24

Helicobacter pylori *Infections*

BARRY J. MARSHALL
ROBERT H. GILMAN

INTRODUCTION

Helicobacter pylori colonizes the mucus layer of the human stomach and causes inflammation termed *active chronic gastritis.*[1] *H. pylori* can easily be identified using simple techniques available in all microbiology laboratories. The bacterium infects more than half of the population of the world, is more common in tropical countries, and in some people it causes peptic ulceration or gastric cancer. In the tropics it may be associated with reduced gastric acid,[2] increased diarrhea, and malnutrition.[3] *H. pylori* can be diagnosed by direct examination of gastric mucosal biopsy tissue obtained at endoscopy, or noninvasively using serology, a urea breath test, or a fecal antigen test. Cure of the infection is possible in most patients with a two-week treatment using combinations of antimicrobial agents and acid-lowering drugs.[4] In the tropics, due to poor sanitation, reinfection is common.[5–7] For this reason there is currently much interest in the development of an oral vaccine to prevent new infections.

AGENT

H. pylori is the type strain of a new genus of spiral-shaped bacteria named *Helicobacter*. Their morphology and sheathed flagella may facilitate motility in the mucus layer of the gastrointestinal tract. *H. pylori* is microaerophilic, which means that it prefers a reduced amount of oxygen for growth, but is not anaerobic. This is probably the environment found in the mucus layer of the gut, a transitional zone between the anaerobic lumen and the oxygenated mucosa. The characteristics and growth requirements of *H. pylori* are listed next.

Morphologically, *H. pylori* is a gram-negative spiral, 3.5 μm long × 0.6 μm thick, with 1.5 wavelengths and four to seven sheathed flagella at one end of the organism, as shown in Figure 24-1. In tissues *H. pylori* appears spiral and lies close to the gastric epithelial cells and in the mucus glands. Squashed or smeared fresh gastric biopsy specimens may be stained by Gram stain or examined by phase contrast microscopy.[8,9] In histologic sections *H. pylori* stains well with Giemsa, toluidine blue, or silver stains. Hematoxylin and eosin (H&E) stain does not adequately demonstrate *H. pylori.*

In culture, *H. pylori* appears longer and spiral forms are not as obvious. Usually comma shapes and U-shapes (unseparated dividing organisms) are seen.[10]

Culture

H. pylori is microaerophilic, growing in reduced O_2 at 37°C in 4 to 6 days on fresh (preferably) chocolate or blood agar. The "*Campylobacter* atmosphere" generated by a commercial *Campylobacter* kit in a gas jar provides atmosphere for *H. pylori* culture. If available, a 10% CO_2 incubator also provides excellent growth conditions. "*Campylobacter*" atmospheres are also available as premixed cylinders that can be used to fill a sealable plastic bag with a moist paper towel inside. If nothing else is available, a candle jar with moist paper towels in the bottom will provide an adequate atmosphere, and even gas-generating methods such as the "steel wool and Alka Seltzer" idea of Pennie and colleagues[11] can be used.

Selective Media

Although *H. pylori* can be isolated easily from gastric biopsy samples onto nonselective media, 20% of patients have bacterial contamination of the biopsy, and overgrowth of commensal flora will make isolation of *H. pylori* difficult. To maximize the isolation rate, a selective medium can be made by adding vancomycin, trimethoprim, and amphotericin to the culture medium.[12] Ready-made selective media for *H. pylori* culture are available,[13] or *Campylobacter* isolation media such as Skirrow's medium may also be used.[14]

Identification

On blood or chocolate agar, transparent or pale-yellow 1- to 2-mm "water spray" colonies appear after 3 to 6 days. They are strongly positive for catalase, oxidase, and rapid urease. In the last-named test, a pink color is observed within 5 minutes of applying a colony to Christensen's urea agar. The organism may also be grown in broth such as shaking tubes of *Brucella* broth[15] or in gas-permeable shaking bags[16] in a CO_2 incubator, or in fermenters.[17]

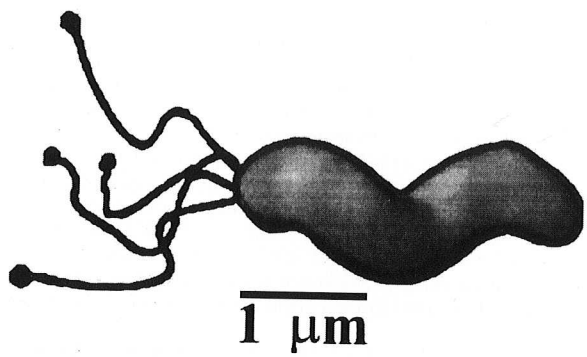

FIGURE 24-1 *Helicobacter pylori* (3.5 × 0.6 μm) has a smooth wall and four to seven sheathed flagella arising from only one end of the cell. These features distinguish it from *Campylobacter* spp., which have rough cell walls and a single, thinner, unsheathed flagellum at each end of the cell. Other *Helicobacter* spp. have distinguishing features such as many flagella and axial filaments (*H. felis* from cats) or flagella sprouting from the sides of the organism (*H. mustelae* from ferrets). Mature organisms appear as spiral forms with 1.5 wavelengths.

Pitfalls

When subculturing *H. pylori,* one should always examine the Gram's stain morphology of *H. pylori* as well as perform the biochemical identification tests described earlier. Contaminating organisms may appear similar to the naked eye and are often urease, oxidase, or catalase positive.

EPIDEMIOLOGY AND TRANSMISSION

H. pylori infects more than 70% of persons in most developing countries and about 30% of persons in developed countries. In societies that have recently emerged to affluence (such as Japan), *H. pylori* is still quite common and infects most persons over the age of 40 years.

H. pylori is acquired in childhood, probably by the fecal-oral route. The bacterium has been isolated from the feces of children in The Gambia[18] and polymerase chain reaction (PCR) techniques have demonstrated the genome of the organism in water from Peru.[5] In developing countries, children may be infected at the rate of 15% per annum so that most of the population is infected by adulthood. The initial infection with *H. pylori* may be somewhat precarious in that some children lose the infection spontaneously for a time but then reacquire it from the environment. This may occur several times before the child maintains a stable permanent gastric infection.[19] Initially the infection may spread from one parent to one of the children in the family, then spread to other family members, siblings, or the uninfected parent by fecal contamination, and even aerosols of vomitus.[20,21] Thus the infection is transmitted from one generation to the next, but young children appear to amplify the infection rate.[22]

The exact mechanism of spread is still somewhat controversial. *H. pylori* DNA is sometimes present in the dental plaque of some infected persons,[23] but actual live organisms are only very rarely culturable from the oral cavity. Thus the organisms might occasionally be carried to the mouth in gastric reflux, but probably do not live in the mouth. Oral-oral spread of *H. pylori* seems possible but has been hard to demonstrate. For example, in Belgium, investigators studied infants born to 67 infected mothers but could detect only one new *H. pylori* infection by breath test during a 12-month period.[24] This may mean that most new infections in developing countries are fecal-oral, that is, from other children, relatives, or from environmental sources. The prevalence rate in developing countries, as compared with Western countries, is shown in Figure 24-2.

