REVIEW
OF
LITERATURE
2. Review of Literature

Gastrointestinal disturbances which include gastritis, duodenitis, gastroduodenal ulcers and other inflammatory conditions are a world wide problem among all age and sex groups. Several causative factors have been identified contributing to the development of these complications. These include age, gender, hyperacidity, smoking habit, alcohol consumption and use of nonsteroidal anti-inflammatory drug. Besides these factors there is strong evidence that bacterial agent *Helicobacter pylori* can cause gastritis, gastric ulcer, duodenal ulcer and non-ulcer dyspepsia. Although this spiral shaped organisms in the gastric mucosa were first seen and reported by Bizzozero in 1893 (Bizzozero, et al., 1893) in the histological sections of the biopsies taken from the patients with gastroduodenal disturbances, it is only with the isolation of this organism by Warren and Marshall (1983) that the relationship with gastroduodenal pathology was established (Marshall, 1983). The organism was previously named as *Campylobacter pylori*, but later it was placed in a new genera and now referred to as *Helicobacter pylori* (H. pylori), (Goodwin, et al., 1989). The association between the presence of this spiral organism in antral mucosa and histological gastritis as well as peptic ulceration has now been well established both in adults (Graham, 1989; Buck, 1990) and children (Cadranel, et al., 1986; Drumm, et al., 1987). However prevalence rate increases with age. The discovery of this organism as an etiological factor of peptic ulcer disease has resolved the enigma which has puzzled physicians for many years. It has been postulated that long term gastritis caused by *H. pylori* sets the scene for the initiation of gastric cancer, a disease that is more common in countries with a very high prevalence of *H. pylori* infection (Correa, et al., 1990).
2.1. Classification of *H. pylori*

Domain : Eubacteria
Kingdom : Bacteria
Phylum  : Proteobacteria
Class   : Epsilonproteobacteria
Order   : Campylobacterales
Family  : Helicobacteraceae
Genus   : *Helicobacter*
Species : *pylori*

2.1.1. Domain Eubacteria

*H. pylori* is in Eubacteria domain because it has no nuclear membrane, no organelles (except for ribosomes), and its genetic material is found within a single strand of circular chromosome.

2.1.2. Kingdom Bacteria

*H. pylori* is considered a bacterium because it is a unicellular microorganism that lacks a nucleus. It also has no membrane bound organelles.

2.1.3. Phylum Proteobacteria

*H. pylori* falls under Proteobacteria phylum because it is a Gram-negative bacterium. Its outer membrane consists of lipopolysaccharides, rather than peptidoglycan as found in Gram-positive bacteria. As seen in *H. pylori*, many of these bacteria use flagella for movement but some are sessile (non-motile), or move via gliding. This phylum gets its name from the Greek god Proteus, who could change his shape because of the wide variety of bacterial forms found in it.

2.1.4. Class Epsilonproteobacteria

Most of the bacteria within this class inhabit the intestinal tract of mammals. They
can either be symbionts (either benefiting or not affecting the host) or parasites such as
*H. pylori*.

2.1.5. Order Campylobacterales

This order is composed of mesophiles, meaning they live in moderate temperatures
(10°-50°C). The human stomach, which is the habitat for *H. pylori*, falls within this
range.

2.1.6. Family Helicobacteraceae

This family is characterized by the helical shape of its members.

2.1.7. Genus *Helicobacter*

Members of this genus live in the acidic mammalian stomach by producing urease.
They are all flagellated and can move quite fast. This genus was once part of the
*Campylobacter* genus but was later made into its own group.

2.1.8. Species *pylori*

*H. pylori* is the most widely known species of the *Helicobacter* genus.

2.2. Epidemiology and Prevalence of *Helicobacter pylori* infections

*H. pylori* exists the world over and its prevalence in the population increases with
age. In developed countries, prevalence increases about 1% per year of age where it is
rare in children, and reaches 70% in the seventh decade. *H. pylori* infection is chronic
and once acquired remains life long, unless eradicated by antibiotics given for some
other conditions. Humoral and tissue immune response by the host is usually not
sufficient to clear the infection (Mendal *et al.*, 1992; Noach *et al.*, 1993).

More than 50% of the world population is colonized with *H. pylori* (Mendall,
inflammatory condition of stomach and duodenum, presenting as recurrent abdominal
pain. It is a major cause of morbidity in infected patients as it is associated with 90% of
duodenal ulcers and 80% of gastric ulcers (Aroori, 2001). The disease has a low mortality but it results in substantial human suffering and hence loss of manpower. *H. pylori* is also associated with gastric mucosa associated lymphoid tissue, lymphomas and gastric adenocarcinoma. Humans appear to be the only reservoir of *H. pylori* infection and therefore human contacts remain the major mode for its transmission. Iatrogenic spread through contaminated gastrointestinal equipment has been documented (Logan and Hiscal, 1996). Water has been shown to be a source for *H. pylori* infection (Klein, *et al*., 1991).

There is mounting evidence that *Helicobacter pylori* infection play an important role in pathogenesis of carcinoma of gastric antrum and fundus. Carcinomas of the gastric cardia are pathogenically related to adenocarcinomas of the esophagus. Other risk factor includes dietary factor, genetic factors, and predisposing conditions. Consumption of a poor diet or lacking in raw vegetables, fruit, and high fiber breads increases the risk of developing gastric cancer. There is definite increased risk within certain ethnic groups (Gold, 1999). Several pathogens processes are thought to be risk factors for gastric carcinoma. These processes include chronic gastric ulceration, atrophic gastritis, gastrojejunal anastomosis, immunodeficiency syndromes, and menetrieras disease (Gold, 1999). Most case of chronic gastric ulcers and many cases of atrophic gastritis are due to *H. pylori* infection (Gold, 1999 and Marshall *et al*., 1989).

