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Ultra-Short Therapy for the Treatment
of
Helicobacter pylori

By
CORINNE CHAHINE

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Pharmacy Doctor degree

Thesis Advisors:

Oussayma Moukhachen, Pharm. D. - LAU
Ala' Sharara, M. D. , FACP - AUBMC

School of Pharmacy
LEBANESE AMERICAN UNIVERSITY

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LEBANESE AMERICAN UNIVERSITY
GRADUATE STUDIES

We hereby approve the thesis of

Corinne Chahine

Candidate for the Pharmacy Doctor degree

Gabriel Maliha, M.D. , Ph. D.

Oussayma Moukhachen, Pharm. D.

Shihadeh N. Nayfeh, Ph. D.

Ala' Sharara, M. D. , FACP

Date: *June 8, 2001*

To my parents

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ABSTRACT

Background: Treatment regimens for *Helicobacter pylori* (*H. pylori*) usually combine an acid reducing agent (proton-pump inhibitor, H₂-antagonist) with two or three antibiotics (macrolide, metronidazole, tetracycline, amoxicillin), with a duration varying from seven to fourteen days. Recently, studies have shown acceptable eradication rates using short-term triple-therapy regimens. Azithromycin, a new generation macrolide with improved pharmacokinetic properties (long t_{1/2} life and large volume of distribution), has been proposed for *H. pylori* treatment with variable eradication rates of 57% to 93%.

Objectives: The purpose of this study is to establish the safety and efficacy of two short-term triple-therapy regimens (lansoprazole, azithromycin and amoxicillin) for *H. pylori* eradication.

Methods: From April 2000 to September 2000 thirty symptomatic patients with a positive rapid urease assay for *H. pylori* were assigned to receive either a 3-day therapy (Group A) or a 5-day therapy (Group B). In both groups, patients received lansoprazole 30mg bid on day 1; lansoprazole 30mg bid, amoxicillin 1g bid and azithromycin on days 2 and 3. Patients in group B received lansoprazole 30mg bid and amoxicillin 1g bid for two additional days (4 and 5). In-vitro antimicrobial susceptibility of *H. pylori* recovered from gastric biopsies was tested using the epsilometer test (E-test). Compliance and side effects were monitored. Evaluation of *H. pylori* eradication was done a minimum of four weeks after end of therapy using the ¹⁴C-urea breath test.

Results: 28 patients were enrolled (15 in Group A and 13 in Group B). Treatment was well tolerated with minimal side effects. Eradication rates were 36% and 22% per-protocol in Groups A and B respectively (26.6% and 15.4% respectively by intent to treat analysis). None of the isolated strains of *H. pylori* showed in-vitro resistance to amoxicillin or the macrolide clarithromycin.

Conclusion: Azithromycin-based ultra-short therapy for 3 or 5 days is not a suitable regimen in the treatment of *H. pylori* infection.

1 HISTORICAL BACKGROUND

In the last quarter of the nineteenth century, when microscope resolution had sufficiently advanced, the investigation for gastric bacteria properly began and alterations in gastric physiology with the associated pathology were documented in details.

The microscope use by early investigators identified the role for bacteria, yeast and fungi in gastric pathophysiology. However, it was unclear yet whether a specific organism was the cause of a gastric disease or whether it was simply an abnormal accumulation of organisms, in the stomach itself, which culminated in gastric disturbances.

By the beginning of the twentieth century, physicians involved in the treatment of gastrointestinal disease were generally familiar with some infective processes of the digestive tract and hyperchlorhydria was attributed to the development of chronic ulcers. In 1910, "no acid, no ulcer", the adage of the Croatian Karl Schwartz, was uniformly accepted and pathological observations in regard to the bacterial origin of gastric ulceration continued to be investigated.^{1,2}

Table 1.1 * Key dates in the history of *H. pylori*

Year	Individual	Observation
1875	G. Bottcher / M. Letulle	Demonstrated bacteria in ulcer margins
1881	C. Klebs	Bacterial colonization and "interglandular small cell infiltration"
1888	M. Letulle	Experimental induction of acute gastric lesions in guinea pigs (<i>S. aureus</i>)
1889	W. Jaworski	Spiral organisms (<i>Vibrio rugula</i>) in gastric washings
1893	G. Bizzozero	Identified spirochetes in gastric mucosa of dogs
1896	H. Salomon	Spirochetes noted in gastric mucosa and experimentally transferred to mice
1906	W. Krienitz	Spirochetes in gastric contents of patient with gastric carcinoma
1908	F.B. Turck	Induced gastric ulcers in dogs by <i>Bacillus (Escherichea) coli</i>
1916	E.C. Rosenow	Described streptococcus induced gastric ulcers
1917	L.R. Dragstedt	Identified bacteria in experimental ulcers, no significant role identified
1921	J.S. Eskins	Experimental physiology of <i>S. regaudi (H. felis)</i>
1924	J.M. Luck	Discovered gastric mucosal urease
1925	B. Hoffman	Described <i>B. Hoffmani</i> - putative ulcerous agent
1930	B. Berg	Partial vagotomy inhibits secondary infections in ulcers
1938	J.L. Doenges	Spirochetes / inflammation in <i>Macacus</i> monkey and man
1940	A.S. Freedberg/L. Barron	Identified spirochetes in man - no etiologic role
1940	F.D. Gorham	Postulated gastric acidophilic bacteria as an etiologic agent in ulcer disease
1950	D. Fitzgerald	Urease in patients with gastric ulceration neutralizes gastric acid via the production of ammonia
1975	H.W. Steer	Polymorphonuclear migration in ulcers - isolated <i>Pseudomonas aeruginosa</i>
1983	J.R. Warren	Identified <i>Campylobacter (Helicobacter) pylori</i> in human gastritis
1983	B. Marshall	Isolated and cultured <i>Helicobacter pylori</i>
1985 - 1987	B. Marshall / A. Morris	Relationship between acquisition of <i>Helicobacter pylori</i> infection and development of gastritis

*Adapted from Kidd et.al.¹ and Buckley et.al.²

By 1980, Robin Warren, a histopathologist in the Royal Perth Hospital, Western Australia, had for many years observed bacteria in the stomach of people with gastritis. Although he was convinced that they somehow played a role in gastric disease, he had been reluctant to discuss this controversial observation in a wider gastroenterologist community. In 1982, a young gastroenterology fellow, Barry Marshall, was desperately looking for a project to complete his fellowship and was attracted by the hypothesis of Warren. He finally convinced this latter to allow him to investigate this hypothesis further in an appropriate clinical setting.¹

They noted both the constancy of bacterial infection, as well as the constancy of the associated histological changes, which they had identified in 135 gastric biopsy specimens studied over a three-year period. Warren commented that these microorganisms were difficult to see with hematoxylin and eosin, but stained well in the presence of silver. Furthermore, he observed the bacteria to be most numerous in an active chronic gastritis where they were closely associated with granulocyte infiltration. Utilizing the knowledge that these bacteria resembled the species of campylobacters rather than spirochetes, Marshall used campylobacter isolation techniques (microaerophilic conditions) to successfully grow isolates on moist chocolate agar³. Subsequent attempts to culture these bacilli were unsuccessful until April 1982. During the Easter weekend, the plates were unintentionally incubated for 5 days and colonies were visible. In order to substantiate that the microorganism was actually a disease-causing agent, it was necessary to determine that it could colonize normal mucosa and induce gastritis. To prove pathogenicity, Marshall decided to be his own "guinea pig".

Marshall, who had histologically normal gastric mucosa and was a light smoker and social drinker, received, per mouth, a test-isolate from a 66-year-old nonulcer dyspeptic man. Over the next 14 days a mild illness, characteristic of an acute episode of gastritis developed, and was accompanied by headaches, vomiting, abdominal discomfort, irritability and "putrid" breath. The infectivity of the agent was then successfully confirmed. After 10 days, histologically proven gastritis was endoscopically documented⁴. The association of these bacteria with gastritis was first presented at the Royal Australian College of Physicians on 22nd of October 1982 and published in a letter form in The Lancet in 1983.³

2 H. PYLORI CHARACTERISTICS

2.1 TAXONOMY

The first official name of those “*Campylobacter*-like-organisms” observed by Warren and Marshall in 1982 was *Campylobacter pyloridis*, since their general morphological and primary characteristics conformed with the description of the genus *Campylobacter*⁵. For grammatical reasons, the name subsequently was changed to *Campylobacter pylori* (*C. pylori*).⁶

Those species comprised curved rod-shaped bacteria that were gram-negative, motile, microaerophilic and grew at 37 °C on conventional culture media with moist chocolate or blood agar as the preferred medium. They exhibited a coccoid non-culturable form and produced catalase and oxidase but were non-saccharolytic.

The principal conventional test differences between *C. pylori* and other campylobacters were in flagella arrangement and cell morphology. Strains also differed from the description of *Campylobacter* as they hydrolysed urea and did not reduce nitrate.⁷

Table 2.1 *Key taxonomic features distinguishing *H. pylori* from campylobacters

- Rapid urease
- Possesses 4-5 unsheathed flagella, glycocalyx and terminal bulbs
- Lacks the respiratory quinone TPQ 6
- Distinctive cellular fatty acid content
- Distinctive protein electrophoretic profile
- Divergent 16 r-RNA sequence

*Adapted from Heatley RV et.al. 7

For the above taxonomic features distinguishing the bacterium from campylobacters, it was apparent that it has been placed in the wrong genus.

Thus, the final change in nomenclature created the genus *Helicobacter* with nine official species identified. *Helicobacter pylori* (*H. pylori*) is the one causing 99% of human helicobacter infection. 8

Table 2.2 ¹*H. pylori* species and related organisms

Species	Host
<i>Helicobacter pylori</i>	Human / pig / rhesus monkey gastric mucosa
<i>Helicobacter mustelae</i>	Ferret gastric mucosa
<i>Helicobacter felis</i>	Cat, gastric mucosa
<i>Helicobacter nemestrinae</i>	Pigtailed macaque, gastric mucosa
<i>Helicobacter (campylobacter) cinaedi</i>	Human, rectal swab
<i>Helicobacter (campylobacter) fennelliae</i>	Human, rectal swab
<i>Wolinella succinogenes</i>	Cow, rumen
<i>Flexispirita rappini</i>	Dog feces
<i>Helicobacter helmanii (Gastrospirillum hominis)</i>	Human gastric mucosa

¹Adapted from Heatley RV et.al. 7

2.2 BACTERIOLOGY

2.2.1 STRUCTURE

H. pylori is a spiral or curved, Gram-negative rod, 2.5-5 μm long and 0.5-1 μm wide, with four to six unipolar flagella which sometimes have a characteristic terminal bulb. ⁷

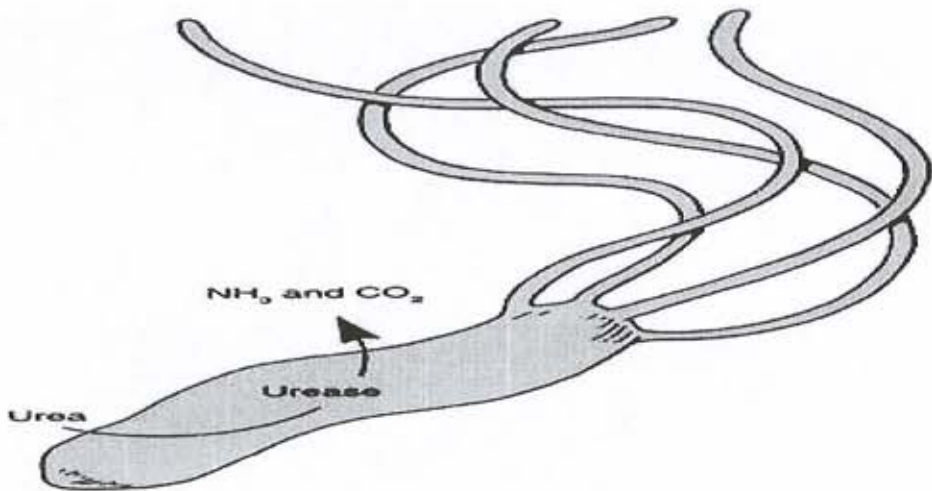


Fig. 2.1 *H. pylori* spiral and flagellated morphology showing the urease activity

