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Helicobacter pylori Infection: Epidemiology, Pathophysiology, and Therapy

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Helicobacter pylori is one of the most commonly encountered human pathogens. It has been shown to be closely associated with peptic ulcer disease (PUD), gastric adenocarcinoma, and the gastric mucosa-associated lymphoid tissue (MALT) that may lead to gastric lymphoma. The current diagnostic methods include histology, microbiological culture, classic serology, urease activity detection, polymerase chain reaction (PCR) and stool antigen detection. Its treatment modality options are multiple; however, a triple regimen consisting of a proton pump inhibitor (PPI), and two antibiotics for 10 to 14 days is preferred. Drug resistance is a growing problem in this organism and new therapeutic options are currently limited.

Key words: *Helicobacter pylori*, PCR, PPI, Peptic ulcer disease (PUD) MALT

INTRODUCTION

Since the initial report of the isolation of a curved bacillus from patients with active chronic gastritis in 1982, this organism, now known as *Helicobacter pylori*, has been recognized as one of the most common human pathogens, probably affecting more than one half the population of the world. It has been associated with significant inflammatory and malignant conditions involving the upper gastrointestinal tract. Although marked progress has been made in the diagnosis and treatment of this infection, further work is needed to understand the full picture of its pathophysiologic mechanisms. This article will review the current status of this widespread infection with special emphasis on its epidemiology, diagnosis, and therapy. The recent increasing problem of emerging antibiotic resistance and potential vaccine development for *H. pylori* infection will also be discussed.

CHARACTERISTICS OF *HELICOBACTER*

Currently, the genus *Helicobacter* consists of 18 species. Some of these organisms have also been isolated from humans and are possibly implicated in disease. *H. heilmannii* has been isolated from gastric mucosa of humans, cats, dogs and pigs and has been associated with various gastric diseases similar to *H. pylori* (Borody et *al.*,1991; Morgner et *al.*, 1995; Stolte et al., 1997). Other helicobacters isolated in humans and of possible clinical significance include *H. cinaedi*, *H. canis* and *H. fennelliae* associated with diarrheal illnesses (Burnens *et al.*, 1993; Stanley *et al.*, 1993). *H. bilis*, *H. hepaticus* and *H. pullorum* (Fox et *al.*, 1999) have been isolated from the biliary tract of rodents and humans, and a potential role in cholecystitis and gallbladder cancer has been proposed. *H. cinaedi* was also found to cause recurrent cellulitis and bacteremia in immunocompromised hosts (Kielhbauch *et al.*, 1994).

The major human pathogen belonging to the genera *Helicobacter*, *H. pylori* was initially described as a campylobacter-like bacterium in 1982 by Marshall and Warren. Originally this organism was named *Campylobacter pyloridis* because of its isolation from patients with chronic gastritis and duodenal ulcers (Skirrow, 1983), and its name was subsequently changed to *Campylobacter pylori* (Marshall et *al.*, 1987). In 1989, after further characterization of this organism by genotypic and phenotypic criteria, a new genus *Helicobacter* was created.

The complete genomic sequence of *H. pylori* strain 26695 was completed in 1997 by Tomb *et al.* The

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organism was found to have a small size genome (1.7 Mb) and fewer regulatory proteins than other organisms that can adapt to different environments. It is also suggested that *H. pylori* may use frequent mutations of a particular gene to preserve functional activity and facilitate survival in a particular environment (Foxall *et al.*, 1992).

H. pylori is a spiral-shaped, microaerophilic, gram-negative bacterium of approximately 0.5 to 1.0 µm in width and 3 to 4 µm in length.This organism has four to six unipolar sheated flagella that facilitate its free movements in environments such as the gastric mucosa (Hazell *et al.*, 1986). *H. pylori* is nutritionally fastidious and needs an increased level of atmospheric carbon dioxide for optimum growth. Selective media with antibiotics (Krajden *et al.*, 1987; Parsonnet *et al.*, 1988; Ansorg *et al.*, 1991) and a variety of supplements, such as starch, horse serum or charcoal (Buck and Smith, 1987), have been used to facilitate its growth.

Biochemical characteristics of this bacterium include urease, catalase and oxidase positivity. It has been reported that as high as 30% of the dry weight of the organism is accounted for by urease. Urease production is one of the major properties of this organism and appears to be an important factor in its survival in an environment such as the gastric mucosa (Eaton *et al.*, 1991). A protective buffering effect of urea hydrolysis by urease has been demonstrated (Marshall *et al.*, 1990). This provides the bacterium with a microenvironment of ammonia thus neutralizing gastric acidity. Damage to the gastric mucosa by urease is also a possible virulence factor of *H. pylori*. Urease production has also been one of the cornerstones in the diagnosis of *H. pylori* associated disease either by direct detection from a biopsy specimen or by the use of labeled urea breath tests. Other enzymes, such as catalase or superoxidase dismutase, are also considered important defense mechanisms against the oxidative damage in areas of mucosal inflammation.

