis of *Helicobacter pylori* Using Invasive and Noninvasive Approaches

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Helicobacter pylori (*H. pylori*) as gram-negative and spiral microorganism is responsible for colonization in the gastric microniche for more than 50% of world population. Recent studies have shown a critical role of *H. pylori* in the development of peptic ulcers, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. Over the past decade, there has been a sharp interest to use noninvasive tests in diagnosis of the *H. pylori* infection. During the years after discovery by Marshall and Warren, it has been frequently declared that the rapid urease test (RUT) is one of the cheapest and rapid diagnostic approaches used in detecting the infection. Although the specificity and sensitivity are durable for this test, clinical experiences had shown that the ideal results are only achieved only if we take biopsies from both corpus and antrum at the same time. Given the diagnosis of the *H. pylori* in clinical samples, gastroenterologists are facing a long list of various molecular and nonmolecular tests. We need more in-depth researches and investigations to correctly generalize rapid and accurate molecular tests determining both bacterial identity and antibiotic resistance profile.

1. Introduction

Following the groundbreaking discovery of *Helicobacter pylori* (*H. pylori*) in 1983, a challenging era in the management of gastroduodenal diseases has been initiated [1]. This usually chronic infection is thought to play an inevitable role in peptic ulcer diseases and gastric adenocarcinoma. *H. pylori*, as the most commonly prevalent and recognized bacterium, is carried by more than half of the world population [2–6]. Once colonized, *H. pylori* induces a persistent, but superficial, inflammation, resulting in duodenal ulcer, gastric ulcer, and gastric cancer [7–11]. As predicted, many recent studies have confirmed a critical role of *H. pylori* in the development of peptic ulcers, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer [12]. Given the causative role of *H. pylori* in duodenal ulcer and gastric cancer, clinicians and microbiologists are eager to find the best diagnostic approach [13–18]. Currently, there are various diagnostic methods used for *H. pylori* infection in different subjects (children and adults), but the only methods with both high sensitivity and high specificity remain useful and

recommendable. In other words, precise detection of this bacterium in different clinical specimens (e.g., urine, stool, saliva, biopsy, and gastric juice) attributed with successful therapeutic practice will be listed in hot topic researches interest globally [19–22]. According to a traditional classification, *H. pylori* infection can be diagnosed by noninvasive tests such as *H. pylori* antigen in stool specimen, UBT (Urea Breath Test), serology, and invasive tests such as PCR (polymerase chain reaction), culture, and histology which require endoscopic surgery and biopsy specimens [23–25]. Invasive tests (e.g., Histological examination, culture, and polymerase chain reaction) require endoscopy and noninvasive techniques (e.g., serology and urea breath) are independent of endoscopic surgery. Nonetheless, for having the best management of *H. pylori*-related diseases, we need to specific and accurate diagnosis, especially for treatment courses (pretreatment and posttreatment of *H. pylori* infection). In fact, the selection of choice method is highly dependent on the availability and feasibility of many circumstances [26]. To now, many tests had been invented for diagnosis of *H. pylori*; however, each one has certain advantages and disadvantages

[26–29]. In current article, we will review the recent advances of invasive and noninvasive methods suggested in diagnosis of *H. pylori*. Moreover, application and priorities of those methods, especially in evaluating the infection following the therapeutic regimen, are secondary goals of our paper.