2.2.2 GROWTH

H. pylori is easy to grow on the appropriate growth medium that is usually blood/agar base containing selective antibiotic supplements to prevent the overgrowth of transient gastric microbiota. ^{6,9}

H. pylori is a microaerophilic organism requiring no or a little oxygen (5%). Special gas mixtures or generating kits are available to achieve appropriate environment for cultures. They have to be incubated for at least 2 to 7 days for primary culture from a biopsy.⁷

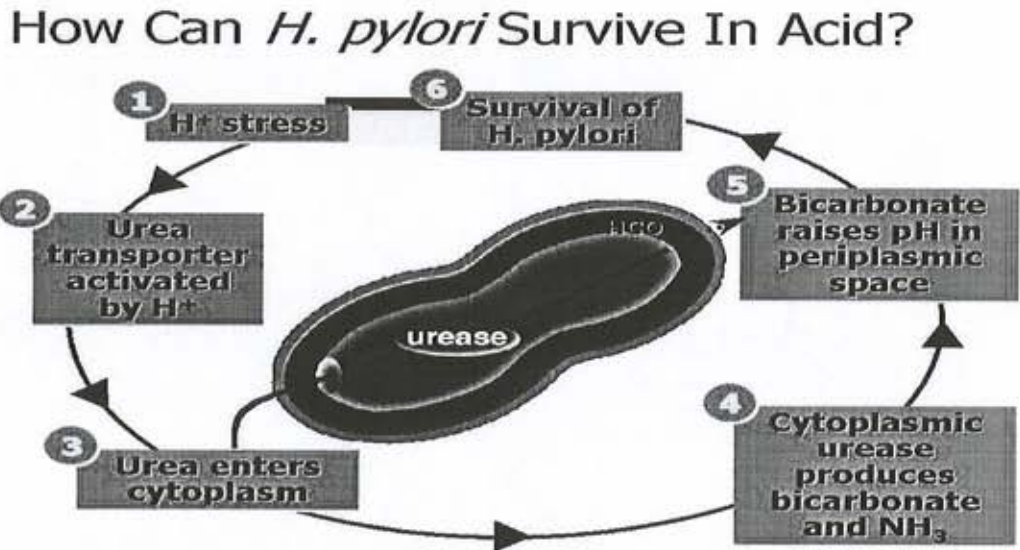
Growth characteristics have been reported as follows: growth occurs in a microaerobic atmosphere, at 33 - 40 °C (optimal 37 °C), and in the presence of 0.5 % glycine or 0.04% Triphenyl-Tetrazolium-Chloride (TTC). There is no growth in air or anaerobically at 37 °C, microaerobically at 25 °C or at 30 °C or 42 °C, in the presence 1% glycine, 1% bile or 1% NaCl. *H. pylori* will grow over a wide pH range of 5.5 - 8.5 with good growth between 6.9 and 8.0.^{7,10}

2.2.3 BIOCHEMICAL CHARACTERISTICS

Some key biochemical characteristics reported are^{6,7,9}:

- Positive reactions in tests for oxidase, catalase, urease, acid and alkaline phosphatase, leucine and gamma-glutamyl aminopeptidases, and hydrogen sulphide detected by lead acetate paper
- Negative reactions in tests for hippurate hydrolysis, reduction of nitrate, utilization of carbohydrates, production of indole, hydrolysis of indole acetate and production of hydrogen sulphide in triple sugar iron agar.
- The sole respiratory quinones present in *H. pylori* are menaquinones (MK6).

Fig. 2.2 Survival of *H. pylori* in acid



- The most studied *H. pylori* enzyme is the urease that breaks down urea to yield ammonia and carbon dioxide. In addition of being the basis of many of the diagnostic tests for the bacterium this property has shown to be important in the colonization and direct damage of the gastric mucosa.

2.2.4 ISOLATION

H. pylori organisms live closely attached to gastric epithelial cells, beneath a protective layer of mucus, where the pH is virtually neutral. The localization of *H. pylori* is limited to cells derived from the gastric mucosa, and they are never related to intestinal-type mucosal cells. Since the bacteria are most frequently found deep to the layer of mucus overlying the epithelial cells; therefore, they are not apparent unless this layer of mucus is removed. ¹¹

There are no reports of culture from sites other than gastric juice and the mucosa of the stomach or the adjacent esophagus and duodenum, and no reports of invasion and spread to other sites.

H. pylori are found in areas of acute and chronic gastritis. They are most numerous in areas of active chronic gastritis when bacteria are frequently associated with migrating polymorphonuclear leukocytes. ¹²

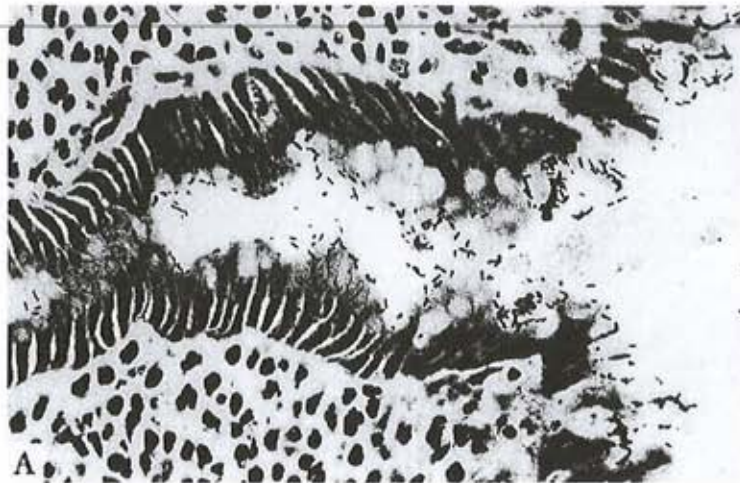


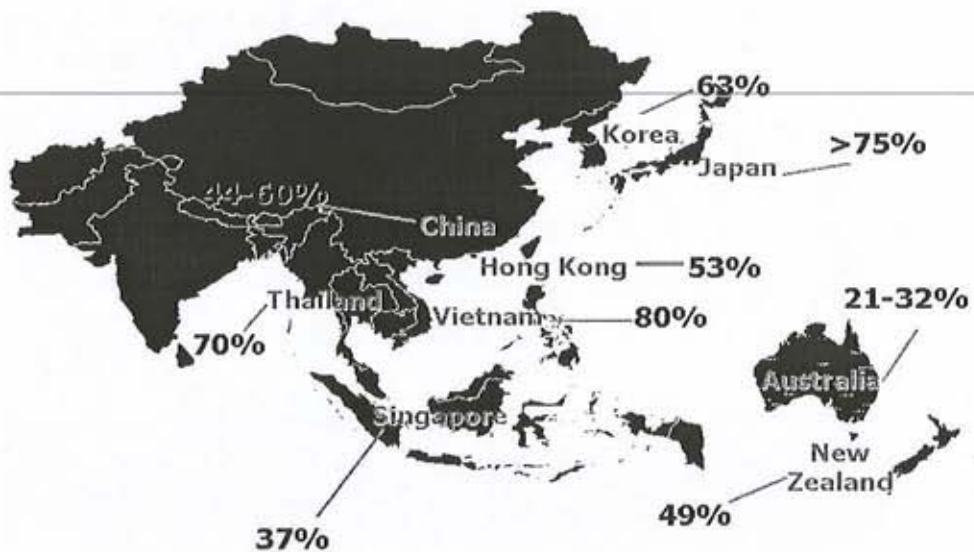
Fig. 2.3 *H. pylori* colonizing the gastric mucosa

3 EPIDEMIOLOGY

3.1 PREVALENCE RATES

Cross-sectional and predominantly serological epidemiologic studies of *H. pylori* showed that the infection was commonly acquired in childhood with a slower acquisition rate in adulthood. Recent data further suggest that childhood is the period of maximum incidence of *H. pylori* infection, however within this same period there is the highest potential for acquisition and loss of infection, particularly in the first two years of life.¹³⁻¹⁵

Fig. 3.1 Geographical incidence of *H. pylori* infection in the Far East and Australia



3.2 OTHER RISK FACTORS

3.2.1 SOCIOECONOMIC STATUS

The next important factor with regard to *H. pylori* infection, is the socio-economic status. The poorer a population is, the sooner in life it will become infected and the higher the cumulative infection rate will be. The differences between the developing and developed world in childhood suggest the possibility of an environmental pool of *H. pylori* to which children are exposed in developing countries. In developed countries, either the pool has been cleared or children are not exposed to it. High rates of seropositivity in children are found in many developing countries.¹⁶

3.2.2 ETHNIC BACKGROUND

It is difficult to claim that some ethnic groups have a particular susceptibility to *H. pylori* infection, and the socio-economic conditions could be a source of bias. Comparisons must be made with the same socio-economic status in the same environment. Some comparative studies have been attempted, but still with no significant conclusive results.^{16,17}

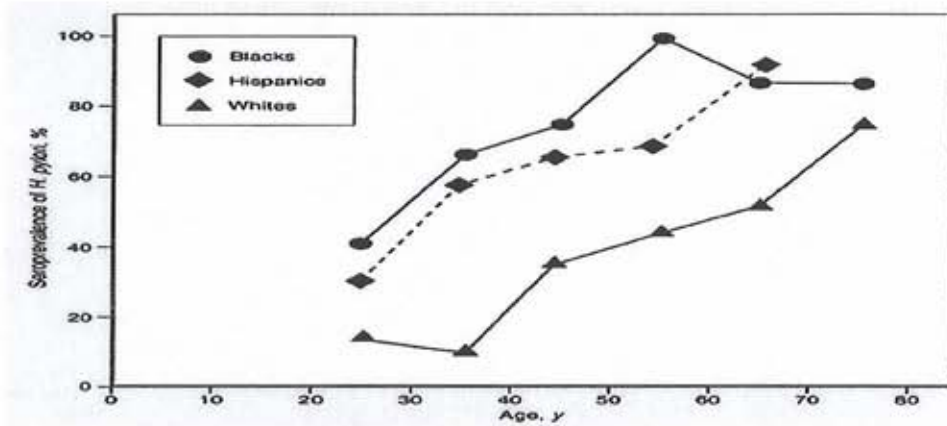


Fig. 3.2 *H. pylori* infection: ethnics and age of acquisition

3.2.3 GENDER

Seroprevalence of *H. pylori* infection is often similar in males and females. It is generally accepted that males and females have the same risk of becoming infected in all age groups considered.^{18, 19}

3.3 TRANSMISSION

No predominant route of acquisition of *H. pylori* has been defined. Possibilities include oral-oral, fecal-oral, iatrogenic, and vector transmission.^{19, 20}

3.3.1 ORAL – ORAL SPREAD

The evidence for and against the oral-oral transmission of *H. pylori* is, at present, equivocal. The oral cavity has been proposed as a reservoir for infection, using data from a variety of cultural and polymerase-chain-reaction (PCR) - based techniques, with varying results. Some studies have shown frequent isolation of *H. pylori* from oral specimens, particularly dental plaque. Others have observed only occasional isolation and many have failed to isolate the organism at all from the mouth. There is evidence for the transmission of *H. pylori* between spouses, although the possibility remains that partners are both exposed to a common source of infection. There have been suggestions that the mouth may be a reservoir for re-infection, even though samples are apparently culture negative.