H. pylori resides only in the gastric epithelium of humans, primates and possibly cats (Krajden *et al.*,1989; Fox *et al.*, 1996).The exact mechanisms by which the bacterium adheres to the gastric epithelium has not been fully elucidated, but it is believed to be associated with both host factors (blood group type) and bacterial factors (production of adhesins and hemagglutinins) (Boren *et al.*, 1994; Lingwood *et al.*, 1993; Valkonen *et al.*, 1994).

EPIDEMIOLOGY

H. pylori is one of the most common infectious pathogens in humans. Approximately 50% of the worlds population is believed to be infected. The prevalence of infection varies worldwide with significantly higher rates in developing countries (Megraud *et al.*, 1989; Dooley *et al.*, 1989). Studies of seroprevalence of *H. pylori* in developing countries have found 50-75% seropositivity in children with a plateau of 80-90% during adulthood (Megraud *et al.*, 1989; Holcombe *et al.*, 1992; Bardhan *et al.*, 1997; Sathar *et al.*, 1997). On the other hand, in the industrialized world, childhood seroprevalence is less than 10% with gradual increase with advancing age to a 40%-60% seropositivity by the age of 60 (Megraud *et al.*, 1989; Graham *et al.*, 1991; Everhart *et al.*, 2000).

Socioeconomic status and race are also closely linked to *H. pylori* infection. In studies of seroprevalence in the United States, infection has been found to be present in 60%-70% of blacks and Hispanics compared with 20%-30% of whites with significant inverse correlations with income, educational level and crowding (Graham *et al.*, 1991; Staat *et al.*, 1996; Everhart *et al.*, 2000). Immigrants from developing countries to industrialized countries also have higher seroprevalence of infection than the population born in the host country and this is maintained in their first-generation (Verdu *et al.*, 1996).

Transmission of *H. pylori* is believed to occur by fecal-oral and oral-oral routes (Everhart *et al.*, 2000). Although neither oral nor fecal exposure has been definitively established as routes of transmission, the higher incidence of infection in children compared with adults favors this assumption. *H. pylori* has been isolated in feces (Thomas *et al.*, 1992), saliva, vomitus, cathartic stools (Parsonnet *et al.*, 1999), the oral cavity and dental plaques (Majumdar *et al.*, 1990). Other routes of transmission, such as sexual contact or by contaminated environmental contact, seem less likely (Everhart *et al.*, 2000).

Humans are the main reservoir of *H. pylori*, although the organism has been isolated from nonhuman species such as primates, pigs, and cats. Human contacts with these animals are not frequent or intimate enough to account for the widespread prevalence of *H. pylori* infection (Bardhan *et al.*, 1997). The organism has been recovered from houseflies (Grubel *et al.*, 1998) but the potential role of insects as vectors has not been established. Endoscopes have been implicated in the transmission of infection (Tytgat, 1995). A higher prevalence of *H. pylori* infection has been noted in gatrointestinal endoscopists, but not in dentists (Bardhan *et al.*, 1997).

H. PYLORI RELATED DISEASE

Acute infection with *H. pylori* results in histologically proven gastritis clinically manifested by epigastric fullness, vomiting, soft stools, irritability and "putrid breath" as described by Barry Marshall *et al* in 1985 while trying to fulfill Kochs postulates with self ingestion of live organisms. This experiment was repeated in 1987 by Morris and Nicholson with similar results and evidence of chronic gastritis. Although spontaneous clearance may occur, the majority of the patients will develop an asymptomatic chronic state in which there is histologic evidence of gastritis with normal gastric acid production (Langenberg *et al.*, 1988).

Infection with *H. pylori* has been linked to many disease states but data support a strong association with only a few conditions, which include peptic ulcer disease, gastric adenocarcinoma, and gastric lymphoma (Parsonnet *et al.*, 1998). Other associations including the role in non-ulcer dyspepsia have yet to be confirmed.

Peptic Ulcer Disease (PUD)

H. pylori is clearly associated with both duodenal and gastric ulcers. Patients with *H. pylori* infection have been shown to have at least a threefold increased risk of developing duodenal ulcers (Kurata *et al.*, 1997). In addition, approximately 90%-95% of patients with duodenal ulcers and 70%-90% with gastric ulcers are infected with *H. pylori* (Parsonnet *et al.*, 1998; Nomura *et al.*, 1994; Cohen, 2000).

