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Review

An update of laboratory diagnosis of *Helicobacter pylori* in the Kingdom of Saudi Arabia

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Abstract

Helicobacter pylori is a micro-aerophilic, slow-growing, Gram-negative spiral bacterium that colonizes the mucous lining of the human stomach. Infection with this bacterium has been identified as a cause of gastritis, peptic ulcer disease, and gastric mucosa-associated lymphoid tissue lymphoma. Globally, the prevalence of *H. pylori*-related infection is high compared to any other infectious diseases, and the rate of prevalence much higher in developing countries than in developed nations.

This review article aims to describe the trend of *H. pylori*-related works in the Kingdom of Saudi Arabia (KSA) and the use of various laboratory tests for the diagnosis of *H. pylori*-related infections in adults and children.

Therefore, published literature was referenced in the explanation and discussion of the different methods used to diagnose *H. pylori*-related disease, including papers published in the KSA and other Middle Eastern countries. The PubMed (http://www.ncbi.nlm.nih.gov/pubmed?cmd=search) search engine was used extensively.

Culture and histopathology tests have been employed widely to detect this pathogen at the early stage. However, over the years, an array of tests including the rapid urease test, serology, the urea breath test, the fecal antigen test, and molecular testing have been developed to diagnose and better manage *H. pylori*-associated diseases since the discovery of this novel pathogen.

Key words: *Helicobacter pylori*; diagnosis; PCR; culture; Urea breath Test; drug susceptibility.

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Introduction

Helicobacter pylori is a non-spore-forming Gram-negative bacterium. The cellular morphology may be curved, spiral, or fusiform, typically 0.5 to 1.0 μm in width and 2.5 to 5.0 μm long. The spiral wavelength may vary with the age, growth conditions, and species identity of the cells [1]. In old cultures or those exposed to air, cells may become coccoid [2].

Although bacteria were seen in stomach tissue (histopathology) a century ago [3], Marshall and Warren (1994) made a phenomenal change in gastroenterology by culturing a novel bacterium from gastric mucosa in 1983 [1], which was a turning point in our understanding of gastrointestinal microbial ecology and disease. This was recognized in 2005 when they won the Nobel Prize in Physiology or Medicine.

The story about how *H. pylori* was discovered is very interesting. The Royal Perth Hospital in Australia where Marshall worked used to discard stomach biopsy culture plates after 48 hours. It was Easter Thursday and plates were supposed to be checked on Saturday; however, technologists did not review them until next Tuesday, which allowed longer incubation and resulted in pure colonies of *H. pylori* [4]. Due to the resemblance of *H. pylori* with *Campylobacter* in several aspects, including its morphological shape and size, microaerophilic nature, and fastidious character, it was initially named *Campylobacter pylorides* at the Second International Workshop on *Campylobacter* Infections held in Brussels, Belgium, in September 1983 [5]. Later, this bacterium was renamed *Campylobacter pylori* and finally *H. pylori* based of its flagellar structures, proteins, fatty acid, and genetic compositions [6-7]. Soon after this, all attention turned

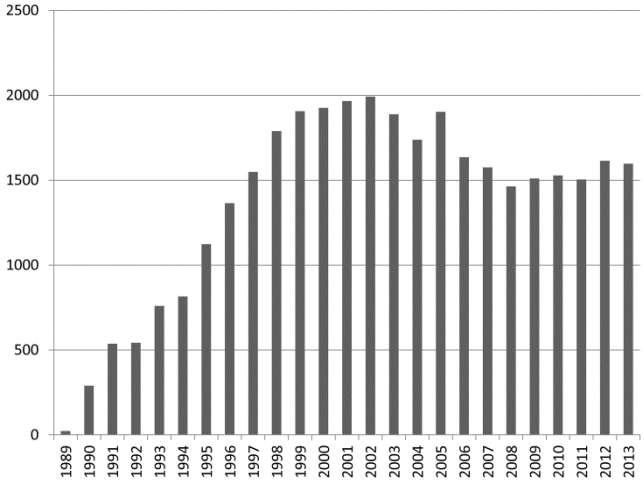
to this novel pathogen. Over last three decades, tremendous progress has been made to understand its biology [8-10], epidemiology [11-12], pathogenicity [13], treatment [14-18], and laboratory diagnosis [19-22]. The number of peer-reviewed articles on *H. pylori* is still growing (Figure 1). Although the whole genome sequence was completed in 1999 [23], a great deal of research is still needed to understand the growing concern of the multiple-drug resistance issue. In addition to drug resistance, further studies are also needed to understand the host pathogen interaction, transmission route, and its relationship with other microorganisms and diseases.