3.3.2 FECAL – ORAL SPREAD

The organism has been cultured from feces by PCR. In addition, data concerning water as a source are based on contamination of water by feces. The possibility of fecal-oral spread remains of great interest, but there are little data to support this concept.

3.3.3 IATROGENIC SPREAD

Gastroscopy units can be considered a privileged area for the transmission of *H. pylori* because of access to the stomach and possible regurgitation of gastric juice. Data suggest that the risks of infection from an endoscope are very small in instruments that are mechanically cleaned and then machine washed.

3.3.4 VECTORS

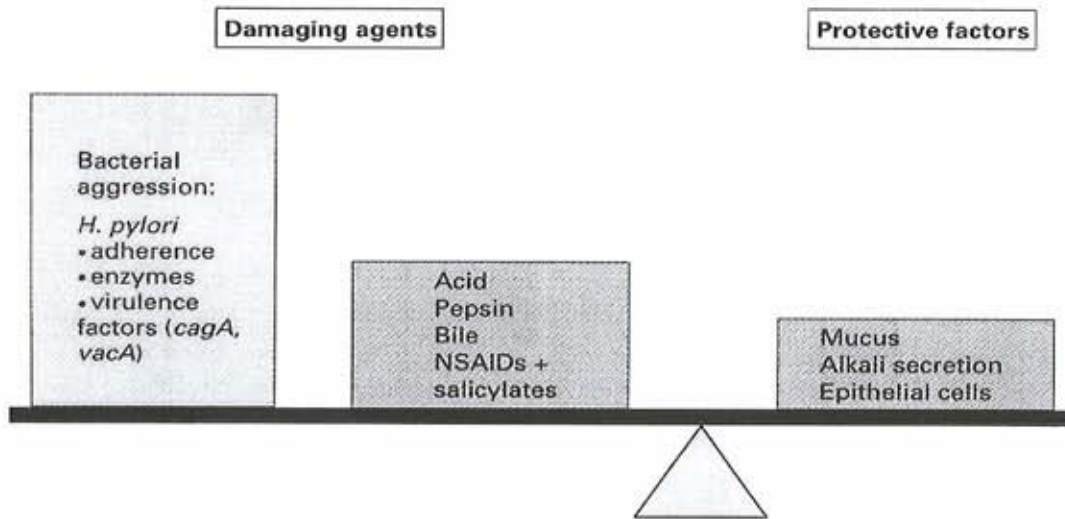
The existence of an animal reservoir of *H. pylori* besides primates is still hypothetical. Recent data suggest that domestic cats can carry *H. pylori*, but no large scale epidemiological data are yet available about cats in a community setting.

4 PATHOGENESIS OF HELICOBACTER PYLORI INFECTION

H. pylori infection of the gastric mucosa causes an active chronic gastritis, characterized by gastric epithelial cell injury, which in some cases progresses to atrophic gastritis. There is debate as to whether the mucosal injury occurs as a direct result of bacteria-induced cell injury or as a result of autoimmune cell damage from the inflammatory response. ²¹⁻²⁴

In-vitro studies have shown that *H. pylori*, by direct contact and by the elaboration of enzymes and cytotoxins, can directly injure gastric cells and may therefore be directly responsible for the majority of gastric cell damage found in infected persons. Also, strain differences have been postulated to account for the different disease outcomes found in persons infected with this bacterium. Strains with the cytotoxin-associated-antigen (*CagA*) pathogenicity island have been found in higher frequency in patients who develop peptic ulcers and gastric cancer as opposed to patients with gastritis alone. ^{21, 25}

Fig. 4.1 * Balance between damaging agents and protective factors of the stomach



* Adapted from Heatley R.²⁶

4.1 DIRECT MECHANISMS

4.1.1 BACTERIAL ENZYMES

A - UREASE

The urease is one of the most abundantly produced proteins by this bacterium. This enzyme helps protect the bacteria from acid by the production of ammonia, which increases the pH in the microenvironment around the organism.

In-vitro, ammonia caused direct injury to gastric epithelial cells and in some studies, it has been shown that ammonia exposure can impede gastric cell cycle progression, causing a delay in cells from progressing through the S-phase in the cell cycle.^{21, 27, 28}

Other studies suggested that the ammonia produced may be a nitrogen source for the synthesis of proteins necessary for bacterial adhesion, supporting the critical role of this enzyme in the pathogenesis.^{21, 29}

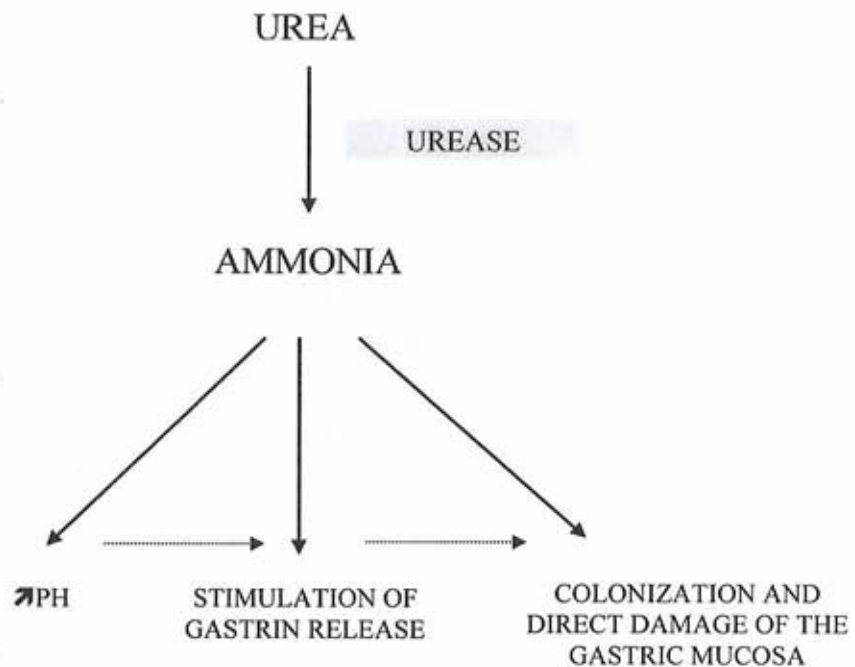


Fig. 4.2 Urease activity and pathogenesis

B - PROTEASE AND LIPASE

Lipase and protease elaborated by *H. pylori* can degrade gastric mucosa shown by the loss of protective qualities of mucus. *H. pylori* protease activity leads to disintegration of the polymeric structure of mucin whereas elaborated lipases and phospholipase A2 in particular result in mucus lipid degradation, loss of mucosal surface hydrophobicity, and lysophospholipid generation.^{30, 31}

The lytic activity of the resulting lysophospholipids is harmful to mucous gel integrity and to gastric epithelial cell membranes. In-vitro studies have also shown that *H. pylori* can inhibit the secretory response of mucous cells, indicating an additional mechanism by which this organism can undermine gastric mucosal cytoprotection. ^{21, 32}

4.1.2 BACTERIAL ADHERENCE

Colonizing bacteria have specialized adhesion molecules allowing them to maintain themselves within their habitat. Adhesion prevents bacteria from being removed rapidly by the local nonspecific host-defense mechanisms, including peristalsis, ciliary activity, turnover of epithelial cells and the mucous layer. An important aspect of adherence, in addition to assisting the organism in colonizing the host, is that it may allow for efficient transfer of bacterial toxins to host cells. ²¹

4.1.3 BACTERIAL VIRULENCE FACTORS

The *Helicobacter* genome sequence was published in 1997 ³³. It is an organism of 1.7 million nucleotides that represent approximately 1600 genes, each of which code for a separate protein. Of these, 55% are found in other organisms, but 45% are unique to *H. pylori*. Mutations in nucleotides give rise to differences between strains and affect the behavior of the organism such that some are selected out in preference to others - for example, in the development of antibiotic resistance. ²⁵

The genetic variation between the different strains of *H. pylori*, can lead to variable inflammatory responses in the host ranging from mild gastritis to severe and complicated ones. The major difference between virulent and avirulent organisms may depend upon the presence of the *Cag* pathogenicity island, a segment of DNA that has been acquired possibly from another organism and is now incorporated in the *Helicobacter* genome. ²⁵

A – PATHOGENICITY ISLAND (PAI)

The reason why it is termed PAI is because organisms that contain this sequence of DNA cause a more severe inflammation in the host mucosa. The island is analogous to the plasmids and insertions found in *Escherichia coli* that cause adhesion invasion and toxin production. It is unclear from which organism the PAI was derived; however, it has features in common with certain sequences in *Escherichia coli*, *Yersinia pestis* and *Vibrio cholerae*. The function of the PAI is not clearly understood, but it is believed to be involved in the export of macromolecules important in parasite/host interactions. ^{25, 34}

B - THE VACUOLATING TOXIN (*VacA*)

This toxin is secreted by *Helicobacter* strains that contain the PAI. It is activated at a low pH and is resistant to acid and pepsin. It is the first proteinaceous toxin to be identified in *H. pylori*. It was named vacuolating toxin because of its ability to induce vacuoles in the eukaryotic cells. Analysis of the *VacA* gene showed the presence of any one of three signal sequences (S1 a, S1 b and S2) and one of two middle-region alleles (m1 and m2). Strains with signal sequence 1 and middle sequence 1 (S1/m1) were associated with the higher levels of vacuolating cytotoxin activity; in addition, strains containing the S1/m1 alleles are more strongly associated with peptic ulcer disease.³⁵

C - THE CYTOTOXIN ASSOCIATED ANTIGEN (*CagA*)

One of the 40 genes contained in the PAI is *CagA* which codes for the protein *CagA* which was initially found in organisms that secrete the vacuolating cytotoxin.

However, even if it is not present, still the PAI is capable of secreting the vacuolating toxin.²⁵

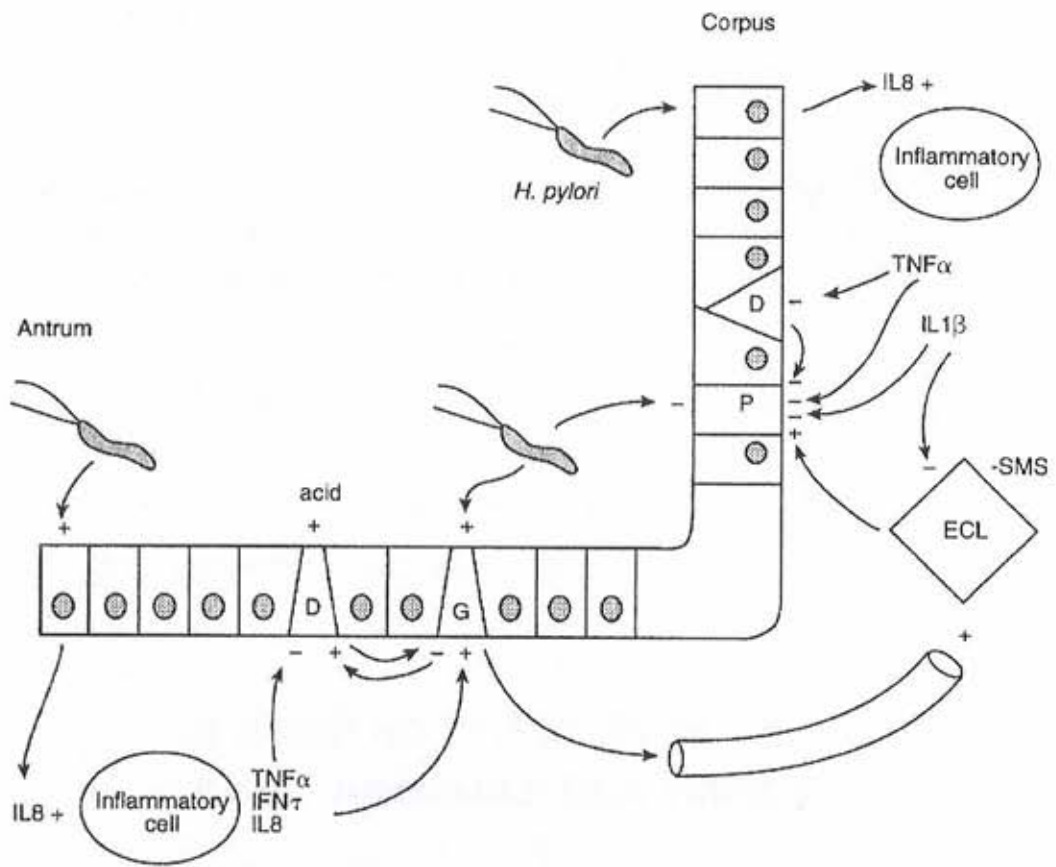
CagA gene is present in the majority of *H. pylori* isolates and its presence has been associated in some studies with an increased risk of gastric cancer and peptic ulcer disease. The role of *CagA* protein in the pathogenesis of the organism is not yet known. Presently, the *CagA* protein serves as a useful marker of the *Cag* pathogenicity island.^{36,37}