The most important evidence for a causal association between *H. pylori* and PUD is that the disease process reverses upon the eradication of the organism. Less than 10% of patients that have received an effective treatment against *H. pylori* have recurrences compared with more than 70% of those that only received acid-suppressive therapy (Graham *et al.*, 1992; Hopkins *et al.*, 1996). The link between *H. pylori* and PUD has also been reinforced by studies done in smokers in which a twofold increase in the risk of ulcerative disease disappears after cure of *H. pylori* infection (Bardhan *et al.*, 1997). The role of *H. pylori* in gastric ulcers, although not as well studied as in duodenal ulcer disease, is similar to duodenal disease (Graham *et al.*, 1992).

Although the exact pathogenesis of PUD remains unclear, the following hypothesis has been proposed. *H. pylori* causes antral endocrine cells to release somatostatin (Graham *et al.*, 1990; Levi *et al.*, 1989) which results in postprandial gastrin release. This hypergastrinemic state increases acid production and predisposes the host to develop gastric metaplasia. Gastric metaplasia is also enhanced by concomitant risk factors such as smoking, alcohol, non-steroidal anti-inflammatory drugs (NSAID) or *H. pylori* pathogenic factors such as *cagA* or *vacA* genotype. It appears that these two genetic loci are relevant to the clinical consequences of *H. pylori* infection. Virtually every patient with PUD is infected with a *cagA* positive strain, and *vacA* positivity determines the interaction with epithelial cells causing the inflammatory reaction and vacuolization reaction.

Gastric adenocarninoma

Although the incidence of gastric cancer has been declining worldwide since the 1930s, it is still one of the most common human malignancies. Evidence for an association between *H. pylori* infection and gastric cancer first came from epidemiological studies. The prevalence of *H. pylori* infection paralleled that of gastric cancer in different populations around the world. There is a three to eightfold increase in the risk of gastric cancer in *H. pylori* infected patients. In addition, *H. pylori* infection preceded gastric cancer in other studies (Nomura *et al.*, 1991; Parsonnet *et al.*, 1991; EUROGAST, 1993). About half of the malignancies involving the gastric body and antrum are linked to *H. pylori* infection but tumors arising in the gastroesophageal junction are not associated with this infection (Parsonnet, 1998). Individuals with infection involving the gastric body have a higher risk than those with infection involving the antrum. These patients seem to have less dense colonization with *H. pylori* and a state of hypochlorhydria as compared with patients with antral involvement (El-Omar *et al.*, 1997). On the other hand, most of the people with *H. pylori* infection will not develop gastric cancer.

A recently published prospective study from Japan that included 1526 patients followed over an average of eight years (Uemura *et al.*, 2001). They found a significantly higher incidence of gastric cancer in the *H. pylori* positive patients with history of nonulcer dyspepsia, gastric ulcers, and hyperplastic gastric polyps, but not among those with duodenal ulcers.

The pathogenesis of gastric cancer is believed to be different than that of PUD. It has been shown that patients with ulcerative disease actually have a lower incidence of gastric cancer (Hansson *et al.*, 1996, Uemura *et al.*, 2001). It is known that chronic epithelial injury has a carcinogenic effect in many tissues and is thought to be one of the mechanisms implicated in the development of gastric cancer in patients infected with *H. pylori*. This organism resides in the gastric mucosa and it causes chronic superficial gastritis. Differences in bacterial virulence and a combination of host factors, such as differences in the immune and reparative responses, may determine the ultimate outcome (Scheiman and Cutler, 1999). Inflammation will induce cell proliferation, mutation and eventually selection of the fittest mutant clone (Murakami *et al.*, 1997; Parsonnet, 1998). There is also a release of free radicals that can damage DNA nucleotides which will lead to mutations and if left unrepaired can result in metaplasia and cancer (Parsonnet, 1998). Finally, in 1994 the World Health Organization declared *H. pylori* to be a type I carcinogen and a definite cause of cancer in humans (IARC, 1994).

The effect of *H. pylori* eradication in preventing gastric cancer is still unclear. Some studies have shown regression of preneoplastic changes in patients successfully treated for *H. pylori* (Borody *et al.*, 1993; Genta *et al.*, 1993), but other studies have failed to show this association (Borody *et al.*, 1995; Sung *et al.*, 1998).

