REVIEW OF LITERATURE

HISTORICAL REVIEW

The presence of spiral organisms in the stomach of man and animals has been known since the last century. Bizzozero saw spiral organisms in the stomach of various animals [1]. Spiral bacteria were first reported in humans by Krienitz in 1906. He isolated ‘spirochaetes’ from the stomach of a patient with gastric carcinoma [2]. In 1938, Doenges found ‘spirochaetes’ in 43% of 242 human stomachs studied postmortem [26]. Doenges drew no conclusions mainly because most of the biopsies were unsuitable for pathological diagnosis. Palmer extensively examined human gastric suction biopsies but was unable to identify ‘spirochaetes’ as he did not use special stains. He suggested that the previously recognized ‘spirochaete like’ structures in the stomach were due to contamination with oral flora [27].

The introduction of fibre-optic techniques permitted large scale sampling of the antral mucosa by biopsies. This allowed Steer and Colin-Jones in 1975 to observe Gram-negative bacilli under the mucous layer of the stomach in 80% of their patients with gastric ulcer but not in normal individuals [28]. Cultures of the endoscopic biopsy specimens in which there was granulocyte infiltration, using non-microaerophilic techniques, yielded only pseudomonas aeruginosa. Their ultrastructural photographs indicated that the bacteria were spiral. Cultured Pseudomonas aeruginosa is not a spiral organism and must therefore be considered
a contaminant. Steer and Colin-Jones did not remark on this discrepancy [28]. They, however, provided evidence suggesting that these micro-organisms were not innocent contaminants because leukocytes appeared to migrate through the epithelium in response to the bacteria under the mucus layer. Soon, the bacteria were once more forgotten.

In parallel with the history of discovery of spiral bacteria in the stomach, research had aimed at unravelling the source and significance of urease activity in animal stomachs. In 1924, Luck and Seth discovered urease in the stomach of cats and other animals [29]. It was considered that gastric urease partly neutralized luminal acid by stimulating alkali secretion or protected the mucosa as part of an intracellular defence mechanism against acid. In 1950, Fitzgerald and Murphy noted in gastrectomy specimens an association between urease production at the surface of the mucosa and peptic ulcer disease [30]. The precise source of this urease remained unknown. Later, it was shown that gastric urease was of bacterial origin. However, once again, as in the case of gastric 'spirochaetes', interest in gastric urease faded [31].

In 1980, Warren in Australia observed curved and S-shaped bacilli in 135 gastric biopsy specimens in patients with active gastritis. On light microscopy, these bacilli resembled Campylobacter jejuni [32]. Using standard Campylobacter media and non-selective media, Warren and Marshall cultured 34 antral mucosal biopsy specimens for 48 hours without success. The thirty-fifth antral biopsy was incubated
before the extended Easter holidays. This culture was examined after 5 days when there was a heavy growth of Campylobacter-like organisms [3]. These microorganisms did not resemble any other known bacterial species either morphologically or biochemically. The provisional name, Campylobacter-like organisms (CLO's), was proposed because of the morphological similarity to other members of the genus. However, studies on the chemical composition and ultrastructure showed fundamental differences between these Campylobacter-like organisms and other Campylobacters. In view of the frequency with which Campylobacter-like organisms were found in the gastric antrum, the name Campylobacter pyloridis was proposed [33]. Subsequently, based on the rules of taxonomy, the name was changed to Campylobacter pylori [34]. In October 1989, the organism was named Helicobacter, because of the presence of sheathed flagella, a unique fatty acid profile, different respiratory quinones and a different 16S RNA sequence [35,36].

The isolation of H pylori from the gastric mucosa and the report of the organism's urease activity generated excitement especially when it was postulated by Marshall that these micro-organisms could be the cause of gastritis and could be a dominant etiological factor in the pathogenesis of peptic ulcer disease [32,37]. With the isolation of H pylori, the floodgates opened to a new era of discovery and understanding of gastroduodenal pathology. We now know that H pylori is the causative agent for most cases of chronic non-specific gastritis and also that H pylori is a common finding in the asymptomatic population of developing nations. Further
research has provided an impetus to the study of this fascinating bacterium to appreciate its clinical significance in various disorders of the upper gastrointestinal tract.

**HELCOBACTER PYLORI: THE ORGANISM**

*Helicobacter pylori* is one of the species of the new genus *Helicobacter*. These organisms are curved or spiral, Gram-negative and multilflagellated, the general characteristics seen in most mucus associated intestinal bacteria. It is unipolar, measuring 0.5 to 1.0 μm in width and 2.5 to 4.0 μm in length. Four to six sheathed flagellae are attached to one pole, each 2.5 μm long and 30 nm thick.*H pylori* grows best in an atmosphere of reduced oxygen (5-15%) with added CO₂ [38]. These conditions are easily obtained in the lab by use of Campylobacter atmosphere generation kits, or CO₂ incubators [39]. The ideal temperature is 37°C rather than the higher temperature of 42°C required by the Campylobacters.

Prolonged culture gives rise to coccoidal forms which also appear after exposure to oxygen. It is assumed that these forms are indicative of a dormant state and assist the survival of the organism in an environment where conditions are not favourable for its growth. The coccoidal forms of *H pylori* are formed when the outer envelope appears to separate and ceases to keep pace with cell growth leading to folding of the inner cytoplasmic rod. The original poles of the organism become adjacent and the outer limiting membrane becomes coccoidal in shape [40]. In 1985, it was reported that the major cellular fatty acids of *H pylori* have a new profile.
The major fatty acids were tetradecanoic acid (14:0 i.e. fourteen carbon atoms and zero double bonds) and 19-carbon cyclopropane fatty acid (19:0 cyc), with a very small amount of hexadecanoic acid (16:0) [41]. In addition, *H pylori* has been found to be unique in possessing 3-hydroxy-octadecanoic acid (3-OH-18:0) [42].

**Gastric Bacteria other than Helicobacter pylori**

Since the culture of *H pylori* from the human stomach in 1982, there has been renewed interest in other bacteria that had been observed in animal stomachs as early as the late nineteenth century. Many of these bacteria have now been isolated and have been shown to belong to the same genus, Helicobacter, which currently includes nine species [43]. Bacteria of special importance include “Gastrospirillum hominis”, a distinctive tightly spiralled bacterium commonly found in cats and dogs, recently shown to be Helicobacter helmanii. This infects a small proportion of human patients and causes a mild chronic gastritis [44,45]. Helicobacter felis, a bacterium isolated from cats has been found to be associated with gastritis in one human patient [46]. It easily colonizes small laboratory animals like mice causing gastritis and thus providing a useful model of the human infection [44,46]. This model of chronic infection mimics that present in humans in that it is not transmitted between mice. Besides, it is difficult to eradicate except with triple therapy regimens. Prevention of *H.felis* infection by oral immunization of mice with urease or *H pylori* surface antigens shows that immunotherapy may have a role in preventing or even treating human infection [47].
Helicobacter mustelae, the natural inhabitant of the ferret gastric mucosa also induces a form of chronic gastritis [48]. This organism also shares important properties with *H pylori*, namely an ability to adhere firmly to gastric mucosa and an association with peptic ulceration. Investigation of these non-*H pylori* gastric bacteria in natural or experimental hosts provides useful models of *H pylori*-associated gastroduodenal disease. This makes possible assessment of potential therapeutic regimens and provides information that may result in the development of novel intervention strategies.

**EPIDEMIOLOGY**

*Helicobacter pylori* infection can be considered an infection acquired in childhood that will last most, if not all, of the individual’s life. In some subjects with associated risk factors that remain to be defined it will contribute to the development of stomach and duodenal diseases. This model of “slow infection”, was proposed by Blaser and is rather unique in the field of bacterial diseases [49]. The major risk factor for *H pylori* infection seems to be the socioeconomic status of the child’s family. In developing countries almost all children are infected by 10 years of age; in developed countries, the children of families at the lowest socioeconomic level are infected [12]. These infected children harbour infection all their life unless treated. Prevalence curves drawn from cross-sectional population studies in the past in different countries reflect the level of infection of each country at various ages [12]. This is the so called cohort phenomenon which shows, in this particular model, that the age factor that is thought to be very important is linked to socioeconomic status.
In Japan, the prevalence rate is around 80% to 90% for those aged 40 to 49 years or older, i.e. born before 1950. Economic development after this period has led to prevalence rates of 45% for those born between 1950 and 1960, 25% for those born between 1960 and 1970 and 20% for those born between 1970 and 1980 [50]. The same observations have been made in other places, such as Republic of San Marino in the Italian peninsula where the development of tourism in the 1960s improved economic conditions [51]. In developed countries, infection before 20 years is unusual. In adults the prevalence rates range from 40% to 60%. Exposure to *H pylori* occurs early in India and is widespread even in the normal population. The reported age related prevalence in the 10-19 years age group in India was 44%, between 20-29 years 55%, between 30-39 years 58%, between 40-49 years 36% and over 50 years, 33% [14].

**Transmission of *H pylori***:

*H pylori* is thought to be spread by the feco-oral route. The organism has been cultured from diarrheal stools of infected children in Gambia [52] and occasionally from infected adults in the U.K. [53]. *H pylori* has been cultured from dental plaques from Indians [54]. However, only rarely can viable organisms be found in the mouth of persons in Western countries [54,55]. This indicates that oro-oral transmission may not be an important mode of transmission in Western countries. Prevalence of *H pylori* in adults was significantly higher if there was more
than one person living per room in the family home during childhood, if a bed was shared or if there was no hot running water in the house [56].

The source of *H pylori* infection in some countries like Peru may be the water supply. Klein et al found that in order to be protected from infection, children in Peru needed to have a water supply from a private well with indoor plumbing [57]. Socioeconomic status or indoor plumbing was not protective if the children drank water from the municipal supply. Nosocomial transmission between patients undergoing endoscopy has been reported. With manual endoscope washing, such transmissions occurs in 1 to 3% of endoscopies but does not appear to occur in modern endoscopy units where endoscopes are mechanically sterilized and washed [58].

**Epidemiology of *Helicobacter pylori* in Children**

Colonization of the gastric mucosa with *H pylori* increases with age. Jones et al found that *H pylori* infection is rarely present in children under 14 years of age, whereas 50% of adults over 60 years of age have serologic evidence of infection [59]. This age related increase in colonization has been reported in many studies [60,61]. Children in developing countries are more likely to be infected than are their counterparts in developed countries [60,62]. In France, only 3.5% of children are infected in the first decade of life. In contrast, 45% of children in Algeria and 55% in the Ivory Coast are infected during the first year of life [60].
The study by Fiedorek and colleagues in Arkansas, USA, demonstrated the relationship between infection rate with *H pylori* and the socioeconomic condition of the families of investigated children [7]. A clear association between the family income and the prevalence of *H pylori* was seen. Children who came from families with a low income had a higher prevalence (50% vs 10%) than those from higher income families. Blacks were more likely to be infected compared to Whites [7].