4.2 INFLAMMATORY RESPONSE

The mucosal immune responses include secretory immunoglobulin (Ig) A, which synthesis and secretion are enhanced by regulatory T-cells that produce cytokines. The pattern of cytokine production can distinguish two subsets of helper T-cells referred to as Th1 and Th2 cells. Th1 cells normally boost cell mediated immunity to cancers as well as intracellular infections, whereas Th2 cells seem to be more important in the generation of secretory immune responses in the mucosa. Paradoxically, during infection with *H. pylori*, an extracellular infection, Th1 cells predominate, whereas Th2 cells are virtually absent. This would not be the ideal response for this type of infection and may therefore account for the persistence of the infection. Moreover, Th1 cells produce cytokines that promote inflammation, auto-antibody formation and cell-mediated damage to the epithelium.³⁸

H. pylori also induces a chronic-active inflammation with a mixture of neutrophils, T-cells as well as B-cells, and plasma cells. These cellular changes are associated with an increase in Interleukin (IL) -1, IL-6, IL-8 and tumor necrosis factor (TNF)- α concentrations and Interferon- γ (IFN γ) producing cells. These responses can induce a number of changes in the gastric epithelium that may promote inflammation and epithelial cell damage. In turn, damaged epithelial cells may increase the risk of peptic ulcer or lead to aberrant repair recognized as gastric atrophy or epithelial cell metaplasia.^{38, 39}

Fig. 4.3 * Gastric physiology and the effects of *H. pylori*



* Adapted from Calam J. ⁴⁰

Gastrin from G-cells (G) stimulates parietal cells (P) to secrete acid by releasing histamine from enterchromaffin-like cells (ECL). All of these cells are normally restrained by release of somatostatin (SMS) from D-cells (D). Bacterial products stimulate G-cells but inhibit parietal cells. Inflammatory cytokines including tumor necrosis factor ($TNF\alpha$), interferon ($INF\gamma$), and interleukins 1 ($IL-1\beta$) and 8 ($IL-8$) influence the various cells as indicated, resulting in changes in acid secretion which depend on the distribution of gastritis.

4.3 EFFECT ON ACID AND GASTRIN PHYSIOLOGY

4.3.1 G - CELLS

Gastrin is a hormone produced from G-cells in the gastric antrum and proximal duodenum. It acts via the circulation to stimulate acid secretion, both by stimulation of the parietal cells (P-cells) and by promoting the release of histamine from the enterchromaffin-like cells (ECL) cells in the gastric corpus. It is now established that *H. pylori* infection elevates plasma gastrin concentrations⁴⁰⁻⁴²:

- *H. pylori*'s enzyme urease produces ammonia, and diets supplemented with ammonium acetate elevate circulating gastrin. However, a urease inhibitor failed to decrease plasma gastrin levels, so other *H. pylori* products might be responsible.
- The bacterium is reported to produce N- α -methylhistamine which by itself releases gastrin from G-cells via histamine H₂-receptors.
- *H. pylori* infection elevates mucosal expression of cytokines including IL-1 β , IL-6 and IL-8, TNF- α , INF γ and platelet activating factor. All of these inflammatory factors stimulate the release of gastrin, which suggests a synergism between bacterial and inflammatory products.

4.3.2 D - CELLS

Somatostatin peptides are released from D-cells in the antrum and the corpus and mediate the major reflexes that inhibit gastrin release and acid secretion. D-cells are stimulated by luminal acid, fasting, gastrin-releasing peptide and by small intestinal peptides such as cholecystokinin. Patients infected with *H. pylori* have less somatostatin peptide and fewer immunoreactive D-cells in their gastric mucosa. This lack of somatostatin may explain increases in gastrin release and acid secretion in infected subjects. ⁴⁰

4.3.3 ECL - CELLS

Histamine is released by ECL-cells in the gastric mucosa and stimulates acid secretion via H₂-receptors on parietal cells. It is produced from histidine by histidine decarboxylase. Mucosal concentrations of histamine have been generally found to be diminished in *H. pylori* gastritis. This probably reflects diminished synthesis because mucosal levels of histidine decarboxylase are also low. It is not known how *H. pylori* diminishes mucosal synthesis of histamine, but some possibilities suggest the effect IL-1 β greatly inhibits histamine from ECL-cells, or the stimulation of histamine H₃-receptors on ECL-cell that also inhibits histamine release. ^{40, 42}

4.3.4 PARIETAL CELLS

Effects of G-, D-, and ECL-cells on acid secretion depend greatly on the number and responsiveness of parietal cells, which varies from patient to patient and thus predicts disease outcomes. Factors that affect parietal cells are:

- Physiological regulators: More gastrin and less somatostatin will increase acid secretion, but the suppression of ECL-cells will diminish acid secretion. ⁴³
- *H. pylori*'s products and unusual fatty acids production have been identified to inhibit parietal cells. ⁴⁴
- Inhibitory cytokines: TNF- α and IL-1 β which are upregulated in *H. pylori* gastritis, also inhibit parietal cells. ⁴⁵
- Gastric atrophy: It is now recognized that *H. pylori* is a major cause of gastric atrophy, which is a loss of specialized cells including parietal cells from the gastric mucosa and thus the infection diminishes acid secretion by changing gastric histology. ⁴⁶

Overall, *H. pylori* gastritis more or less restricted to the gastric antrum in duodenal ulcers patients is associated with increased acid secretion. This is because gastritis increases the release of the antral acid-stimulating hormone gastrin and diminished mucosal expression of the inhibitory peptide somatostatin. Bacterial products and inflammatory cytokines also contribute to these changes in the endocrine function.

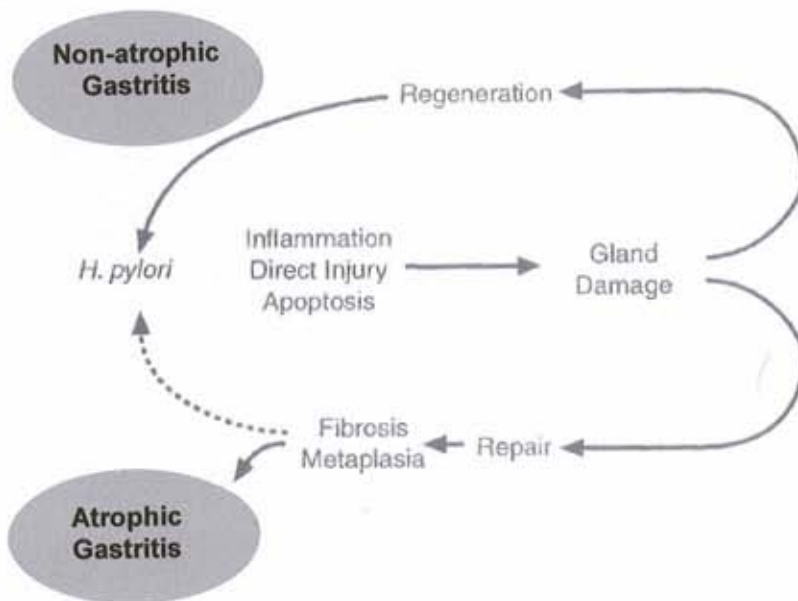
5

H. PYLORI AND RELATED DISEASES

5.1 GASTRIC ATROPHY AND INTESTINAL METAPLASIA

Irrespective of the mechanism of damage, the gastric mucosa may either regenerate and return to normal, or undergo adaptive reparative processes that lead to the replacement of the normal functional epithelium. When the destroyed glands fail to regenerate, the space occupied in the lamina propria may be replaced by fibroblasts and extracellular matrix. The end-result of this process is an irreversible loss of functional structure, which can lead to atrophy. In another pathway, often overlapping with the first one, the specialized gastric glandular epithelial cells are substituted by an intestinal-type epithelium containing goblet cells, intermediate, so-called absorptive cells, and in most case are lined by a brush border. This is known as intestinal metaplasia. During chronic *H. pylori* infection, all three of these types of repair occur, the respective proportion of each probably being modulated by environmental, genetic, and bacterial factors.⁴⁷

Fig. 5.1 * Possible scenarios of damage and repair



* Adapted from Genta R. ⁴⁷

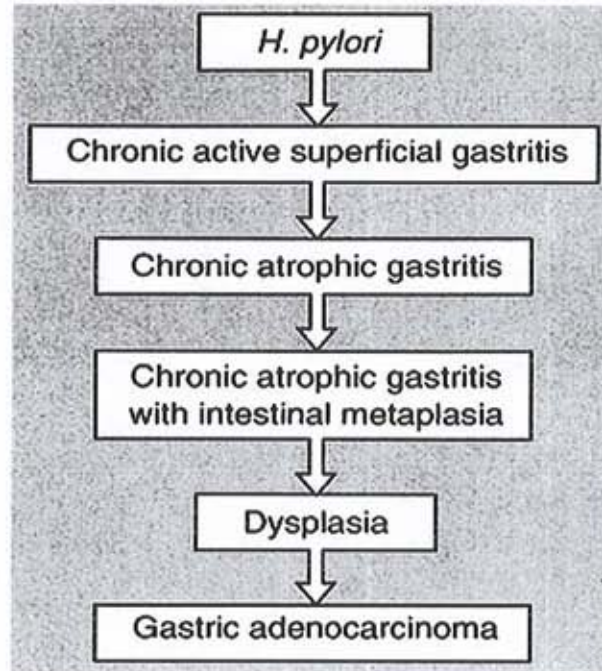
5.2 GASTRIC CANCER

Infection with *H. pylori* leads to changes in many factors that are important in the pathogenesis of gastric cancer, including the vitamin C content of gastric juice, reactive oxygen metabolites and epithelial cell proliferation. Ascorbic acid, the reduced form of vitamin C, is present in the gastric juice of healthy subjects in concentrations considerably higher than those in plasma. The importance of this mechanism may lie in the ability of ascorbic acid to protect against the development of gastric cancer. It was also found that high gastric juice ascorbic acid concentrations are found after successful eradication of *H. pylori*. ⁴⁸

Another theory is that the *CagA* gene and its product are more prevalent in *H. pylori* strains from patients with gastric cancer than in those obtained from individuals with histological gastritis alone. However, other reports showed that there was no significant difference in the prevalence of *CagA* gene between gastritis and gastric cancer strains. These results suggest that the significance of the *CagA* gene association with the development of gastric cancer will remain controversial ⁴⁹.