Colonisation of *H. pylori* occurs by producing urease and gastric acid inhibitory protein. It can colonise only in gastric type epithelium and cannot stay anywhere else in the GI tract in absence of gastric mucosa. Metaplasia, which is present in more than 90% of patients of duodenal ulcer, occurs by replacing the columnar cells, normally covering the duodenal villi, by gastric type epithelium. Adhesion of *H. pylori* to the
gastric epithelium occurs by tissue specific proteins. Colonisation of the duodenal bulb by *H. pylori* leads to mucosal inflammation which makes it vulnerable to attack by acid or pepsin or bile resulting into ulceration, however, factors leading to gastric metaplasia in the duodenal bulb are not known. Stimulation of the immune system of *H. pylori* contributes to host damage and it evades the immunological clearance (Noach *et al.*, 1993).

The World Health organization recently declared *Helicobacter pylori* a class I carcinogen because of the association of *H. pylori* and gastric malignancies. More than two-thirds of patients diagnosed with gastric cancer die of the disease. Worldwide, this makes gastric cancer the leading cause of death from cancer. The prevalence of gastric cancer in the United States decrease significantly in the last several decades from effecting 33 in 100,000 persons in 1935 to 9 in 100,000 in the 1990 (Gold, 1999). In Asia and Japan, gastric cancer is the leading cause of death from malignancy.

2.2.1. Hemagglutination

*In vitro* studied have shown that *H. pylori* has very strong hemagglutination abilities, although enormous variation exists among strains. *In vivo*, *H. pylori* does not interact with red blood cells, but interaction with neutrophils and monocytes occurs. Studies show that the strong hemagglutinating strains have increased resistance to phagocytosis compared to weak hemagglutinating strains (Evans *et al.*, 1988).

2.2.2. Lewis B Binding Adhesins

Another adhesion for *H. pylori* is Lewis B binding adhesions. Boren (1993) has reported that during a stationary phase of growth, *H. pylori* can bind to Lewis B structure. As with hemagglutination, great diversity is present among strains. Even with Lewis B binding adhesions, *H. pylori* does not always bind to epithelial cell types with Lewis B antigens. Many other adhesions are present in *H. pylori*, but not all are useful
in vivo condition.

2.2.3. Adhesins for obtaining Iron

To obtain the critical nutrients, iron, *H. pylori* uses its main mechanisms. *H. pylori* can secrete siderophores to obtain iron from the surrounding environment. It binds iron from the environment and returns it to the bacterium. In addition *H. pylori* has lactoferrin binding protein on the cell wall of the bacterium (Nelians, 1995). The lactoferrin binding protein binds lactoferrin, a protein in the human body that holds iron, to obtain iron from the environment. With these mechanisms, *H. pylori* can obtain iron from mucosal surfaces and nutrophills to provide sufficient nutrients for growth.

2.2.4. Urease

Urease maintains an important role in metabolism and evading the immune response. Urease is involved in protein synthesis by providing nitrogen, converting glutamate to glutamine. Urease, commonly found unbound from *H. pylori*, might indicate autolysis of some cells or active transport (Dunn et. al., 1997). This may prevent secretory immunoglobin (IgA) from binding to *H. pylori*. The secreted urease enzyme would bind antibodies preventing binding to cell bound urease, thus stopping opsonization.

2.3. Diseases associated with *H. pylori*

*H. pylori* infection is found to be associated with gastritis, non-ulcer dyspepsia (NUD), duodenal ulcer, gastric ulcer, gastric cancer, and gastric lymphoma of mucosa associated lymphoid tissue (MALT), non-Hodgkin’s lymphoma and even coronary heart disease. It has now been well established that *H. pylori* is the cause of almost all duodenal ulcers (DU) and chronic benign gastric ulcers (GU). More than 95% of DU and 90% of GU are associated with *H. pylori* infection and there is a dramatic decrease in their relapse rate after the *H. pylori* eradication. Prevalence of *H. pylori* infection is

Association between *H. pylori* and gastric cancer has been reported in several retrospective epidemiological studies. It is postulated that starting with acute gastritis, *H. pylori* infection leads to chronic atrophic gastritis, intestinal metaplasia, dysplasia and ultimately progression to gastric adenocarcinoma. High *H. pylori* infection rate has been reported in patients with gastric cancers compared to healthy subjects. The WHO has put *H. pylori* in group I, a definite carcinogen. *H. pylori* has also been found to be associated with development of MALT and subsequent transformation to malignant lymphoma. Eradication of *H. pylori* has shown regression of low-grade b cell gastric lymphoma of MALT type. There is some epidemiological evidence that *H. pylori* infection is associated with non-Hodgkin’s lymphoma which is comparatively rare in stomach (Parsonnet *et al.*, 1994 and Parsonnet, 1993).

2.4. Survival and Persistence of *H. pylori*

One of the important aspects of the pathogenicity of *H. pylori* is the remarkable ability of the organism to remain in the stomach for long period of time. *H. pylori* have acquired several sophisticated mechanisms for long survival, it is supposed that *H. pylori* may survive in patient for entire life.

2.4.1. Inaccessibility

Under normal conditions host defense mechanisms are able to eliminate *H. pylori*, however due to certain mechanisms *H. pylori* escapes from these defense mechanisms and produce disease. *H. pylori* is a motile and microaerophilic organism it infect gastric epithelium and is present deep inside the tissues, so inaccessible to host defense
mechanisms. The adhesins keep an area of tissue continuously colonized, and the urease allows the bacteria to survive when mucus is washed into the stomach or gastric cells are sloughed off.

2.4.2. Immune Evasion

Major humoral and cellular response develops against \textit{H. pylori} infection, but these immune response are unable to eliminate the organism from host (Blaser, 1992), indicating that \textit{H. pylori} may have immune evasion mechanisms.