The aim of this article is to briefly review the Kingdom of Saudi Arabia (KSA) literature on *H. pylori* and describe the utilization of different diagnostic methods by laboratory workers and allied healthcare workers.

Helicobacter pylori literature in the Kingdom of Saudi Arabia

Since writing this review, 121 articles have been indexed by PubMed when the terms "*Helicobacter pylori* and Kingdom of Saudi Arabia" were searched for, and the status has not significantly changed since Abdulaziz A Bin Saeed wrote in his editorial that the Saudi contribution was very minimal [24]. In 2007, he found only 70 articles related to *H. pylori*, where the worldwide contribution was over 26,000 articles globally (Figure 1). He also compared the Saudi contribution with Australia's, which has a similar population size with a lower prevalence rate. Australia published over 500 articles in the same period of time [24]. Most of the studies have been done in universities, research centers, or hospital settings. There are many sero-epidemiological studies that were done in the KSA for both the asymptomatic and symptomatic population since the late 1980s [25-31]. In both asymptomatic and symptomatic populations, the rate of infection in the Saudi population is comparable to that in North America and greatly varies by age groups. A gastritis study was initiated in the KSA in the late 1980s immediately after the discovery of this pathogen. Few studies had been conducted to determine the efficacy of different methods [32-33] for the detection and isolation of *H. pylori*. Since 1980, a number of studies aimed to understand the pathogenesis of this pathogen as well [34-36]. Al-Khattaf [34] reported the presence of *cagA* and *vacA* genes in 100% of patients with adenocarcinoma. Marie [35] showed that *H. pylori* strains of *vacA* s1 and the combinations of s1/m1 were

Figure 1. Number of peer-reviewed publications per year on *Helicobacter pylori* using PubMed search (key words used were *Helicobacter pylori* or *H. pylori* in anywhere in the paper)



associated with peptic ulceration and the presence of the *cagA* gene. Momenah and Tayeeb [36] studied the *iceA* gene. Very few or no studies were done in the areas of cytokines, Lewis antigens, or adhesins to understand more about the pathogenesis of Saudi strains of *H. pylori*. Recently, King Saud University launched molecular studies on this novel pathogen [35,37]. Momenah and Tayeb (2006) studied *iceA* genes [38] to understand the relationship between the *H. pylori vacA* genotype and the risk of peptic ulcer disease. There are several untapped areas in the field of *H. pylori* and its diseases. Saudi Arabia took the lead among Middle Eastern countries on studying pathogenesis, the gut microbiome, bioinformatics, molecular characterization, and sequencing of the whole genome to understand more about the host pathogen interaction and the transmission route of the *H. pylori* strains in the Middle East.

Laboratory methods

There are several laboratory methods that have become available to detect *H. pylori* infection over the last three decades [39]. The tests are divided in two main groups: those that are invasive and others that are not. Invasive methods include histology (microscopy), rapid urease test, culture, and brush cytology; all these tests require endoscopy to obtain biopsy materials. In rare circumstances, biopsy could harm patients, as could brush in an ulcerated area to do cytology in order to diagnose *H. pylori* infection. Non-invasive methods include testing of urine, stool, blood, saliva, and breath samples (Table 1).