On the other hand, gastritis involving the gastric corpus tends to diminish acid secretion because bacterial products and cytokines inhibit parietal cells. *H. pylori*-associated mucosal atrophy will also contribute to acid hyposecretion. Such diminished acid allows overgrowth of carcinogen-producing bacteria in the stomach lumen. It is also now established that *H. pylori*'s associated chronic active gastritis, progresses to chronic atrophic gastritis with intestinal metaplasia, and finally to dysplasia and gastric carcinoma. ⁴⁰

Fig. 5.2 Involvement of *H. pylori* in gastric ulcer and gastric cancer



5.3 GASTRIC MALT - LYMPHOMA

Mucosa-Associated-Lymphoid-Tissue (MALT) in the stomach is the consequence of an infection with *H. pylori* infection. Other agents may also result in gastric MALT, but it is believed that in more than 90% of the cases, infection with *H. pylori* is responsible for the induction of lymphoid tissue in the stomach. MALT is an immunologic defense system to control local infection caused by bacteria such as *H. pylori*. It is composed of *H. pylori* reactive T-cells, plasma cells and other B-cells and mimics lymphoid follicles known from typical nodal tissues.^{50, 51}

Localization of gastric MALT parallels the site of *H. pylori* infection, the antrum. In addition, the number of lymphoid follicles in the stomach correlates well with the grade of *H. pylori* induced inflammation. Given the close relationship between *H. pylori* infection and the development of gastric MALT, studies determined the role of *H. pylori* in gastric MALT - lymphomas. It was proven that patients with early gastric MALT - lymphomas went into complete histological and endoscopic remission of the lymphoma after eradication of *H. pylori*.⁵⁰⁻⁵²

6 HELICOBACTER PYLORI DETECTION

Many diagnostic methods have been developed over the past 15 years to detect *H. pylori* infection. Some are invasive because they cannot be performed without endoscopy or biopsy, and others non-invasive, each presenting advantages and disadvantages.

Table 6.1 Diagnostic methods for H. pylori infection

INVASIVE TESTS	NON-INVASIVE TESTS
Endoscopy-based tests - Rapid Urease test - Histology - Culture - PCR	Labeled Urea-Breath-Test Serology

6.1 INVASIVE TESTS

6.1.1 UREASE TEST

The test detects the presence of urease produced by *H. pylori* in the biopsy specimen. A gastric biopsy specimen is placed in urea broth or agar. The urease present hydrolyses urea in the broth, with the production of ammonium ions, which raise the pH. This pH change is detected by a pH indicator, phenol red, which changes color from yellow-brown at pH 6.9 to pink at pH 8.4.

The ingredients for Christensen's urea broth are: urea 20g/l; phenol red, 0.012g/l; KH_2PO_4 , 2g/l; peptone, 1g/l; NaCl, 5g/l; glucose, 10g/l.

A buffer may be present in the broth to increase its stability and this reduces false positives. A color change from yellow brown to pink is considered positive⁷. The test, although highly specific was slow (3 to 6 hours), therefore new urease tests are now available (*e.g.* CLO tests, 1-min tests...) where the sensitivity and speed could be increased by omitting the buffer; by increasing the indicator concentration, and by crushing the biopsy specimen before inoculation^{53,54}. A variety of urease tests are commercially available, at least 10^5 bacteria are needed for a positive result.⁵³

- Tests should be read according to the manufacturer's instructions.
- The agar-based tests are designed to be read after 24 hours; if read after one or two hours their sensitivity may be low.
- Use of 2 biopsy specimens increases the sensitivity of the test.
- With longer reading times, the risk of false positive reactions increases due to the production of urease by other bacteria present in the stomach, but does not exceed 5 %.
- The optimum temperature for urease activity is 45 °C. The test is most efficient, therefore when performed above room temperature.
- Strip tests are designed to be read within 2 hours. Their specificity is good without loss in specificity. There is no need for incubation at temperature higher than room temperature.

Table 6.2 Advantages v/s Disadvantages of the Urease Test

Advantages	Disadvantages
<ul style="list-style-type: none"> • Can be performed simply • Results obtained before the patient leaves the endoscopy department • Tests are cheap compared to cultures or microscopy of histology 	<ul style="list-style-type: none"> • Despite the high sensitivity some positives can be missed, because <i>H. pylori</i> infection can be patchy and organisms may be present in one specimen and not another • The test is not as sensitive when performed with specimens from patients who have received antimicrobial or bismuth treatment

6.1.2 HISTOLOGY

The size and orientation of the biopsy specimen are crucial for correct diagnosis. Ideally, endoscopic biopsy specimens should contain the full mucosal thickness. As *H. pylori* are almost exclusively found on the superficial and foveolar epithelium, the presence of this type of epithelium is essential for the evaluation of *H. pylori* colonization.⁵³

None of the currently used staining methods is specific for *H. pylori*. Of all the special stains available the most frequently proposed are Warthin-Starry, modified Giemsa, gimenez, half Gram, acridine orange, and cresyl violet. Although the choice of stain should be made on the basis of reproducibility, cost and personal experience, in most of the published studies Giemsa was used, as it is an easy, rapid, effective and inexpensive staining method.

Histology testing depends largely on the observer's experience and skill and the extent of biopsy sampling, so it is difficult to give reliable data on the sensitivity of histology compared with other techniques.

In addition, careful attention has to be paid to any persistence of active gastritis, as it may represent an indirect sign of eradication failure in apparently *H. pylori* negative biopsy samples. *H. pylori* is unable to colonize intestinal type metaplastic epithelium, attempts must be made to obtain biopsy specimens from non-metaplastic mucosa as well.⁵⁵

Sometimes, the observer is unable to distinguish *H. pylori* from other bacterial species that are occasionally present in the stomach as a consequence of achlorhydria or of a passive transport from the upper digestive pathways, therefore, immuno-histochemical techniques may be required to clarify the situation.⁵³

Table 6.3 Advantages v/s Disadvantages of Histology

Advantages	Disadvantages
<ul style="list-style-type: none"> • Possibility of estimating the extent of <i>H. pylori</i> infection simultaneously with inflammatory and degenerative mucosal lesions. • Availability in almost all medical institutions 	<ul style="list-style-type: none"> • Endoscopy needed to get the sample • The overall performance depends on the skill and experience of the pathologist • Limited power for the differentiation of <i>H. pylori</i> from other similar looking bacteria
<ul style="list-style-type: none"> • Permanence of preparations allowing retrospective evaluation of specimens 	<ul style="list-style-type: none"> • Impossibility of studying antimicrobial resistance and typing of the bacteria • Delayed result

6.1.3 CULTURE

Appropriate transport conditions are important in order to avoid desiccation of biopsy samples and long exposure to ambient air. The choice of a particular medium will usually depend on the local facilities and also on personal preferences. It is mandatory to grind the biopsy specimens with a tissue homogeniser in a small volume of saline solution (0.25-0.5 ml), or use an electrical homogeniser. Grinding yields higher isolation rates of bacteria than simply rubbing the biopsy specimens on an agar plate; a more homogenous growth and higher number of colonies are also obtained^{56,57}. Incubation must be carried out at 37 °C in a microaerobic atmosphere (5-6% O₂, 8-10% CO₂, 8–85% N₂) with a relative humidity of greater than 98%. Supplementation of the atmosphere with 4-5% H₂ may accelerate the growth rate and yield larger colonies. Incubation is usually for 7 days, with daily inspection from the second day, this is extended for up to 12 days in the event of post-treatment evaluation.⁵³

Table 6.4 Advantages v/s Disadvantages of Culture

Advantages	Disadvantages
<ul style="list-style-type: none"> • Maximum specificity as the bacteria grow and can be accurately identified • Possibility of antimicrobial susceptibility testing • Permits typing of the strains by all the methods currently available • Potentially available in almost all medical institutions 	<ul style="list-style-type: none"> • Endoscopy needed to get sample • Results take several days • Sensitivity may be impaired by inappropriate sampling, transport, or processing conditions • Does not give insight into the status of the mucosa

6.1.4 POLYMERASE CHAIN REACTION (PCR)

The PCR can be used to detect bacteria which are slow-growing or difficult to identify in body fluids or other specimens. At present, it is used only as a research tool, because no kits are available commercially as yet. This test may be particularly useful in the post-treatment period when the bacterial load may be low. PCR can also be used in molecular epidemiology for fingerprinting *H. pylori* isolates. This may be useful in the post-treatment period to distinguish recurrence of infection (implying treatment failure) from reinfection (that is, new infection with another strain of *H. pylori*).⁵³

Table 6.5 Advantages v/s Disadvantages of PCR

Advantages	Disadvantages
<ul style="list-style-type: none"> • High specificity which is maximized when two sets of primers are used • No need for specific transport conditions • Results can be available in a day • Allows typing of the strain 	<ul style="list-style-type: none"> • Endoscopy needed to get the sample • Does not permit antimicrobial susceptibility testing • Does not give insight into the status of the mucosa • Not generally available • Risk of contamination (false positive) or reaction inhibitors (false negative)

6.2 NON - INVASIVE TESTS

6.2.1 UREA BREATH TESTS

The ^{13}C and ^{14}C -urea breath test (UBT) are straightforward, easy and accurate non-invasive tests for *H. pylori* infection. They are based on the principle that, in the presence of *H. pylori* urease, labeled carbon dioxide is exhaled in the expired breath. Both UBTs are semi-quantitative tests of active *H. pylori* infection and, in contrast to biopsy based tests, are not liable to sampling errors^{53, 58, 59}. The test consists of giving to the patient a test meal, followed 10 min later by the labeled urea solution (50-75ml), after which they lie horizontally on each side for two to three minutes in attempt to get the labeled urea evenly distributed over the lining of the stomach. The test exploits the large amount of urease produced by *H. pylori*, as this enzyme hydrolyses the orally administered, labeled urea into ammonia and labeled CO_2 , which is absorbed through the mucus layer of the stomach and then transported to the lungs via the blood stream for excretion. Isotope enrichment is then measured in breath samples collected at appropriate by constructing an excretion curve or calculating the percentage of the dose expired.^{53, 60}

A - ^{13}C -UBT vs ^{14}C -UBT

Urea can be labeled with two different carbon isotopes: ^{14}C and ^{13}C . The main difference between them is that the former is radioactive whereas the latter is stable. The advantages of using ^{14}C -urea is that it is cheap, so rapid that administering ^{14}C -urea in a gelatin capsule allows an accurate response to be obtained from a single 10 min, breath sample, and does not require a test meal. However, although the dose of ^{14}C has become progressively smaller and the test can now be performed with 1 μCi , which is equal to the natural background radiation received in one day, the main problems are still the availability of nuclear medicine department or centers licensed for storage and disposal of radioactive substrates^{60, 61}. In contrast, ^{13}C is a non-radioactive isotope that can be used safely for repeated testing, which is frequently required in clinical practice and for detecting *H. pylori* infection in children and women in childbearing age. The major drawbacks of ^{13}C -urea are the higher cost compared with ^{14}C -urea, and the need for expensive mass spectrometry, which is the most preferable device for measuring ^{13}C enrichment in breath samples of subjects infected with *H. pylori*.

B - ACCURACY OF UBT

False negative results may occur if the UBT is performed within 7-14 days of taking antibiotics, bismuth salts, or proton pump inhibitors. It is therefore recommended that these drugs are stopped at least 28 days before testing.

The ^{13}C and ^{14}C are of particular value in assessing eradication of *H. pylori* four weeks post-treatment. Both tests may also be used to screen patients before endoscopy.⁵³

Table 6.6 Advantages v/s Disadvantages of UBT

Advantages	Disadvantages
<ul style="list-style-type: none"> • Non-invasive method • High sensitivity • High specificity • No need for specific transport conditions • Tests the whole of the stomach 	<ul style="list-style-type: none"> • Does not permit antimicrobial susceptibility testing and strain typing • Does not provide insight into the status of the mucosa • Not available in all medical institutions

6.2.2 SEROLOGY

Serological follow-up is useful for long-term treatment monitoring in an adult population. This test could reduce the number of endoscopic interventions and lead to greater patient compliance and cost reduction.⁵³

Some patients do not return to seronegativity even years after eradication. A falling antibody level and not seronegativity is therefore the criterion for successful treatment; collection of pretreatment serum samples is thus a requirement.