2.4.3. Inflammatory Mediators

Histological examination of biopsies of patients with \textit{H. Pylori} associated gastritis revealed that tiny inflammatory events are deep in the tissue, removed from the area of colonization, It is postulated that the organism sheds large amounts of extra cellular products which diffuse into the mucosa and it is against these bacterial products that the inflammatory response and possibly a local immune response are directed (Newell, 1990). \textit{H. Pylori} associated gastritis either could be a direct consequence of these products or results from other immune activities occurring concurrently in the mucosa and lamina propria. Chronic gastritis (i.e. accumulation of lymphocytes, plasma cells and macrophages) is thought to be the result of an immune response to the antigens of gastric bacteria.

Little is known about the immune responses in the stomach, and it is not known whether the local immune response occur directly via the mucosa or whether the responses are due to recruitment of systematic responses stimulated via the traditional small-bowel antigen-capturing mechanisms Heat Shock Protein.

2.4.4. Ulcerogenic Factors

Peptic ulcers are caused by acid following an alteration in the normal mucosal protective mechanisms. \textit{H. pylori} somehow create a change in the local environment
that results in increased vulnerability of the mucosal barrier.

2.4.5. Effects on the Mucus Layer

The natural habitat of *H. pylori* is gastric mucosa. It has been found that mucus layer of infected patients was thinner than that found in control uninfected persons. Routine histological sections also show mucus depletion. The findings indicate that bacterium could stimulate increased mucus turnover, thus creating a favorable physiological environment for the bacterium. Over stimulation of the mucus may accelerate the expulsion of mucus into the gastric lumen, resulting in thinner mucus layer. The hydrophobicity of isolated gastric biopsies is different for infected and non-infected persons, indicating changes in the mucus coating of these surfaces (Spychal *et al.*, 1990; Goggin *et al.*, 1991).

2.4.6. Cytotoxins

*H. pylori* is known to produce several toxins. A cytotoxin is present in up to 60% of isolates, the cytotoxin produce vacuolation in cultured epithelial cell lines. It has been shown that the *H. pylori* strains isolated from patients with ulcers produced cytotoxin more often than strains isolated from patients with chronic gastritis only (Figura, *et al.*, 1989).

The vacuolating toxin, a protein of about 130 KDa, is also produced *in vivo*, and is most probably involved in the development of ulcer. In addition, practically all patients with ulcerative or erosive gastroduodenal mucosa lesion had antibodies to toxin compared to 64% of patients without ulcers or gastric erosions (Figura, *et al.*, 1990). A cytolethal toxin, which also caused death in mice after intraperitoneal injection, has been described (Hupertz and Cinn, 1998). Another toxin prevents cultured parietal cells from secreting acid (Cave and Vargas, 1989). Acid secretion inhibitors *H. pylori* strains can inhibit gastric acid secretion from parietal cells by the production of a non-diffusible
protein, favouring the colonization process.

2.4.7. Endotoxin

Lipid A contained in *H. pylori* lip polysaccharide acts like an endotoxin, and can cause an inflammatory response, and the release of mediators by mononuclear cells which damage the gastric epithelium (Fumarola, and Migliarotta, 1988).

2.4.8. Urease

*H. pylori* produces ammonia by breaking down the plasma urea which transduces into the stomach. There is much evidence that urease production, as well as facilitating bacterial colonization, can also damage the gastric mucosa, and predispose to the development of ulcers. *In vitro*, the urease activity induces cytopathic effects on mammalian cells (Barer *et al.*, 1988). Other studies suggest that ammonia itself exerts a toxic effect (Kawano *et al.*, 1991; Triebling *et al.*, 1 991). In animals, ammonia inhibits mitochondrial oxygen consumption, and impairs energy metabolism (Kawano *et al.*, 1998). A further effect of the rapid urea hydrolysis carried out by *H. pylori* at intercellular junctions is the gradient of a transmucus ammonium gradient which prevents the normal passage of hydrogen which prevent the normal passage of hydrogen ions form the gastric glands to the gastric lumen, and the back ion diffusion (Hazell and Lee, 1986). This interference in hydrogen ion diffusion could result in acid-induced damage (Hazell and Lee, 1986). Ammonia can also cause defective biosynthesis or breakdown of mucus at the mucosal surface (Sidebotham and Baron, 1990).

2.4.9. Catalase

*H. pylori* has very high catalase activity. Since this enzyme protects against the damaging effects of oxygen metabolites it could protect the bacterium from endogenous hydrogen peroxide produced by polymorphonuclear Leukocytes which
participate in great number in a primary infection. Thus, it could be considered a virulence factor (Hazell, 1991; Hazell et al., 1991; Marshall, 1991).

2.4.10. Hemolysin

The hemolytic activity of *H. pylori* strains varies greatly, and it is not clear whether the differences observed correspond to various degrees of *H. pylori* pathogenicity (Wetherall and Johnson, 1989). Whether hemolysin is produced in vivo is not yet been determined.

2.4.11. Proteases

*H. pylori* produces a protease capable of weakening the gastric mucus, impairing its viscoelastic properties *in vitro* (Sarosick et al., 1991), and most probably causing a reduction of the mucus thickness in *H. pylori* positive patients (Shida et al., 1989).

2.4.12. Lipase and Phospholipase

*H. pylori* can elaborate lipase and phospholipase, which may after some substances involved in the maintenance of the gastric hydrophobic lining, which are also part of the gastric epithelium cell membranes (Slomiany et al., 1991). The first phospholipase A2 may also produce lysolecithin, which is cytotoxin, from biliary lecithin refluxed into the stomach (Readseh et al., 1989). Digestive enzymes of *H. pylori* proteases, lipases, and phospholipases, may destroy the integrity of the gastric mucus layer.