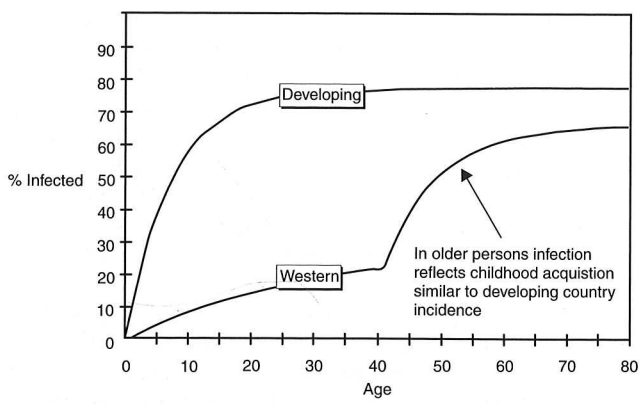

FIGURE 24-2 The prevalence of *Helicobacter pylori* in developing and Western countries. In developed countries, *H. pylori* is decreasing in prevalence so that most of the infections are in those over the age of 50 years who likely acquired the infection during childhood. The infection in young persons is only seen in immigrants from high-risk countries.

PATHOGENESIS

Acute Infection

Immediately after ingestion by a healthy person, it is thought that the urease enzyme of *H. pylori* enables the bacterium to survive in acid by generating ammonia and bicarbonate from urea present in the gastric juice:[25] Vague symptoms of epigastric discomfort then commence 72 hours after ingestion of the organism. Gastric acid secretion may initially increase, associated with varying degrees of epigastric discomfort[26] followed by vomiting episodes.[20,27] Symptoms usually settle after several days as the bacterium induces achlorhydria. The cause of the achlorhydria is unknown; however, it might involve the action of a bacterial toxin,[28] ammonia, or the presence of cytokines, especially interleukin-1β.[29]

In the tropics, achlorhydria has long been associated with malnutrition and susceptibility to enteric infections such as *V. cholerae* and enterotoxigenic *E. coli* infections.[30,31] So far, *H. pylori* infection is the only known environmental agent proven to cause achlorhydria. Since the acute infection is associated with long periods when the gastric acid barrier is impaired, *H. pylori* may increase susceptibility to the other enteric pathogens and thus be an important pathogen in the pediatric age group.[32] Perhaps related to the achlorhydria, acute *H. pylori* infection is associated with a mild growth arrest,[33] and in a few studies infected children grow up shorter than controls.[34,35]

The achlorhydria of acute *H. pylori* is "chemically induced" and is reversible, unlike that seen after many years of the chronic *H. pylori* infection, where atrophy of gastric mucosa causes acid secretion to be irreversibly diminished (see following discussion).[32]

Attachment

H. pylori has several attachment mechanisms that enable it to selectively colonize gastric mucosa but not intestinal mucosa. To various degrees, *H. pylori* is adherent to Lewis B antigen,[36] phosphatidylethanolamine,[37] and ganglioside GM_3,[38] all of which are present on the gastric mucus epithelial cells. In addition, *H. pylori* synthesizes Lewis X antigen which may be a mechanism of "molecular mimicry" to make the organism similar to the host tissue, thus attenuating the immune response.[39] Once attached, *H. pylori* induces the production of interleukin-8 (IL-8) which in turn attracts neutrophils.[40] If deep colonization occurs in the acid secreting mucosa, secretion of IL-1 by inflammatory cells may inhibit acid secretion

Cytotoxins

The effect of *H. pylori* on the gastric mucosa is more severe when the bacterium produces cytotoxins. The most notable of these is CagA, which is one component of about 30 genes in the cagA pathogenicity island. This structure, present in most

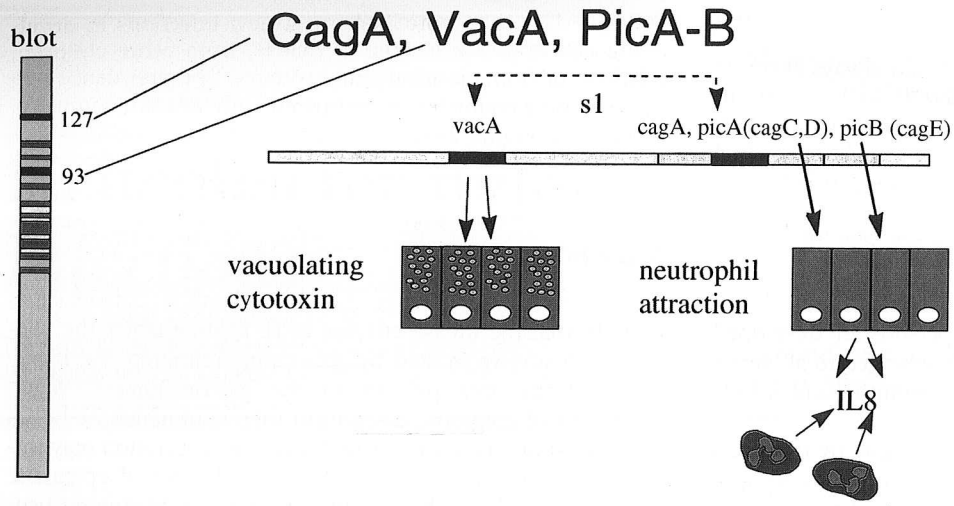

FIGURE 24-3 Relationships of cytotoxin genes and proteins. The left side diagrams a representative immunoblot pattern of a patient with a duodenal ulcer and *Helicobacter pylori* infection. The 127-kDa band is the cytotoxin-associated gene A (*cagA*) product. The cagA pathogenicity island has a higher guanine + cytosine content than the rest of the *H. pylori* genome. This suggests that the *cagA* gene group has long ago been imported from a different genus. The *vacA* (vacuolating toxin A) gene has two main subunits (s and m), and these can each be in two subtypes (s1, s2, and m1, m2). When s2 is present, the cytotoxic potential of the organism is very weak and is not usually associated with peptic ulcer, and the CagA island is usually absent.

strains, codes for a "Type IV" secretion system. This causes a hollow pilus-like structure to form which then injects the CagA toxin protein into the epithelial cell. CagA toxin emulates at least two growth-factors causing the epithelial cell to adopt a less structured, more mobile, primitive form. Thus the integrity of the gastroduodenal epithelium is compromised and nutrients are able to leak up from between the cells and towards the *H. pylori*. In developed countries, CagA is present in only 60% of strains, whereas in most tropical or developing countries (Peru is a good example) more than 90% of strains are CagA-positive.

Linked to the cagA island is a propensity for such isolates to produce VacA toxin of a more virulent subtype. VacA is a membrane pore which leads to leaky intracellular organelles and the appearance of "vacuoles" in the cells. The two *vacA* subunits "s" and "m" can each exist in two forms, s1 or s2, and m1 or m2; only s1 (m1 or m2) are associated with active cytotoxin (Fig. 24-3).[41] Other toxins are BabA (Blood-Group Antigen Binding Toxin A)[42] and iceA (Induced with Contact to Epithelium). Toxin-producing strains (cagA, vacA, BabA) are more likely to be present in persons with duodenal ulcer or with gastric cancer because they upregulate the degree of mucosal inflammation. This effect is also modulated by various interleukin polymorphisms in the patient, such as IL-1β and its receptor so that some individuals with a virulent strain could experience a 5–50 fold increased risk of cancer from a *H. pylori* infection.[43,44]

DISEASE ASSOCIATIONS AND CLINICAL MANIFESTATIONS

Active Chronic Gastritis

Chronic gastritis refers to the histological presence of mononuclear cells (lymphocytes and plasma cells) in the gastric mucosa. Histologic chronic gastritis is associated very closely with *H. pylori* and there are few patients with this finding who do not have the organism.[45] There is also a variable amount of neutrophilic infiltration of the mucosa, typically invading the necks of mucous glands. This latter appearance gives the name "active" or "acute" to the typical histologic appearance of "active chronic gastritis."[46] Because the cause of chronic gastritis was unknown before the discovery of *H. pylori*, the terminology was confusing and nonstandardized. In most of the literature, terms such as "atrophic gastritis," "superficial gastritis," "simple gastritis," "antral gastritis," and "type 1 gastritis" all refer to the histology of *H. pylori* infection. The various classifying and descriptive terms for the lesion have been well described in several papers.[47–49]