In order to use serological tests for treatment monitoring, a number of factors must be considered.^{53, 62}

- A small proportion of infected patients do not mount a systemic immunoglobulin G (IgG) response. In such patients serology will not yield helpful results.
- IgG should be tested (IgA is useful only in a small number of patients).
- A titer reduction of greater than 50% is a reliable indicator of *H. pylori* eradication.
- Long-term follow-up of patients after *H. pylori* eradication therapy produces three common patterns:
 - Patients with permanent eradication of *H. pylori*: in these patients, a noticeable and consistent fall in anti-*H. pylori* IgG can be observed. Although this fall may be detectable within 3 months of successful treatment, it is recommended that serology should be performed after 6 to 9 months (reduction of > 50% compared with pretreatment titers).

- Patients without eradication of *H. pylori*: in this group of patients, titers remain in the range of pre-treatment titers (+/- 20%). Irrespective of treatment success, a slight decrease in IgG antibody levels can be seen a few weeks after the end of treatment. (This phenomenon reflects suppression of *H. pylori* infection).
- Patients with reinfection: reinfection can be detected by a significant increase in *H. pylori* specific IgG titers.

Given the possible variability of tests, the choice of which one to use depends on ⁵³:

- The aims and the end points of the study
 - The antimicrobial agents to be used
 - The inclusion or follow-up period
 - The population to be tested (adults or children, developed or developing countries)
-

When comparing the accuracy of the tests, the most specific technique is culture, but sensitivity may suffer from inappropriate sampling, transport conditions or processing. Histology is valuable, but sensitivity and specificity vary according to the pathologist's experience and skill.

Non-invasive tests also have good sensitivity and specificity. UBT is the test of choice for assessment for eradication.

The rapid urease test is not recommended for post-treatment evaluation because it is an endoscopy-based test and may have a relatively low sensitivity, especially in patients treated with urease inhibitors, such as proton-pump inhibitors.⁵³

7

MANAGEMENT OF *H. PYLORI* INFECTION

7.1 RECOMMENDATIONS AND GUIDELINES

Although *H. pylori* has been described in association with a number of clinical conditions, there are currently relatively specific cases where eradication treatment is indicated. These indications were first designed on an international level but now protocols and recommendations are filtering down to both national and local levels. In February 1994, the National Institute of Health (NIH) convened a Consensus Development Conference Panel that reviewed information relating to *H. pylori* infection⁶³. This conference was an important beginning, and information relating to *H. pylori* continued to evolve as expert panels have been meeting and issuing updated guidelines and consensus statements.

Table 7.1 *NIH consensus statement*

- Ulcer patients with *H. pylori* infection require antimicrobial and antisecretory drugs
- Maintenance acid suppression may also be wise in those who have suffered a bleeding ulcer
- Value of treating *H. pylori* in non-ulcer dyspepsia remains uncertain
- The relationship between *H. pylori* and gastric cancer requires further exploration

In February 1997, the Digestive Health Initiative (DHI) Update Conference was organized in a format similar to that of the NIH, 3 years earlier, to update the status of *H. pylori*.⁶⁴

Table 7.2 DHI consensus statement

- Testing and treatment should take place for:
 - o patients with an active or documented past history of duodenal ulcer, gastric ulcer and complicated duodenal or gastric ulcer
 - o those who have had a resection of early gastric cancer or low grade MALT - lymphoma
- Testing and treatment not recommended for patients with undiagnosed dyspeptic symptoms

Synchronously, the European *Helicobacter pylori* Study Group (EHPSG) held a Consensus conference in Maastricht. In addition to outlining the indications for eradication therapy, recommendations were given at one of three levels (strongly recommended, advisable or uncertain), reflecting mainly the strength of the available evidence in the literature.⁶⁵

Table 7.3 EHPHG Indications for *H. pylori* eradication therapy ⁶⁵

DIAGNOSIS	STRENGTH OF RECOMMENDATION	STRENGTH OF EVIDENCE
PUD (active or not)	Strong	Unequivocal
Bleeding PUD	Strong	Unequivocal
MALT - lymphoma (low grade)	Strong	Unequivocal
Gastritis (severe abnormalities)	Strong	Supportive
After early gastric cancer resection	Strong	Supportive
Long term PPI therapy for GERD	Advisable	Supportive
After surgery for PUD	Advisable	Supportive
Nonulcer dyspepsia	Advisable	Equivocal
Family history of gastric cancer	Advisable	Equivocal
NSAID therapy	Advisable	Equivocal
Patient's wishes	Advisable	Equivocal
Prevention of gastric cancer (no risk)	Uncertain	Equivocal
Asymptomatic subjects	Uncertain	Equivocal
Extra-alimentary tract disease	Uncertain	Equivocal

In 1998, The American College of Gastroenterology (ACG) developed guidelines for the management of *H. pylori* infection in adult population. Those ACG guidelines consist of eight recommendations that explore the following issues: principles of testing, non-ulcer dyspepsia, diagnostic strategies, treatment and follow-up. ⁶⁶

Table 7.4 ACG guidelines for the management of *H. pylori* infection ⁶⁶

Principles of testing for *H. pylori* infection

- *Diagnostic testing for H. pylori infection should only be performed if treatment is intended*
- *Testing for H. pylori infection is indicated in patients with active PUD, a past history of documented peptic ulcer, or gastric MALT - lymphoma*
- *Testing for H. pylori is not indicated in asymptomatic individuals without a past history of PUD, or in patients on long-term treatment with a proton pump inhibitor (PPI) for GERD*

Nonulcer dyspepsia

- *There is no conclusive evidence that eradication of H. pylori infection will reverse the symptoms of nonulcer dyspepsia. Patients may be tested for H. pylori on a case-by-case basis, and treatment offered to those with a positive result*

Diagnostic strategies for *H. pylori* infection

- *The diagnostic test to use in a particular patient depends on the clinical setting, particularly if the upper GI endoscopy is performed*
- *When endoscopy is indicated, the test of first choice is a urease test on an antral biopsy. If a biopsy urease test is negative, H. pylori infection may be diagnosed by histology or serology. Biopsy urease tests have reduced sensitivity in patients taking PPIs and in patients with recent or active bleeding. Histology is not generally necessary and is expensive. When endoscopy is not performed, an office-based serological test is the least expensive means of evaluating for evidence of H. pylori infection. A urea breath test is the best non-endoscopic test for documenting H. pylori infection*

Treatment of *H. pylori* infection

- *The highest eradication rates are achieved with the following regimens:*
 - *a PPI, clarithromycin, and either amoxicillin or metronidazole for 2 wk*
 - *ranitidine bismuth citrate, clarithromycin, and either amoxicillin, metronidazole, or tetracycline for 2 wk*
 - *a PPI, bismuth, metronidazole, and tetracycline for 1 or 2 wk*

Follow up of patients after treatment for *H. pylori* infection

- *Currently, routine post-treatment testing is only recommended in patients with a history of ulcer complications, MALT - lymphoma, or early gastric cancer. Patients with recurrent symptoms after treatment of H. pylori infection will also need further evaluation*

7.2 H. PYLORI TREATMENT STRATEGIES

Therapeutic strategies for peptic ulcer disease historically have focused on decreasing intra-gastric acidity. Recently, with the acceptance of the causative role of *H. pylori* infection in peptic ulcer disease, current therapy comprises antibiotic regimens in combination with acid decreasing drugs to eradicate *H. pylori* and to achieve enhanced healing of ulcers. The bacterium *H. pylori* cannot be eradicated effectively using one drug only and the ideal therapy to cure the infection is still lacking.

7.2.1 BACTERIOLOGICAL TREATMENT

The first step in the search for a suitable antimicrobial agent to treat any infection is to determine the in-vitro susceptibility of the organism to suitable therapeutic agents. In-vitro, *H. pylori* is sensitive to a wide variety of antimicrobial agents, including β -lactams, macrolides, tetracycline, metronidazole, tinidazole, rifampicin, nitrofurantoin and some quinolones. However, in-vivo attempts to eradicate *H. pylori* with any of these agents administered as monotherapy have generally proved to be unsuccessful.⁷

Table 7.5 * In-vivo efficacy of antibiotics monotherapy in eradicating *H. pylori*

Treatment	Duration (days)	Eradication %
Amoxicillin caps 1 g bid	7	0
Amoxicillin caps 375 mg tid	28	30
Clindamycin caps 300 mg qid	21	0
Doxycycline caps 200 mg	10	0
Erythromycin ethylsuccinate susp 500 mg qid	21	7
Metronidazole 500 mg tid	10	10
Ofloxacin 200 mg bid	21	20
Tetracycline 500 mg bid	28	0

* Adapted from Heatley RV et.al. ⁷

Those clinical failures may be explained partially by a lack of diffusion of those agents into the mucus and by the reduced activity in the acid environs of the gastric mucosa . Moreover, many drugs are unstable under acidic conditions, or achieve only sub-inhibitory concentrations in the gastric crypts leading to in-vivo failure. ⁷

Table 7.6 * Effect of pH on the activity of antimicrobial agents against *H. pylori*

Agent	Isolates (n)	MIC ₉₀ (mg/l)		
		pH 7.5	pH.6.0	pH 5.5
Penicillin	20	0.03	0.5	0.5
Ampicillin	20	0.06	0.25	0.5
Cephalexin	17	2	16	32
Erythromycin	20	0.06	2	8
Ciprofloxacin	20	0.12	0.5	2
Tetracycline	17	0.12	0.25	0.5
Ntrofurantoin	17	1	2	2
Metronidazole	15	2	2	2
Bismuth subcitrate	17	16	8	-

* Adapted from Heatley RV et.al. ⁷

7.2.2 BISMUTH COMBINATIONS

Marshall *et.al* ⁶⁷ were the first to report on the in-vivo bactericidal effect of bismuth subcitrate. Bismuth preparations are effective in suppressing or clearing *H. pylori* from the stomach. This may be due to the bismuth's antimicrobial activity, its cytoprotective effects or both.