Gastric lymphoma

H. pylori infection appears to lead to development of gastric lymphoid tissue that is not usually found in normal mucosa. This mucosa-associated lymphoid tissue (MALT) can undergo malignant transformation into a rare low-grade B cell lymphoma of the stomach. This organism has been found in the majority of patients with this type of lymphoma (Isaacson and Spencer, 1993) and what is even more remarkable is that 70% of patients with MALT lymphoma have shown to have a complete regression after successful treatment for *H. pylori* infection (Bayerdorffer *et al.*, 1995). Patients with large tumors or with deep invasion into the gastric wall are less likely to respond to therapy (Parsonnet, 1998). Reinfection with *H. pylori* can cause recurrence or the tumor process (Carlson *et al.*, 1996).

A causative role of *H. pylori* in the development of non-Hodgkins lymphoma of the stomach, the most common form of primary gastric lymphoma, has also been suggested (Parsonnet *et al.*, 1994). Chronic antigenic stimulation by *H. pylori* has been proposed as the mechanism (Isaacson, 1994).

Role in nonulcer dyspepsia

Nonulcer dyspepsia is defined as the presence of pain or discomfort in the epigastrium, associated with nausea, vomiting, heartburn, early satiety, anorexia and belching, and with no evidence of structural or biochemical abnormalities in the gastric mucosa. The annual prevalence in western countries is approximately 25%, and it accounts for about 5% of office visits (Talley *et al.*, 1998). A possible role of *H. pylori* in the etiology of this entity has been suspected since the organism was first linked to gastritis. However, current evidence does not seem to support this relationship. Some studies, including meta-analyses, have found a slight benefit in terms of symptomatic relief in patients who have received therapy against *H. pylori* compared with those treated only with acid suppressive therapy (McColl *et al.*, 1998; Jaakkimainen *et al.*, 1999). These studies have been found to have methodologic weaknesses in the definition of nonulcer dyspepsia, the regimens used, and the documentation of *H. pylori* eradication was not well documented. A recently published meta-analysis of seven randomized controlled trials, using combination therapy against *H. pylori* and with adequate follow-up to assess therapeutic response, did not find a significant trend towards a beneficial effect of therapy (Laine *et al.*, 2001).

Role in other diseases

H. pylori has been linked to several other clinical conditions, such as hypertrophic gastropathy, bronchiectasis, rosacea, chronic urticaria, sudden infant death syndrome and coronary artery disease (Parsonnet, 1998). Some these associations may not actually represent a causative effect of *H. pylori* and several confounding factors may be implicated.

DIAGNOSIS

Diagnostic tests for *H. pylori* infection can be divided into two categories, invasive and noninvasive methods. Invasive tests involve an upper gastrointestinal endoscopy with gastric mucosal biopsy and either rapid urease testing, histology, culture or polymerase chain reaction (PCR) tests. The noninvasive tests include antibody detection, carbon labeled urea breath tests and stool antigen detection. When determining the most appropriate test for a given situation, it is important to consider several factors including: 1) if an endoscopy is planned for any other reasons, 2) is it a follow-up test for a residual infection, and 3) prior history of gastric cancer.

Invasive diagnostic tests

Rapid urease tests

Rapid urease tests are relatively inexpensive assays based on the principle that a pH change brought on by ammonia produced by *H. pylori* urease is detected by the use of an indicator (Marshall *et al.*, 1987). These tests are highly specific and moderately sensitive (Cutler *et al.*, 1995 and 1996).

Several different test procedures are commercially available. CLOtest derived from Campylobacter-like organism (Ballard Medical Products, Draper, Utah) employs direct placement of urease specimen on an agar gel. A change in color from yellow to red signifies the presence of *H. pylori*. Results are obtained about 24 h after tissue placement, although most reactions can be detected within 3-4 h. This test has a sensitivity of 75% to 95% and a specificity of 75% to 100% (Brown and Peura, 1983) (Table I). Two biopsies are recommended to optimize the interpretation, usually one from the antrum and one from the body of the stomach. Other available tests include PyloriTek (Serim Research Corp., Elkhart, Indiana) which uses a semipermeable membrane through which gaseous ammonia can diffuse, accelerating the reaction to about one hour with similar sensitivity and specificity. Also available is the hp*fast* (GI Supply, Camp Hill, Pennsylvania), the newest test, in which a cell-wall detergent is added to the agar in an attempt to improve test performance but clinical evaluations have demonstrated similar results to the CLO test.

The rapid urease tests are based on the presence of adequate numbers of bacteria in the specimen. The sensitivity of these tests can be adversely affected by the recent use of antibacterial agents or medications that could alter the urease activity, such as proton pump inhibitors (PPI) or bismuth compounds (Cutler *et al.*, 1996).