A study from Southern China investigated the prevalence of *H pylori* using ELISA. They reported a significant positive correlation between the role of *H pylori* infection and the density of housing and age. The prevalence increased with age. By the age of 5 years, 23% of all children living in Southern China were infected with *H pylori*. In children more than 5 years of age, in both urban and rural areas, the seroprevalence rates of *H pylori* increased by approximately 1% per year [63].

Studies in children have demonstrated marked clustering of infection within families. More than 80% of the siblings of *H pylori* colonized children have serologic evidence of infection, in comparison to only 13% of age matched controls [64]. Also children of infected patients are more likely to be colonized than are those whose parents are not colonized [65]. Such clustering could be due to a common source of infection, person to person transmission or a genetic predisposition to infection.

Person to person transmission of *H pylori* can be implicated if an identical strain were isolated from members of the same family. Strains isolated from two
sets of siblings in Canada were all different when DNA restriction endonuclease digest patterns were examined [66]. This implies that the same strain is not clustered within families. However, individuals are also known to simultaneously harbour more than one strain of the organism [67].

*H pylori* is the major cause of gastritis in children and adults [68,69]. Colonization of the gastric mucosa with *H pylori* is also important in relation to the natural history of duodenal ulcer disease. Duodenal ulcers do not appear to relapse if *H pylori* is cleared from the gastric mucosa. Because of the low incidence of *H pylori* in children, a multicenter study is required to demonstrate whether the above statement is also true of children with duodenal ulcers. Future studies on children will be of importance in determining whether *H pylori* gastritis in them is a cause of specific symptoms, elucidating the epidemiology of *H pylori* infection in children and clarifying the possible role of this organism in the natural history of gastric cancer.

**DIAGNOSIS OF HELICOBACTER PYLORI**

There are many methods to detect the presence of *H pylori* infection. An ideal test for detecting *H pylori* should have (a) a high degree of sensitivity and specificity, (b) be inexpensive, (c) be easy to perform using routine equipment and techniques and (d) be minimally invasive with good patient acceptance. Though such an ideal test is yet to be evolved, the available diagnostic methods have
yielded important information regarding the natural history and epidemiology of 
\textit{H pylori} infection.

Methods for detecting \textit{H pylori} can be categorized as direct or indirect. Histologic demonstration of the organism or its identification by microbiologic means from cultured tissue constitutes direct evidence of its presence. Indirect techniques rely on detecting a property of the bacteria (e.g. the ability to hydrolyse urea) or the response of the immune system to the organism's presence (i.e. specific antibodies). Because of their high degree of specificity, one or both of the direct tests are commonly regarded as the standard with which the indirect tests are compared [70]. However, the use of this standard as a measure is limited by the fact that the sensitivity of these tests particularly culture are less than ideal.

\textbf{Histology}

\textit{H pylori} can be identified histologically in gastric mucosal biopsy specimens using several staining techniques. The characteristic histologic appearance of \textit{H pylori} is a 3.0 x 0.5 \(\mu\)m spiral rod located adjacent to the gastric epithelium. The organism is gram negative. Gram's stain can provide a rapid diagnosis when performed on smears from heavily colonized samples. The routinely used haematoxylin and eosin stains may also demonstrate \textit{H pylori}. However, the sensitivity of this technique depends to a considerable extent on the experience of the observer. The Warthin Starry stain advocated by Warren tends to focus attention on the organisms by making them more prominent. The technique is time
consuming because sections need time to develop. *H pylori* stains selectively black because the organisms get impregnated with silver and are therefore easily detectable in biopsy specimens. Silver precipitation in the yellow-stained mucus covering the mucosa may occasionally interfere with correct interpretation. Despite these limitations, there is excellent correlation between positive staining and bacterial culture [71].

Many authors have found the inexpensive Giemsa stain to be equivalent to the Warthin-Starry stain. Potters et al compared the Warthin-Starry stain with the Giemsa stain and found the latter cheaper, easier to perform and superior in detecting *H pylori* [72]. Ethidium bromide, Giemnez and Hopps-Brown stains have also been used [73]. In addition to the stain used, a second factor that influences the histologic detection of *H pylori* is the uneven distribution of the organism throughout the mucosa. Particular emphasis needs to be placed on the patchiness of Helicobacter colonization of the body and fundus, although the antrum appears to be more uniformly involved. There has been some concern that a single biopsy may lead to a false negative result. This sampling error, however, is thought to be minor, provided the biopsies are taken within 5 cm of the pylorus from the antrum [74].

Histology being a highly sensitive and specific test, has the advantage that specimens can be re-examined, used for different staining techniques and preserved to provide a permanent record. Although the requirement for upper gastrointestinal endoscopy is a disadvantage of this method, biopsies can be readily
obtained during endoscopies performed for clinical indications. Further limitations include the need for special stains and a skilled observer to read the sections.

Culture

Identification of *H pylori* by culture from the antral mucosa obtained by endoscopy is considered the most accurate method of diagnosis because it uses biochemical and morphologic characteristics for identification of *H pylori*. Unfortunately it is also more difficult. The procedure is technically demanding and time consuming. It usually takes 3 to 6 days to grow the bacteria. In addition, even in well established laboratories an isolation rate of only 70% to 90% can be achieved. In other laboratories, the yield is much less. This makes culture a highly specific test but one of only moderate sensitivity [75]. Biopsied material for culture should be kept moist in a minimal amount of saline and plated within 2 hours of retrieval. Growth is enhanced by a moist microaerophilic atmosphere at 37°C [3]. Minced or homogenized tissue is streaked on culture plates. Although blood agar and chocolate agar are commonly used, a number of other media support *H pylori* growth. The organism is identified by the presence of gram negative curved bacteria with positive urease, catalase and oxidase reactions.

As with histology, the problem of uneven distribution of the organism may contribute to false negative cultures. However, this is less of a problem with culture because very few bacteria can still yield a positive culture. Other factors that have been implicated for unsuccessful culture of *H pylori* are recent antibiotic use,
ingestion of a topical anaesthetic or simethicone during endoscopy and contamination of the biopsy forceps with other organisms or glutaraldehyde [76]. For these reasons, culture appears to add little to the sensitivity of histologic examination [74]. An important application for culture in selected settings is the determination of antibiotic susceptibility profile of treatment resistant organisms [74].

**Urease Test**

A characteristic feature of *H pylori* is the production of large quantities of urease [37,77]. Urease catalyses the degradation of urea to ammonia and bicarbonate. This reaction causes an increase in the pH of the surrounding medium detectable by a pH indicator. The presence of urease is signalled by a change of colour of the indicator. This method can be used for rapid diagnosis of *H pylori* colonization. Christensen's 2% urea broth is a standard medium for detecting urease activity [78]. A number of variations on this theme have been developed ranging from simple home made solutions of urea, water and phenol red to the commercially produced CLO test (Delta West Ltd., Canning Vale, Western Australia). This consists of a gel pellet containing urea, a bacteriostatic agent and phenol red as a pH indicator. The indicator turns from yellow to pink when urease is present. Marshall reported results after only 20 minutes of incubation in about 75% of the culture positive patients and in all specimens after 24 hours [79]. The CLO test detects only preformed urease activity. Further urease production by *H pylori* or other contaminating organisms is prevented by the bacteriostatic agent in the gel [74].
Some other modifications of the urease test permit even faster detection of *H. pylori* in gastric biopsies. A one minute endoscopy room test was reported by Arvind et al who used an unbuffered urea in water solution at pH 6.8 including two drops of 1% phenol red [80]. This medium changed from yellow to pink in more than 90% of *H. pylori* culture-positive individuals within one minute of inserting the biopsy. However, unbuffered urea solution may lead to false positive results if read later due to contaminants such as Proteus and Pseudomonas giving false-positive results.

The sensitivity and specificity of the urease test seems to be comparable to histology and culture. Moreover, urease tests are cheaper and quicker. False negative urease test results can occur in patients with widespread intestinal type metaplasia of the stomach or in patients with low bacterial density. Intestinal metaplastic epithelium, in contrast to gastric type epithelium, is not colonized by *H. pylori*. There may also be false negative results within 2-3 weeks after stopping antibacterial therapy [79]. Since biopsy specimens for histological and microbiological examination are simultaneously collected, it is unlikely that infected individuals will be missed if both tests are simultaneously used. False positive results are never seen within 4 hours of inoculation. Delayed false positive urease test results can occur if other contaminating bacteria produce urease, which after prolonged incubation may hydrolyse urea. However, if rigorously cleaned endoscopes and biopsy forceps are used and if a bacteriostatic agent is added to the urease medium, false positive tests are very unlikely [81]. The need for invasive
endoscopy is a disadvantage of this method as it is with other direct methods. However, on the basis of its excellent sensitivity and low cost compared with that of histology and culture, urease testing should be considered the endoscopic method of choice for diagnosis of *H. pylori* infection [74].

**Breath tests**

Another type of test based on *H. pylori*'s efficient hydrolysis of urea is the labelled carbon breath test. In this test, urea that has been labelled with a carbon isotope is administered orally. In infected individuals, the urea is metabolized to ammonia and labelled bicarbonate by the urease produced by the organism and the latter is excreted in the breath as labelled carbon dioxide. The amount of labelled carbon excreted can then be quantified. The original description by Graham et al. used the stable naturally occurring isotope carbon-13 [82]. Although $^{13}$C has the advantage of being non-radioactive, its measurement requires a gas isotope ratio mass spectrometer, which is not widely available. An adaptation of this method using carbon-14 has been devised which can be easily quantified using a scintillation counter [83]. Although radioactivity of $^{14}$C is a theoretic concern, the actual level of exposure appears to be minimal because of the rapid excretion of urea and CO$_2$. Nevertheless, $^{13}$C breath tests should be used in children and pregnant women and in patients in whom multiple tests are required.

A variety of modifications exist in the methodology of this test in terms of the dose of the labelled urea used, types of standard meals given at the onset of testing.
or the amount of the non-radioactive carrier added to the labelled urea [84]. The intervals of sampling and the length of the test and the method of expressing the results are also variable [84]. The duration of the study ranges from 30 to 80 minutes. Usually, either a single sample is collected at 20 minutes after ingestion or two measurements are performed at 30 and 60 minutes [85].