In the antrum of the stomach, *H. pylori* is most numerous on the surface of the epithelium (beneath the mucus layer) but it also lives in the mucus-secreting glands. In the body of the stomach (corpus), almost all of the organisms are found on the surface. The inflammation tends to collect near the bacteria. Thus in the corpus, the appearance is that of a "superficial gastritis," whereas in the antrum, the inflammation is deeper. In either place the lesion can be associated with lymphoid follicles.[50]

In the long-standing case of chronic active superficial gastritis, the superficial inflammation includes a predominance of PMNs. In contrast, in the deep portion of the glands below the necks the inflammation is predominantly chronic with lymphocytes surrounding destroyed remnants of gastric deep glands; an appearance that has been called the glandular lymphocytic adherence lesion. As this deep inflammation becomes more extensive, the deep glands become islands separated by chronic inflammation, they are less tightly packed, and their depth decreases. This decrease is called chronic atrophic gastritis. Another way deep glands are replaced is through intestinal metaplasia. The degree of deep glands lost through either inflammation or intestinal metaplasia determines how extensive is the chronic atrophic gastritis.[51] Chronic atrophic gastritis is a precancerous lesion probably due to the effects of long-term hypochlorhydria and the resultant presence of abnormal gastric flora. In general, inflammation, both superficial and deep, is more severe in the antrum, less in the corpus, and least in the cardia.[52] In both the antrum and the corpus, the preceding lesions may be associated with lymphoid follicles. Lymphoid follicles are rarely seen in the gastric mucosa except after *H. pylori* infection.[53]

Attachment of the bacterium causes damage to the cytoskeleton of the epithelial cells so that they bulge out rather than maintain a flat luminal surface. Under periodic acid–Schiff staining, the apical mucus content of infected gastric mucosa is less than normal and cells are shorter. These changes have been termed the destructive mucin lesion of the covering gastric epithelium.[49,54]

Over the lifetime of the infected person, inflammation may destroy the glandular elements (atrophy) and intestinal cells often replace gastric mucus-secreting epithelium (intestinal metaplasia). The resulting atrophic gastritis is the final "burned out" phase of *H. pylori* infection, usually seen in older persons. In tropical countries, however, where *H. pylori* may have been present from a very early age, atrophic gastritis may be seen in young adults and is believed to be a major risk factor for gastric cancer.[55]

Duodenal and Gastric Ulcer

The most obvious disease associated with *H. pylori* is peptic ulceration (Fig. 24-4). More than 90% of duodenal ulcers are associated with toxin-producing *H. pylori*.[56] When a patient with a duodenal ulcer does not have *H. pylori* infection, etiologic factors such as Zollinger-Ellison syndrome or nonsteroidal anti-inflammatory drug (NSAID) use are likely.[57]

In gastric ulcer, two causes prevail, and many patients will exhibit both. Most gastric ulcers have *H. pylori* and these can be identified by presence of the bacterium or chronic gastritis. The stomach is also directly exposed to ingested agents such as an NSAID and is more likely than the duodenum to ulcerate in response to these agents. Therefore, in the United States, about 50% of gastric ulcers are not associated with histologic chronic gastritis or *H. pylori* but are caused by NSAIDs.[48]

In tropical countries where NSAIDs are less widely used and *H. pylori* is very common, most gastric ulcers are caused by *H. pylori*.[58] Perhaps because of this, gastric ulcers are more likely to be malignant and require endoscopy and biopsy for histologic examination.

The proposed causation of duodenal ulcer is as follows. Persons with *H. pylori,* but with colonization mainly in the antrum, have high acid secretion but a defective mucosal barrier. Inflammation in the antrum impairs the growth of D cells (which make somatostatin) and thus decreases their inhibitory effects on the gastrin-producing G cells. This results in higher gastrin production, which may in turn, over the lifetime of the patient, cause a hyperplasia of the acid-secreting mucosa. Gastric mucus cells normally present in the duodenal bulb become colonized with *H. pylori,* seeded from the infection present in the antrum. Neutrophil invasion of the duodenal epithelium (duodenitis) increases susceptibility to ulceration. Inflammation is more severe when the *H. pylori* secretes CagA, thus associating the cytotoxin with duodenal ulcer.[59,60]

All persons with peptic ulcer should be tested for *H. pylori* and treated with antimicrobial agents when evidence of the infection exists.[4,61] Ulcer recurrence is less than 10% when *H. pylori* is eradicated, whereas more than 90% of ulcers recur when the bacterium persists.[62] Thus, most patients are cured of their ulcer disease with effective antibiotic treatment.[63]

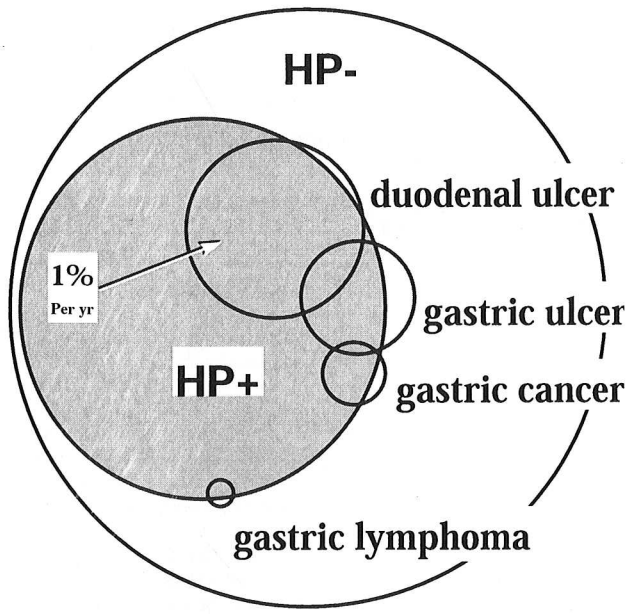

FIGURE 24-4 Disease associations with *Helicobacter pylori* (HP). The large circle represents a typical population in a developed country where 60% of persons are *not* infected with *H. pylori.* The darker circle represents the 40% of persons who *are* infected with *H. pylori.* Even so, nearly all the duodenal ulcers and gastric ulcers occur in the *H. pylori*–positive group. Each year, 1% of infected patients undergo transition from asymptomatic gastritis to symptomatic peptic ulcer. Note that most gastric adenocarcinomas and gastric mucosa–associated lymphoid tissue (B-cell) lymphomas also occur in the *H. pylori*–positive persons. Controversy reigns as to the role of *H. pylori* in persons with dyspepsia but in whom ulcers are not found: Should *H. pylori* be treated in these persons, or ignored?

Gastric Cancer

Adenocarcinoma

Worldwide, gastric cancer is the second most common cancer, the high prevalence areas being Brazil, Colombia, Korea, China, and Japan. *H. pylori* infection affects more than half the population in these countries.[63] The incidence of gastric cancer has declined in the United States since 1930. It was the most common cancer, but now it ranks about ninth.[64]

H. pylori confers an approximately sixfold risk of gastric cancer, accounting for about half of all gastric cancers.[63] Thus, in most tropical countries where *H. pylori* is prevalent, gastric cancer is also common. In India, Bangladesh, the Middle East, southern China, and some African countries, however, *H. pylori* is prevalent but gastric cancer is not. This paradox suggests that genetic, dietary, and other unknown environmental factors are also important in the etiology of adenocarcinoma.[64,65]

The proposed chain of events in gastric carcinoma starts with a very early (age 1 to 5 years) infection with *H. pylori* so that the corpus mucosa is damaged during childhood. In this setting, ulcers are unlikely but a large area of the stomach is involved in the process. Damaged areas coalesce into chronic atrophic gastritis and acid secretion diminishes, eventually allowing other organisms to colonize the stomach.[66–68] In this setting, nitrates can be changed to nitrites and then to nitrosamines, which are carcinogenic. In the presence of ammonia, inflammation itself can cause nitrosamines to form in the mucosa. Inflammation is more severe when the *H. pylori* secretes toxin, thus associating several cytotoxins with gastric cancer.