Histology

Although biopsy of the gastric mucosa with histologic

Table I. Comparison of diagnostic tests for *H. pylori* infection

Test	Sensitivity (%)	Specificity (%)	Need for endoscopy
Histology	85-100	85-100	Yes
Culture	50-95	100	Yes
Rapid urease test	75-95	75-100	Yes
Polymerase chain reaction (PCR)	93-100	86-100	Yes [a]
Urea breath test	90-100	95-100	No
Serology	94-99	91-100	No
Stool antigen test	80-100	91-97	No

[a]PCR has been used to detect *H. pylori* from other sites such as saliva, stool, gastric juice, dental plaque and bile.

examination may be considered the gold standard test for diagnosis of *H. pylori* infection, this test is easily affected by factors such as the site, number and size of specimens as well as the stain used and the expertise of the pathologist (el-Zimaity *et al.*, 1995; Andrew *et al.*, 1994). The presence of *H. pylori* in the gastric mucosa can be patchy (Cutler, 1996) and at least two antral biopsies with hematoxylin and eosin are recommended to increase the yield. Other stains used include Genta, Giemsa and Warthin-Starry, and can be useful when the diagnosis is unclear. The presence of intestinal metaplasia or gastric atrophy can be difficult to interpret (Karnes, 1991). On the other hand, the absence of chronic inflammation excludes the diagnosis of *H. pylori* infection (Cutler *et al.*, 1995).

A recent use of antibiotics, proton-pump inhibitors or bismuth containing compounds, can improve the histologic appearance of the gastric mucosa without microbiological cure (Cutler, 1996; Kuipers *et al.*, 1995). In patients that have received treatment, specimens from both the antrum and the body are recommended. The presence of chronic active gastritis in a follow up specimen may represent slow resolution and not necessarily treatment failure (Cutler, 1996).

Culture

Identification of *H. pylori* by biochemical and morphological markers is probably the most specific method of diagnosing infection by this organism. This method also allows the determination of antibiotic sensitivities which may be necessary in certain settings, such as areas with a very a high rate of antibiotic resistance. However, the limited availability of this method, its cost, and the fastidious nature of the organism, are major limiting factors of this test (Cutler, 1996)). The sensitivity and specificity of this test ranges from 77% to 92% and 100%, respectively (Brown and Peura, 1983) [Table I]. Culture of *H. pylori* is currently performed in very few institutions and its use is probably reserved for research purposes.

Polymerase chain reaction (PCR) tests

This method is based on the amplification of short regions of the *H. pylori* DNA. Any region of the gene can be used, provided that the DNA sequences of the template are known. Special care is needed to ensure that the targeted gene is well conserved in the organism of interest and will not cross-react with genes from similar or related bacteria, yielding false positive results (Ho and Windsor, 2000).

Compared with histology and cultures, PCR has a sensitivity of 93% and a specificity of 100% with a threshold of 10 to 100 organisms per specimen (Ho and Windsor, 2000). It allows the detection of DNA in samples that are too small or too degraded for other types of analysis. There are no requirements in terms of previous treatment and transport or storage of the specimen (van Zwet *et al.*, 1993). The main disadvantage of this method is the risk of false positive findings due to contamination of the specimen in the clinical or laboratory settings (Roosendaal *et al.*, 1994). This technique may be reserved for situations when a highly sensitive and objective method is desired, when rapid results are needed or when transport conditions cannot be controlled (Ho and Windsor, 2000). Other potential uses of this method include identification of *H. pylori* from other sites (i.e., saliva, stool, water supply), antibiotic susceptibility testing, epidemiologic studies, detection of potentially more pathogenic strains carrying the *cagA* and *vacA* genes, and the possibility of large scale international retrospective analysis (Ho and Windsor, 2000).

Noninvasive diagnostic tests

Carbon-labeled urea breath tests

The carbon-labeled urea breath tests (UBT) are based on the fact that the carbon dioxide generated by the action of *H. pylori* urease is detected by isotope assay. These tests utilize orally administered carbon-13 (^{13}C) or carbon-14 (^{14}C) labeled urea. Urease cleaves the labeled urea to ammonia and labeled CO_2 that is rapidly absorbed into the bloodstream and eventually expelled in the breath. Using mass-spectroscopy for ^{13}C or scintillography for ^{14}C labeled CO_2 is measured in the exhaled air. The test takes less than 30 min. These tests have a sensitivity of 90% to 100% and a specificity that ranges from 95% to 100%

(Graham *et al.*, 1987; Marshall *et al.*, 1991) (Table I).