2. Endoscopy: A Pivotal Approach in Diagnosis of *H. pylori*

Although various methods had been attempted to accurately detect the *H. pylori* infection, noninvasive methods were preferred by gastroenterologists for many reasons [30–32]. The whole advantages and disadvantages of invasive and noninvasive methods are listed in detail (see Table 1). In a short sentence, the main rationale for choosing the noninvasive methods is to avoid endoscopy. Relatively high numbers of guidelines were recommending the noninvasive tests as first choice [33–35]. What should not be forgotten is that the endoscopy surgery is an unpleasant and uncomfortable approach for investigating the *H. pylori* in dyspeptic patients [36, 37]. Additionally, there are other drawbacks which limit using the invasive methods such as endoscopy; (i) patients need for 1–3 days off for this surgery, (ii) high cost for disposable forceps and other stuffs, and (iii) high risk of contamination by some viruses such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV) [38, 39]. Of course, valid evidence indicating transmission of HIV and HCV among the subjects for endoscopy is not well-documented, but many patients declined this surgery just for this unpleasant probability released in media over the last years [40, 41]. Disparate distribution (patchy) of *H. pylori* in stomach is causing the bias in sampling (false negative) [1]. Indeed, taking a biopsy specimen (maximally 3–4 mm²) cannot guarantee the existence of *H. pylori*-colonized in stomach environment (500–1000 mm² in different persons). A solution would be to increase the number of taken gastric biopsies, but for ethical limitations, gastroenterologists are highly prohibited to take 6 or more biopsies from a patient. Lastly, endoscopy is an impossible procedure for subjects such as pregnant women, children, and elderly patients [26, 42]. There are two major approaches to noninvasive tests to diagnosis the *H. pylori* infection: UBT and serological examinations [26, 43]. The main superiority of these methods is their easy applications in epidemiological studies. Furthermore, their easy application is affecting their high popularity in studies investigating the eradication rate following the antibiotic therapy [42, 44, 45]. In next paragraphs, we discuss each method separately to see their priorities and limitations in clinical settings.

3. Urea Breath Test

The first report of the application of UBT was about 60 years ago by Kornberg et al. investigating the intravenous injection of ¹⁴C urea into the cat and determining the amount of decreased ¹⁴C-CO₂ in animal breath [46, 47]. Their finding was due to the colonization of *Helicobacter felis* in the cat. When ingesting ¹³C- or ¹⁴C-labeled exposed to the bacterial

urease in *H. pylori* positive patient, hydrolyzation results in the production of CO₂ in the stomach. Thereafter, labeled CO₂ enters into the bloodstream and is exhaled in the lung. Consequently, the amount of trapped labeled CO₂ will be measurable in the exhalation [48]. As mentioned above, the principle of this test is based on the intrinsic ability of *H. pylori* to break down absorbed ¹³C or ¹⁴C-labeled urea into CO₂ in acidic gastric condition. If one is colonized actively with *H. pylori*, the urea is metabolized to the ammonia and labeled bicarbonate [¹⁴C-CO₂]. Thereafter, labeled bicarbonate is transferred to the lung and produces labeled carbon dioxide. This product is detectable by the machine to confirm the existence of the infection. Because of high sensitivity and specificity, UBT is a very attractive method to measure the *H. pylori* active infection by microbiologists and clinicians. At least for asymptomatic subjects, the UBT is a gold standard method [49, 50]. Another preference of UBT is that the method is free of sampling errors (lack of endoscopic surgery). This superiority made it very popular for clinicians to confirm bacterial eradication, especially in asymptomatic, elderly, and pediatric subjects. Clinicians need to wait 1–2 months for performing UBT to confirm successful bacterial eradication. As noted, the false-positive result is a minor problem with UBT, and clinicians need to take care of other urease-producing organisms which may be able to change the result. Overall, the specificity and sensitivity of the UBT are mostly more than 95%. Although these high rates for both sensitivity and specificity are an advantage for this test, lack of data on antibiotic resistance and further analysis is the main limiting feature of this popular method to detect active *H. pylori* infection [51, 52]. A major consideration for this test is about its radiation. So far, decreased dosage of radiation made it a bit convenient for children but is still prohibited for pregnant women. In recent years, stool antigen test (SAT) and UBT became more acceptable diagnostic tests to detect active *H. Pylori* Infection. Lacking a universal protocol to perform UBT is an unsolved problem. Till now, only the manufacturer's experiences guided current standards in order to perform this method. Given high sensitivity (>95%) in posttreatment procedures [53], one of the disadvantages with UBT is the chance of colonization by urease-forming pathogens than *H. pylori* [54]. This probability is existing by the relatively low rate of current reports which increased our hopes to generalize application of UBT in routine and posttherapy *H. pylori* detection.