2.5. Substances Elicited or Increased by Helicobacter pylori as Virulence Factors

The presence of *Helicobacter pylori* can stimulate the production of substances which could have detrimental effects on the gastric mcosa.

2.5.1. Platelet-Activating Factor (PAF) Production

PAF is a proinflammatory mediator produced by a variety of bacteria and cells
which can cause gastroduodenal ulceration in animals (Sobhani et al., 1989). *H. pylori* is able to enhance PAF production at the gastric mucosa level (Sobhani et al., 1989; Pretolani et al., 1989), possibly causing the development of gastroduodenal erosive lesions.

2.5.2. Leukotriene Production

Increased levels of leukotrienes, substances produced by neutrophils, having cytotoxic activity, were found in gastric mucosa infected by *H. pylori*. The more severe the neutrophil infiltration, the higher the levels of leukotrienes (Arakawa et al., 1989).

2.5.3. Cytochrome P-450 Activation

Cytochrome P-450 is part of the intestinal mucosa oxidative systems which catalyze biotransformation reactions. As a result of these reactions, some toxic metabolites, which can be detoxified by the epoxide hydrolase enzyme, are formed. *H. pylori* associated with gastritis patients had both an enhancement of P-450 metabolizing enzyme, and a decreased mitochondrial epoxide hydrolase activity (Dworkin et al., 1989).

2.6. *Helicobacter pylori* Infection and Gastric Cancer

In the recent years it has been postulated that *H. pylori* infection may have some role in development of gastric cancer. This hypothesis is supported by the fact that prolonged presence of the organism in progression from the relatively benign chronic gastric is with neutrophil activity to atrophic gastritis, a condition in which inflammatory in Infiltration and lymphoid follicle aggregate disrupts the mucosa to such a degree that ablation of function occurs. This tissue destruction results in intestinalization of gastric mucosa and achlorhydria followed by overgrowth of intestinal bacteria in the stomach (Correa and Ruiz, 1989). If other conditions are present for example consumption of certain dietary factors (e.g., salts) or reduction of
protective antioxidant such as vitamin C, then the scene is set for induction of cancer (Reeavarren-Arec et al., 1991). *H. pylori* probably does not itself induce gastric cancer but may promote either the production of carcinogens or mutagenic events, e.g., the atrophic gastritis which has been known for many years to be an essential prerequisite in certain forms of gastric cancer (Correa, 1988). Should this hypothesis prove to be true, and at present all the epidemiological study support (Forman et al., 1991; Nomura et al., 1991; Parsonnet et al., 1991), then intervention strategies resulting in prevention of *H. pylori* infection could eliminate a disease of major morbidity mortality in certain development countries.

2.7. Effects or *Helicobacter pylori* infection on Gastric Physiology

Infection with *H. pylori* can influence normal gastric function. Infection results in increased meal stimulated serum gastrin levels (Graham et al., 1990). Investigation of infected volunteers or persons who have been studied by chance during early infection shows that *H. pylori* infection can result in hypochlorhydria (Ramsey et al., 1979). *In vitro* studies have suggested this could be due to the production of an antisecretory protein by this organism (Cave and Vargas, 1989). In patients with iatrogenic *H. pylori* infection a marked diminution of gastric ascorbic acid was noted (Sobala et al., 1991), these findings could have broad implications with respect to the role of *H. pylori* in gastric carcinogenesis (Graham et al., 1988).

2.8. Immune Response to *Helicobacter pylori* Infection

The presence of *H. pylori* on human gastric mucosal surfaces, especially of the antral region, is usually accompanied by signs of local inflammation and a measurable systemic and local immune response. It is demonstrated that *H. pylori* infection seem to be long-lasting and the level of systemic immune response remains constant during the presence of the causative agent (Langenberg et al., 1988). A soluble bacterial
component attracts phagocyte cells (neutrophils and macrophages) into the glands and lamina propria leading to the typical gastritis appearance (Craig et al., 1989). These phagocytes migrate through the epithelial cell layer and may generate toxic products directed at the organism. It is possible that this process is the cause of some of the epithelial cell damage seen in patients with gastritis. The lamina propria also contains many mononuclear cells. These immunologically competent plasma cells produce specific IgG and IgA. Complement activation occurs in the presence of IgG (presence in high titre in most patients) and H. pylori antigens. The lymphocytes present in H. pylori gastritis are mainly T-cells. They induce the expression of class II antigens on epithelial cells (Engstrand et al., 1989), indicating activation of a potentially cytotoxic Local immune response

2.9. Non-ulcer Dyspepsia

Treatment for H. pylori associated with symptoms of non-ulcer dyspepsia is also controversial and at present there is no convincing evidence that H. pylori is responsible for these symptoms (Kemmer et al., 1994). Moreover, prevalence of H. pylori in this group of patients is not higher than the general population without symptoms. Although a subset of patients may have their symptoms due to presence of H. pylori, there is no clear evidence that they benefit after H. pylori eradication. At present there is no indication that patients with non-ulcer or functional dyspepsia should be treated for H. pylori, till the results from large trials are available (Valdhuerzen van Zanten et al., 1995; Moayyedi et al., 2002; Moayyedi et al., 2003).

2.10. Gastritis and Dyspepsia

The symptoms are discomfort, bloating, nausea and perhaps vomiting. The person may also have symptoms that suggest ulcers burning or pain in the upper abdomen, usually occurring about an hour or so after meals or even during the night. The
symptoms are often relieved temporarily by antacids, milk, or medications that reduce stomach acidity. Yet, the physician does not find an ulcer when the patient is tested by x-ray or endoscopy. When \textit{H. pylori} is found in the stomach, it is tempting to believe that it is the cause of the symptoms, although this connection is not yet clear cut. The physician will usually prescribe antibiotic therapy to see if clearing the infection relieves symptoms (Malaty \textit{et al.}, 1996; Marshall, 1983).