The sensitivity of UBTs can be adversely affected by the recent use of antibiotics, proton-pump inhibitors, and to a lesser extent by H_2-receptor blockers or antacids (Chey, 2000). The sensitivity can also be reduced in patients with previous gastrectomy but this effect has not been clearly established (Lotterer *et al.*, 1993). UBTs have been shown to be accurate in confirming eradication of *H. pylori* when performed four to six weeks after completion of therapy (Slomianski *et al.*, 1995) and they are currently recommended for this purpose. The use of UBT in the primary diagnosis of *H. pylori* infection may be limited because it is more expensive than antibody detection methods.

Serologic testing

Chronic infection with *H. pylori* produces local and systemic immunologic responses that lead to the production of IgG and IgA antibodies. Measurement of IgG is the preferred test since levels of this antibody are a more accurate indication of infection status (Cutler *et al.*, 1995). These tests are inexpensive, easy to perform and have a sensitivity that ranges from 94% to 99% and a specificity of 91% to 100% (Table I). Several modalities have been used to detect these antibodies, but the commonly used methods are the quantitative enzyme-linked immunosorbent assays (ELISA) and the qualitative in-office immunoassays (Feldman and Evans, 1995; Anderson *et al.*, 1997).

The most accurate test uses serum antibodies from clotted blood obtained by venipuncture because the dilution of the serum is constant (Ho and Marshall, 2000). Blood samples obtained from a fingerstick can be less sensitive due to technical difficulties that may change the antibody concentration in the serum. Whole-blood serum test results have sensitivities in the 90% range (Enroth *et al.*, 1997), while those obtained with fingerstick procedure show only 75% to 90% range (Ho and Marshall, 2000). A saliva test is also available with accuracy similar to that of fingerstick-based tests.

The gold standard of serologic tests is the immunoblot in which a visual representation of multiple antigens can be obtained in an individual patient. It has a sensitivity of 95% to 97%. A recent study comparing this test with other commercially available noninvasive and invasive methods found the immunoblot to have excellent levels of sensitivity and specificity. In addition there is an added benefit of detecting *cagA* and *vacA* antibodies that have been found in more pathogenic strains (Monteiro *et al.*, 2001). Although commercially available, this test is considerably more expensive (Ho and Marshall, 2000).

Serologic testing is the preferred diagnostic method in previously untreated patients with clinical symptoms suggestive of *H. pylori* infection because of its low cost, noninvasiveness, and high sensitivity and specificity. Some studies (Lerang *et al.*, 1998; Feldman *et al.*, 1998) have found it useful to follow antibody response to evaluate effectiveness of treatment with levels that fall about 50% after six months. However, others have found persistence of positive serology even 12 months after completion of treatment (Cutler *et al.*, 1998). The role of serology in confirming eradication of *H. pylori* is still uncertain and should not be used for this purpose.

Stool antigen test

A newly developed enzyme immunoassay for the detection of *H. pylori*-specific antigen in stool is commercially available. The commercial kit is Premier Platinum HpSA (Meridian Diagnostics, Cincinnati, Ohio) and utilizes immunoaffinity-purified polyclonal anti-*H. pylori* rabbit antibody adsorbed to microwells for detection of *H. pylori* (Makristathis *et al.*, 1998). Recent studies have found that this test has a sensitivity of 80% to 100% and a specificity of 91% to 97% (Monteiro *et al.*, 2001; Makristathis *et al.*, 1998; Vaira *et al.*, 1999; Vakil *et al.*, 2000) (Table I). Stool testing has shown to be a useful technique in the diagnosis of *H. pylori* infection in certain settings such as in children (Oderda *et al.*, 2000; Konstantopoulos *et al.*, 2001) and in hemodialysis patients (Wang *et al.*, 2001). The role of HpSA in posttreatment follow up is still uncertain with some studies showing results similar to the UBTs (Konstantopoulos *et al.*, 2001; Wang *et al.*, 2001) while others showing less accuracy (Forne *et al.*, 2000; Roth *et al.*, 2001).

Who should be tested for H. pylori?

Diagnostic testing for *H. pylori* should only be done if treatment is intended. Currently, treatment has proven to have value only in patients with active or documented PUD and MALT lymphoma, thus the testing is indicated only in patients with these conditions. Patients with a previous history of PUD on the basis of endoscopic or radiographic studies but have not yet received treatment for *H. pylori* should have a serological test done (Howden and Hunt, 1998). Those with symptoms of ulcerlike dyspepsia should also have a diagnostic test done; a serological test would be the first choice if an endoscopy were not indicated for other reasons. Testing in asymptomatic individuals, patients with nonulcer dyspepsia, those on long term treatment with a PPI for gastroesophageal reflux disease or those with an increased risk of gastric cancer, is still controversial (Battle and Peura, 1999).

Posttreatment testing to document the eradication of *H. pylori* is recommended in patients with complicated ulcer disease, MALT lymphoma, and after resection of early gastric cancer. The posttreatment test should be performed at least four weeks after treatment is completed (Battle and Peura, 1999).