Table 1. Diagnostic tests for *Helicobacter pylori*

Invasive (tissue biopsy required)	Non-invasive
Histology/microscopy	Serum antibody (serology)
Rapid urease test (CLO)	Urea breath test (UBT)
Culture and susceptibility	Stool antigen test
Brush cytology	Rapid stool antigen test
Molecular test (PCR)	Saliva/dental plaque PCR
	Urine antibody test

Sample collection

For invasive methods, tissue biopsies from the stomach are considered to be preferred samples. A sterile endoscopic apparatus should be used for each patient, and the patient must adhere to a pre-endoscopy protocol in order to get the best sample. The patient should not take antibiotics, bismuth substances, and proton pump inhibitors for at least two weeks. The physician should target the ulcerated area of the antrum of the stomach to allow optimal recovery of *H. pylori*. Other methods have been used, including an oro-gastric brush to collect mucosal tissue [40], the string test to obtain gastric mucous [41], and a nasogastric tube to obtain nasogastric juice [42]. None of these methods have been widely used among gastroenterologists.

Sample transport

Since *H. pylori* is known as a microaerophilic organism sensitive to drying, it has been recommended that rapid transportation using a transport media (Stewart's transport media) is crucial for successful isolation [43]. If transport media is not available, normal saline containing 20% glucose and glycerol can act as a transport media as well [8]. Bedside sampling would be ideal but is not feasible in most instances.

Invasive methods

Histology/microscopy

Since the discovery of *H. pylori*, histopathology (tissue biopsy from the stomach) becomes the number one test of choice of laboratorians. Histopathologists consider this to be the gold standard in the diagnosis *H. pylori*-related infection in both developed and developing countries. This test also provides additional information such as the degree of inflammation and associated pathology related to metaplasia, lymphoma, mucosa-associated lymphoid tissue (MALT), or cancer. It has now been established that biopsy samples from the affected area of the stomach are optimal for demonstrating *H. pylori* infection. This method may also have a few shortcomings, the major one being patient compliance. Patients should not have any antibiotic at least two weeks prior to their endoscopy and must follow pre-endoscopic procedures. Accurate test results also depend on many factors such as collection of biopsy from the ulcerated antrum sites of the stomach [44], sample preparation and staining for histopathology slides, and expertise in reading slides and interpreting them accurately [45]. If on-site histopathologists are unavailable, the tissue sample should be blocked in paraffin and then shipped to a site where the expertise is available.

Rapid urease testing

H. pylori is one of the best urease enzyme-producing organisms in the bacterial kingdom. Scientists used that marker to develop urea breath tests that were marketed as CLO tests ("CLO" was used to describe *Helicobacter* as *Campylobacter*-like organisms). This has been especially popular in developed countries because of its easy-to-use nature.

This CLO test can be performed at the time of endoscopy because of the desire to know a patient's *H. pylori* status before discharge from the endoscopy suite, and the result can be made available within one hour. To perform this test, a biopsy sample from the stomach is placed into a media containing urea, phenol red (pH indicator), buffers, and bacteriostatic agents. If *H. pylori* is present in the sample, it will produce urease enzyme, which will hydrolyze urea to ammonia. As a result, the pH of the medium will increase and the color will change from yellow to red. Sensitivity and specificity were found to be acceptable (98% and 97%, respectively), provided the biopsy sample was collected from an ulcerated antrum site of the stomach [46-47]. False-negative results may occur when very low numbers of *H. pylori* are present or if the bacteria have a patchy distribution [48].

Brush cytology

Brush cytology is not a common method and is rarely used in clinical practice. This test can be considered only in patients with bleeding disorders that make biopsy difficult and risky [49]. The sample obtained from the antrum or body of the stomach can be examined using standard Gram stain techniques or special staining if the results of the Gram stain are inconclusive. Brush cytology has a reported sensitivity of 95% to 98% and specificity of 96% [50].