4. Stool Antigen Test (SAT)

Historically, serology approach was the first suggestion in order to diagnose *H. pylori* infection. Although the SAT is an accurate and precise method this accuracy is influenced by several limiting factors: upper gastrointestinal bleeding, antibiotic consumption, bowel movement, and also proton pump inhibitors (PPIs) uptake [55]. This noninvasive and almost cheap test became recommended whenever UBT was not available (Table 1). The superiority of UBT versus SAT was also found by Perri et al., while they showed that the diagnosis with UBT is undertaken with higher

TABLE 1: Overview of *H. pylori* diagnosis with invasive and noninvasive methods.

Name	Type	Reference method	Characteristics	Advantages	Disadvantages
UBT*	Noninvasive	No	Sensitivity: >95% Specificity: >95%	(i) High specificity and sensitivity (ii) Useful to confirm <i>H. P</i> eradication (iii) Useful to detect gastroduodenal bleeding (iv) Relatively cheap, simple and safe (v) A gold standard only for asymptomatic patients (vi) No sampling errors, good for epidemiological studies (vii) practically useful for children ~100% sensitivity	(i) Rarely false positive results refer to urease positive organisms (ii) Radiation in the case of application of ¹⁴ C-UBT (iii) No data about antibiotic resistance
Serology	Noninvasive	No	Sensitivity: >96% Specificity: 60–90%	(i) Has no false negative result (ii) Cheap, simple and safe (iii) Highly recommended for initial <i>H. pylori</i> screening (iv) Not affected by gastric bleeding (v) No false negative result in the case of PPI** consumption (a unique character)	(i) No data about antibiotic resistance (ii) Failure in distinguish between active and past infection (iii) No application in clinical practice and hospitals
SAT****	Noninvasive	No	Sensitivity: >95% Specificity: >95%	(i) High specificity and sensitivity (ii) Good popularity among patients (iii) Relatively fast and simple (iv) Easy modification to produce better results (v) No need to skilled staffs	(i) No data about antibiotic resistance (ii) The false positive result in the case of PPI and antibiotics (iii) Variation in specificity and sensitivity over the different clinical circumstances
Culture	Invasive	Yes	Sensitivity: 50–95% Specificity: >95%	(i) Existing the data about antibiotic resistance (ii) High specificity but low sensitivity (the most specific method existing) (iii) The possibility of having the pure bacterium and chance of preservation for a long time	(i) Need optimal incubation conditions and highly skilled operators (ii) Fast processes after endoscopy in necessary to avoid bacterial death (iii) Risk of the false negative result in the case of PPI and antibiotic consumption (iv) Need strict condition in transport before culturing (cool temperature) (v) Time-consuming and also the most expensive method
Histology	Invasive	Yes	Sensitivity: 60–90% Specificity: >95%	(i) The gold standard for direct <i>H. pylori</i> detection (ii) Almost cheap method for using in the universal scale (iii) Simple method	(i) Contradictory results following the PPI consumption (ii) Need extra biopsy sample and facing with ethical limitations (iii) Fluorescent microscope required method (limiting wide-spread usage) (iv) The relatively high rate of false negative reports

TABLE 1: Continued.

Name	Type	Reference method	Characteristics	Advantages	Disadvantages
RUT****	Invasive	No	Sensitivity: 95% Specificity: 80–90%	(i) Rapid, simple and cheap method (ii) High specificity (~99%), but low sensitivity (~80%) (iii) The most handfull test in a clinical setting	(i) No data about antibiotic resistance (ii) Decreased sensitivity in patients with gastric bleeding (iii) Increased false negative results in the case of antibiotics & PPI consumption and achlorhydria (iv) Not useful for screening the eradication in epidemiologic studies
PCR	Invasive	No	Sensitivity: 80% Specificity: 100%	(i) Existing data about antibiotic resistance (ii) High specificity and sensitivity (iii) Tracking the mutations involved in antibiotic resistance (iv) The possibility of virulence typing (v) Useful to detect the bacterium in environmental samples (vi) Rapid and accurate results	(i) High cost (ii) Risk of contamination (iii) Time consuming and requirement to skilled staff (low feasibility in all laboratories) (iv) Lack of data about phenotypic antibiotic susceptibility profile

*Urea breath test; **proton pump inhibitor; *** stool antigen test; **** rapid urease test.