The accuracy of the test can be influenced by several factors. The most common reason for a false negative result is testing too soon after a course of antibiotics, bismuth salts, or omeprazole [85]. Breath tests should not be performed until at least one month after completing such a treatment. False negative findings can also arise in conditions leading to emptying of urea from the stomach or with late sampling [86]. False positive results are possible if other urease producing bacteria are present in the stomach or from urease activity of oral bacteria. The latter can be avoided by not sampling too early and by rinsing the mouth prior to the test meal. The small dose of radioactivity exposure and the cost of the test are its primary drawbacks. Otherwise, its speed, accuracy and non-invasiveness makes it appropriate for evaluation of therapy directed at eradication of the organism.

Serology

Being a chronic infection, H. pylori elicits both a local and a systemic immune response. This response has formed the basis for diagnostic efforts aimed at identification of the organism in gastric biopsies as well as attempts to non-invasively diagnose and evaluate treatment. The antibodies to H. pylori can be detected by
bacterial agglutination, complement fixation, and enzyme linked immunosorbant assays (ELISA). Of these three methods, ELISA is the technique of choice because it is simple, quick, inexpensive and sensitive [87].

In patients colonized with *H pylori*, both IgG and IgA antibodies are significantly elevated. IgM titres, however, are similar in both *H pylori* positive and negative persons, probably because the IgM seroconversion which occurs in the early phase of infection is transient and will fall to negative levels very soon. IgG and IgA serum antibodies detect *H pylori* colonization of the stomach with a fair degree of accuracy without the need for endoscopy [81]. Though measurement of antibodies of both immunoglobulin classes appears to improve sensitivity, with the use of antigenic material of higher specificity such as high-molecular-weight cell membrane associated protein, IgG ELISA alone has yielded a sensitivity and specificity of 98.7% and 100% respectively [88]. Since *H pylori* is a chronic infection that does not resolve spontaneously, elevated IgG titres indicate active infection, unless the patient is known to have been treated in the recent past i.e. less than 2 years [38]. In population based studies, serodiagnosis remains one of the methods of choice for detecting the prevalence of infection [89].

Two types of ELISA are currently available. The "rapid office test" (using commercial kits) can be performed not only on serum but also on saliva or gingival secretion making it particularly appropriate for children [90]. This is not a quantitative test and the results are expressed either as positive or negative. The
main disadvantage of this test is the high rate of false positive results. Consequently, the rapidity and sensitivity of this test can be used more advantageously for exclusion of infection, whereas positive results need to be supplemented by another confirmatory method. The “machine-read, laboratory-based serology tests” are more accurate and provide quantitative results. Though serology cannot be the sole diagnostic test used, in combination with a direct test it represents a reliable and valid method for diagnosis of *H pylori* infection [91].

Serology cannot monitor immediate effects of antibacterial therapy because of the delay between bacterial eradication and the decline in antibody levels. Cutler et al reported that a 20% fall in IgG concentration after therapy has a sensitivity of 93% for determining *H pylori* eradication at 12-21 months after *H pylori* treatment [92]. They concluded that serology is an alternative to endoscopy or urea breath tests in monitoring patients after *H pylori* treatment and suggested that serum IgG levels should not be expected to reach a seronegative range after successful *H pylori* eradication. Wang et al reported that seroconversion of IgG titres from a seropositive to seronegative state usually occurs after 1 year indicating complete eradication of *H pylori* [93]. They also suggested that fall of IgG titres or seroconversion to a seronegative state can be judged without endoscopic examination 8 months after treatment for *H pylori* eradication and rise in titres may be a warning of the possibility of relapse or reinfection with *H pylori.*
Polymerase Chain Reaction (PCR)

This is another method of direct detection of the presence of the organism but without the need to grow it [94]. Furthermore, PCR does not require the use of tissue with the highest degree of contamination such as gastric mucosa, because sources such as feces or saliva are sufficient and can replace endoscopic biopsies [95]. Polymerase chain reaction permits rapid detection of *H. pylori* from fresh gastric biopsy specimens and can also detect *H. pylori* from paraffin-wax-embedded biopsy specimens. The primer is derived from the nucleotide sequence of the urease A gene of *H. pylori* [94]. Other primers used are nucleotides derived from *H. pylori* 16S rRNA [96]. These primers are specific to all strains of *H. pylori* and are not detected in other bacterial species making PCR, therefore, highly specific. PCR is also one of the most sensitive procedures compared to rapid urease test, culture or histology. The endoscope and the biopsy forceps need extensive cleaning and disinfection before PCR to avoid “DNA contamination”.

PCR based techniques are also used for fingerprinting of the strains. The first technique to be used in this respect was restriction endonuclease DNA analysis (REA) following the work of Langenberg et al [97]. After extraction, the DNA is digested by a restriction enzyme and the DNA fragments are separated on an agarose gel. The endonuclease commonly used is Hind III but Hae III can also give satisfactory results. Ribotyping with Hae III and Hind III provides a reliable, reproducible and discriminatory basis for distinguishing one strain from the other.
Other techniques are in the process of development. They include analysis of PCR products, arbitrary primer PCR and the analysis of DNA fragments by pulse field gel electrophoresis [94,96]. Table R1 shows the tests for detection of \textit{H pylori} (next page).

**PATHOPHYSIOLOGY OF \textit{HELICOBACTER PYLORI} INFECTION**

\textit{Helicobacter pylori} infection is almost always associated with an inflammatory response. However, peptic ulcer disease and gastric carcinoma occur only in a subset of individuals chronically infected with \textit{H pylori}. Presumably, both bacterial and host factors contribute to this differential response. The role of \textit{H pylori} as a gastric pathogen is dependent on virulence factors and pathogenic mechanisms. Virulence factors are those that allow \textit{H pylori} to survive in the hostile environment of the gastric lumen. These include its spiral shape, motility, adaptive enzymes, proteins and ability to adhere to gastric mucosal cells and mucus [98]. Pathogenic mechanisms are those that lead either directly to disruption of the gastric mucosal barrier including toxins and mediators of inflammation, or those that contribute to gastric acid peptic activity [98].

**Virulence and Pathogenicity**

The spiral shape and flagella of the organism allows efficient motility in the mucus and in the gastric juice [99]. The enzyme urease by breaking down urea in the gastric juice appears to generate enough bicarbonate and ammonium ions around the organism to allow its safe passage through the gastric acid barrier to
TABLE R1. TESTS FOR DETECTION OF HELICOBACTER PYLORI

<table>
<thead>
<tr>
<th>TEST</th>
<th>SPECIMEN</th>
<th>SENSITIVITY (%)</th>
<th>SPECIFICITY (%)</th>
<th>NEED FOR ENDOSCOPY</th>
<th>RELATIVE COST</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histology (Giemsa stain)</td>
<td>Two mucosal biopsies</td>
<td>93-99</td>
<td>95-99</td>
<td>Yes</td>
<td>+</td>
<td>Simple. Very accurate Specimen can be re-examined in equivocal cases. Permanent record.</td>
</tr>
<tr>
<td>Culture</td>
<td>One mucosal biopsy</td>
<td>72-92</td>
<td>100</td>
<td>Yes</td>
<td>+++</td>
<td>Technically demanding. Antibiotic sensitivity information may guide Therapy</td>
</tr>
<tr>
<td>Urease test</td>
<td>Two mucosal biopsies</td>
<td>89-98</td>
<td>93-98</td>
<td>Yes</td>
<td>+</td>
<td>Endoscopic method of choice for diagnosis</td>
</tr>
<tr>
<td>Urea breath test</td>
<td>Breath Sample</td>
<td>95-98</td>
<td>95-98</td>
<td>No</td>
<td>++</td>
<td>$^{13}$C test has no radiation but costs more. $^{13}$C is less expensive and simpler. Small radiation exposure</td>
</tr>
<tr>
<td>Serology ELISA</td>
<td>Serum</td>
<td>88-99</td>
<td>86-95</td>
<td>No</td>
<td>+</td>
<td>Provides titres so that fall in antibody can be assessed. Patients with rapid fall are cured</td>
</tr>
<tr>
<td>Rapid office test</td>
<td>Serum</td>
<td>95</td>
<td>85</td>
<td>No</td>
<td>+</td>
<td>Performed in 10min Qualitative positive/ Negative result</td>
</tr>
<tr>
<td>Polymerase chain reaction</td>
<td>Stool, gastric juice, Biopsy of the Stomach</td>
<td>95</td>
<td>95</td>
<td>No if biopsy</td>
<td>++</td>
<td>False positive reactions limit use as gold standard</td>
</tr>
</tbody>
</table>

reach the protective mucus layer [100]. The pathogenic effects of ammonia has been documented in many studies. Ammonia elevates the pH of the gastric mucous layer from about 6 to 7 [101]. It is known to deplete aerobic cells of alpha ketoglutarate, an essential substrate for the tricarboxylic acid cycle [38]. Ammonia in high concentration induces vacuoles exactly the same as those seen when cells are exposed to the Vac A toxin of *H pylori* [102]. Once within the gastric mucus, *H pylori* is able to attach to phospholipids such as phosphatidyl ethanolamine, sialated glycoproteins such as ganglioside GM3 and Lewis B antigens present in persons with blood group O [103-105]. Once attached to the mucus layer and the mucosa, *H pylori* secretes soluble proteases and phospholipase which may be harmful to both the integrity of the mucus layer and the underlying cells [38]. The "wettability" of gastric mucus is increased when *H pylori* is present perhaps due to partial lysis of the phospholipid component [106].

One of the most important aspects of *H pylori* pathogenicity is the "vacuolating cytotoxin". This 87 kDa protein is expressed in about 65% of *H pylori* strains and is responsible for creating vacuoles in the epithelial cells [107]. Nearly all patients with *H pylori* associated duodenal ulcer yield an isolate that produces vacuolating cytotoxin [108]. The gene for the cytotoxin protein is called Vac A and has been cloned by Cover et al [108]. The Vac A gene is present in all *H pylori* bacteria but only produces an active protein in 65% of the isolates. A second protein at 127 kDa is called cytotoxin associated gene A, or Cag A. Cag A is a
marker for the vacuolating toxin effect and the gene for Cag A is only present when Vac A cytotoxin effect is present. The organisms have been classified into type I organisms which have Cag A and Vac A which are more ulcerogenic and type II organisms that lack Cag A and do not produce cytotoxins [107,108]. Antibodies to the toxin are present in nearly all duodenal ulcer patients.