Bacterial culture and sensitivity testing

H. pylori is one of the most fastidious and slow-growing bacteria. Although culture is considered the gold standard, very few laboratories routinely culture *H. pylori* due to its complex nature, slow growth, and special growth requirements. Routine culture as a diagnostic tool is not recommended. However, in patients in whom standard second-line antimicrobial therapy has failed, culture is essential to determine which antibiotics the organism is sensitive to. *H. pylori* requires 85% N_2, 10% CO_2, and 5% O_2 for its optimal growth. Growth occurs at 34°C to 40°C, with an optimum temperature of 37°C, and requires three to five days. It can also be grown in a candle jar, although sensitivity is low [22]. Although its natural habitat is the acidic gastric mucosa, *H. pylori* grows best at a neutral PH (7.0), although it will survive brief exposure to pHs of less than four [51].

H. pylori is inherently resistant to nalidixic acid, trimethoprim, sulfonamides, and vancomycin; those antibacterial agents can be used in culture media to avoid contamination and allow selective isolation. For this reason, *H. pylori* culture facility should be available in teaching hospitals or reference laboratories.

Molecular methods

Detection and drug susceptibility testing

H. pylori is sensitive to penicillin, ampicillin, cephalothin, kanamycin, gentamicin, rifampin, clarithromycin, tetracycline and metronidazole; however, over the years, resistance to these antibacterial agents has been reported to varying degrees. These variations differ significantly in different regions or countries. Since culturing of this microorganism is difficult, it is often hard to achieve optimal concentrations (McFarland standard 2) do an antibiogram. As a result, traditional drug susceptibility testing is not available in most laboratories, and physicians treat patients with drug regimens known to be effective. The most commonly used anti-*H. pylori* drugs are macrolides (clarithromycin), amoxicillin, floroquinolones, rifamycin, tetracycline, and metronidazole. Resistance to all of them has become an alarming issue. The most popular drug regimens are combinations of clarithromycin, metronidazole, and a proton pump inhibitor, or amoxicillin, metronidazole, and a proton pump inhibitor [52].

Polymerase chain reaction (PCR) has not been considered to be practical tool for the routine diagnosis of *H. pylori*; however, many laboratories are now moving to this as a result of convenience and efficacy. A few laboratories also use real-time PCR to determine drug resistance against commonly used drugs (clarithromycin, tetracycline, metronidazole) by detecting point mutations (Table 2). PCR has also been found to be useful in detecting the organism when ordinary culture is difficult, as with testing environmental samples such as drinking water [53]. Drug resistance monitoring in communities or regions allow healthcare providers to better define susceptibility patterns and establish optimal treatment regimens [54]. *H. pylori* is resistant to nalidixic acid, trimethoprim, sulfonamides and vancomycin; however, it is also capable of acquiring resistance to any drug that is overly used, and this has become a major issue in treating patients successfully. The most commonly used drug, metronidazole, showed resistance of 80%–90% in tropical countries, 50% in European countries, and 80% in Saudi Arabia [55]. Bakri (2013) [56] found a higher percentage of clarithromycin-resistant genes in Saudi Arabia. These results indicate that physicians should be careful

Table 2. Antibiotic groups and genes involved in point mutation or other genetic changes leading to antimicrobial resistance in *Helicobacter pylori*

Antibiotic group	Antibiotic target	Gene involved
Macrolides	Binds to P site on 50S subunit of bacterial ribosome; inhibits protein synthesis	*23S rrn*
Metronidazole	Inhibits nucleic acid synthesis by disrupting DNA	*rdxA*
Quinolones	Inhibit DNA gyrase (in many Gram-negative bacteria)	*gyrA*
Rifamycins	Affinity for prokaryotic RNA polymerase; inhibits DNA-dependent RNA synthesis	*rpoB*
Amoxicillin	Inhibits peptide crosslinking during PG synthesis	*pbp-1A*
Tetracycline	Binds to 30S ribosome subunit; inhibits amino-acyl tRNA binding	*16S rrn*

prescribing metronidazole and clarithromycin for *H. pylori*-related dyspepsia. Megraud (2004) provided an excellent summary on the prevalence of *H. pylori* resistance to antibiotics, its consequences, and the advances in detecting it so that physicians can make informed decisions about treating patients [52].