accuracy [56]. Sequentially, SAT was introduced following the UBT into the clinic. Polyclonal antibodies-EIA gave useful reports on the diagnostic practices but occasional inconsistent findings (mostly false-positives) forced clinicians to start application of the monoclonal antibody-based approach. An actual improvement in this technique was the higher specificity which reduced the false-positive findings [57–59]. As the constructive shift in this immunologic assay, using monoclonal antibodies provided improved sensitivity and specificity rates in comparison with UBT. There are two major preferences for SAT in comparison with UBT; less expensive chemicals and materials and also not high-technology equipment are necessary. Another advantage of this method was that patients could have stored the samples at home and send it to laboratories at a suitable time. The partial insufficient condition of preservation of the stool sample in-house (optimal temperature, shaking, and transport medium to the laboratory) beside that applied cut-off value in final measurement can determine any bias in the diagnosis of the infection. To detect *H. pylori* infection, there are two main types of SAT: based on enzyme immunoassay (EIA) and immunochromatography (ICA) [60]. In clinical practices, the ICA-based test is more convenient to run in the small clinics and hospitals since so complex procedures are involved. In 1997, this test was suggested for the first time and the found acceptable sensitivity and specificity (88% and 94%, respectively) initiate other groups to apply it over the clinical practices [61]. A very attractive advantage of this method is to measure successful eradication of the infection using a simple laboratory examination [62]. Of course, to target pediatrics, SAT using monoclonal antibodies can give better feasibility since it is of low cost and is easy to handle by regular laboratories personals. In recent years, a new generation of stool antigen tests was invented. The Testmate pylori antigen EIA and Testmate rapid pylori antigen are the major examples [63, 64]. Testmate rapid pylori antigen is an immunochromatography based approach which is located on the commercially patented test strip. This easy application is to drop of suspended stool sample into the test strip. In the case positive result, an immunologic complex with the red latex-labeled MAb 21Ge will be produced and then it moves till the red color line becomes visible in the test strip. Currently, there is a good agreement between published guidelines and consensuses that SAT using monoclonal antibodies is one of the best approaches in the measurement of successful eradication of the bacterium and also for primary detection of this microorganism in clinical settings [33, 43, 55, 65–67].

5. Serological Tests

In general, detection of specific-antibody following the exposure to the various *H. pylori* antigens can be a useful method in clinical practices. As application and logic procedure was undertaken for many other pathogenic microorganisms, *H. pylori* discovery was not far from serological diagnosis [68, 69]. To date, different bacterial components include whole cell lysate, specific outer membrane proteins, LPS, heat shock protein (HSP), catalase, and cagA protein and many of the

adhesions were applied to induce specific antibodies in the host for facilitating the serological assay [60, 70–73]. Broadly defined, human immune response to the *H. pylori* is very complicated since many surface antigens are contributed. Routine *H. pylori* serologic methods can only detect specific IgG antibodies. The clinical importance of this test emerges when antibiotics and PPIs consumption are reported. Indeed, false negative results observed for other methods can have different response using serologic analysis. In addition to those drug uptakes, gastric bleeding and gastritis atrophic condition were also caused by false negative results for other methods; again, the serologic assay can be helpful for clinicians [74, 75]. The highlighted problem with the serologic approach is the weak distinguishing power of this test to discriminate active and inactive infection. Due to the different backgrounds in host genetics, it can be expected that various *H. pylori* strains induce different levels of antibodies and it may be a considerable item in explaining the reported findings [76]. Because of acceptable sensitivity and specificity rates observed in many commercial IgG-bases tests exist and have been validated in recent years [77–81]. One of the interesting aspects of serology method is following the antibiotic therapy; the long-lasting antibodies are still existing and it may cause the false-positive result [82–84]. This point should be cautiously considered by epidemiologists and gastroenterologists in their examinations in populations. In total, serologic tests are inexpensive; thus the application of these antibodies-based tests in some geographical area such as developing countries is highly acknowledged. A major consideration for the regions with a low prevalence of *H. pylori* is that suboptimal specificity can increase the false-positive results. Moreover, IgA-based measurement was also suggested but noted that the test is less trustful and reliable than IgG-based assays [85–88]. In some interesting studies, examinations of *H. pylori*-specific antibodies in other sample sources than serum were investigated [89, 90]. In brief, saliva and urine were checked but because of the lower titer of antibodies in these samples in comparison with serum, clinicians are not so eager to check this sort of samples for *H. pylori*. Taking together, the antibody-based examination cannot guarantee the accuracy of reported *H. pylori* status following the antibiotic treatment; thus further analysis is needed [91].