The host reaction to \textit{H. pylori} may be an important cause of mucosal incompetence because large numbers of neutrophils and lymphocytes are attracted to the bacterium. The attraction is related to the presence of chemotactic proteins that are liberated by \textit{H. pylori} [109]. Mononuclear cells release interleukins, tumor necrosis factor and oxygen free radicals. Neutrophils also liberate oxygen free radicals in response to the bacterium. The link between Vac A toxin and duodenal ulceration is the intense neutrophil reaction with subsequent increased tissue damage and ulceration. The inflammatory response produced by the organism does not result in its eradication. This may be because the bacterium produces superoxide dismutase (SOD) and catalase, which protects it from being killed in the neutrophil phagocytic vacuoles. SOD converts superoxide into hydrogen peroxide and catalase then breaks down the H$_2$O$_2$ into oxygen and water interrupting the normal chain of events [38].

The chronic inflammation seen in the lamina propria of infected patients stimulates the secretion of IgG and IgA antibodies. The level of both antibodies in serum and saliva have been used for diagnosis.
Goodwin had proposed that *H pylori* contributes to gastroduodenal injury by impairing local mucosal defence mechanisms. This was described as the "leaking roof hypothesis"[110]. The intact gastric mucosal barrier formed by the epithelial cells and the mucin gel represents the "roof" that protects the underlying submucosal tissue from gastric acid ("rain"). Decreased mucosal integrity ("leaking roof") predisposes to back diffusion of H⁺ ions ("puddle formation") leading to submucosal injury and ulcer formation [110]. The two major mechanisms leading to decreased mucosal integrity include elaboration of toxins by *H pylori* and induction of mucosal inflammation [98].

Levi and associates initially reported that individuals with duodenal ulcer and antral *H pylori* infection had significantly higher basal and meal stimulated plasma gastrin concentrations and higher peak, but not basal acid output than did individuals with duodenal ulcer not infected with *H pylori* [111]. In their view ammonia produced from hydrolysis of urea by *H pylori* urease increases the pH of the mucus layer overlying the gastric epithelium thus interfering with the normal feedback inhibition of gastrin release by intraluminal acid. Increased gastrin levels induce increased gastric acid secretion either directly by stimulating the parietal cells or indirectly by exerting a trophic effect on the parietal cell mass or both. The resultant increase in acid secretion promotes duodenal ulceration. Eradication of *H pylori* abolishes the hypergastrinemia suggesting that this is due to *H pylori* infection [112].
Even though patients with duodenal ulcer secrete more acid, Chandrakumaran et al reported that maximal gastric secretion in response to histamine was decreased in patients with duodenal ulcer and *H pylori* infection. He suggested, therefore, that induction of duodenal ulceration by the organism may not be through increasing maximal gastric secretion [113].

The distribution of *H pylori* in the stomach can predict which patients with infection would develop duodenal ulcer. Though organisms are seen in the body of the stomach in patients with duodenal ulcer, they are seen in lesser number than the pyloric antrum [114,115]. It is likely that the higher the acid output, the more the infection and the resultant inflammation would be concentrated to the antrum. This is because, *H pylori* induced hypergastrinemia would increase acid output and consequently the mucosa of the body of the stomach would become increasingly hostile to *H pylori* infection. Conversely individuals with low acid output and *H pylori* infection would lack the protection of the body mucosa afforded by acid and would develop pan gastritis. This over a period would lead to glandular atrophy and further decline in acid output (115). These subjects are highly unlikely to develop duodenal ulcer but are at increased risk of developing gastric ulcer and gastric cancer. The majority of infected individuals will occupy an intermediate position with normal or moderately elevated acid output. They will exhibit lesser degree of antral predominance and have a low risk for duodenal ulceration. Such individuals may never manifest clinical diseases associated with *H pylori* infection (114).
Somatostatin deficiency is seen in the gastric antrum in patients infected with *H pylori* [112]. Although these patients had hypergastrinemia, acid output was not increased [51]. Subsequently, it was discovered that immuno-reactive somatostatin, D cells, and somatostatin message were all decreased in patients with gastritis [112]. This abnormality was related more to the inflammation than to the actual presence of *H pylori* [38]. A proposed mechanism integrating the effect of acid and *H pylori* in the development of duodenal ulcer is presented in Fig.R1.

Fig.R1. *H pylori* and duodenal ulcer: a proposed pathogenic mechanism

D cells = Somatostatin secreting cells
G cells = Gastrin secretin cells
As a result of either genetic predisposition or an alteration in G-cell or D-cell function due to \textit{H pylori} infection, some patients will develop an increased parietal mass. The increased parietal mass results in an increased acid load that leads, in some patients to gastric metaplasia in the duodenum. \textit{H pylori} associated antral gastritis appears to be pre-requisite for colonization of areas of duodenal metaplasia and the appearance of duodenitis and duodenal ulceration [113]. El Omar et al reported a 6-fold increase in basal acid secretion in duodenal ulcer patients which fell to normal 6 months after \textit{H pylori} eradication [116]. These factors provide a link between \textit{H pylori}, gastritis, acid hypersecretion and peptic ulceration.

\textbf{\textit{H pylori} and Pathogenesis of Gastric Cancer}

It is now certain that at the least, \textit{H pylori} infection is a marker for an increased risk of gastric adenocarcinoma. The link is strong for the intestinal type of gastric carcinoma. Several potential mechanisms of gastric carcinogenesis by \textit{H pylori} have been postulated [117]. These are;

1. Metabolic products of the organisms which directly transform the mucosa.
2. Analogous to carcinogenesis by viral pathogens, \textit{H pylori} DNA is incorporated in the host cells causing transformation.
3. \textit{H pylori} induces an inflammatory response that by itself is genotoxic.
The current hypothesis is that chronic inflammation selects nongastric (intestinal) type epithelium for progress of the inflammatory response in the stomach [38]. As intestinal type of mucosa replaces the functioning parietal cell mucosa, acid secretion falls and commensal bacteria intermittently colonize the stomach. These other bacteria may reduce nitrate to nitrite and further promote the formation of carcinogenic nitrosamines [118]. Another mechanism suggests that the chronic inflammatory cells generate superoxide and nitric oxide, which can form both reactive oxygen species and nitrosamine with subsequent carcinogenic effects [119]. The carcinogenic effect of *H pylori* infection is modulated by dietary and perhaps other environmental factors. Sobala et al have documented a fall in gastric vitamin C levels in *H pylori* infected patients. Vitamin C is an antioxidant and prevents the formation of nitrosamines in the stomach [120].

A model for gastric carcinogenesis associated with *H pylori* is shown in Fig.R2.

![Fig. R2. Model for Gastric Carcinogenesis by *H pylori*](image-url)
*H. pylori* causes cell proliferation. It thus increases the risk for DNA damage by replication error, endogenous inflammation related mutagens and exogenous dietary mutagens [117]. Most of the DNA damage is corrected by the body’s normal protective mechanisms, but the capacity to survey and repair damage is less than perfect. Hence, some DNA damage accumulates with time. The longer the duration of infection, the higher the likelihood of inadequate repair and malignant transformation. People infected with *H. pylori* at a younger age who generate a marked inflammatory response would be at risk of cancer [117]. If these people consumed diets high in potential carcinogens and low in antioxidants, their risk would be compounded [117].

**ROLE OF *HELCOBACTER PYLORI* IN UPPER GASTROINTESTINAL DISORDERS**

**Duodenal Ulcer**

*Helicobacter pylori* is undoubtedly the dominant factor in the multifactorial etiology of peptic ulcer disease. A link between *H. pylori* infection and duodenal ulcer disease is now clearly established. We should not ignore the other contributing factors but rather try to identify how they interact with the organism and initiate the ulcerative process. The interplay of acid attack and mucosal defence is modulated by genetics, gender, blood group, smoking, age and various physiologic considerations, including acid output [121]. These and other considerations explain the discrepancy between the high frequency of *H. pylori* infection in the population and a less than 10% overall lifetime prevalence of duodenal ulcer disease [121].
The prevalence of H pylori infection in duodenal ulcer has consistently been found to be between 90% and 100% [122-124]. The almost universal occurrence of H pylori infection in duodenal ulcer should be viewed against age-adjusted prevalence in that specific population. Most agents used for treatment of duodenal ulcer are aimed at reducing acid secretion and promote healing by minimizing acid attack. Such treatments, however have no effect on the H pylori status and do not correct the underlying state of gastroduodenitis. The mucosa, therefore, remains abnormal and vulnerable. After cessation of acid suppressive therapy ulcer relapse is likely. Goodwin had likened the inflammed mucosa to a leaking roof, in which temporary dryness (healing) is assured if the rain (acid) stops but permanent protection can be achieved only by mending the roof by complete healing of the mucosa [110]. Therefore, therapy that fails to address the role of H pylori in the causation of the mucosal inflammation which predisposes to ulceration is likely to confer only short-term benefit.

Eradication of infection has been shown beyond doubt to markedly alter the natural history of duodenal ulcer disease. A number of series have shown either low or no recurrence of ulcer at the end of one year compared with a natural recurrence rate of more than 70% [125,126]. Several studies have demonstrated (Table R2 – next page) that ulcers recur in only a small percentage of patients following
<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Ref. No.</th>
<th>Number</th>
<th>Follow-up (months)</th>
<th>H. pylori +ve</th>
<th>H. pylori -ve</th>
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<tr>
<td>Coglan et al[91A]</td>
<td>1987</td>
<td>127</td>
<td>39</td>
<td>12</td>
<td>22/29(76%)</td>
<td>1/10(10%)</td>
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<tr>
<td>Marshall et al[91]</td>
<td>1988</td>
<td>126</td>
<td>70</td>
<td>12</td>
<td>38/47(81%)</td>
<td>5/23(22%)</td>
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<tr>
<td>Borody et al[91B]</td>
<td>1988</td>
<td>128</td>
<td>21</td>
<td>12-15</td>
<td>3/3(100%)</td>
<td>0/18(0%)</td>
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<tr>
<td>George et al[91C]</td>
<td>1990</td>
<td>129</td>
<td>62</td>
<td>12-48</td>
<td>-</td>
<td>0/62(0%)</td>
</tr>
<tr>
<td>Patchett et al[91D]</td>
<td>1990</td>
<td>130</td>
<td>51</td>
<td>12</td>
<td>5/18(28%)</td>
<td>0/33(0%)</td>
</tr>
<tr>
<td>Logan et al[91E]</td>
<td>1991</td>
<td>131</td>
<td>20</td>
<td>9</td>
<td>12/17(71%)</td>
<td>0/3(0%)</td>
</tr>
<tr>
<td>Fiocca et al[91F]</td>
<td>1991</td>
<td>132</td>
<td>144</td>
<td>6</td>
<td>55/114(48%)</td>
<td>3/30(10%)</td>
</tr>
<tr>
<td>Mannes et al[91G]</td>
<td>1993</td>
<td>133</td>
<td>178</td>
<td>12</td>
<td>42%</td>
<td>6%</td>
</tr>
</tbody>
</table>
successful \textit{H pylori} eradication in comparison to a recurrence rate of 50\% or greater within the course of one year when the organism persists [126-133]. The economic savings after eradication or even suppression of \textit{H pylori} in duodenal ulcer disease is estimated to be enormous [134]. However, eradication has proved to be difficult and reinfection rates are high in developing countries. Recrudescence or reinfection with \textit{H pylori} is common and may be an important factor in recurrence of peptic ulcer disease since successful eradication virtually abolishes recurrence of duodenal ulcer [135]. There is, therefore, a compelling need for improvement in therapy directed against \textit{H pylori}. Once such an optimal therapy is available, large scale eradication studies with long term follow up will become possible. Such studies are essential to discover the effects of eradication of the organism and histologic normalization of duodenal gastric metaplasia.