Non-invasive methods

Serology

It is now well understood that *H. pylori* requires a longer time than do other bacteria to cause infection. As a result, IgM is virtually absent. However, IgG and IgA are found in the patient's serum and saliva. IgA and IgG are found in 80% and over 95% patients [57-59], respectively. Different methods of serological tests such as complement fixation, hemagglutination, immunochromatography, enzyme-linked immunosorbent assay (ELISA)/enzyme immunoassay (EIA), and western blot have been described in the literature [60], and among them, the ELISA/EIA method achieved greater acceptability by laboratorians due to cost, ease of use, and full automation [58-59]. The sensitivity and specificity of ELISA/EIA depends primarily on the nature of the antigen bound to the solid support.

However, in 1999, chemiluminescent-based EIA showed higher sensitivity as well as specificity compared to traditional colorimetric-based ELISA/EIA [61]. Serology showed the highest sensitivity (92%) compared to histology (82.2%), rapid urease tests (55.6%), and culture (51.1%) in a developing country setting [21]. If any laboratory would prefer to use the serological test for clinical use, local validation is needed because if the prevalence rate is low, the positive predictive value (PPV) will be significantly different in the areas where the prevalence rate is very high. The serological test also has an advantage for the group of patients who cannot afford to go off proton pump inhibitors because of their medical conditions. One of the key questions in using the serological test is whether it can predict cure of the infection. Researchers discovered, in 1995, that the IgG titer decreased 50% between six and nine months after treatment [62]. At present, due to easy access to the urea breath test (UBT), clinicians mostly rely on UBT rather than on serological tests. Serological testing is an excellent tool to rule out infection, and in a rural area where UBT is unavailable, serological testing still could be useful if used diligently. Even in modern settings, serological testing can be done using an algorithmic approach.

Urea breath test (UBT)

UBT is based on the hydrolysis of urea by *H. pylori* to produce CO_2 and ammonia. A labeled carbon isotope (^{14}C or ^{13}C) is given by mouth; *H. pylori* liberates ^{14}C- or ^{13}C-tagged CO_2 that can be detected in breath samples by a scintillation counter. The UBT was discovered long before *H. pylori* by Kornberg *et al.* (1954), who measured $^{14}CO_2$ in cat's breath [63]. Graham *et al.* (1987) [64] used the same principle to measure ^{13}C, followed by Marshall *et al.* (1988) [65] for ^{14}C for diagnosing *H. pylori* infection in humans.

Both the non-radioactive ^{13}C test and the radioactive ^{14}C test obtained Food and Drug Administration (FDA) approval and are commercially available. Both tests have similar cost and accuracy. Some physicians prefer the ^{13}C test to ^{14}C since it does not use a radioactive isotope. Although the dose of radiation in the ^{14}C test is minimal, the use of ^{14}C should be avoided in children, pregnant women, and possibly women of childbearing age since no long-term data are available on its safety in these groups [66].

A similar method testing blood instead of breath has been developed. ^{13}C-labeled bicarbonate can be measured in blood before and one hour after administration. The urea blood test (Ez-HBT) samples were processed using gas isotope ratio mass spectrometry (Metabolic Solutions Inc., Nashua, USA). This test is not as popular as the breath test. This test has a reported sensitivity of 92% to 100% and a reported specificity of 96% to 97% [67].

Among the non-invasive tests, the UBT is highly accurate and reproducible in both establishing the disease diagnosis and measuring the treatment outcome. The sensitivity and specificity of ^{14}C UBT are approximately 88% to 95% and 95% to 100%, respectively [68]. False-positive and false-negative results are unlikely but may be observed in patients who are not compliant with the direction provided by the laboratory, such as the withdrawal of any proton pump inhibitor, bismuth, or antibiotic two weeks prior to testing [69].