6. Invasive Methods

No need to mention that having genomic data from the clinical samples increases our knowledge of susceptibility patterns and virulence factors. Bacterial culture, Rapid urease test, PCR assay, and histology are the invasive methods applied to diagnose the *H. pylori* infection in different biologic sources. The criteria for this nomination (invasive methods) are referring to undertaking the endoscopic surgery. In patients with two complaints, the gastric endoscopy will be necessary: (i) no response to antibiotic therapy, and (ii) signs indicating problematic and symptomatic gastric conditions. Usually, clinicians take biopsies from antrum, but PPI consumption will reduce diagnostic value; thereafter, stomach body would be the next place for biopsy sampling. In next paragraphs, we

discuss invasive methods used to diagnose *H. pylori* infection in clinical samples.

7. Histology

Histology was the first method unconsciously used to detect the *H. pylori* in clinical samples. Our main evidence is reported by Warren in his laboratory before his collaborations with Barry Marshall ended in their great discovery. However, application of Warthin–Starry silver stain by Warren eventually caused a big triumph to visualize bacterial colonization in a biopsy sample from a patient with severe gastritis [19]. Current diagnosis of *H. pylori* infection is highly influenced by the histological report [92, 93]. Since typical morphology of *H. pylori*, histopathological confirmation of this infection can be easily achieved while further histologic changes in patterns of gastritis can be helpful to characterize the digestive diseases properly. An accurate histopathological observation can give us a detailed report of possible *H. pylori* colonization (and also bacterial density), and degree of inflammation and histopathology (e.g., severe atrophic gastritis, intestinal metaplasia, and malignancy [93, 94]). The identification of the bacteria in the histopathological analysis is based on different staining including hematoxylin and eosin (A&E). In order to increase specificity in detection of the *H. pylori*, different dyes such as Gimenez, Toluidine blue, Romanowski, Genta, Warthin–Starry silver, and Giemsa can be also used [95, 96]. The specific application of Warthin–Starry silver is in the chance of coccoid forms of the *H. pylori*. Histopathology examination is basically time-consuming and relatively expensive. Thus, the requirement of trained staffs besides being a consuming process resulted in a not handy method to detect *H. pylori* infection. Both sensitivity and specificity of the histology are nearly around the 94% [97–100]. We have searched the databases (Web of Sciences, Scopus, Medline, and Google scholar) and found not that much-published reports are investigating the specificity and sensitivity of the commercially available stains. From a scientific point of view, there is no superiority for any of those tests; however, some other aspects such as rapidity, reproducibility, cost, and being less time-consuming can be favorable for some of the tests. Rotimi et al. used modified McMullen's staining as a novel approach in comparison with other staining methods but found no significant differences (P value > 0.05) [95]. Although we have found relatively high sensitivity for this test, patchy distribution of this bacterium in the stomach can reduce the chance of histopathological changes in the taken biopsy from antrum [101–104]. Accordingly, to maintain this high rate of sensitivity and avoid sampling errors, we need to increase the number of taken biopsies [105, 106]. Last but not least, being an experienced pathologist can increase the sensitivity of this approach. Regardless of our limitations in reduced sensitivity for histology, the typical shape of the *H. pylori* and its expectable location in the luminal surface of the cells can help pathologists for easy diagnosis of this S-shaped bacterium. There is a general agreement among the pathologists that in the case of the existence of the *H. pylori*, all staining methods are good, but modified Giemsa is the first

choice because of less expensive materials and reproducible and sensitive results.