\textbf{\textit{Helicobacter pylori} and Complicated Duodenal Ulcer Disease}

The major complications of duodenal ulcer disease are bleeding, perforation and gastric outlet obstruction.

\textbf{Bleeding}

Bleeding is by far the most frequent complication of chronic duodenal ulcer disease. 30\% of bleeding ulcers bleed massively [136]. Among patients who present with a bleeding ulcer, approximately one-third will have recurrent bleeding in the following 1-2 years if left untreated by definitive therapy after achieving initial
ulcer healing [16]. The prevalence of *H pylori* in bleeding duodenal ulcer is high. Many studies report a prevalence rate of up to 100% which is comparable to the colonization rate seen in uncomplicated duodenal ulcer [137-139]. Kadayifei reported a higher prevalence rate of *H pylori* infection in patients with bleeding duodenal ulcer (88%) compared to patients with uncomplicated duodenal ulcer (67.2%) [137]. He concluded that *H pylori* eradication therapy is indicated for all patients with *H pylori* positive duodenal ulcer to prevent recurrent bleeding. In a prospective study of seventy consecutive patients with acute bleeding peptic ulcer Henriksson et al noted that 85% of patients with bleeding duodenal ulcer have antibodies to *H pylori* as determined by the ELISA test [138]. They concluded thus that *H pylori* infection plays a major aetiological role in patients with acute bleeding peptic ulcer.

Rokkas et al reported thirty one patients with bleeding duodenal ulcer who were positive for *H pylori* infection [139]. After achieving healing in all patients by administration of 20 mg of omeprazole daily for four weeks, patients were randomized to receive omeprazole 20 mg b.i.d. alone (Group O) or the combination of omeprazole 20 mg b.i.d. plus amoxicillin 500 mg q.i.d. (Group O+A) for 2 weeks. Endoscopy was performed at 4 weeks after the treatment ended to check for eradication of *H pylori* and again when rebleeding or symptomatic relapse occurred. Eradication was achieved in 13.3% in patients in Group O and in 81.3% in Group O+A. Five patients rebled during followup and all of them belonged to Group O and were patients in whom eradication had failed [139].
Labenz et al reported the rebleeding rate from bleeding duodenal ulcers at 17 months following eradication to be zero, whereas in patients with persistent *H pylori* infection the rebleeding rate was 37% [17]. A number of other studies too have shown that recurrence of bleeding from duodenal ulcer is virtually abolished if patients receive *H pylori* eradication therapy [138,139]. Santander et al feel that antimicrobial therapy for *H pylori* reduces recurrence of peptic ulcer and of recurrent hemorrhage from the ulcer more effectively than long-term maintenance antisecretory therapy [140]. Even though there is growing evidence that successful eradication of *H pylori* reduces recurrent duodenal ulcer bleeding, more data with double blind, randomised clinical studies with longer follow up are needed to clearly elucidate the role of *H pylori* in bleeding peptic ulcers.

Gastric Outlet Obstruction

Long standing duodenal ulcers on healing may lead to cicatricial stenosis of the lumen of the pyloric channel and the duodenum besides altering intragastric milieu. Gastric emptying, which is modulated by mechanical, chemical and neurohormonal mechanisms is impaired in patients with gastric outlet obstruction [141]. Apart from conventional surgery for this complication of duodenal ulcer, some authors have advocated endoscopic balloon dilatation and *H pylori* eradication for this group [18,142]. Boer et al successfully treated two patients with pinpoint pyloric stenosis by quadruple therapy aimed at eradication of *H pylori* [18]. They recommended this modality for all patients with gastric outlet obstruction who test
positive for *H pylori* infection instead of surgery or endoscopic dilatation [18]. However, it is unlikely that firm intramural fibrosis would respond to antimicrobial therapy. Endoscopic balloon dilatation followed by eradication therapy for *H pylori* has also been reported [143]. This may be possible only in a subset of patients in whom obstruction is primarily due to spasm and/or oedema due to an active ulcer rather than fibrotic stenosis.

**Perforation**

There are two components of the surgical treatment of perforated duodenal ulcer. Immediate surgery with oversewing of the perforation and omental patch repair has been proven beyond doubt to be safe and effective [144]. The mortality rate is less than 1 percent in the absence of associated major medical illness and in patients with perforations of less than 24 hour duration [145]. The second component relates to the management of the ulcer diathesis. Bomman et al reported an overall recurrence rate of 42 percent with simple closure alone [146]. These rates are higher in patients with chronic rather than acute ulcers. Illingworth et al in 1946 in a meticulous long term follow up study of 733 patients who had a simple closure of a duodenal perforation found that more than half the patients had relapse of ulcer disease within 5 years of perforation [147]. Ananthakrishnan and Angami had an endoscopic recurrence rate of 42.2% after simple closure with the majority of patients remaining asymptomatic [148].
Since recurrence is unacceptably high following simple closure, it is necessary to identify whether there are any predictors for recurrence. The difficulty in achieving this has arisen largely because of the inability to select, at the time of primary laparotomy, those patients who require definitive surgery as opposed to others in whom a simple closure would suffice. The distinction between acute and chronic ulcer based upon pre-perforation duration of dyspepsia is poor. Selection on the basis of preceding history or whether the ulcer is judged acute or chronic by the surgeon at laparotomy has failed to identify patients who would require definitive surgery [149]. Operative evidence of chronicity is frequently difficult to recognize due to oedema around the ulcer. Besides, definitive surgery has the disadvantage that in the long term, it might cause side-effects which are troublesome especially in those patients who might otherwise have been cured by simple closure alone [20].

The poor results of simple closure and the problems of definitive surgery have led to the use of histamine-2 (H₂) receptor blockers after simple closure of perforation. Sevvel et al in a prospective study of 100 patients with perforated duodenal ulcers treated by simple closure, found that the endoscopic rate of persistent or recurrent ulcer at 6 months was 33% and 30% for the ranitidine and placebo group respectively. Ranitidine, therefore, did not appear to promote healing or prevent recurrence of a perforated duodenal ulcer after simple closure [20].

Attention has recently been focussed on the role of H pylori in perforated duodenal ulcer. Little is known at present, about the significance of H pylori
infection in perforated peptic ulcer and in the subsequent relapse or persistence of ulceration. Jensen et al reported a prevalence rate of *H pylori* of 48% in patients with acute perforated duodenal ulcer [23]. The corresponding prevalence rate in controls was not reported. Sebastian et al reported *H pylori* in 24 out of 29 patients with perforated peptic ulcer tested by the $^{13}$C-urea breath test (UBT) done on the eighth postoperative day [24]. Per-operative samples were also taken from the duodenal mucosa and from the pyloric antrum with a flexible choledochoscope threaded blindly through the perforation. Duodenal mucosal biopsy specimens were obtained in 25 patients and six of these were positive for *H pylori* by the urease test. Antral mucosal biopsy samples were available from only nine out of 25 patients because of practical difficulties. Five of these nine patients had a positive urease test. At six weeks followup all patients who had persistent duodenal ulcers were positive for *H pylori*. The authors, therefore, recommended eradication of *H pylori* in all patients with perforated peptic ulcer associated with *H pylori* infection [24].

In contrast, Reinbach et al in a study of the prevalence of *H pylori* in 80 patients with acute perforated duodenal ulcer by IgG serology, reported that 47% of perforated duodenal ulcer patients were positive for *H pylori*. This was similar to the 51% seen in controls [25]. They concluded that this similar rate of infection in patients with perforated duodenal ulcer and controls suggests that the pathogenesis of perforated duodenal ulcer is different from chronic duodenal ulcer [25]. Ozmen et al recommended that patients with perforated ulcers after surgery should have their *H pylori* status checked by using either serology for anti-*H pylori* IgG or $^{13}$C-UBT.
UBT should be done 4 weeks after completion of therapy. Serial serology titres should be done 6 monthly to detect fall in titres. All patients with *H pylori* infection should be treated by eradication therapy as it speeds up healing and decreases the relapse rate of ulcer disease [150].

**Gastric Ulcer**

The relationship between *H pylori* and gastric ulcer is less clear than with duodenal ulcer. It has been shown that gastric ulcer is associated with *H pylori* in over 70% of patients. If drug induced ulcers are excluded, the prevalence of *H pylori* in gastric ulcer approaches 96% [124,151]. Chronic gastritis, which generally accompanies gastric ulceration, is characteristically a diffuse pan-gastritis and exhibits both multifocal glandular atrophy and intestinal metaplasia. *H pylori* cytotoxins, high ammonia concentrations, formation of cross-reacting antibodies and liberation of neutrophil proteases and toxic oxygen radicals have all been suggested as contributors to mucosal atrophy [152].

As in duodenal ulcer, a weakening in the mucosal defences may render the gastric wall susceptible to acid-peptic attack. It has been shown that eradication of *H pylori* speeds gastric ulcer healing with a six week healing rate of about 85%. Refractory ulcers heal within 6-10 weeks after eradication of *H pylori* [151]. Eradication also diminishes the recurrence rate of gastric ulcer indicating that *H pylori* plays a major etiological role in gastric ulcer disease [153]. Bayerdorffer et al found a relapse rate of 33% in *H pylori* positive patients as opposed to 3% in the
H pylori negative group [154]. Other studies also showed a significant decline in the recurrence rate of gastric ulcers with H pylori therapy [155,156].