Stool antigen assay

The stool sample has had a place in the clinical laboratory for centuries. Since *H. pylori* has to pass through stool, scientists invested ample time to develop a stool test for it. Although there are few reports on successful culture of *H. pylori* in stool [70] or detection of *H. pylori* in stool by PCR [71], none of those procedures have been very successful. *H. pylori* often becomes non-cultivable due to biliary salts and

other inhibitors [72]. Stool PCR was also challenging due to the inhibitors affecting it [73]. Stool antigen tests have been extensively evaluated and accepted as routine clinical tests in many laboratories where scintillation counters are unavailable or patients are not willing to ingest isotopically labeled urea. These tests can also be used before and after treatment [74], similar to UBTs, which are widely available and require no special setup [75]. A commercially available enzyme immunoassay is the recommended method for the primary diagnosis of *H. pylori* in stool [76]. The accuracy of the test was evaluated in a study involving 270 patients in whom the diagnosis of *H. pylori* was established by endoscopy and UBT [77]. The sensitivity and specificity of the test were 94% and 86%, respectively. Similar results were found in a comparably designed study involving 272 infected patients (sensitivity 94%, specificity 92%) [78]. This test has been extensively evaluated by several groups [79-81].

Rapid stool antigen tests

The stool antigen described above is performed in a laboratory, which delays diagnosis. A rapid *H. pylori* stool antigen test that can be performed during a clinic visit is available. This method can be helpful in a rural setting where laboratory facilities are not available [82].

Other assays

Salivary assays

H. pylori has been found in the oral cavity [83], and this organism was found in both saliva and dental plaque detected by PCR [84]. Serology tests were also developed for saliva and found to be very useful for children. However, because of the availability of stool antigen test assays and the reduced sensitivity of saliva IgG, this test lost ground and no laboratories use it.

Urinary assays

Similar to salivary assays, a urine-based ELISA was also developed [85] because of the difficulties in obtaining samples from children [86]. Due to the higher sensitivity and specificity of chemeluminescent-based serology, ELISA of urine is no longer used for the diagnosis of *H. pylori* infection [61].

Test recommendations

No recommendation has been made by the Saudi Medical Council based on local data; gastroenterologists and other physicians mostly rely on test recommendations from either European or Western countries. Briefly, diagnosis of *H. pylori* for adult patients should be made preferably with the UBT or the stool antigen test. Serology can be used in patients who have never been treated for *H. pylori* since this test cannot differentiate between active and past infection. For the relapsing patient, endoscopy followed by histology, culture, and antibiogram should be performed. PCR is becoming a common test in many laboratory settings and could be used as alternate for culture and antibiogram for detection and to determine drug resistance genes for commonly used antimicrobial drugs. It is worthwhile to confirm *H. pylori* eradication at least four to eight weeks following completion of antibiotic therapy because of increased resistance to antibiotic therapy [14,16,18,68,76].

Children differ from adults in all aspects except the etiological agent *H. pylori*. The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) and the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) developed guidelines that were revised recently [17]. These are meant only for children in Europe and North America, where the prevalence of disease in this population is low. Canada also had similar guidelines [87]. In developing countries and some industrialized countries, *H. pylori* infection is usually acquired during the first year of life [14,18]. For children with persistent or severe upper abdominal symptoms, upper endoscopy with biopsy should be the investigations of choice, and the ^{13}C-UBT is recommended as the best non-invasive diagnostic test that has the added advantage of being able rule out non-*H. pylori* related complications. The stool antigen test has also been recommended, although it is difficult to obtain stool from teenaged children.

Consensus guidelines for the management of *H. pylori* infection need to be established in the Middle East for both adults and children based on local data and clinical and laboratory practices.

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