TREATMENT

H. pylori seemed to be difficult to eradicate at the time of its initial discovery although the reasons for this were not clear. Most current therapies were developed through the process of trial and error. After poor results were obtained with single antibiotic therapy, the necessity of combination therapy for successful eradication of this organism was recognized (Shiotani *et al.*, 2000). The highest rates of eradication were obtained with combinations of antibiotics and antisecretory agents or a bismuth formulation. Antibiotic resistance is a growing problem and, as discussed below, need to be taken into account when deciding a treatment regimen.

The outcome of therapy is influenced by several factors and these include compliance with the regimen, antibiotic resistance, dosage and duration of treatment. Accept-able goals are ≥ 90% cure rate on *per-protocol* basis and ≥ 80% cure rate on *intent-to-treat* basis (Megraud et *al.*, 1997).

Current regimens approved by the Food and Drug Administration (FDA) are listed in Table II. These are two classes regimens: dual therapies (approved but not recommended due to their low cure rates) and more efficacious triple therapy regimens. Triple regimens consist of a PPI, and two antibiotics such as clarithromycin and amoxicillin or tetracycline and metronidazole. In practice, many different combination regimens are available. Some examples of these regimens may contain triple therapies containing bismuth, PPI, or ranitidine bismuth citrate (RBC). Bismuth quadruple therapy is also available (Shiotani *et al.*, 2000). Regimens that include a PPI or RBC are usually twice-a-day combination of two antibiotics of the following: clarithromycin (500 mg), amoxicillin (1 g), or metronidazole (500 mg). The success rate is similar using a PPI or RBC when used for 10 to 14 days in patients with duodenal ulcer and antibiotic-susceptible *H. pylori.* Cure rates of 95% to 99% have been achieved with either regimen (Shiotani et *al.*, 2000). Patients with nonduodenal ulcer disease seem to have lower cure rates and this may be related to the *cagA* status of the organism involved (Broutet *et al.*, 2001). The efficacy of regimens with a shorter duration (5 to 10 days) is still under scrutiny and although some studies have found acceptable eradication rates (Laine *et al.*, 1996; Dajani *et al.*, 1999; Cammarota *et al.*, 2000; Calvet *et al.*, 2001), others have failed to show a favorable outcome (Bhasin *et al.*, 2000; Garcia *et al.*, 2000). An increased incidence of antibiotic resistance may result with shorter duration of therapy but this theory needs further studies (Pilotto *et al.*, 2000).

Quadruple therapies that include a bismuth compound; tetracycline, 500 mg; metronidazole, 250 mg or 500 mg; and an antisecretory agent, are given four times a day and have been associated with the highest cure rates (Shiotani *et al.*, 2000). Because of the significant pill burden and the higher incidence of related side effects, these highly effective regimens are usually reserved for patients that have failed initial treatment with a simpler regimen.

Repeated failures to eradiate *H. pylori* are difficult to manage and in these cases culture of the organism with subsequent antibiotic sensitivities should be performed before deciding on the next regimen.

H. pylori eradication is also recommended in patients with low grade gastric MALT lymphoma, since complete remission has been seen in the majority of successfully treated patients (European, 1997; Howden and Hunt, 1998). A highly effective regimen, as used in patients with PUD, should be chosen in this setting, such as the PPI and bismuth subsalicylate regimens described above (Steinbach *et al.*, 1999).

Antibiotic-resistant *H. pylori*

Antibiotic resistance of *H. pylori* is a growing problem, both in the industrialized world and in developing countries. Efficacy has been shown to decrease when resistance to one of the antibiotics is present (Houben *et al.*, 1999). Resistance to metronidazole has been rather high at 15% and 39% in Europe and the United States (Cabrita *et al.*, 2000; Pilotto *et al.*, 2000; Osato *et al.*, 2001), while in developing countries it has been even higher at 45% and 70% (Vasquez *et al.*, 1996; Valdez *et al.*, 1998; Salcedo and Al-Kawas, 1998). Resistance to clarithromycin was