Non-Ulcer Dyspepsia (NUD)

Non-ulcer dyspepsia, also termed functional dyspepsia, is defined as a chronic or recurrent abdominal pain or discomfort centered in the upper abdomen lasting for more than 1 month with symptoms for 25% or more of the time in subjects who have no other organic disease likely to explain symptoms as evidenced by clinical examination or radiological, biochemical and endoscopic investigations [157].

Data in literature is conflicting on whether NUD is associated with gastritis or H pylori infection. Mechanisms by which gastritis and H pylori infection could result in symptoms of dyspepsia are unclear. However, there is some evidence that inflammatory changes of the gastric mucosa result in motor derangement and gastric emptying abnormalities which may cause upper gastrointestinal tract symptoms [158]. The other evidence to support the role of H pylori in NUD is the higher prevalence of H pylori seen in subjects with NUD than in the control population in countries with low seroprevalence [159,160]. There are also reports of clinical improvement and corresponding mucosal restoration in patients after eradication of the bacterium [157].

H pylori is found in 43% to 87% of subjects with NUD [159-162]. The wide variation in occurrence of H pylori in patients with NUD reflects both differences in
the criteria used to diagnose NUD and also the difference in populations evaluated. Few studies have compared *H. pylori* prevalence in NUD subjects with an age, sex and ethnically matched representative population. In developed countries, the prevalence of *H. pylori* infection in patients with NUD is consistently higher than that in controls [159-162]. In children with chronic abdominal pain, a higher rate of *H. pylori* infection was seen than in controls [163]. An *H. pylori* prevalence rate of 44 to 76% has been reported from India in patients with NUD [164-167]. Joshi et al found a lower prevalence in Western India compared to studies from Southern India [167].

Several authors have studied the effect of *H. pylori* suppression or eradication on dyspeptic symptoms. Some studies have shown symptom improvement although the type of symptom ameliorated has been variable and the results are often conflicting [168,169]. Sheu found improvement in symptoms one year after eradication of *H. pylori* in NUD [170]. A similar result was found by McCarthy [171] and Borody [172]. A review of 89 studies on patients with NUD and *H. pylori* infection found symptomatic improvement in 73% of those following eradication compared to only 45% in those with persistent *H. pylori* infection. This suggests a beneficial role for *H. pylori* eradication in NUD [173]. Balamourougane found that in nearly two thirds of patients in whom *H. pylori* was eradicated there was good symptomatic improvement [174]. This, however, declined to only 31.6% at 6 months as a result of regrowth of *H. pylori*. On the contrary, other reports have shown that eradication of *H. pylori* with subsequent resolution of gastritis does not alleviate
dyspeptic symptoms [175,176]. These conflicting reports, therefore, mandates further clinical trials to determine the role of \textit{H pylori} in non-ulcer dyspepsia.

\textbf{Gastritis}

\textit{Helicobacter pylori} is the most common cause of non-specific gastritis, also called chronic gastritis. Two major forms of gastritis are recognized; Type A gastritis, which involves the fundus, is associated with pernicious anaemia, antibodies to parietal cells and other autoimmune conditions. Type B gastritis which is much more common appears to mainly affect the antrum. Autoimmune phenomena are absent and it has so far been considered to be idiopathic. It is the latter which is associated with \textit{H pylori} infection [177]. Though endoscopic examination may show congestion of the mucosa in patients with antral gastritis, essentially the diagnosis is histologic. Prevalence of \textit{H pylori} in subjects with type B antral gastritis approaches 100%.

While the prevalence of \textit{H pylori} in patients with specific types of gastritis, such as that related to alcohol or nonsteroidal anti-inflammatory drug (NSAID) abuse is low, the high prevalence in type B gastritis lends credence to the association of \textit{H pylori} with this condition [177]. There are, in addition other evidences to support the hypothesis that \textit{H pylori} causes antral gastritis. First, acute inflammatory gastritis was seen to develop in volunteers after ingestion of \textit{H pylori} [178,179]. Secondly, animal studies using a gnotobiotic pig model resulted in chronic inflammation and a
histologic picture similar to human chronic active gastritis [180]. Finally, the gastritis resolves after successful treatment of \textit{H pylori}.

The prevalence of \textit{H pylori} in chronic gastritis ranges from 48 to 100% [32,167,181-183]. Its prevalence is not high in other types of gastritis. \textit{H pylori} is not found in atrophic gastritis accompanied by intestinal metaplasia. Balan et al reported a low prevalence (40%) of \textit{H pylori} in patients with portal hypertensive gastropathy whereas ninety percent of patients with chronic active gastritis were infected with \textit{H pylori} in this study [184]. There are several studies on \textit{H pylori} in postoperative bile gastritis. O'Connor et al retrospectively studied a group of 35 patients with active duodenal ulcers and 54 patients who had undergone surgical treatment for duodenal ulcers [185]. Patients with active duodenal ulcers and those treated with highly selective vagotomy had an \textit{H pylori} prevalences of 97% and 94% respectively, whereas patients with Billroth I or Billroth II partial gastrectomies or truncal vagotomies and gastroenterostomy had a significantly lower prevalence [185]. After a gastroenterostomy or a partial gastrectomy patients develop a specific histologic type of gastritis in which the characteristic finding is foveolar hyperplasia. This is attributed to reflux of bile or other duodenal contents [186]. It is believed that this reflux decreases the frequency of \textit{H pylori} infection by creating a hostile environment [185,187].

The prevalence of \textit{H pylori} infection in patients taking NSAIDs has been reported to range from 22% to 63% [188,189]. Most studies show no difference in
*H. pylori* occurrence in NSAID users and nonusers [189]. Although NSAIDs and alcohol cause gross gastric injury they do not produce an inflammatory cell infiltration of the gastric mucosa. If there are histological features of gastritis in NSAIDs or alcohol abusers this is probably related to the presence of *H. pylori* and not due to the drug [189]. Kulkami et al studied the frequency of occurrence and the effect of *H. pylori* on the gastroduodenal mucosa in patients on long term NSAID use [190]. In a group of 65 patients more than 6 months on NSAID therapy, *H. pylori* infection did not increase the risk of gastroduodenal damage (52% vs 45%). They concluded that patients on long-term NSAIDs are not at increased risk of *H. pylori* infection and presence of *H. pylori* infection in patients on NSAIDs is not associated with an increased risk of gastroduodenal damage [190].

In specific types of gastritis, such as that seen in Crohn's disease, studies suggest that *H. pylori* prevalence is either decreased or not increased [191]. Lymphocytic gastritis is a newly recognized type of non-erosive nonspecific gastritis associated with chronic erosive gastritis, celiac disease and Menetrier's disease [192-194]. *H. pylori* prevalence is not increased in this condition [194].

**Gastric Cancer**

The evidence in support for a role for *H. pylori* in gastric cancer comes from three sources; i) studies comparing the incidence of gastric cancer with that of *H. pylori* infection, ii) cross-sectional studies of *H. pylori* infection in patients with cancer and iii) prospective studies of patients with *H. pylori* infection [195].
i) Geographic studies of *H pylori* prevalence and gastric cancer incidence reveal striking epidemiologic parallels between the two. Internationally, *H pylori* infection like gastric cancer of the intestinal type, has foci of high prevalence usually in countries with lower socioeconomic development [60]. In regions of Peru, Mexico, and Columbia where gastric cancer rates reach almost epidemic proportions, virtually all adults are infected with this organism [117,195].

ii) Cross sectional studies show that the rate of infection with *H pylori* ranges between 50% to 100% in people with adenocarcinoma of the stomach [196-198]. In the Netherlands, 61% of patients with gastric cancer were found on biopsy or gastric resection to be infected compared to 34% of age-matched blood donor controls. This difference was statistically significant [198]. Talley et al used an ELISA test to compare *H pylori* prevalence rates in patients with gastric cancer with rates in cancer-free controls. Patients with adenocarcinomas of the antrum, body and fundus had a significantly higher prevalence of *H pylori* infection when compared to healthy controls [199].

Reports from India are conflicting. Prabhu et al in a study from Western India reported a *H pylori* prevalence rate of 38% in patients with gastric carcinoma [166]. They concluded that although *H pylori* infection and chronic atrophic gastritis are common in Indians, the incidence of intestinal metaplasia is low thus raising a doubt on the role of *H pylori* in gastric carcinogenesis in India. On the other hand,
Sivaprakash et al in a study from South India, reported the prevalence of *H pylori* infection as determined by the urease tests, ELISA and culture to be between 56.0% to 62.6% in patients with gastric carcinoma and only 37.3% to 46.6% in controls [200]. This difference was statistically significant suggesting an association of *H pylori* infection with gastric carcinoma.

Lin et al reported an *H pylori* seroprevalence rate of 60.3% and 58.8% in gastric adenocarcinomas and healthy volunteers respectively [201]. Although some of these cross-sectional studies show a higher prevalence of *H pylori* in cancer that exceeds the prevalence in the normal populations, they do not establish temporal sequence links between infection and cancer and can at best be termed circumstantial rather than causal evidence [195].

iii) Three nested case control studies in well-defined, different populations have shown remarkably consistent evidence that *H pylori* infection increases the risk for later development of gastric malignancy [202-204]. The study populations from Great Britain, California and Hawaii were all cohorts that had been followed over many years for clinical outcomes including cancer. All cohort subjects had banked serum at the onset of a clinical observation period. Cases with gastric cancer that were identified within the cohorts in subsequent years were matched to controls by age and date of serum donation and the sera were tested in a blinded fashion for *H pylori* antibodies. Despite the ethnic diversity, subjects with cancer in the three studies showed a similar higher risk of prior infection with *H pylori* than did controls.
This increased risk was restricted to adenocarcinoma of the antrum, body and fundus [202-203]. Subjects who developed cancer soon after serum donation were excluded to rule out occult malignancy.

Thus, it is certain that \textit{H pylori} infection is at least a marker for increased risk of gastric adenocarcinoma at a later date. In an extensive review of gastric cancer and \textit{H pylori}, the Eurogast Study Group felt that presence of \textit{H pylori} confers an approximately six-fold increase in relative risk of gastric cancer incidence [205]. Definitive proof of cause, however, will be established only when double-blind placebo control trials demonstrate that elimination or prevention of infection prevents malignancy.