2.11. Stomach Ulcers

With stomach ulcers, \textit{H. pylori} infection is found in 60 to 80 percent of the cases. Again, it is still uncertain how the infection acts to cause the ulcer. It probably weakens the protective mucous layer of the stomach. This allows acid to seep in and injure the underlying stomach cells. However, there is still a great deal of research to be done to unravel this relationship (Marshall and Warren, 1984; Megraud, 1993).

2.12. Duodenal ulcers

In times past, physicians were taught "no acid, no ulcer." The medical profession felt the single most important factor causing duodenal ulcers to form was strong stomach acid. Research has now shown that over 90% of all patients who develop duodenal ulcers have \textit{H. pylori} infection in the stomach as well. Medical studies are under way to determine the relationship between the two and how an infection in the stomach can be related to a duodenal ulcer. Acid is still important; patients without acid in the stomach never get duodenal ulcers. However, physicians now accept the fact that the infection is directly related to the development of duodenal ulcers. It is now rather easy to clear duodenal ulcers with the strong acid-reducing medicines available. But, the ulcers will usually recur unless the \textit{H. pylori} infection is also cleared from the stomach (Malfertheiner and Bode, 1993; and Marshall \textit{et al.}, 1988).
2.13. Stomach Cancer and Lymphoma

These two types of cancer are now known to be related to *H. pylori* bacteria. This does not mean that all people with *H. pylori* infection will develop cancer; in fact, very few do. However, it is likely that if the infection is present for a long time, perhaps from childhood, these cancers may then develop. This is another reason why it is important to treat *H. pylori* infection (Graham, 1997; Peterson, 1991).

2.14. Diagnosis

A number of invasive and non-invasive tests with almost comparable sensitivity and specificity are available. Invasive tests require upper GI endoscopy and biopsy from stomach for histology, bacterial culture, rapid urease test (RUT) and PCR (Cutler *et al.*, 1995).

Biopsy is fixed in 10% formalin and stained with haematoxylin and eosin or by modified Giemsa stain. Biopsy can also provide additional information on gastritis, metaplasia and dysplasia. Biopsy specimen can also be used for bacterial culture in selective or non-selective media. Though the sensitivity and specificity of this test is >95% and >80% respectively, it is time consuming, expensive and also it is not easy to culture this bacteria (Cutler *et al.*, 1995; Peura, 1995).

RUT is 90% sensitive and 100% specific, inexpensive and provides results within 20 minutes. Urease produced by the bacteria hydrolyses urea into ammonia. A change in pH changes colour of the indicator from yellow to pink. In case of low urease activity it may take as long as 24 hours to change the colour. False negative result may be there if the number of bacteria in the specimen is less or if the antral biopsy is taken after one week of proton pump inhibitors, antibiotics or bismuth treatment, when *H. pylori* colonize in body or fundus (Peura, 1995).

PCR is highly sensitive and specific but it may detect DNA of non-viable bacteria
also giving false positive results and also has a limited role in confirming eradication of \textit{H. pylori} after treatment. It is usually used for molecular typing of \textit{H. pylori} and for research (Cutler \textit{et al.}, 1995; Peura, 1995).

Non-invasive tests include serology and urea breath test (UBT). Commercially available ELISA kits detect IgG antibodies in sera. This test is useful to screen the patients for \textit{H. pylori} infection, usually to find out prevalence of \textit{H. pylori} infection in the community. It is a relatively sensitive and specific test and also inexpensive, but it has a limited role in diagnosing acute infection and in confirming eradication. UBT is a good non-invasive test. Although it is expensive, it is highly sensitive (95\%) and specific (100\%) and also ideal to check the post-treatment eradication. Detection of labelled CO$_2$ (13C or 14C) in expired air indicates hydrolysis of urea and presence of urease producing organism in stomach (Peura, 1995; Artherton and Spiller, 1994).

At present, no single test is the gold standard for diagnosis of \textit{H. pylori}. The combination of two or more of above mentioned tests are used for this purpose (Artherton and Spiller, 1994).

\subsection*{2.15. Treatment of Helicobacter pylori infection}

The discovery of \textit{Helicobacter pylori} in the early 1980s revolutionized the management of many gastrointestinal diseases. Our understanding of \textit{H. pylori} infection and its associated gastrointestinal diseases continues to evolve, with new indications for anti-\textit{H. pylori} treatment being constantly added. The availability of simple, accurate, and non-invasive diagnostic tests, such as the urea breath test and serological analysis, facilitates the screening and eradication of \textit{H. pylori} by primary care physicians. However, confusion still exists regarding the indication and treatment regimens.
2.16. *H. pylori* eradication

Guidelines on who should receive *H. pylori* eradication therapy were first published by the National Institutes of Health (NIH) Consensus Development Panel on *H. pylori* in 1994 (NIH Consensus Conference, 1994). The panel concluded that patients with *H. pylori* infection and peptic ulcer disease, regardless of disease stage (first presentation or recurrent ulceration) or use of non-steroidal anti-inflammatory drugs (NSAIDs), require eradication therapy. But whether or not *H. pylori* infection in those with non-ulcer dyspepsia should be treated was unclear. The routine detection of *H. pylori* in the absence of an ulcer was not recommended. The NIH Consensus Panel did not give a definite recommendation on the need for *H. pylori* eradication in patients with complicated peptic ulceration, peptic ulcer disease in children, or as a preventive measure against gastric cancer. Two years later, in 1996, the European *H. pylori* Study Group formulated further guidelines on the management of *H. pylori* infection (The European *Helicobacter pylori* Study Group, 1997). This group confirmed that all *H. pylori* positive patients with peptic ulcer disease, whether the condition is active or not, should receive anti-*H. pylori* therapy. The most remarkable feature was that it recommended that dyspepsia be screened for and treated at the primary care level. This European Consensus suggested that screening for *H. pylori* followed by eradication therapy should be given to all dyspeptic patients younger than 45 years with no alarm symptoms. The group suggested that therapy should also be extended to patients with mucosa-associated lymphoid tissue (MALT) lymphoma, gastritis with severe intestinal metaplasia (or gastric atrophy), and those who have had early gastric cancer resected. It should be pointed out that some of these recommendations were based on relatively weak scientific evidence, but more by voting of the participants.