8. Rapid Urease Test

During the years after discovery by Marshall and Warren, it has been stated that the rapid urease test (RUT) is a one of the cheapest but rapid diagnostic tests used in detecting this infection [107]. The main biologic basis of this diagnostic test is to evaluate the presence of urease enzyme in clinical specimens shipped to the laboratories [108, 109]. Due to the historic dogma, it has hypothesized that detected gastric enzyme is a production by the human stomach to protect its epithelial cells from the acidic condition. Interestingly, following the groundbreaking introduction of *H. pylori*, it became widely accepted that the enzyme does not have a human origin [110]. Consequently, the detection of this bacterial product was used to identify this chronic infection. Interestingly, the specificity and sensitivity are durable for this test, but clinical experiences had shown that the optimal results are only achieved only if we take biopsies from both corpus and antrum. Some factors are influencing the final result of RUT: (i) bacterial urease concentration, (ii) temperature, (iii) waiting time for optimal reaction condition, and (iv) substrate concentrations. *Staphylococci* and *streptococci* are the other major urease-producers present in the gastric mucosa and may interfere with the detection of *H. pylori* based on the urease activity. There is an underestimated issue in the diagnosis of the *H. pylori* infection using the RUT. In some clinical settings mostly in developing countries, gastroenterologists ask the patients to keep the RUT test tube for 1–2 days and inform the hospitals' personals to add the data about likely positivity of the test [107, 111, 112]. In the case of oral colonization by *H. heilmannii*, the test will be positive, while it can interfere with positivity or negativity of the true *H. pylori* infection [113]. However, this reaction needs both higher load of *H. heilmannii* and longer time for positive urease reaction which mostly do not occur. Another solution to avoid this false-positive result is to check histopathological observation which is partially informative to identify *H. heilmannii* [114]. However, the necessary time to make RUT positive is quite different among numbered microorganisms; so far, a good specificity is promising news for gastroenterologists. Most of *H. pylori* contained-biopsies become red or pink within first fifty minutes after the endoscopy and placement of the biopsy in the medium [1]. The shift in the color of the medium is an indicator for the produced ammonium ion and increased pH (determined with pH indicator, e.g., phenol red). Although there are many commercial but specific mediums recommendable for detecting the urease positive organisms, some of other commonly used mediums such as Christensen medium are also useful in clinical settings [115–118]. Increasing the urea concentration (4–6 times) and reaction temperature were two potential modifications to increase the sensitivity of RUT [119]. Moreover, it has been firmly addressed that at least 10^5 *H. pylori* isolates are required to cause a positive result in RUT (changing the color into pink). The sensitivity rates range (85–100%) made the RUT one of the highly recommendable

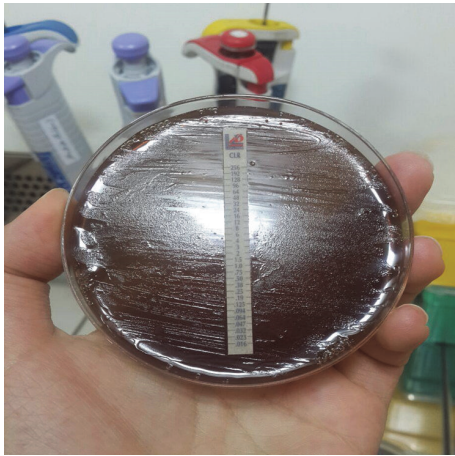


FIGURE 1: Successful isolation of *Helicobacter pylori* on Brucella agar supplemented with 5% horse blood.

methods in the diagnosis of the *H. pylori*. Similarly, relatively 100% specificity was another favorable item to make RUT popular among the gastroenterologists for rapid diagnosis of this bacterium in clinical settings [120].