The Ulcer-Cancer Controversy

It has been well demonstrated that CagA toxin–positive strains of *H. pylori* are associated with both duodenal ulcer (a high-acid state) and stomach cancer (a low-acid state). Paradoxically, patients who have duodenal ulcer are protected from developing stomach cancer. This implies that acid protects from the carcinogenic effects of *H. pylori*. Even in Japan, where stomach cancer is common, it is quite rare for a person with duodenal ulcer to develop the malignancy.[69]

One proposed explanation for this is the age of acquisition of the infection. In a tropical country where *H. pylori* is acquired in early childhood and nutrition may be poor, the infection causes severe damage to the acid-secreting area of the stomach.[55] Poor nutrition probably also assists this tendency to develop an asymptomatic low-acid state.[70]

Various factors are associated with the development of gastric cancer associated with *H. pylori* infection. These include the presence of hypochlorhydria due to infection, virulence of the infecting strain, genetic factors of the host, micronutrients in the diet, and geographical location of the patient. Thus, even if CagA toxin is present, the patient may never develop a duodenal ulcer but may be susceptible to gastric cancer in later life.[3]

If *H. pylori* is acquired in late childhood or in the adult years, then the infection tends to affect mainly the antrum of the stomach, leaving the acid-secreting part of the stomach (the corpus) intact. High acid secretion allows the development of duodenal ulcer disease.

Lymphoma

H. pylori eradication therapy should be the initial step in the treatment of proven or suspected gastric lymphoma. Up to 90% of mucosa-associated lymphoid tissue (MALT) lymphomas are associated with *H. pylori*.[71] These indolent B-cell lymphomas are sometimes driven by continuing *H. pylori* antigenic stimulus and regress when *H. pylori* infection is treated.[72,73] Apparent cure of MALT lymphoma occurs in 70% of patients in whom *H. pylori* is eradicated.[74]

DIAGNOSIS

The diagnosis of *H. pylori* may be by invasive or noninvasive methods, or both, as shown in Table 24-1. Endoscopic biopsy of gastric mucosa is the usual invasive method, although invasive methods can include blind biopsy, nasogastric aspiration, or the gastric string test.[75] Noninvasive tests are primarily serologic tests that detect IgG antibody to *H. pylori*, urea breath tests (UBT) to detect gastric urease, and fecal antigen tests.

Invasive Tests

Histology

For histologic study from intact mucosa, mucosal biopsy specimens are taken away from any visible lesion. This allows the pathologist to separate true inflammation (active chronic gastritis) from changes due to acute ulcer healing. Biopsies for

Table 24-1 *Accuracy of Diagnostic Tests for* Helicobacter pylori *Infection in 268 Patients Undergoing Esophagogastroduodenoscopy*

Tests	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
Invasive				
Biopsy: Chronic inflammation*	100	66.3	84.4	100
Biopsy: Acute inflammation†	86.7	93.7	96.2	79.5
Biopsy: Warthin-Starry silver stain‡	93.1	99.0	99.4	88.7
CLOtest rapid urease test§	89.6	100	100	84.1
Noninvasive				
^{13}C-urea breath test‖	90.2	95.8	97.5	84.3
Fecal Antigen Test**	94.1	91.8	93.4	92.6
Serum IgG¶	91.3	91.6	95.2	85.3
Serum IgA	71.1	85.3	89.8	61.8

*Chronic inflammation present in gastric antral biopsies.
†Acute inflammation present in gastric antral biopsies.
‡Warthin-Starry stain of gastric antral biopsy.
§Urease test conducted on gastric antral biopsy with results ascertained at 24 hours.
‖^{13}C-urea breath test 60 minutes after administration of 150 mg ^{13}C-labeled urea.
**Data from Vaira D, Malfertheiner P, Megraud F, et al: Diagnosis of *Helicobacter pylori* infection with a new non-invasive antigen-based assay. HpSA European study group. Lancet 354(9172):30–33, 1999.
¶Serum antibodies to *H. pylori*.
Data from Cutler AF, Havstad S, Ma CK, et al: Accuracy of invasive and noninvasive tests to diagnose *Helicobacter pylori* infection. Gastroenterology 109:136, 1995.

H. pylori should be in addition to biopsies taken to exclude malignancy. At least two or, preferably, three biopsies need to be taken to detect *H. pylori* histologically. One biopsy can be taken from the greater curve of the antrum, one from the mid-corpus, and a third from the lesser curve angular notch.[75] In addition to routine H&E sections, the specimens should be stained with Giemsa or a silver stain such as Warthin-Starry or Genta stain.[76]

Culture

Culture methods have been described earlier. Cultures may be moistened with a single drop of saline and transported to the laboratory in a sterile tube the same day. In some studies, organisms have remained viable in snap frozen biopsies.[77]

Culture has also been performed from blind gastric mucosal biopsies, from gastric aspirates, and from gastric string tests. These methods are less sensitive than culture of biopsies and are not widely used. Biopsy material, gastric mucus, and gastric juice can, of course, also be examined with polymerase chain reaction (PCR) or immunologic methods to detect *H. pylori,* but at present these are not reproducible in different laboratories and have no particular advantage over culture or histology.

Urease Test

The ability of *H. pylori* to produce urease allows one to rapidly detect the organism in gastric biopsy material. Typically, the mucosal biopsy specimen is placed in a medium containing urea and a pH indicator. If urease (or *H. pylori*) is present, urea is converted to ammonia and the pH rises with a subsequent color change.[78] *H. pylori* is a prolific urease producer so that the reaction occurs in a few minutes in biopsies from infected patients. Since the test is so specific for *H. pylori,* once a positive urease test has been noted, other diagnostic material is often unnecessary and may be discarded to save expense.[79]

Noninvasive Tests

Infection is almost always accompanied by a rise in specific IgG antibody to *H. pylori.* IgM is present in some persons during the acute infection but has not been well studied because acute infections are rarely documented. IgA is present in 80% of persons with *H. pylori* so it can be used to diagnose the infection when present, but the absence of IgA does not exclude infection. Thus, in most cases, IgG is the best predictor of *H. pylori* infection (Fig. 24-5).

Tests to detect IgG come as a laboratory-based multi-well enzyme-linked immunosorbent assay (ELISA) kit, which is the most accurate serologic test. In properly selected patients, sensitivity and specificity reach 95%. In patients who have been treated for *H. pylori* infection in the preceding two years, IgG may remain positive and give an incorrect positive result even in persons who no longer have *H. pylori* infection.[80] For this reason the urea breath test is a better choice for follow-up. However, if carefully validated methods are used, serology can be used as a somewhat less accurate predictor of bacterial eradication. In Mexican patients, a 10% drop in ELISA absorbance at one year predicated *H. pylori* eradication in 84% of treated patients.[81]

A fecal antigen test for *H. pylori* is less sensitive than the urea breath test at detecting *H. pylori* after treatment, but fecal tests are ideal when the UBT is unavailable, and in very young children.

As well as being present in blood, small amounts of IgG may be detected in urine and gingival secretions. The latter two sources allow initial diagnosis from urine specimens and saliva.