In 1998, the Asia-Pacific Consensus established guidelines for use in this region
Lam et al., 1997). The consensus statement shares much with the European guidelines. It extends recommendations regarding the treatment of H. pylori infection to patients with complicated ulcer disease. For patients requiring long-term NSAIDs, prophylactic H. pylori eradication therapy is recommended. Routine screening and eradication in asymptomatic patients is still not implemented. The role of H. pylori eradication in patients with premalignant lesions such as intestinal metaplasia was not addressed by the Asia-Pacific Consensus despite, the high incidence of gastric cancer in the region (Sung et al., 1998).

2.17. Cytokines

In general cytokines are polypeptide hormones secreted by a cell that affects growth and metabolism either of same (autocrine) or of another (paracrine) cell. First cytokine to be discovered was interferon (IFN) in 1957 by Issacs and Lindenmann (Issac and Lindenmann, 1957), as a soluble factor produced by cells following exposure to heat-inactivated influenza virus.

Cytokines was originally coined to designate the molecules produced by the cells of the immune system and the biological response modifiers of the same system, it quickly became apparent that molecules outside the immune system have similar modes of production and action. Currently, lymphocytes and monokines, which are the secreted products of lymphocytes and monocytes, respectively, but also secreted products of neutrophils, mast cells, endothelial cells, fibroblasts, astrocytes, and other cell types are included among the cytokines.

Cytokines function through a cytokine network and exert their function through their receptors on target cells (Masuda et al., 1993). Cytokines regulate growth, differentiation, and function of hematopoietic lineages, as well as mediate a large variety of normal and pathological immunological responses. The key to the immune
responses elicited by viral, bacterial, protozoan, or a neoplastic antigen is the nature of the T-cell activity elicited. Cell-mediated immune responses involve activation of macrophages and induction of CD4\(^+\) cells and CD8\(^+\) cytotoxic cells. Helper T cells, B cells, and macrophages respond to antigenic stimulation by production of soluble factors identified as cytokines, which regulate the immune response. These cytokines may stimulate certain effector cells and may inhibit others. The balance of these complex interactions determines the overall response, and any imbalance may result in pathological responses.

2.18. The best combination drugs for \textit{H. pylori} infection in cases of treatment failure

Following are attempted individually or in combination for treatment for \textit{H. pylori} infection.

2.18.1. Pantoprazole

Pantoprazole sodium is a proton pump inhibitor, a substituted benzimidazole: viz. 5-Difluoromethoxybenzimidazole-2-yl-3,4- dimethoxy-2-pyridylmethyl sulphoxide. It is used for inhibition of gastric acid secretion. Pantoprazole is extensively metabolized, mainly via hepatic cytochrome P450 (CYP) 2C19 isoenzyme (Anderson \textit{et al.}, 1978; Klastersky and Zinner, 1982).

On achieving hemostasis by treating patients with active bleeding ulcers or ulcers with major signs of recent bleeding with distilled water injection (Hsu \textit{et al.}, 2004 ). They were randomized to receive intravenous Pantoprazole. They reported that Pantoprazole was superior adjunct treatment to endoscopic injection therapy in high-risk bleeding ulcers (Hsu \textit{et al.}, 2004; Huggins \textit{et al.}, 2003). Pantoprazole (PPIs) to be superior to the \textit{H}\textsubscript{2} receptor antagonists (Huggins \textit{et al.}, 2003). Metz \textit{et al} have elaborated the utility and safety of Pantoprazole in controlling the acid output of
patients with Zollinger-Ellison syndrome, albeit at higher doses than the normal ones (Metz et al., 2001). It has also been used in those patients with pathological hypersecretion associated with Zollinger-Ellison syndrome (Louw et al., 1998; Metz et al., 2001; Pisegna, 2001).

Pantoprazole is a substituted benzimidazole which accumulates in acidic environment of patient cells after absorption, which binds to the $\text{H}^+/\text{K}^+$ ATPase, thus inhibiting the proton pump and causing potent long lasting suppression of basal and stimulatal gastric acid secretion (autylcho-line, histamine, gastrine) (Anderson et al., 1978; Klastersky and Zinner, 1982). Pantoprazole becomes active in higher acidic condition and get inactive in higher pH i.e in alkaline environment.

2.18.2. Clarithromycin

Clarithromycin is an macrolide antibiotic usual to treat the $H.\text{ pylori}$ infection (Klastersky and Zinner, 1982; Labenz et al., 1995). Treatment usually involves a combination of antibiotic and acid suppressors proton pump inhibitor which provides protection against $H.\text{ pylori}$ infection. Clarithromycin regiment recommended for patients may differ across regions of the world because different areas have begun to show. (Anderson et al., 1978; Klastersky and Zinner, 1982).

Clarithromycin prevents bacteria from growing by interfering with their protein synthesis and its binds to the subunit 50S of the bacterial ribosome and thus inhibits the translation of peptides. Clarithromycin is more effective against certain gram-negative bacteria $H.\text{ pylori}$ (A. Malhotra-Kumar et al., 2007; Graham, 1995; Graham, 1993; Hunt, 1996).