\textbf{\textit{H pylori} and MALT Lymphoma}

Mucosa-associated lymphoid tissue (MALT) may undergo malignant change, leading to a low grade lymphoma of the stomach. Retrospective biopsy studies show that 90% of such MALT lymphomas are associated with \textit{H pylori} [206]. Histologically, many lymphoid follicles are seen in the mucosa which if stained for immunoglobulins are shown to be monoclonal. In the usual \textit{H pylori} associated gastritis, lymphoid follicles are seen but they regress after effective therapy of \textit{H pylori}.

Wotherspoon et al found that 92% of 110 patients with MALT lymphomas were associated with \textit{H pylori} infection compared to 50% seen in controls [207].
Published reports have suggested that these tumors are driven by a continuing \textit{H pylori} antigenic stimulus and regress when \textit{H pylori} is treated effectively [208]. The German MALT Lymphoma Study Group reported that apparent cure of MALT Lymphoma occurred in half the patients in whom \textit{H pylori} was eradicated [209]. Thus, \textit{H pylori} therapy may be the initial step in the treatment of proven or suspected gastric lymphoma.

**TREATMENT OF \textit{HELICOBACTER PYLORI} INFECTION**

The introduction of effective \textit{Helicobacter pylori} eradication therapy has made possible the eradication of \textit{H pylori} in patients with gastroduodenal diseases and active infection. Therapy should not be given unless a diagnostic test for \textit{H pylori} has been performed and a positive result obtained [38]. Even duodenal ulcers are not universally infected with \textit{H pylori}. Also, if a test is negative for \textit{H pylori} in a patient with peptic ulcer, it is a diagnostic pointer to an unusual etiological factor such as NSAID abuse or Zollinger-Ellison syndrome [210]. Several factors should be taken into account in the consideration of therapeutic regimens; viz. efficacy, compliance, side effects, antibiotic resistance and cost. Many eradication regimens have been evaluated in numerous clinical trials worldwide over the past 10 years. However, these have been of variable quality in study design, number of patients studied, application, diagnosis of eradication, analysis and reporting. The ideal regimen is still awaited [211-212].
The aim of therapy in treating patients with *H pylori* infection is complete eradication of the organism. Efficacy is the most important criteria for choosing a regime. Previously, regimens that achieved more than 80% cure were considered useful; however, currently only those combinations that achieve more than 90% cure rate are acceptable in clinical practice.

Numerous agents are effective in vitro but are ineffective in vivo because of patient non-compliance, altered drug distribution, bacterial resistance or poor drug bioavailability [213]. When treating patients, clinicians need to be aware of antibacterials which induce resistance (Table R3 – next page). Antibiotics that inhibit *H pylori* but are often associated with resistance are metronidazole, tinidazole, erythromycin, clarithromycin and quinolones. Those that very rarely lead to bacterial resistance include bismuth, amoxicillin, tetracycline, doxycycline, furazolidine and nitrofurantoin [214]. It follows, therefore, that the former group of drugs cannot be reused if eradication attempts with them have previously failed but the latter can be reused several times.

The optimum duration of treatment is still unclear. However, there is no justification for treating patients longer than 14 days. Cure rates have been less with shorter duration of therapy and a longer course of drugs has not been shown to produce higher cure rates [211-212]. If a 14-day therapy fails, the bacterium is probably resistant to the antibiotic combination used and future therapy may need to be guided by antimicrobial sensitivity testing of the cultured organisms.
<table>
<thead>
<tr>
<th>DRUGS THAT LEAD TO ANTIBIOTIC RESISTANCE</th>
<th>DRUGS THAT DO NOT LEAD TO ANTIBIOTIC RESISTANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>Colloidal bismuth subcitrate</td>
</tr>
<tr>
<td>Tinidazole</td>
<td>Bismuth subsalicylate</td>
</tr>
<tr>
<td>Erythromycin base</td>
<td>Amoxicillin or ampicillin</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Doxycycline</td>
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<tr>
<td>Ofloxacin</td>
<td>Furazolidine</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>Nitrofurantoin</td>
</tr>
</tbody>
</table>

Source: Borody et al (1993)
Compliance decreases with longer or more complicated regimens [212,215]. If a compliant patient develops severe side effects, the therapy may be terminated at 7 days by which time the infection might have been eradicated in most patients [38].

**Drug Regimens for H pylori Eradication**

**Single Antimicrobial Regimens**

Numerous regimens have been studied for their effectiveness in eradicating *H pylori* infection either alone or in combination. Single drug usage has not been successful and results from clinical trials are disappointing irrespective of the class, doses and duration of antimicrobials used. The highest eradication rate reported with a single drug was 54% for clarithromycin at a dose of 500 mg four times daily for fourteen days [216]. Single antimicrobial regimens are not advocated in clinical practice because of their lower efficacy and the potential for development of antimicrobial resistance to nitroimidazoles, macrolides and the quinolones.

**Bismuth Based Regimens**

Bismuth based triple therapy using colloidal bismuth subcitrate (CBS) 120 mg q.i.d., tetracycline 500 mg q.i.d. and metronidazole 400 mg t.i.d. for 2 weeks is considered the gold standard and the first practical choice if antibiotic sensitivity testing is not feasible [153,217,218]. This triple therapy has the advantage of both luminal activity and systemic activity. Bismuth can be seen to precipitate in and around *H pylori* organisms in the gastric mucus layer, to collect beneath the cell wall.
of *H pylori*, to cause detachment of the organism from the mucosa and to lead to lysis of most gastric mucosal *H pylori* within two hours of ingestion [219]. Tetracycline is acid stable and active at an acid pH, achieves high concentrations in the mucosa, and exceeds the MIC of *H pylori* for several hours [220]. This therapy has provided consistent with predictably high *H pylori* eradication rates [221,222]. In a meta-analysis reported by Chiba et al in 1992, the highest mean eradication rate was 94.1% [221]. If tetracycline was replaced with amoxicillin, the pooled eradication rate dropped significantly to 73.1% [221]. Colloidal bismuth subcitrate when combined with amoxicillin and metronidazole is more effective in eradication of *H pylori* than with ranitidine replacing CBS in the above regimen [222].

Major disadvantages of the classical bismuth based triple therapy include a complicated treatment regimen, poor compliance and frequent occurrence of side effects [221]. Approximately 30-50% of treated patients experience side effects, but none of these are disabling or long lasting. Low cost also makes patients prefer this therapy [38].

The use of metronidazole containing triple regimens may be limited by the increasing antimicrobial resistance to the nitroimidazoles which is being reported worldwide [223]. This seems to be an important issue in developing countries where there are studies indicating that 80-90% of clinical isolates are resistant to metronidazoles, compared with a mean resistance rate of 25.6% in Europe [223-225]. Such a high primary resistance rate may be associated with previous
treatment with metronidazole for other infectious diseases or parasitic infestations in patients already infected with *H pylori* [215]. The sensitivity of *H pylori* to metronidazole is pivotal to treatment success. In a pooled analysis, 11 studies involving 699 patients with nitroimidazole sensitive strains treated with metronidazole containing triple therapies achieved an overall eradication rate of 92%, whereas eradication treatment failed in up to 56% of patients infected with resistant strains [135].

**Combinations of Acid-Suppressing Agents and Antimicrobials**

Many antimicrobials inhibit *H pylori* growth in vitro, but are ineffective at eradicating the organism in vivo. This is considered most likely to be a problem of insufficient drug delivery to the gastric mucosa and the mucus bicarbonate layer as a result of degradation of antibiotic activity by gastric juice or failure of the antibiotic to concentrate at the site of *H pylori* colonization [226,227]. Many currently used antibiotics are acid labile and their efficacy is critically pH dependent. Example of these are erythromycin, clarithromycin, amoxicillin and ampicillin. The MIC₉₀ values for erythromycin, clarithromycin and ampicillin reduce dramatically when the pH increases from 5.5 to 7.5 [228]. Metronidazole which is a base should work at a low pH. However, it is ineffective at eradicating *H pylori* when given alone.

Proton pump inhibitors (PPI) such as omeprazole, lansoprazole and pantoprazole are the strongest acid-suppressing agents among current antisecretory drugs. Therefore, PPIs may have a synergistic effect with antimicrobials by offering
an optimal pH environment in which the effect of acid-labile antimicrobials would be maximized [229]. In addition, the PPIs themselves have a direct, though minimal, antimicrobial effect upon \( H \) \( pylori \). This is reported to be maximal with rabeprazole. In vitro the urease activity of \( H \) \( pylori \) is inhibited by omeprazole and lansoprazole in a selective dose dependent manner [230]. This inhibition is probably, fatal to the organism since its survival in the hostile milieu depends upon the neutralizing activity of urease. Furthermore, a reduction in the volume of gastric acid secretion due to the PPIs may increase the local concentration of antimicrobials in the stomach which in turn can enhance the bactericidal effect of these drugs. Raising the intragastric pH above 5 with omeprazole also markedly inactivates pepsin activity. This may reduce the degradation of \( H \) \( pylori \) specific immunoglobulins secreted across the gastric mucosa resulting in a prolongation of the half life of \( H \) \( pylori \)-specific immunoglobulins in the gastric mucosa and gastric juice [231,232].

PPI Based Dual Regimens

Results from early studies employing a combination of omeprazole plus amoxicillin were inconsistent with eradication rates ranging from 0 to 84% [226,227]. The pooled eradication rates from two comprehensive reviews are almost the same at 60% and 61% and is independent of drug dose and treatment duration [226,233]. Short term treatment (less than 7 days) with this combination has been tried and shown to be much less effective [234,235]. Labenz et al have shown that a therapeutic gain of over 20% in eradication rates was achieved when the duration of treatment was extended from one week to two weeks with similar regimens [235].
The omeprazole-amoxicillin combination has the advantages of being simple with few side effects and no antimicrobial resistance. However, the major disadvantages with this combination are the widely inconsistent eradication rates, dose and duration dependency, adverse effect of smoking on eradication and the significant proportion of patients who are allergic to penicillins making them unsuitable for this therapy [235].