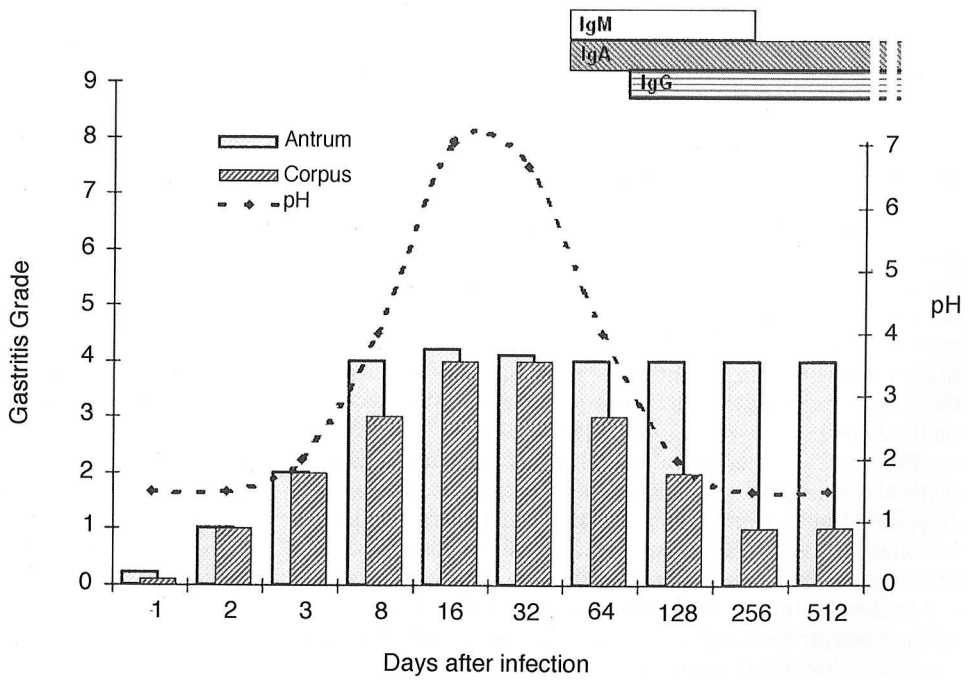

FIGURE 24-5 The natural history of *Helicobacter pylori* infection. The horizontal axis shows the time scale in days over 18 months. The left vertical axis is gastritis grade as represented in the columns showing antral and corpus mucosa inflammation graded 0 to 4. The right vertical axis shows the pH of gastric juice on a scale of 0 to 7. Note that corpus mucosa inflammation (*hatched vertical bars*) subsides after 3 months, whereas antral inflammation remains. As the corpus mucosa returns to near normal, acid secretion revives and gastric pH (*dotted line*) falls to normal acidic levels. Variable early responses are seen in IgA and IgM. IgG is present after the fourth week and remains as the most stable antibody response in nearly all infected persons.

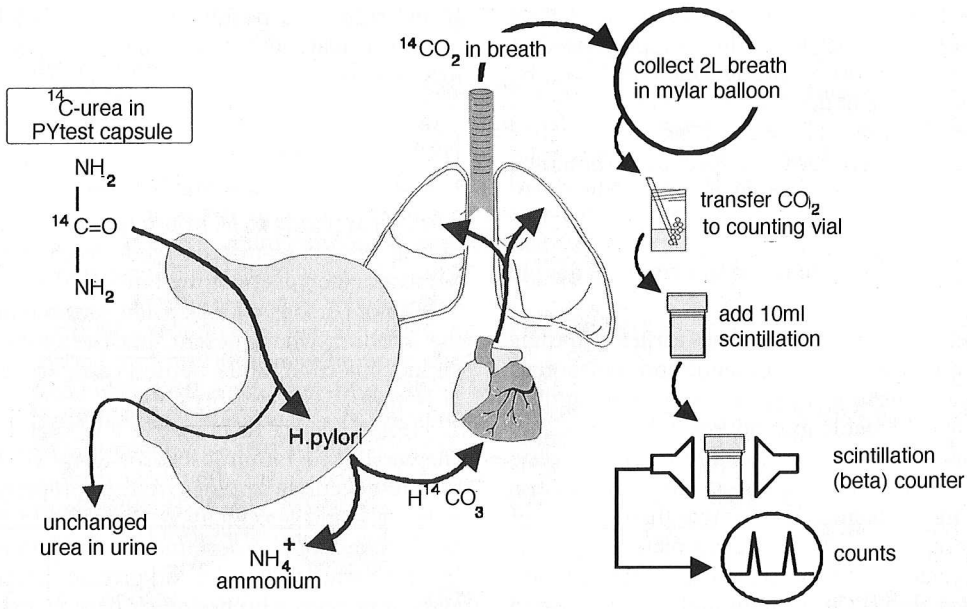

FIGURE 24-6 The urea breath test. Urea labeled with an isotope of carbon (a capsule of ^{14}C-urea in this illustration) is swallowed by the fasted patient. Ten to 15 minutes later, a breath sample is collected into a balloon, processed as shown, and then counted in a scintillation counter. $^{14}CO_2$ can be detected in the breath of a patient infected with *Helicobacter pylori*. When *H. pylori* is not present, the urea remains intact and there is no $^{14}CO_2$ in the breath. Unchanged ^{14}C-urea is excreted in the urine. Since more than 90% of the isotope is excreted within 3 days, radiation exposure is exceedingly small, about the same as natural background in 24 hours (0.3 mrem). In the ^{13}C-urea breath test, the patient first swallows a high-fat meal or drink, which serves to delay gastric emptying. Ten minutes later, a baseline breath sample is collected and a solution of isotope is swallowed. Diagnostic breath samples are collected 20 to 40 minutes later. Breath samples are analyzed in an isotope ratio mass spectrometer.

Breath Tests

Urea breath tests rely on the breakdown of isotope-labeled urea by urease (from *H. pylori*) in the stomach (Fig. 24-6). The C^{14} UBT only takes 10 minutes and requires a single breath sample. The C^{13} test takes 40 minutes and requires a baseline plus a 20–30 minute test sample. Both tests are highly accurate and the choice of which to use depends on availability and cost. Although the C^{14} UBT uses a trace of radioactive carbon, the dose is very small—equal to less than 24 hours of background exposure, so it is not excluded from use in women or children.[82]

Diagnostic Criteria for Research Studies

Table 24-1 gives comparative data on the accuracy of various diagnostic tests as observed by Cutler and associates and Vaira and coworkers.[83,84] Although histology is accurate, these data reflect the results obtained by an expert pathologist with considerable experience in *H. pylori* diagnosis. Accuracy is quite variable among community pathologists, depending on their experience and the technical excellence of the staining methods used.

For clinical research, concordance between two different methods is necessary to prove the presence or absence of *H. pylori*. Good combinations for initial diagnosis would be histology and culture, urease test and culture, histology and serology, or urease test and serology.

In developing countries, noninvasive "indirect" methods of diagnosis are less helpful in demonstrating cure after therapy, probably due to frequent exposure and reinfection with *H. pylori*. Positive serology in a highly endemic site frequently does not reverse. In addition, even "direct" tests such as UBT and stool antigen tests are more difficult to interpret due to false-positive results.

Proof of cure requires demonstration of sterile gastric mucosa, by two tests, 4 weeks after completion of therapy. Alternatively, two negative tests (UBT or fecal antigen) will suffice, the first at 4 weeks post therapy and the second at 6 to 8 weeks post therapy.