2.18.3. Amoxicillin

Amoxicillin is an penicillin group antibiotic usual to treat the $H.\text{ pylori}$ infection particular gram negative infection (Dore et al., 1999). Treatment usually involves a
combination of antibiotic and acid suppressor’s proton pump inhibitor protection (Louw et al., 1998 and Noophun et al., 2004). Very high resistance rates to amoxicillin have been reported in some prevalence studies (Godoy et al., 2003 and Wu et al., 2000).

2.18.4. Tetracycline

Tetracycline is a cheap and effective antibiotic with better broad spectrum activity against \textit{H. pylori} infections, a gram negative organism (Megraud and Marshall, 2000; O’Morain and Montague, 2000). Tetracycline prevents bacteria from growing by interfering with their protein synthesis and its binds to the subunit 30S of the bacterial ribosome and thus inhibits the translation of peptides. Tetracycline is more effective against certain gram-negative bacteria \textit{H. pylori} (Brodersen et al., 2000; Piolett et al., 2001).

Treatment with a combination of an antibiotic Tetracycline plus proton pump inhibitor pantoprazole has shown increased efficacy. Such combination therapy ensured in increased bacterial activity and rate of killing \textit{in vitro} and prevention of the emergency of drugs resistance (Chopra et al., 1992; Roberts, 1996; Schnappinger and Hillen, 1996; Speer et al., 1992).

2.18.5. Metronidazole

Metronidazole is selectively toxic to anaerobic microorganisms \textit{H. pylori}. After entering the cell by diffusion its nitro group is reduced by certain redox proteins operative only in anaerobic microbes to highly reactive nitro radical which exerts cytotoxicity by damaging DNA halix (Tripathi, 2003). Metronidazole is a better broad spectrum antibiotic usual to treat the \textit{H. pylori} infection particular gram negative infection (Tripathi, 2003; Pavicic et al., 1993). Treatment usually involves a combination of antibiotic and acid suppressors proton pump inhibitor which provides

Treatment with a combination of an antibiotic Metronidazole plus PPI Pantoprazole has shown increased efficacy. Such combination therapy ensured in increased bacterial activity and or rate of killing *in vitro* and prevention of the emergency of drugs resistance (Menbonca *et al.*, 2000).

2.18.6. Cefotoxime

Cefotaxime (proposed generic name of 3- acetoxyethyl-7-[(2-(2-amino-4 thiazolyl)-2- methoxy-iminoacetyl) amino]-ceph-3-eme-4-carboxylic acid, sodium salt) is a novel parenteral cephalosporin with activity against gram negative organisms (Anderson, 1978; Barza *et al.*, 1976).

Cefotaxime is a cephalosporin with a spectrum of activity which may make it appropriate for use in pediatric patients. Cefotaxime is a semi synthetic parenteral cephalosporin with exceptional activity against gram-negative organisms and considerable stability against their β-lactamases (Bennett *et al.*, 1966; Fong *et al.*, 1976).

2.18.7. Ceftazidime

Ceftazidime is a broad spectrum, third generation parenteral cephalosporin effective against many gram negative organisms. Serious infections of the respiratory tract and gastrointestinal tract infections in immunocompromised patients were found (Ayrton, 1981).

Ceftazidime is a semisynthetic, broad-spectrum, beta-lactam antibiotic for parenteral administration. It is the pentahydrate of pyridinium, 1-[[7-[[[(2-amino-4-thiazolyl)][1-carboxy-1-methylethoxy)imino]acyethyl]amino]-2-carboxy-8-oxo-5-thia-1 zabicyclo [4.2.0] oct-2-en-3-yl]methyl]-, hydroxide, inner salt, [6R-6α,7β (Z)] (Barza, 1982).
The pharmacokinetic properties of ceftazidime, a third generation cephalosporin, and MICs of ceftazidime for some gram-negative strains isolated from clinical cases were determined. An efficacy predictor, measured as the time over which the active drug exceeds the bacteria MICs was calculated. Serum ceftazidime disposition was best fitted by a bi-compartmental and a mono-compartmental open model with first-order elimination after IV and IM dosing, respectively (Cars and Qgren, 1985; Dawson, 1981).

2.18.8. Ceftriaxone

Ceftriaxone is a new semisynthetic cephalosporin which has a broad spectrum of activity in vitro (Allaz et al., 1979). Its activity against most gram-negative bacteria was greater than those of some of the other new broad-spectrum cephalosporins. The in vitro activity of ceftriaxone against gram-negative anaerobic organisms compares favorably with those of other cephalosporins (Angehrn et al., 1980).

Ceftriaxone inhibits the synthesis in the bacterial cell wall. Like other cephalosporins of the 'third generation, it has a very broad anti-bacterial spectrum and it is stable against most β-lactamases. Ceftriaxone owes its popularity mainly to its relatively long plasma half-life. Its renders an intensive cephalosporin therapy possible on an out-patient basis. The daily short infusion only necessitates half an hour. Without unambiguous and clear indications therapy is not advisable (Craig, 1980).

2.18.9. Piperacillin

Broad spectrum semi-synthetic penicillin antibiotic bactericidal against gram positive and gram negative aerobic and anaerobic organisms. Exerts its bacteriocidal action by inhibiting cell wall synthesis. Piperacillin is inactivated by beta lactamases produced by staphylococci and some gram negative bacteria (Auclair and Ducharme, 1999; Derendorf and Dalla Costa, 1996).
2.18.10. Tazobactam

Tazobactam is a derivative of penicillinic acid sulphone, which functions as an irreversible inhibitor of many beta-lactamases. It is added to the extended spectrum beta-lactamases antibiotic piperacillin. It broadens the spectrum of piperacillin by making it effective against organisms that express beta-lactamase and would normally degrade piperacillin (Daniel and Krop, 1996).