Clarithromycin is one of the most effective single agent against *H pylori* as it binds tightly to *H pylori* ribosomes which allows for a prolonged antibacterial effect [236]. Clinical trials using dual therapy with omeprazole and clarithromycin have shown consistent and predictable eradication rates [233]. They were significantly higher than the eradication rates with omeprazole and amoxicillin [233]. A disadvantage with this combination is the potential for the development of bacterial resistance to clarithromycin which may contribute to treatment failure. It would also compromise retreatment, if initial antimicrobial therapy fails. A newer regimen of 14 days treatment with ranitidine bismuth citrate plus clarithromycin is effective and has few side effects [215]. Moreno et al reported a significant increase in eradication rate of *H pylori* with two consecutive dual therapies consisting of omeprazole and amoxicillin followed by omeprazole and clarithromycin [237]. A PPI with another antibiotic in the second course improved the eradication rates significantly.
PPI based Triple and Quadruple Regimens

As a result of inconsistent and modest eradication rates with dual therapies, investigators have attempted to improve the efficacy of treatment for \textit{H pylori} infection by adding a second antibiotic. As nitroimidazoles have proven to be effective in the 'classic bismuth triple therapy, metronidazole or tinidazole has been added to a dual therapy containing a PPI and amoxicillin or clarithromycin. The usual dose is omeprazole 20 mg OD or b.i.d., plus clarithromycin 250 mg or 500 mg b.i.d. plus either tinidazole 500 mg b.i.d. or metronidazole 250-500 mg b.i.d. for 7-14 days. The pooled results from 21 treatment arms involving 1119 patients showed a mean eradication rate of 88.7\% [238]. In the presence of antimicrobial resistance to either clarithromycin or nitroimidazole, amoxicillin 1000 mg b.i.d. can be used as a substitute. This yielded a mean eradication rate between 79.7-88\% in a meta analysis of 14 trials with a total of 1272 patients [238].

Lansoprazole has shown effects similar to omeprazole when used in triple regimens. A large multicentre, randomized trial has shown that one week triple therapy combination using lansoprazole (L) with amoxicillin (A) and clarithromycin (C) eradicated \textit{H pylori} infection in approximately 90\% of patients. This was significantly better than the LCM eradication rate of 72.5\% obtained with lansoprazole, clarithromycin and metronidazole, but not significantly different from a combination therapy consisting of omeprazole (O), amoxicillin and metronidazole which yielded an eradication rate of 81.7\% [239].
OAM triple therapy has been shown to be less effective than OAC and OMC for eradicating *H pylori* infection in patients with duodenal ulcer [240]. Eradication rates over 90% were achieved in OAC and OMC regimens [240]. The lower efficacy observed with OAM was probably related to the bacterial resistance to metronidazole [239]. As PPI-based triple therapies are usually given for 7-10 days only, side effects are few, especially with the combination of omeprazole, clarithromycin and amoxicillin [241].

In an attempt to achieve a 100% rate of eradication of *H pylori* infection, omeprazole plus 'classic bismuth triple therapy' has been studied by several investigators [238,242]. Huang et al reported a pooled cure rate as per protocol analysis of 95% [243]. The major advantages of this combination are high and consistent eradication rates, a one week treatment offering a cure rate similar to a 2 week treatment and a significant reduction in the incidence of complications seen with the two week bismuth triple therapy [242]. Disadvantages include a very complicated treatment regimen and poor patient tolerance to metronidazole and tetracycline containing regimens both of which might lead to poor compliance. This regimen is best used in patients after previous treatment failures. The eradication rate for various drug regimens for *H pylori* is shown in Table R4 (next page).
<table>
<thead>
<tr>
<th>REGIMENS</th>
<th>DOSAGE</th>
<th>DURATION</th>
<th>H. PYLORI ERADICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole + Amoxicillin</td>
<td>20-40 mg b.i.d.</td>
<td>2 weeks</td>
<td>50-85%</td>
</tr>
<tr>
<td></td>
<td>500 mg q.i.d. or 1 g b.i.d.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omeprazole + Clarithromycin</td>
<td>40 mg o.d.</td>
<td>2 weeks</td>
<td>60-80%</td>
</tr>
<tr>
<td></td>
<td>500 t.i.d.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colloidal Bismuth Subcitrate (CBS)</td>
<td>120 mg q.i.d.</td>
<td>2 weeks</td>
<td>30-95%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>500 mg q.i.d.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metronidazole</td>
<td>400 q.i.d.</td>
<td></td>
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</tr>
<tr>
<td>Proton pump inhibitor (PPI) Amoxicillin Metronidazole</td>
<td>40 mg o.d.</td>
<td>1 week</td>
<td>75-90%</td>
</tr>
<tr>
<td></td>
<td>500 mg t.i.d.</td>
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</tr>
<tr>
<td></td>
<td>400 mg t.i.d.</td>
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<tr>
<td>PPI Amoxicillin Metronidazole</td>
<td>40 mg o.d. or 20 mg b.i.d.</td>
<td>1 week</td>
<td>85-95%</td>
</tr>
<tr>
<td></td>
<td>250 mg b.i.d.</td>
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<tr>
<td></td>
<td>400 mg b.i.d.</td>
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<tr>
<td>PPI Amoxicillin Clarithromycin Metronidazole</td>
<td>40 mg o.d. or 20 mg b.i.d.</td>
<td>1 week</td>
<td>85-95%</td>
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<tr>
<td></td>
<td>1 g b.i.d.</td>
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<tr>
<td></td>
<td>250-500 mg b.i.d.</td>
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<tr>
<td>Omeprazole CBS Tetracycline Metronidazole</td>
<td>20 mg o.d. or b.i.d.</td>
<td>1 week</td>
<td>86-98%</td>
</tr>
<tr>
<td></td>
<td>120 mg q.i.d.</td>
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</tr>
<tr>
<td></td>
<td>500 mg q.i.d.</td>
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<tr>
<td></td>
<td>400-500 mg q.i.d. or t.i.d.</td>
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</table>

Source: Harris (1997)
RECRUDESCENCE AND REINFECTION OF HELICOBACTER PYLORI INFECTION

Successful eradication of Helicobacter pylori with antimicrobial agents prevents relapse of duodenal ulcer and gastric ulcer besides resulting in regression of primary low grade B cell lymphoma. The currently accepted definition of successful eradication is failure to detect the organism in gastric biopsy material (rapid urease test, cytology, histology or culture) or by a $^{14}$C or $^{13}$C-urea breath test 4 weeks after the end of antimicrobial therapy [38]. It is believed that recrudescence of H pylori infection will occur within four weeks after completion of treatment if the organism is not completely killed [245]. This is not acceptable universally since some studies have reported a considerably higher recurrence rate of H pylori infection within 12 months in patients who were defined as having the organism eradicated at four weeks [126,236-247]. It has been suggested that this early recurrence of H pylori infection in the first 12 months after apparent “cure” represents recrudescence of persistent infection undetectable at 4 weeks after the end of the treatment, rather than reinfection with a new strain [247,248]. The currently accepted definition of eradication of H pylori infection may, therefore, need to be revised. Langenberg et al have shown that recurrent strains of H pylori after apparently successful eradication are genetically identical to the pretreatment strains using restriction endonuclease analysis (REA) of genomic DNA [97]. Thus, recurrence of H pylori may represent recrudescence of the original infection rather than reinfection with a fresh strain. Borody et al reported a true reinfection rate of
0.36% per patient per year by following patients up to 4-9 years using a $^{14}$C-urea breath test [248]. Rollan et al in a paper from Chile reported a relatively lower reinfection rate after following eradication of *H pylori* in patients with duodenal ulcer [249]. In this study, the cumulative reinfection rate was 8% at one year, 11% at 2 years and 13% at 3 years.

The method by which recurrence occurs is uncertain at present. Two possibilities exist, either incomplete eradication of the bacteria by drug treatment or incomplete elimination of the bacteria from the host as the organism can inhabit a reservoir other than stomach such as saliva or dental plaque [90,250,251]. The subgingival area in the mouth is believed to provide an ideal microaerophilic environment conducive for the growth of *H pylori*. Desai et al could culture the organism from dental plaques of healthy volunteers [54]. Oshowo et al too could detect *H pylori* in dental plaques. However, whether the plaque was a resident or transient reservoir was not clear [252]. Support for these explanations is provided by several studies. *H pylori* can live in human gastric pits where it might avoid the bactericidal activity of antimicrobial agents and recolonize the rest of the stomach after the treatment regime is over [182]. *H pylori* can also change its morphology from the typical helical to the coccoidal form under hostile conditions found in the gastric environment and can revert to its original shape under favourable conditions [40].
Reinfection and recrudescence are both more common in developing countries than in developed countries. The pooled one year reinfection rate was 22% in developing countries and 13% in developed countries [226]. However, true reinfection is seldom seen in developed countries [247]. The high reinfection rate seen in some developing countries makes it necessary to consider whether permanent eradication of *H pylori* infection is feasible.

**HELCOBACTER PYLORI ASSOCIATED GASTODUODENAL DISEASE – ROLE OF ERADICATION**

The course of duodenal ulcer disease varies from patient to patient. It has been shown that only a quarter of patients do not relapse within one year after stopping treatment with only an acid-inhibitory drug [253]. It can therefore be said that this subgroup of patients who have low activity ulcer diathesis might not require *H pylori* eradication therapy. Presently, there are no predictors for selecting these patients with low activity disease [253]. Thus, two strategies can be adopted: (i) ulcer healing treatment together with anti-*H pylori* therapy once endoscopic diagnosis of duodenal ulcer is made or (ii) ulcer healing with conventional therapy followed if necessary by conventional therapy and anti-*H pylori* treatment only in case of early ulcer relapse.

The National Institute of Health (NIH) USA, consensus is that all patients with gastric or duodenal ulcers who are infected with *H pylori* should be treated with antimicrobials regardless of whether they are suffering from initial presentation of the disease or recurrence [254]. *H pylori* infected peptic ulcer patients who are receiving
maintenance treatment with antisecretory agents or who have a history of complicated or refractory disease should also be treated for the infection.

However, in asymptomatic \textit{H pylori} infected patients without ulcers, the evidence is not sufficient at present to support antimicrobial therapy to prevent ulcer disease in future or to reduce the likelihood of developing a gastric neoplasm [254].

There is no convincing data to support routine treatment of patients with non-ulcer dyspepsia who are infected with \textit{H pylori}.

Though there is sufficient evidence to link \textit{H pylori} with gastric carcinoma, eradication is not recommended in all subjects. However, in those infected patients with atrophy, intestinal metaplasia or dysplasia who are in the younger age group chemo-prevention could be used to decrease the risk of adenocarcinoma [195]. Also, due to a close association of \textit{H pylori} with low grade B-cell MALT lymphoma and a good response following eradication of \textit{H pylori} in these patients, \textit{H pylori} therapy may be the initial step in the treatment of proven or suspected gastric lymphoma [207].