Table II. FDA-approved therapies for *H. pylori* infection

Regimen
• Lansoprazole 30 mg plus clarithromycin 500 mg plus amoxicillin 1 g, all twice daily for 10-14 days
• Omeprazole 20 mg plus clarithromycin 500 mg plus amoxicillin 1 g, all twice a day for 10 days
• Omeprazole 40 mg once a day plus clarithromycin 500 mg three times a day for 14 days, followed by omeprazole 20 mg once a day for 14 days
• Ranitidine bismuth citrate (RBC) 400 mg twice a day plus clarithromycin 500 mg three times a day for 14 days, followed by RBC 400 mg twice a day for 14 days
• Bismuth subsalicylate 525 mg plus metronidazole 250 mg plus tetracycline
• 500 mg, all four times a day, plus H_2-receptor antagonist therapy, all for 14 days, followed by H_2-receptor antagonist therapy for 14 days
•Lansoprazole 30 mg plus amoxicillin 1 g, all three times a day for 14 days

reported in 1.8% of the isolates in a study from Italy (Pilotto *et al.*, 2000), 12% in a recent study from the United Sates (Osato *et al.*, 2001), 22% in a report from Portugal (Cabrita *et al.*, 2000) and 50% in a study with Peruvian subjects (Vasquez *et al.*, 1996). Resistance to macrolides seems to be slowly increasing worldwide while resistance to tetracyclines and amoxicillin is still rare. Although initially susceptible, a rapid development of resistance is the rule in case of all the quinolone antibiotics thus far. Both metronidazole and clarithromycin resistance have been documented in young women from urban areas and in those that have been previously treated with these agents.

Several drugs and different combinations have been under investigation for the treatment of antibiotic resistant *H. pylori*. A new PPI, used in combination with amoxicillin and rifabutin, has shown eradication rates of 71% and 87% when used as salvage therapy in cases of documented antibiotic resistance, including clarithromycin-resistant isolates (Perri *et al.*, 2000 and 2001). A new class of antibiotics known as ketolides, HMR 3647 or telithromycin; and HMR 3004, has shown significant *in vitro* activity against *H. pylori* but clinical data are not yet available (Gustafsson *et al.*, 2001).

VACCINE DEVELOPMENT

The search for a vaccine against *H. pylori* is based on the fact that a large population is involved, and the potential role in the prevention *H. pylori* associated disease. When available, the vaccine would best be applied in areas where childhood infection is endemic to prevent further complications.

Various approaches have been followed in the development of a vaccine against *H. pylori*. Several preclinical studies using selected antigens known to be involved in the pathogenesis of the infection, such as urease, *vacA*, *cagA*, the neutrophil-activating protein (NAP), and others, have been carried out (Ghiara *et al.*, 1997; Kleanthous *et al.*, 1998; Del Giudice *et al.*, 2001). Results from clinical trials performed on volunteers have also been reported. There is one study from Switzerland involving an oral vaccine with urease and *Escherichia coli* heat-labile enterotoxin (LT). 26 *H. pylori*-infected individuals received this vaccine and had a significant decrease in gastric *H. pylori* density as well as a marked immunologic response, although eradication of *H. pylori* was not observed (Michetti *et al.*, 1999). Another study from the United States involved an oral inactivated whole-cell (HCW) vaccine with or without LT. 41 healthy volunteers with or without *H. pylori* infection received this vaccine and had a significant immunologic response based not only on the rise of mucosal anti-HCW antibodies but also on the marked lymphoproliferative response. Again, there was no evidence of *H. pylori* eradication (Kotloff *et al.*, 2001). Although these study results are encouraging, the development of an effective and safe *H. pylori* vaccine requires a better understanding on host immune response to *H. pylori* infection.

CONCLUSIONS

H. pylori is one of the most common human pathogens. Several disease states have been associated with the infection by this organism. Current evidence strongly supports a causal effect for peptic ulcer disease, gastric adenocarcinoma and MALT lymphoma. The role in other diseases including nonulcer dyspepsia is less clear. The diagnosis can be made by either invasive and noninvasive techniques. If an endoscopy is going to be performed, the rapid urease test is the preferred diagnostic method. In patients with typical ulcerlike symptoms a serological test is the most convenient tool if endoscopy is not indicated for other reasons. The UBTs are the test of choice to document eradication of *H. pylori* infection. The role of newer diagnostic techniques, such as polymerase chain reaction and stool antigen test, still need to be determined.

The most effective treatment regimens include a PPI along with amoxicillin and clarithromycin or the combination of bismuth subsalicylate with tetracycline and metronidazole. Resistance to metronidazole and clarithromycin is a growing problem worldwide. A combination of a new PPI, pantoprazole, with amoxicillin and rifabutin, has shown to be effective in cases of recurrent failure due to antibiotic resistance. Several preclinical and a few clinical trials of *H. pylori* vaccines have been performed with promising results. Further research is still needed to clarify other aspects of this infection. These areas include the precise pathogenic mechanism, the immune response of the affected host, the causal effect in other diseases, the role of antibiotic susceptibility testing, the best treatment for eradication especially in cases of antibiotic resistance, and the best target for a vaccine.

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