Tazobactam M1 metabolite maximum plasma concentration increased as renal function declined. The terminal elimination half life and area under the plasma concentration-time curve of the tazobactam M1 metabolite increased as renal function declined (Perry and Markham, 1999).

2.18.11. Ciprofloxacin

Ciprofloxacin is one of a second generation of fluorinated quinolones structurally related to nalidixic acid. The primary mechanism of action of ciprofloxacin is inhibition of bacterial DNA gyrase. It is a broad spectrum antibacterial drug to which most gram-negative bacteria are highly susceptible in vitro. It attains concentrations in most tissues and body fluids which are at least equivalent to the MICs designated as the breakpoint for bacterial susceptibility in vitro. The results of clinical trials with orally and intravenously administered ciprofloxacin have confirmed the potential for its use in a wide range of infections, which was suggested by its in vitro antibacterial and pharmacokinetic profiles (Egerbacher et al., 2000).

Ciprofloxacin generally appeared to be at least as effective as alternative orally administered antibacterial drugs in the indications in which they were compared, and in some indications, to parenterally administered antibacterial therapy. The drug is also well tolerated. Thus, as an orally active, broad spectrum and potent antibacterial drug, ciprofloxacin offers a valuable alternative to broad spectrum parenterally administered
antibacterial drugs for use in a wide range of clinical infections, including difficult infections due to multiresistant pathogens. Ciprofloxacin is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (Egerbacher et al., 2000).

2.18.12. Erythromycin

Erythromycin can be considered the prototype of macrolide antibiotics. These drugs inhibit the ribosomal protein synthesis in bacteria and thus have a bacteriostatic and bactericidal effect. Erythromycin has a similar action spectrum as penicillin and includes in particular many gram negative organisms (Carson et al., 1993).

Orally administered erythromycin base and its salts are readily absorbed in the microbiologically active form. Erythromycin is largely bound to plasma proteins. After absorption, erythromycin diffuses readily into most body fluids. In the absence of meningeal inflammation, low concentrations are normally achieved in the spinal fluid but the passage of the drug across the blood-brain barrier increases in meningitis. Erythromycin crosses the placental barrier, but fetal plasma levels are low. The drug is excreted in human milk. Erythromycin is not removed by peritoneal dialysis or hemodialysis (Latare and Setness, 1989; Seppala et al., 1992).

2.18.13. Streptomycin

To reduce the development of drug-resistant bacteria and maintain the effectiveness of streptomycin and other antimicrobial drugs, streptomycin should be used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria (Hinshaw and Feldman 1945).

Streptomycin is a bactericidal antibiotic in therapeutic dosage. The mode of action is the interference with normal protein synthesis and production of "faulty proteins". Streptomycin is active against susceptible strains of many gram-negative organisms. When used alone, bacterial resistance has been shown to develop rapidly
Gentamicin is an important agent for the treatment of many serious gram-negative bacillary infections. It is the aminoglycosides of first choice because of its low cost and its reliable activity against all but the most resistant gram-negative aerobes (Kaye et al., 1974; Riff and Jackson 1971).

Gentamicin is a broad spectrum aminoglycoside antibiotic. Aminoglycosides work by binding to the bacterial 30S ribosomal subunit, causing misreading of t-RNA, leaving the bacterium unable to synthesize proteins vital to its growth. Aminoglycosides are useful primarily in infections involving anaerobic, gram-negative bacteria. Aminoglycosides like Gentamicin "irreversibly" bind to specific 30S-subunit proteins and 16S rRNA. Specifically Gentamicin binds to four nucleotides of 16S rRNA and a single amino acid of protein S12. This interferes with decoding site in the vicinity of nucleotide 1400 in 16S rRNA of 30S subunit. This region interacts with the wobble base in the anticodon of tRNA. This leads to interference with the initiation complex, misreading of mRNA so incorrect amino acids are inserted into the polypeptide leading to nonfunctional or toxic peptides and the breakup of polysomes into nonfunctional monosomes (Jao and Jackson, 1964; Riff and Jackson 1971).

Tobramycin is in a group of antibiotics called aminoglycosides. Tobramycin fights infections that are caused by anaerobic bacteria. Tobramycin is used to treat bacterial infections of the stomach. Tobramycin is rapidly absorbed following intramuscular administration. Peak serum concentrations of Tobramycin occur between 30 and 90 minutes after intramuscular administration. Tobramycin act by inhibiting synthesis of protein in bacterial cell. Tobramycin acts by inhibiting synthesis of protein in bacterial
cells. In vitro tests demonstrate that Tobramycin is bactericidal (NCCLS, 1993).

2.18.16. Amikacin

Amikacin is an aminoglycoside antibiotic used to treat different types of bacterial infections. Amikacin works by binding to the bacterial 30S ribosomal subunit, causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth (Edson et al., 1999; Begg et al., 1995).

Amikacin has high resistance against bacterial inactivation. It resists attacks by most bacterial inactivating enzymes, this is accomplished by the L-hydroxyaminobuteryl amide (L-HABA) moiety attached to N-3 which inhibits acetylation, phosphorylation and adenylation in the distant amino sugar ring (C-2,C-3,C-4). To prevent the development of bacterial resistance to this very powerful antibiotic, its use is tightly regulated (Bauer and Blouin, 1983; Chow et al., 1982).

2.18.17. Netilmicin

It is not easily inactivated by aminoglycoside-inactivating enzymes produced by bacteria which have become resistant to gentamycin or tobramycin. It is less cytotoxic and nephrotoxic than other drugs. It is given in doses of 2-7 mg/kg per day. Netilmicin is useful for the treatment of serious infections due to susceptible Enterobacteriaceae and other aerobic gram negative bacilli. It has shown to be effective against certain gentamycin-resistant pathogens (Carpenedo et al., 1971; Furlanut et al., 1977).