TREATMENT OF *H. PYLORI* IN THE TROPICS

What is the role of treatment of *H. pylori* infection in the tropics? In many ways, treatment is similar to that in the United States, where eradication is prescribed for patients with peptic ulcer disease and the rare patient with gastric lymphoma.

In the tropics, regimens containing metronidazole are much less effective. Furazolidone is an alternative to metronidazole in a 10-day regimen that includes bismuth and amoxicillin. In some countries (i.e., Peru, Bangladesh, and Turkey), there is rapid recurrence even after successful eradication of *H. pylori* from the stomach. *Eradication* is defined as the absence of *H. pylori* at least 4 weeks after the last dose of antimicrobial therapy was given.[6] Reinfection is most likely caused by fecal contamination of water or food.

By sheer experience, several rules can be stated about eradication of *H. pylori*:

- In vitro sensitivity to an antibiotic does not predict in vivo efficacy. Therefore, use tested antibiotic combinations that are proven to work.

- Acid appears to protect *H. pylori* from antibiotics; therefore, the most successful treatments include strong acid suppression with proton pump inhibitors.
- Eradication rates peak between seven and 14 days of treatment. Do not use treatments shorter than seven days (cure rate is lower) or longer than 14 days (cure rate does not increase, but side effects increase).
- *H. pylori* does not develop resistance to the following drugs: amoxycillin, bismuth, tetracycline, and furazolidone. Therefore they may be reused, and sensitivity testing for them is not required.
- *H. pylori* quickly becomes resistant to the following drugs: metronidazole (and other "idazoles"), clarithromycin (and other "thromycins"), and rifabutin. Box 24-1 outlines doses used in therapy, while Table 24-2 gives treatment options.

PREVENTION AND CONTROL

In developing countries, *H. pylori* recurrence after successful treatment occurs at rates 10 to 20 times higher than in developed countries. In addition, bacterial counts of *H. pylori* in gastric biopsies taken in recurrence are similar to those in biopsies taken prior to treatment. The high reinfection rate and lack of change in the *H. pylori* bacterial counts suggest there is little natural immunity after infection.

The rapid recurrence of *H. pylori* infection in some areas means that its treatment in impoverished tropical areas needs to be based on objectives that have a likelihood of some success. Goals for control of infection will have to be tempered with the additional need for improved sanitation. Thus, for example, antimicrobial treatment of the majority of the world's population living in tropical, developing areas to eradicate *H. pylori* for the purpose of preventing cancer would be unrealistic at present. On the other hand, prevention of gastric ulcer and duodenal ulcer recurrence for periods of a year or two might have some utility even where recurrence is high. Ethnic and cultural factors are also worth considering. For example, in Malaysia, prevalence rates among the Malays ranged from 12% to 29%, while the Chinese ranged from 27% to 58%, and Indians were between 49% and 52%.[85]

The role that treatment will have in reversing the progress of precancerous lesions to cancer is not known. Evidence suggests that treatment may reverse dysplasia but not intestinal metaplasia. In patients with severe intestinal metaplasia associated with atypical changes or dysplasia, it is probably worthwhile at present to attempt to eradicate *H. pylori* from the mucosa. In patients with gastric lymphoma, a condition seen more commonly in developed countries, eradication is mandatory because tumor regression occurs when *H. pylori* infection is treated. There is no evidence to date, however, that eradicating *H. pylori* in patients with gastric cancer will affect the course except to prevent further new cancers.

Reinfection with *H. pylori* makes vaccine use highly attractive in some countries yet the high reinfection rates suggest that finding an effective vaccine may be quite difficult. Vaccines need to work in children to prevent early infection in order to protect against ulcer disease and gastric cancer. As noted previously, natural immunity to reinfection is weak, thus another possible use for the vaccine will be to prevent recurrence of *H. pylori* infection after effective antimicrobial therapy.

Box 24-1 Treatments in Common Usage

- Omeprazole 20 mg BID, clarithromycin 500 mg BID, and amoxycillin 1g BID (7–10 days)
- Omeprazole 20 mg BID, clarithromycin 250 mg BID, and metronidazole 400 mg BID (7 days)
- Bismuth (subsalicylate or citrate) 1 tablet QID*, tetracycline 500 mg QID, metronidazole 250 mg QID (1 g to 1.5 g daily) (10–14 days)
- Rabeprazole 20 mg BID, amoxicillin 1 g BID, levofloxacin 250 mg BID (10 days)
- Omeprazole 20 mg BID, amoxicillin 1 g BID, furazolidone 200 mg BID, and bismuth subsalicylate (De-Nol) 240 mg (2 tabs) BID (14 days)

*Addition of a proton pump inhibitor probably enhances the cure rate.

Table 24-2 *Treatment Options for* Helicobacter pylori*

Group	Description	Duration
A†	**Bismuth**	
	Ranitidine bismuth citrate (RBC) 400 mg BID	14 days
	Bismuth subsalicylate (Pepto Bismol) 525 mg (2 tabs) QID	14 days
	Bismuth subcitrate (DeNol) 120 mg (1 tab) QID	14 days
B	**Penicillin**	
	Amoxicillin IG BID	7 or 10 or 14 days
C	**Macrolide**	
	Clarithromycin 500 mg BID‡	7 or 10 or 14 days
	Josamycin 1000 mg BID	7 days
D	**Nitroimidazole**	
	Metronidazole 500 mg BID or TID‡	7 or 10 or 14 days
	Tinidazole 1000 mg daily	7 or 10 or 14 days
E	**Tetracycline**	
	Tetracycline 500 mg QID	14 days
F	**Quinolone**	7 or 10 or 14 days
	Ofloxacin 500 mg BID	7–14 days
	Levofloxacin 250 mg BID	7–14 days
	Ciprofloxacin 500 mg BID	14 days
G	**Nitrofurans**	
	Furazolidone 200 mg BID‡	7 or 10 or 14 days
H	**Ansamycin**	
	Rifabutin 150 mg BID	14 days
I	**Proton Pump Inhibitors (use double a normal dose)**	
	Omeprazole 20 mg BID	
	Esomeprazole 40 mg BID	
	Lansoprazole 30 mg BID	
	Pantoprazole 40 mg BID	
	Rabeprazole 20 mg BID	

*Treatment priorities are normally: IBC→IBD→IBEG. For penicillin allergy choose ICD or IAED→IFH.
†When Pepto Bismol is not available, substitute DeNol 1 tablet QID. RBC is not available in all countries.
‡Side effects are likely as doses of clarithromycin, metronidazole and furazolidone increase.

H. pylori infection is one of the most common infections in the tropics, significantly associated with peptic ulcer disease, gastric cancer, and possibly with other enteric infections. New diagnostic, therapeutic, and preventive measures will undoubtedly determine the evolving approaches to better controlling the consequences of *H. pylori* infections worldwide.