9. Culture

Since increasing body of evidence showed the long-last colonization of the *H. pylori*, microbiologists started to culture this bacterium in several media. The main superiority of bacterial culture for *H. pylori* is the possibility of antibiotic susceptibility tests to choose proper antibiotics in the treatment of subjects and avoiding a new generation of antibiotic resistance among the symptomatic patients [121–124]. Successfulness of this culture process made this approach as the gold standard in the diagnosis of this infection. Recent international guidelines still insist on performing the bacterial culture in the case of failure in antibiotic susceptibility testing as next action. Based on Sydney classification, clinicians need at least three biopsies (two biopsies from the anterior and posterior corpus and one from antrum) necessary to accurately determine positive bacterial infection in gastritis patients [87, 93, 101, 105, 121, 125]. This kind of recommendation was another factor which initiates ones to run the routine culture for *H. pylori* in diagnostic laboratories. *H. pylori* can grow slowly on many solid media under microaerophilic condition. As a general rule, *H. pylori* needs blood or lysed blood supplements to grow optimally on agar plates (Figure 1).

Currently, Wilkins Chalgren agar, Brain heart agar, and Columbia and Brucella agars are most used base media to propagate *H. pylori* culture in routine diagnosis [26, 44, 126]. Because of the high risk of contaminating microorganisms including gram-positive microorganisms, fungi, and yeasts, using selective medium became a universal rule to have typical *H. pylori* colonies on the plates [1]. In order to increase the sensitivity and specificity of culture in the diagnosis of the *H. pylori*, we need multiple biopsies samples rather than a single antral biopsy [127]. As an additional suggestion to improve

the sensitivity and specificity, the endoscopic surgery should not be performed in less than 3 months for patients who state the record of the consumption of PPI, antibiotics, and antisecretory drugs [26, 36]. Adil et al. used microcapillary, as a novel culture-based approach in detecting the *H. pylori* infection [128]. In this paper, the authors stated that microcapillary method is statistically more sensitive compared with CLO test and HE tests ($P < 0.01$). Although many attempts had been performed to optimize culture media for *H. pylori*, Adil et al. showed a novel results to standardize *H. pylori* culture using new ingredients and conditions. As one of the critical problems in the endoscopic surgery, contamination of the biopsy specimens was an annoying issue. The rationale for this consideration is about the risk of transmission of some agents including human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) during the endoscopy [40]. However, using the disposable forceps in last decade decreased the chance of contamination with these infectious agents very much [129, 130]. Ecologically, *H. pylori* can survive in all sites of them stomach, but, in some cases (e.g., consumption of antisecretory drugs), the corpus will be the likely location to give us a successful culture following the endoscopy. Many medical societies are recommending that it is better to take first biopsies for culture prior to taking samples for histopathological examination. Indeed, this superiority can decrease the chance of exposure to the fixative and infectious agents influencing the chance of *H. pylori* positive culture. There is a challenging discussion among the microbiologists which is do we need to grind the biopsies before transferring the specimens into the agar plates or not? Indeed, nonhomogeneous distribution of the *H. pylori* in biopsies caused this problem. Moreover, many studies showed that there are significant differences if we use the grinded samples. The main explanation for this phenomenon is that grinding the biopsies is increasing the exposure of more *H. pylori* isolates to the favorable condition of growth, so multiple colonies will appear. In other words, isolation of *H. pylori* single colonies is feasible only if we grind the biopsies and then streak it on the plates. The importance of current recommendation is disclosed when researchers were faced with a variable pattern of genotyping from isolated DNA from a single colony in the nongrinded biopsy sample. Therefore, using the mechanical grinder and further application of single colony bacteria can increase the accuracy of the test in the diagnosis of the *H. pylori*. Meanwhile, we nicely avoid DNA-contamination by other possible agents [131]. Culturing the antral biopsy specimens is a leading item to reproduce the one of the highest sensitivities and specificities in the diagnosis of the *H. pylori*. The problems with this test are (i) likely exposure to the oxygen and temperature, expensive materials and consumables, and (iii) it is strict transport conditions. Last but not least, in close future, improving those items can help us to have better molecular techniques needing the isolated single colonies of this rouge bacterium [128].