Table 7.7 Suggested mechanisms of bismuth preparations in ulcer healing

- Inhibition of the bacterial urease activity
- Inhibition of the adherence of the bacteria to the gastric mucosa.
- Binding to ulcer craters
- Inhibiting the pepsin activity,
- Increasing mucosal prostaglandin production,
- Increasing mucus and bicarbonate secretion

Bismuth formulations: colloidal bismuth subcitrate (CBS) also known as tri-potassium di-citrate bismuthate (TBD), bismuth subsalicylate (BSS) and ranitidine-bismuth citrate (RBC)

In-vitro, bismuth subcitrate inhibits the growth of *H. pylori* at concentrations in the range of 4-32mg/l ⁶⁸. Clearing of *H. pylori* using bismuth preparations was exciting, but permanent eradication using bismuth monotherapy was rather disappointing. When bismuth is combined with antibiotics, particularly nitroimidazoles, higher eradication rates can be achieved. Dual therapy of this kind however, is undesirable because many patients remain infected and the residual organism is becoming resistant to the nitroimidazole. ⁶⁹

The triple therapy combination of bismuth, a nitroimidazole plus either amoxicillin or tetracycline is an effective eradication therapy, with variable eradication rates. However, its complicated dosing regimen involving up to 18 tablets per day, long duration of treatment and high incidence of side effects have limited its usage. H2-antagonists were added to the classical bismuth triple therapy in attempt to enhance symptom relief and ulcer healing making a form of quadruple therapy. However, quadruple therapy did not change significantly the eradication rate, and compliance remained the limiting factor for treatment success.⁷⁰⁻⁷⁵

Table 7.8 Bismuth/ Antibiotic therapy combinations for *H. pylori* eradication

Author	Drug combination (dose / duration)	Patients (n)	Eradication (%)
Marshall <i>et al</i> ⁷²	BSS 500 mg qid (wk 1 - 3) Metronidazole 1 - 1.5 gm (wk 2 to 3)	19	79
Marshall <i>et al</i> ⁷²	BSS 500 mg qid (wk 1 - 3) Amoxicillin 500 mg qid (wk 2 to 3)	25	28
Borody <i>et al</i> ⁷³	CBS 1 tab qid (4 wk) Amoxicillin 250 mg qid (4 wk) Tetracycline 500 mg qid (4 wk) Metronidazole 200 mg qid (2 wk)	100	94
Graham <i>et al</i> ⁷⁴	Ranitidine 300 mg qd (4 - 16 wk) Bismuth 8 tabs qd (2 wk) Metronidazole 250 mg tid (2 wk) Tetracycline 500 mg qid (2 wk)	47	85
Borsch <i>et al</i> ⁷⁵	BSS 2 tab tid (2 wk) Amoxicillin 500 mg tid (2 wk) Metronidazole 500mg tid (2wk)	23	90

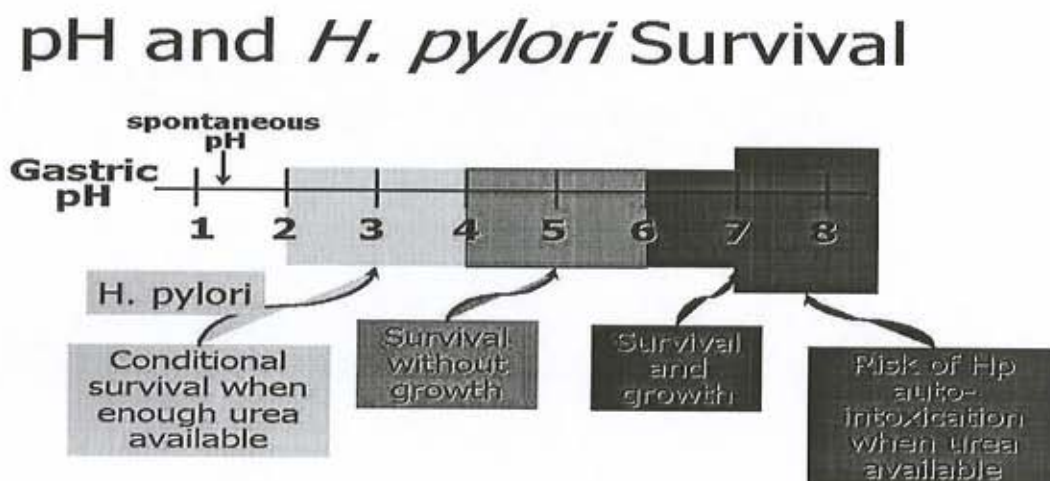
7.2.3 ANTISECRETORY AGENTS. COMBINATIONS

H. pylori is suited to an acid environment therefore when acid secretion is reduced, as in atrophic gastritis or by treatment with a PPI or H₂-antagonist, *H. pylori* colonization diminishes. This is explained possibly by the build up of ammonia within the organism, from the continued action of the urease, unopposed by gastric acid. Yet, acid inhibition alone does not eradicate the bacteria, which recolonizes when the acid secretion returns to pretreatment levels.⁷⁶⁻⁷⁸

Studies have evaluated the role of acid lowering agents such as H₂-antagonists and PPI's in combination with antibiotics for the eradication of *H. pylori*.

The result of some recent studies suggest that antibiotic/antisecretory drug combinations that include a PPI as opposed to H₂-receptor antagonist, provided better eradication of *H. pylori*. Now it is generally accepted that PPIs have greater efficacy than other classes of acid suppressing drugs in the management of peptic ulcer disease and achieve rapid symptom relief and ulcer healing.^{74, 79-81}

Fig. 7.1 pH and *H. pylori* survival



PPIs may augment the action of antimicrobial agents through several possible mechanisms, including a direct bactericidal effect and creation of an unfavorable gastric environment. Unlike H₂-antagonists, all the PPIs, including omeprazole, lansoprazole, pantoprazole and rabeprazole, have in-vitro activity against *H. pylori*.

82, 83

In vitro, both lansoprazole and omeprazole selectively inhibit *H. pylori* urease, which is essential for gastric colonization by the bacteria. However, it is not yet clear how this activity applies to the in-vivo situation, as in-vitro activity of antimicrobials against *H. pylori* in general does not predict in-vivo efficacy.⁸⁴

More recently it has been suggested that the PPIs are also active against a "p" type ATP-ase identified in *H. pylori*. It remains to be determined whether this is an important additional mode of action of the proton-pump-inhibitor acting in synergy with antimicrobials in-vivo.⁸⁵

The pH elevation by PPIs may reduce the degradation of both acid labile antibiotics and *H. pylori* specific immunoglobulins, while also decreasing the pH gradient across the mucus bicarbonate layer enhancing the environment at the epithelial cell surface for neutrophil function. Furthermore, the reduction of gastric acid juice volume produced by high-dose PPIs will enhance the concentration of antibiotics in the stomach.

A wide range of antibiotics combinations has been used in many different studies and on various number of patients. A dual therapy with omeprazole and amoxicillin for 2 weeks gave in some studies consistent eradication rates of 85 %, comparable with bismuth triple therapy ⁸⁷. However, this was not reproducible in other studies, which lead to the combination of a PPI with two antibiotics usually a nitroimidazole and amoxycillin or tetracycline for 2 weeks. Such combinations have proven to be very effective with eradication rates of around 90%. ^{80, 87-90}

Table 7.9 Acid suppressive / Antibiotic therapy combinations for *H. pylori* Eradication

Author	Drug combination (dose/ duration)	Patients (n)	Eradication (%)
Hentschel <i>et.al</i> ⁸⁰	Ranitidine 300 mg (6-10 wk) Amoxicillin 750 mg (12 day) Metronidazole 500mg tid (12 days)	52	89
Labenz <i>et al</i> ⁸⁷	Omeprazole 20 mg bid (2. wk) Amoxicillin 1 g bid (2 wk)	19	85
Bazzoli <i>et al</i> ⁸⁸	Omeprazole 20 mg (1 wk) Clarithromycin 250 mg bid (1 wk) Tinidazole 500 mg bid (1 wk)	40	100
Burette <i>et al</i> ⁸⁹	Lansoprazole 30 mg (4wk) Roxithromycin 300 mg (2Wk) Metronidazole 500 mg bid (2 wk)	17	82
Cayla <i>et al</i> ⁹⁰	Lansoprazole 30 mg bid (2wk) Amoxicillin 1 g bid (2wk) Clarithromycin 500 mg bid (2wk)	21	95

However, the determination of the optimal combination and duration of the treatment remains an active area of investigation, as most of the studied regimens have shown variable eradication rates. Currently treatment regimens for *H. pylori* usually combine an acid-reducing agent (PPI, H2-antagonist) with two or three antibiotics (macrolide, metronidazole, tetracycline, amoxicillin), with a duration varying from seven to fourteen days. Current treatment recommendations for *H. pylori* associated peptic ulcer disease are summarized in table 7.10.

Table 7.10 ASHP treatment recommendations ⁹¹

Regimen	Eradication (%)
BSS 525 mg qid + metronidazole 250 mg qid + tetracycline 500 mg qid each for 14 days + H2-receptor antagonist in ulcer treatment doses for 28 days	77 - 82
Clarithromycin 500 mg bid + amoxicillin 1 g bid + lansoprazole 30 mg bid each for 10 - 14 days	84 - 92
Clarithromycin 500 mg bid + amoxicillin 1 g bid + omeprazole 20 mg bid each for 10 days	78 - 90
BSS 525 mg qid + metronidazole 500 mg tid or 250 mg qid + tetracycline 500 mg qid + PPI (omeprazole 20 mg or lansoprazole 30 mg) bid each for 14 days	98
Clarithromycin 500 mg bid + amoxicillin 1 g bid + omeprazole 20 mg bid each for 14 days	92
Clarithromycin 500 mg bid + metronidazole 500 mg bid + PPI (omeprazole 20 mg or lansoprazole 30 mg) bid each for 14 days	89 - 91

8 LIMITED EFFICACY OF AZITHROMYCIN-BASED ULTRA-SHORT THERAPY FOR HELICOBACTER PYLORI

8.1 INTRODUCTION

Infection with *Helicobacter pylori* (*H. pylori*) has been linked with chronic active gastritis and peptic ulcer disease ⁹²⁻⁹⁷. This ubiquitous organism has also been reported in large case - controlled studies to be strongly associated with adenocarcinoma and non-Hodgkin's lymphoma of the stomach ⁹⁸⁻¹⁰⁰. Eradication of *H. pylori* has been clearly shown to result in accelerated ulcer healing as well as considerable reduction (almost elimination) in the risk of ulcer recurrence ^{93, 96, 101}. The determination of the optimal combination and duration of treatment for *H. pylori* infection remains an active area of investigation, as most studied regimens have shown variable eradication rates, at times with striking differences ¹⁰²⁻¹⁰⁷.

Treatment regimens for *H. pylori* usually combine an acid-reducing agent (e.g. a proton pump inhibitor (PPI), or an H₂-antagonist) with two or three antibiotics (e.g. a macrolide, metronidazole, tetracycline, or amoxicillin), for a duration varying from seven to fourteen days ¹⁰⁸⁻¹⁰⁹. Antimicrobial resistance plays an important role in the success or failure of the therapy ¹¹⁰.

More importantly, however, long-term therapy with multiple dose regimen constitute real barriers to patient compliance and may consequently contribute to failure of therapy and possibly to the development of *H. pylori* resistant strains. The search for a short-duration simple therapeutic regimen has hence been relentless. It is unclear, however, whether any therapy for less than seven days will consistently preserve efficacy (>90% eradication) while improving patient acceptability, compliance and reducing cost. Due to its unique pharmacokinetic properties, azithromycin is anticipated to be the most attractive combination partner for *H. pylori* eradication regimens. This antimicrobial agent represents a new generation macrolide of the azalide class. It consists of a 15-membered lactone ring containing a tertiary amino group, attached to two sugar moieties, and differs from erythromycin in having an amino-substituted, expanded macrolide nucleus. These structural differences provide azithromycin with a good acid stability and special pharmacokinetic properties, consisting of high extraction from the blood into target tissues for prolonged periods. Its concentration in the gastric tissues is largely above the minimal inhibitory concentration (MIC₉₀) for *H. pylori* (0.25 mcg/ml), is rapidly reached and maintained for at least 120 hours^{111,112}. Based on these favorable characteristics, trials were conducted to evaluate short-term azithromycin-based therapy (for 3-6 days) in combination with a proton-pump inhibitor and either amoxicillin, metronidazole, or tinidazole yielding conflicting results with eradication rates ranging widely from 57-93%^{109, 113-125}. The purpose of this study is to investigate the efficacy and safety of two azithromycin-based ultra-short therapy for *H. pylori* eradication.

8.2 METHODS

8.2.1 PATIENTS

From April 2000 to September 2000, patients with dyspepsia referred for outpatient endoscopy at the American University of Beirut Medical Center (AUBMC) and having a positive rapid urease assay (CLOtest®, Ballard medical products, Draper, Utah, USA) for *H. pylori* infection, were enrolled in this pilot, open label study. Exclusion criteria included: age under 18 years, allergies to any of the drugs used, recent antibiotic therapy (within 2 weeks of enrollement), severe ulcers or bleeding, gastric perforation or obstruction, previous gastrectomy, gastric cancer, pregnancy or lactation, or severe concomitant diseases. The protocol was approved by the AUBMC Investigation and Research Board and all patients gave an informed consent.