REFERENCES

1. Marshall BJ, Warren JR: Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1:1311, 1984.
2. Braga LL, Marshall BJ, Moreno H, et al: *Helicobacter pylori*: Gastritis, peptic ulcer and gastric cancer in developing countries. In Guerrant RL, de Souza MA, Nations MK (eds): At the Edge of Development: Health Crises in a Transitional Society. Durham, NC, Carolina Academic Press, 1996.
3. Sullivan PB, Thomas JE, Wight DG, et al: *H. pylori* infection in Gambian children with chronic diarrhea and malnutrition. Arch Dis Child 65:189, 1990.
4. Soll AH: Medical treatment of peptic ulcer disease. JAMA 275:622, 1996.
5. Hulten K, Han SW, Enroth H, et al: *Helicobacter pylori* in the drinking water in Peru. Gastroenterology 110:1031, 1996.
6. Ramirez-Ramos A, Gilman RH, Leon-Barua R, et al: Rapid recurrence of *Helicobacter pylori* infection in Peruvian patients after successful eradication. Clin Infect Dis 25:1027, 1997.
7. Soto G, Bautista CT, Roth DE, et al: *Helicobacter pylori* reinfection is common in Peruvian adults after antibiotic eradication therapy. J Infect Dis 188:1263, 2003.
8. Marshall BJ, McGechie DB, Rogers PAR, et al: Pyloric campylobacter infection and gastroduodenal disease. Med J Aust 149:439, 1985.
9. Pinkard KJ, Harrison B, Capstick JA, et al: Detection of *Campylobacter pyloridis* in gastric mucosa by phase contrast microscopy. J Clin Pathol 39:112, 1986.
10. Buck GE, Smith JS: Medium supplementation for growth of *Campylobacter pyloridis*. J Clin Microbiol 25:597, 1987.
11. Pennie RA, Zunino JN, Rose E, et al: Economical, simple method for the production of the gaseous environment required for the cultivation of *Campylobacter jejuni*. J Clin Microbiol 20:320, 1984.
12. Krajden S, Bohnen J, Anderson J, et al: Comparison of selective and nonselective media for recovery of *Campylobacter pylori* from antral biopsies. J Clin Microbiol 25:1117, 1987.
13. Hachem CY, Clarridge JE, Evans DG, et al: Which is the best medium for isolation of *Helicobacter pylori* from gastric mucosal biopsies? (abstract). Am J Gastroenterol 89:1287, 1994.
14. Tee W, Fairley S, Smallwood R, et al: Comparative evaluation of three selective media and a nonselective medium for the culture of *Helicobacter pylori* from gastric biopsies. J Clin Microbiol 29:2587, 1991.
15. Shadowen RD, Sciortino CV: Improved growth of *Campylobacter pylori* in a biphasic system. J Clin Microbiol 27:1744, 1989.
16. Secker DA, Tompkins DS, Alderson G: Gas-permeable lifecell tissue culture flasks give improved growth of *Helicobacter pylori* in a liquid medium. J Clin Microbiol 29:1060, 1991.
17. Mendz GL, Hazell SL: Amino acid utilization by *Helicobacter pylori*. Int J Biochem Cell Biol 27:1085, 1995.
18. Thomas JE, Gibson GR, Darboe MK, et al: Isolation of *Helicobacter pylori* from human faeces. Lancet 340:1194, 1992.
19. Klein PD, Gilman RH, Leon-Barua R, et al: The epidemiology of *Helicobacter pylori* in Peruvian children between 6 and 30 months of age (abstract). Am J Gastroenterol 89:1306, 1994.
20. Mitchell JD, Mitchell HM, Tobias V: Acute *Helicobacter pylori* infection in an infant, associated with gastric ulceration and serological evidence of intra-familial transmission. Am J Gastroenterol 87:382, 1992.
21. Fecal and oral shedding of *Helicobacter pylori* from healthy infected adults. JAMA 282:2240, 1999.
22. Mitchell JD, Mitchell HM, Tobias V: Acute *Helicobacter pylori* infection in an infant, associated with gastric ulceration and serological evidence of intra-familial transmission. Am J Gastroenterol 87:382, 1992.
23. Nguyen AM, Engstrand L, Genta RM, et al: Detection of *Helicobacter pylori* in dental plaque by reverse transcription–polymerase chain reaction. J Clin Microbiol 31:783, 1993.
24. Blecker U, Lanciers S, Keppens E, et al: Evolution of *Helicobacter pylori* positivity in infants born from positive mothers (abstract). Am J Gastroenterol 89:1334, 1994.
25. Marshall BJ, Barrett LJ, Prakash C, et al: Urea protects *Helicobacter (Campylobacter) pylori* from the bactericidal effect of acid. Gastroenterology 99:697, 1990.
26. Morris A, Nicholson G: Ingestion of *Campylobacter pyloridis* causes gastritis and raised fasting gastric pH. Am J Gastroenterol 82:192, 1987.
27. Graham DY, Alpert LC, Smith JL, et al: Iatrogenic *Campylobacter pylori* infection is a cause of epidemic achlorhydria. Am J Gastroenterol 83:974, 1988.
28. Hoffman JS, King WW, Fox JG, et al: Rabbit and ferret parietal cell inhibition by Helicobacter species. Dig Dis Sci 40:147, 1995.
29. McGowan CC, Cover TL, Blaser MJ: *Helicobacter pylori* and gastric acid: Biological and therapeutic implications. Gastroenterology 110:926, 1996.
30. Shahinian ML, Passaro DJ, Swerdlow DL, et al: *Helicobacter pylori* and epidemic *Vibrio cholerae* O1 infection in Peru. Lancet 355:377, 2000.
31. Evans C, Gilman RH, Rabbani GH, et al. Gastric acid secretion and enteric infection in Bangladesh. Trans R Soc Trop Med Hyg 91:681, 1997.
32. Gilman RH, Partanen R, Brown K, et al: Decreased gastric acid secretion and bacterial colonization of the stomach in severely malnourished Bangladeshi children. Gastroenterology 94:1308, 1988.
33. Passaro DJ, Taylor DN, Gilman RH, et al. Growth slowing after acute *Helicobacter pylori* infection is age-dependent. J Pediatr Gastroenterol Nutr 35:522, 2002.
34. Takahashi M, Kimura H, Watanabe K: *Helicobacter pylori* infection in patients with idiopathic short stature. Pediatr Int 44:277, 2002.
35. Bravo LE, Mera R, Reina JC, et al: Impact of *Helicobacter pylori* infection on growth of children: A prospective cohort study. J Pediatr Gastroenterol Nutr 37:614, 2003.
36. Borën T, Falk P, Roth KA, et al: Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science 262:1892, 1993.
37. Lingwood CA, Huesca M, Kuksis A: The glycerolipid receptor for *Helicobacter pylori* (and exoenzyme S) is phosphatidylethanolamine. Infect Immun 60:2470, 1992.
38. Kamisago S, Iwamori M, Tai T, et al: Role of sulfatides in adhesion of *Helicobacter pylori* to gastric cancer cells. Infect Immun 64:624, 1996.
39. Chan NW, Stangier K, Sherburne R, et al: The biosynthesis of Lewis X in *Helicobacter pylori*. Glycobiology 5:683, 1995.
40. Crabtree JE, Xiang Z, Lindley IJ, et al: Induction of interleukin-8 secretion from gastric epithelial cells by a cagA negative isogenic mutant of *Helicobacter pylori*. J Clin Pathol 48:967, 1995.
41. Atherton JC, Cao P, Peek RM Jr, et al: Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific *vacA* types with cytotoxin production and peptic ulceration. J Biol Chem 270:17771, 1995.
42. Pride DT, Meinersmann RJ, Blaser MJ: Allelic variation within *Helicobacter pylori* babA and babB. Infect Immun 69:1160, 2001.
43. El Omar EM, Carrington M, Chow WH, et al: Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 404:398, 2000.
44. Figueiredo C, Machado JC, Pharoah P: *Helicobacter pylori* and interleukin 1 genotyping: an opportunity to identify high-risk individuals for gastric carcinoma. J Natl Cancer Inst 94:1680, 2002.
45. Drumm B, Sherman P, Cutz E, et al: Association of *Campylobacter pylori* on the gastric mucosa with antral gastritis in children. N Engl J Med 316:1557, 1987.
46. Warren JR, Marshall B: Unidentified curved bacilli on gastric epithelium in active chronic gastritis (letter). Lancet 1:1273, 1983.
47. el-Zimaity HM, Graham DY, al-Assi MT, et al: Interobserver variation in the histopathological assessment of *Helicobacter pylori* gastritis. Hum Pathol 27:35, 1996.
48. Maaroos HI, Kekki M, Villako K, et al: The occurrence and extent of *Helicobacter pylori* colonization and antral and body gastritis profiles in an Estonian population sample. Scand J Gastroenterol 25:1010, 1990.
49. Recavarren-Arce S, Gilman RH, León-Baruá R, et al: Chronic atrophic gastritis: Early diagnosis in a population where *Helicobacter pylori* infection is frequent. Clin Infect Dis 25:1006, 1997.
50. Genta RM, Hammer HW, Graham DY: Gastric lymphoid follicles in *Helicobacter pylori* infection: Frequency, distribution and response to triple therapy. Hum Pathol 24:577, 1993.