10. PCR

As usual for all pathogenic microorganisms, PCR-based methods were applied to detect *H. pylori* infections in large

variety of environmental and clinical samples including water, food, vegetables, human saliva, stool, gastric juice and biopsies, and dental plaques [132]. In most of those essays, housekeeping genes were used frequently to design a sensitive and specific PCR to detect *H. pylori* [133–139]. Current evidences are indicating the requirement of at least one positive test (i.e., SAT, RUT, serology, and histology) in addition to the culture positive result before we can entitle a sample as *H. pylori* positive [26]. However, having positive result from a specific PCR approach can easily replace those time-consuming and expensive tests. The main gap to achieve this point is that optimization of DNA extraction and suitable genes pattern is not validated already. For example, there are more than twenty commercial kits for DNA extraction (i.e., Yekta Tajhiz Azma, Bioneer, Sina-clon, Qiagen, and Sigma) with large variety of the DNA yields which confuse researchers in selecting the proper product. Due to the presence of *H. pylori* DNA in various biologic samples, PCR-based detection was widely used to identify this problematic infection [140–143]. Biopsy samples, saliva and gastric juice, stool, and dental plaques were frequently applied to PCR detection. The main problem with stool sample is due to the existence of billions of bacteria including chemicals, gram-positive and gram-negative, which can mostly act as inhibitor for our detection. Because of variability in different DNA extraction methods, a universal approach needs to be recommended to produce reliable results, at least in clinical settings. Another important issue is the target gene to design the primer sets. As one of global housekeeping genes, 16S rRNA was used, but many mutations have been reported which disappoint clinicians to continuous application for further analysis [144, 145]. Recently, 23S rRNA gene has been suggested due to the high sensitivity in detection of *H. pylori* in clinical samples [146, 147].

11. Gold Standard Methods

Currently, urease and histological analysis are considered a gold standard approach in many clinical circumstances. In other words, there is no unique method acting as a gold standard in the diagnosis of *H. pylori* infection. However, we can use UBT and SAT as highly recommended tests available among the noninvasive methods. Further shreds of evidence are necessary before we can nominate any diagnostic methods as the gold standard in various clinical circumstances of patients attributed to *H. pylori* infections.

12. Different Diagnostic Strategies Useful in the Detection of Bacterial Eradication

Precise identification of *H. pylori* infections among symptomatic and asymptomatic individuals was the focus of many discussions. Following increased importance of eradication of the bacteria and its positive effects ending in gastro-duodenal diseases, now another question regarding the best approach to be the gold standard for detecting the *H. pylori* in treated patients is disclosed [148, 149]. Using current

knowledge of antimicrobial resistance and availability of various machines, equipment, and skilled staffs [150, 151], we need more noninvasive methods to be sued for following the eradication of *H. pylori* in different targets. Thus, researches in this area will be covered by more interest.

13. Conclusive Remarks

Since the accurate diagnosis of *H. pylori* is idealistic view for both gastroenterologists and microbiologists, using synergistically invasive and noninvasive methods will be a future challenge in medical research topics. It is clear that recent advances in invasive and noninvasive methods for accurate diagnosis of the *H. pylori* can drastically change upcoming guidelines attributed with the management of this infection. No doubt that diagnosis of *H. pylori* infection due to its strange microniche is difficult and hard to optimize, especially for routine diagnostic. The fragility of the microorganism is another limiting factor to access necessary information on various aspects of this bacterium. Indeed, the gastroenterologists are facing a long list of various molecular and nonmolecular tests, but the problem is to optimize and design an accurate test to produce information on (i) presence of the infection and (ii) antibiotic susceptibility profile. These are the main difficulties causing complex diagnosis of *H. pylori* even if for developed countries. According to the recent European guideline, the ^{13}C -UBT is nominated as the best method in the diagnosis of *H. pylori* infection. This approach shows acceptable specificity and sensitivity in clinical practice [65]. Concerning the serological examinations, the results are generalizable if local antigens were applied in the tests; otherwise, many discrepancies need further analysis. Using current knowledge of antimicrobial resistance and availability of various machines, types of equipment and skilled staffs, we need more noninvasive methods to be sued for following the eradication of *H. pylori* in different targets. Thus, researches in this area will be covered by intense interest. To achieve this point, in-depth experiments are necessary to generalize rapid and accurate molecular tests determining both bacterial identity and antibiotic resistance profile.

Disclosure

The contents of current review are the sole responsibility of the author and do not necessarily represent the official views of the affiliated institutes.

Conflicts of Interest

The author declares that they have no conflicts of interest.

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