Table 8.1 Patients demographics at entry

	GROUP A	GROUP B
Number of patients	15	13
Age	Mean 46 (22-71)	Mean 43 (24-62)
Gender	Male 12 (80%) Female 3 (20%)	Male 9 (70%) Female 4 (30%)
Current or recent use of:		
NSAID	2 (13%)	1 (8%)
Alcohol	1 (7%)	4 (31%)
Smoking	5 (33%)	9 (69%)

NSAID = Non-steroidal anti-inflammatory drugs

Enrolled patients were interviewed and counselled regarding appropriate medication use to ensure better compliance. Daily phone calls over the period of treatment evaluated compliance and potential side effects. After a minimal period of four weeks following completion of therapy, patients underwent a ^{14}C -urea breath test (UBT) in order to evaluate for eradication of *H. pylori*. This four-week period between treatment and UBT testing is used to eliminate false negative results whereby *H. pylori* may not be detected due to suppression of growth rather than successful eradication. Patients were instructed to discontinue any PPI therapy a minimum of one week prior to UBT testing.

8.2.2 TREATMENT

The patients were divided into two treatment protocols and assigned to receive either a 3-day therapy (Group A) or a 5-day therapy (Group B). Assignment was non-randomized and done consecutively for Group A followed by Group B. In both groups, patients received lansoprazole (Lanzor® 30 mg capsules, Marion Hoechst Roussel, France) 30mg bid on day 1; lansoprazole 30 mg bid, amoxicillin (Amoxil® 500 mg tablets, SmithKline Beecham, UK) 1 g bid, and azithromycin (Zithromax® 250 mg tablets, Pfizer, USA) 500 mg bid on days 2 and 3. Patients in group B received lansoprazole 30 mg bid and amoxicillin 1 g bid for two additional days (4 and 5).

Table 8.2 Treatment protocol

DAY	GROUP A	GROUP B
1	Lansoprazole 30mg bid	Lansoprazole 30 mg bid
2,3	Lansoprazole 30 mg bid Azithromycin 500 mg bid Amoxicillin 1 g bid	Lansoprazole 30 mg bid Azithromycin 500 mg bid Amoxicillin 1 g bid
4,5	—	Lansoprazole 30 mg bid Amoxicillin 1 g bid

8.2.3 IN - VITRO CULTURE AND SUSCEPTIBILITY TESTING

Antral gastric biopsies were collected in 0.9% isotonic saline prior to enrollment from 9 randomly selected patients, and immediately transported to the microbiology laboratory for culture and susceptibility testing after patient enrollement. The biopsies were ground in 0.5 ml of physiologic saline and plated on brucella agar containing Dent's supplement (Oxoid, Unipath Ltd, Hampshire, UK) and 7% horse or sheep blood. The plates were incubated in microaerobic atmosphere using Campy Pak system (Oxoid, Unipath Ltd, Hampshire, UK) at 37 °C for a maximum of 10 days. Colonies suspected to be *H. pylori* were identified by the presence of urease, catalase, and oxidase activities and by the API Campy commercial identification system (bio Mérieux, Cedex, France). Once identified, colonies were subcultured on brucella agar supplemented with 7% sheep blood. Determination of MICs of amoxicillin and clarithromycin against *H. pylori* by the Epsilometer test (E-test, AB Biodisk, Solna, Sweden) was done as previously described ¹²⁶.

8.3 RESULTS

Baseline comparative characteristics of the two groups were similar (Table 8.1). Eleven of 15 patients from group A and 9 of 13 patients from group B completed their respective therapies and follow-up as per protocol. There was 1 protocol violation and 3 lost to follow-up in each group. Both regimens were well tolerated and side effects were limited to diarrhea (3 and 2 in groups A and B respectively) and nausea (in one patient in group B).

H. pylori was eradicated in 4 of 11 (36%) patients in group A, and in 2 of 9 (22%) patients in group B (per-protocol analysis) [26.6% and 15.4% respectively (intent-to-treat analysis)]. In vitro susceptibility data on gastric biopsies from 9 patients showed no resistance to amoxicillin or clarithromycin. Of these patients, only 1 out of 9 had successful eradication of *H. pylori*.

8.4 DISCUSSION

Studies evaluating azithromycin-based short-term therapies (i.e. for ≤ 7 days) for *H. pylori* have yielded conflicting results. Table 8.3 summarizes the published trials to date examining azithromycin-based therapy in combination with a PPI and another antibiotic for *H. pylori* infection. Eradication rates on a per-protocol basis have been reported to reach a high of 93.3% and a low of 57-61% for practically identical regimens involving the use of azithromycin for 3 days.

The dose and duration of use of the second antibiotic (amoxicillin, metronidazole, or tinidazole) were similar in most of these studies ranging from 3-7 days (Table 8.3).

Two studies have suggested, however, that the efficacy of azithromycin-based therapy for *H. pylori* is augmented with higher doses of azithromycin ^{127, 128}.

This study was designed to address whether an ultra-short azithromycin therapy is effective against *H. pylori* in our study population. The choice and length of therapy was based on the following: (1) the prolonged tissue half-life of azithromycin thereby allowing for 2 day-dosing using 1 g daily, (2) the improved bioavailability of amoxicillin and azithromycin after potent acid inhibition and (3) the evidence of synergistic in-vitro effect of PPIs with azithromycin in the eradication of *H. pylori* ¹²⁹.

Therefore, high-dose azithromycin and amoxicillin was started on the second day of therapy taking advantage of a higher intra-gastric pH achieved after 1 day of twice daily lansoprazole therapy. Similarly, high-dose azithromycin was given for only 2 days based on high intragastric tissue concentration achieved and maintained for at least 5 days ^{111, 112}. Furthermore, in group B, amoxicillin and lansoprazole were added on day 4 and 5 to maintain dual antibiotic therapy to make use of pre-existing high levels of azithromycin achieved in gastric tissues in a low acid medium.

The limited eradication rate in our study is unlikely to be secondary to patients' non-compliance because of the short duration of therapy and the daily phone interviews with patients. Furthermore, there is no evidence to suggest that antimicrobial resistance or increased virulence may have influenced the results.

In fact, our eradication rates using standard *H. pylori* treatment regimens and our local in-vitro susceptibility studies are similar patterns to those described in developed nations (unpublished data).

There is increasing evidence, however, that ultra-short therapy, and even 7 day eradication therapy, is suboptimal in selected patient populations.^{130, 131} An additional theoretical concern in the case of azithromycin-based therapy is the possible induction of resistance to azithromycin with increasing tissue concentrations. Kalenic *et al*¹³² showed that serial in vitro passage of *H. pylori* strains with increasing azithromycin concentrations resulted in the development of resistance in 26.9% of strains. This propensity to develop resistance in a high proportion of strains may have an important impact on the choice of the right combinations of macrolides with other agents as well as the dosage of the macrolide antibiotics.

To date, there are no full proof treatment regimen for *H. pylori* and failure rates of 5-15% are still common with the currently accepted superior 10- or 14-day regimen of clarithromycin, amoxicillin, and a PPI. The intriguing fact is that, in our study, 36% of patients in group A did respond to a 3-day regimen (containing only 2 days of antibiotic therapy). The exact factors that contribute to successful eradication of *H. pylori* in a given individual using a certain drug regimen remain largely unclear. Until such factors are identified, and based on the results of our study as well as widely conflicting results from previous trials, azithromycin-based ultra-short therapy for *H. pylori* cannot be recommended.

Table 8.3 Summary of published clinical trials using combination azithromycin with a proton-pump inhibitor and amoxicillin or an imidazole (metronidazole or tinidazole) in the treatment of *Helicobacter pylori*.

Authors	Year	Treatment protocol	Patients (#)	% Eradication (per-protocol)
Marchi et al ¹¹³	1994	OAz (1 g/d x 7d)	10	90
Bertoni et al ¹¹⁴	1996	OA x 14d + Az 500 mg/d x 3d	48	91.6
Di Mario et al ¹¹⁵	1996	OM x 7d + Az 500 mg/d x 3d	69	72.3
Cammarota et al ¹¹⁶	1996	LA x 7d; Az 500 mg/d x 3d	33	61
Caselli et al ¹¹⁷	1997	M + Az (500 mg/d) x 3d; L x 7d	60	93.3
Vcev et al ¹¹⁸	1997	OMAz; Az 500 mg/d x 5d	25	72
Vcev et al ¹¹⁹	1998	OA x 10d + Az (500 mg/d x 6d)	50	85
Trevisani et al ¹²⁰	1998	LT+ Az (500 mg/d on days 2-4)	80	80.8
Trevisani et al ¹²¹	1998	LTAz (500 mg/d x 3d)	70	86.3
Vcev et al ¹²²	1999	OA + Az (500 mg x 6d)	57	82.5
Cammarotta et al ¹²³	1999	LT or PT + Az (500 mg/d) x 3d	69	82.6
Vcev et al ¹⁰⁹	2000	PA + Az (500 mg/d x 6d)	59	78
Flores et al ¹²⁴	2000	OA x 7 + Az 500 mg/d x 3d	27	57
Calabrese et al ¹²⁵	2000	PT + Az 500 mg/d days 1-3 or 4-7	100	87

A=amoxicillin; Az=azithromycin; L=lansoprazole; M=metronidazole; O=omeprazole; P=pantoprazole; T=tinidazole

9 ROLE FOR VACCINATION

H. pylori infection meets two important criteria that give it a high priority for vaccine development: the infection is widespread in both developing and developed countries, and the emergence of antibiotic resistance limiting the efficacy of combination therapy is on the rise. The elevated cost of combination therapy provides also further incentives for vaccine development.

Two types of vaccines are potentially possible: 1) a prophylactic vaccine, which prevents new infections and 2) a therapeutic vaccine, which eliminates an existing infection. A cheap and potent therapeutic vaccine, which eradicates infection and associated disease and simultaneously secures long lasting immunity, is thus highly desirable. On the other hand, caution should be considered in the development of prophylactic vaccines since in some infected individuals, the colonization with *H. pylori* might be beneficial and protective against other disease symptoms.^{133, 144}

Recent studies have suggested that *H. pylori* infection might be beneficial for the human host as it may protect from reflux esophagitis and that colonization by *CagA* positive strains may reduce the risk of developing esophageal cancer. Thus, eradicating *H. pylori* might reduce the risk of developing peptic ulcers, MALT - lymphomas and cancer of the distal stomach while increasing the risk of developing the reflux esophagitis and cancers of the esophagus and proximal stomach.

Yet, such observations require thorough confirmation in large-scale longitudinal

studies.¹³⁵ It has been shown that *H. pylori* infection elicits strong antibody and T cell response, that cannot clear the infection probably because of possible immune escape mechanisms, which the bacterium has evolved as to thrive in its host.

Understanding the nature of these mechanisms might lead to rational approaches to vaccine design. As most studies have examined donors with signs of *H. pylori* associated pathology, attention has thus focused on how T-cells and antibodies could induce this pathology. It may be argued that inducing immune responses which oppose pathological T and B-cell activity should be a major role of vaccination, as such responses could modulate the disease and also result in the elimination of the bacterium. The hypothesis that a vaccine should induce Th2 immunity suppressing pathological Th1 responses is in line with this goal. However the basis of such a hypothesis remains to be more thoroughly investigated.¹³³

It is possible that the different pathologies associated with *H. pylori* infection could be promoted by different mechanisms, and thus a vaccine opposing ulcerogenic immunity may be ineffective or even pathogenic in a way leading to atrophy or MALT - lymphoma. In addition, infected but asymptomatic donors should be investigated to allow an understanding of the immune mechanisms permitting the harmonious coexistence between the host and the bacterium. Several candidate vaccine antigens including urease, flagellin, flagellar sheath protein and *CagA*, are recognized frequently by antibodies from infected subjects. All of these antigens may be important candidate vaccine antigen but so far vaccination studies have achieved only limited success, and a vaccine shown to be successful in animal model might take several years to be proven safe and efficacious in man.¹³³

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