51. Kuipers EJ, Uyterlinde AM, Pena AS, et al: Long-term sequelae of *Helicobacter pylori* gastritis. Lancet 345:1525, 1995.
52. Logan RP, Walker MM, Misiewicz JJ, et al: Changes in the intragastric distribution of *Helicobacter pylori* during treatment with omeprazole. Gut 36:12, 1995.
53. Warren JR: Gastric pathology associated with *Helicobacter pylori*. Gastroenterol Clin North Am 29:705, 2000.
54. Recavarren-Arce S, Leon Barua R, Cok J, et al: *Helicobacter pylori* and progressive gastric pathology that predisposes to gastric cancer. Scand J Gastroenterol 181(Suppl):51, 1991.
55. Recavarren S, León Baruá R, Rodriguez C, et al: *Helicobacter pylori*–associated chronic gastritis in Peruvian adolescents is very common and severe. J Clin Gastroenterol 20:335, 1995.
56. Rauws EA, Langenberg W, Houthoff HJ, et al: *Campylobacter pyloridis*–associated chronic active antral gastritis: A prospective study of its prevalence and the effects of antibacterial and antiulcer treatment. Gastroenterology 94:33, 1988.
57. Sonnenberg A, Townsend WF: Testing for *Helicobacter pylori* in the diagnosis of Zollinger-Ellison syndrome. Am J Gastroenterol 86:606, 1991.
58. Weir WR, Goodgame R, Kiire CF, et al: Campylobacter-like organisms and gastritis in Africa. Trans R Soc Trop Med Hyg 82:172, 1988.
59. Eaton KA, Krakowka S: *H. pylori* strain differences and their role in early, transient neutrophilic inflammation in gnotobiotic piglets (abstract). Gastroenterology 100(Suppl 4):A100, 1996.
60. Wyatt JI, Rathbone B, Dixon MF, et al: *C. pyloridis* and acid induced gastric metaplasia in the pathogenesis of duodenitis. J Clin Pathol 40:841, 1987.
61. Cotton P: NIH consensus panel urges antimicrobials for ulcer patients, skeptics concur with caveats (news). JAMA 271:808, 1994.
62. Hopkins RJ, Girardi LS, Turney EA: Relationship between *Helicobacter pylori* eradication and reduced duodenal and gastric ulcer recurrence: A review. Gastroenterology 110:1244, 1996.
63. Rauws EA, Tytgat GN: Cure of duodenal ulcer associated with eradication of *Helicobacter pylori*. Lancet 335:1233, 1990.
64. Munoz N, Franceshi S: Epidemiology of gastric cancer and perspectives for prevention. Salud Publica Mex 39:318, 1997.
65. León-Baruá R, Berendson-Seminario R, Recavarren-Arce S, et al: Geographic factors probably modulating alternative pathways in *Helicobacter pylori*–associated gastroduodenal pathology: A hypothesis. Clin Infect Dis 25:1013, 1997.
66. Siurala M, Isokoski M, Varis K, et al: Prevalence of gastritis in a rural population. Biopsy study of subjects selected at random. Scand J Gastroenterol 3:211, 1968.
67. Correa P, Cuello C, Duque E, et al: Gastric cancer in Colombia. III. The natural history of precursor lesions. J Natl Cancer Inst 57:1027, 1976.
68. Kuipers EJ, Uyterlinde AM, Pena AS, et al: Long term sequelae of *H. pylori* gastritis. Lancet 345:1525, 1995.
69. Uemura N, Okamoto S, Yamamoto S: *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med 345:784, 2001.
70. El-Omar EM, Oien K, El-Nujumi A, et al: *Helicobacter pylori* infection and chronic gastric acid hyposecretion. Gastroenterology 113:15, 1997.
71. Wotherspoon AC, Ortiz Hidalgo C, Falzon MR, et al: *Helicobacter pylori*–associated gastritis and primary B-cell gastric lymphoma. Lancet 338:1175, 1991.
72. Hussell T, Isaacson PG, Crabtree JE, et al: The response of cells from low-grade B-cell gastric lymphomas of mucosa-associated lymphoid tissue to *Helicobacter pylori*. Lancet 342:571, 1993.
73. Stolte M, Eidt S: Healing gastric MALT lymphomas by eradicating *H. pylori*? Lancet 342:568, 1993.
74. Bayerdorffer E, Neubauer A, Eidt S, et al, for the Malt-Lymphoma Study Group, Germany: Double-blind treatment of early gastric malt-lymphoma patients by *Helicobacter pylori* eradication. Gastroenterology 106(Suppl 2):370, 1994.
75. El-Zimaity HMT, Al Assi MT, Genta RM: Confirmation of successful therapy of *Helicobacter pylori* infection: Number and site of biopsies or a rapid urease test. Am J Gastroenterol 90:1962, 1995.
76. Genta RM, Robason GO, Graham DY: Simultaneous visualization of *Helicobacter pylori* and gastric morphology: A new stain. Hum Pathol 25:221, 1994.
77. Peterson WL, Graham DY, Marshall B, et al: Clarithromycin as monotherapy for eradication of *Helicobacter pylori*: A randomized, double-blind trial. Am J Gastroenterol 88:1860, 1993.
78. Marshall BJ, Warren JR, Francis GJ, et al: Rapid urease test in the management of *Campylobacter pyloridis*–associated gastritis. Am J Gastroenterol 82:200, 1987.
79. Szeto ML, Pounder RE, Hamilton Dutoit SJ, et al: Rapid urease test provides specific identification of *Campylobacter pylori* in antral mucosal biopsies. Postgrad Med J 64:935, 1988.
80. Cutler AF, Prasad VM: Long-term follow-up of *Helicobacter pylori* serology after successful eradication. Am J Gastroenterol 91:85, 1996.
81. Mohar A, Ley C, Guarner J: Eradication rate of *Helicobacter pylori* in a Mexican population at high risk for gastric cancer and use of serology to assess cure. Am J Gastroenterol 97:2530, 2002.
82. Leide-Svegborn S, Stenstrom K, Olofsson M: Biokinetics and radiation doses for carbon-14 urea in adults and children undergoing the *Helicobacter pylori* breath test. Eur J Nucl Med 26:573, 1999.
83. Cutler AF, Havstad S, Ma CK, et al: Accuracy of invasive and noninvasive tests to diagnose *Helicobacter pylori* infection. Gastroenterology 109:136, 1995.
84. Vaira D, Malfertheiner P, Megraud F, et al: Diagnosis of *Helicobacter pylori* infection with a new non-invasive antigen-based assay. HpSA European study group. Lancet 354:30, 1999.
85. Goh KL, Parasakthi N: The racial cohort phenomenon: Seroepidemiology of *Helicobacter pylori* infection in a multiracial South-East Asian country. Eur J Gastroenterol Hepatol 13:177, 2001.