REVIEW ARTICLE

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Current Concepts and Drug Therapy of *Helicobacter Pylori* Infection

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Helicobacter pylori first identified in 1906 in the human gastric mucosa has now been established in the etiology of belching, indigestion, chronic superficial gastritis, chronic peptic ulceration, adenocarcinoma of stomach, primacy gastric non-Hodgkins lymphoma, low grade B cell mucosa associated lymphoid tissue lymphoma (MALT lymphoma).This article discusses the current concepts about the involvement of the bacteria including the pathophysiology and drug therapy for the infection.

Traditionally, the stomach was considered to be a sterile organ, due to the presence of gastric acid bathing the mucosa. It was thought that peptic ulcers occur due to the loss of endogenous balance between the aggravating factors (acid, pepsin) and the local mucosal defenses (secretion of bicarbonates, mucus and prostaglandins) that may occur due to various factors as emotional stress, dietary status (eating spices, irritants), hormonal status (gastrin stimulants) and external stimulus (smoking, alcohol, caffeine and NSAIDS). All these factors modify the gastric acid secretions and gastric defense against acid. But the relapse rates of duodenal ulcers were found to be very high even after successful healing with the traditional antacid therapy. In response to this hidden cause of ulcer relapse, a pathogen has been traced which is a bacteria called Helicobacter pylori[1].

Today over 50% of the world population is chronically infected by this pathogen[2] and has been implicated in the etiology of belching, indigestion, chronic superficial gastritis, chronic peptic ulceration[3,4], adenocarcinoma of stomach, primary gastric non-Hodgkins lymphoma, low grade B cell mucosa associated lymphoid tissue lymphoma (MALT lymphoma)[5] and laryngitis in certain patients[6]. A recent study from England has suggested that eradication of *H. pylori* may lead to the development of reflux esophagitis in duodenal ulcer patients[7].

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DISCOVERY

H. pylori was first identified in 1906 and rediscovered by Warren at the Royal Perth Hospital in Australia in 1979 while studying the gastric biopsies. At the same time Berry Marshal (internist) started taking interest on the same and pursued the link between *H. pylori* and gastric disorders[8,9]. In 1983, he first presented his theory at a gathering of Infectious Disease Specialists at Brussels. But his theory was not accepted because nobody was ready to believe that the gastric juice that has digestive enzymes and hydrochloric acid, that can dissolve iron nails, can harbor bacteria for decades together. To convince people, in 1984, he started work at free mental hospital in Perth by taking a broth containing millions of *H. pylori.* On the first week, he did not develop any symptoms but in the second week, his breath was foul smelling and endoscopy results were positive (showing gastric inflammation) and proved gastric inflammation associated with *H. pylori.* Now, NIH AIDS CONSENSUS report on *H. pylori* in 1994 has declared that it is necessary to diagnose and eradicate *H. pylori* in every ulcer patient. The strange discovery of Marshal has changed the therapy of peptic ulcers from antisecretory drugs to antibiotics.

MICROBIOLOGY

H. pylori is a gram negative, spiral shaped organism with a smoother outer coat with 4-6 bulbous tipped

sheathed flagella at one end, which help it to penetrate the mucosa and colonize on the surface of gastric antrum. An important factor of the bacterium is the production of a protease that aids its movements through mucus by the digestion of mucus and decrease of local viscosity[10]. It has some characteristics similar to the genus *campylobacter* e.g. similar morphology, media requirements, antibiotic sensitivity and DNA base content that led to its earlier nomenclature as *Campylobacter pylori*. But some properties like urease property, multiple sheathed flagella, atypical protein content and unique fatty acid profile are different from the genus *compylobacter* and responsible for its nomenclature as *Helicobacter pylori*[11]. (Fig. 1).

It naturally infects humans and monkeys. It is non-invasive, living in the mucus that overlies the gastric type mucosa and some adherent to mucosa[5]. Its strong urease property causes the release of ammonia which provides the environment of increased pH in its surroundings, enabling the organism to survive in highly acidic gastric atmosphere; thus playing a role in the colonization and maintenance of infection[12,5]. Increase in pH is favorable for replication. *H. pylori* is able to survive at the pH of 2.3 but replicate at pH 4.3[13]. Marshal first reported the presence of presence of *H. pylori* in gastric mucosa, but now, it has been detected in feces, saliva, dental plaques and also in drinking water[14].

At present, there are nine identified species of the Helicobacter genus and all excluding *H. pylori* are of animal origin[15]. These animal helicobacters are of considerable importance to produce intense colonization and inflammation of stomach[16] to study the antibacterial effect of single or combination of drugs and vaccines against *H. pylori*.

EPIDEMIOLOGY

H. pylori infection is present in 90-95% of the patients with duodenal ulcer and 70% of those with gastric ulcer. Remaining 30% of the ulcers are attributable to the use of NSAIDS. There is more likelihood of dyspepsia, peptic ulcers and erosions in patients on long term NSAIDS when *H. pylori* is present than when it is absent[17]. In gastric ulcers with *H. pylori,* NSAIDS may lead to bleeding but not duodenal ulcers[18]. The presence of infection varies with age. It is about 50% in 60 year old and 25% in 30 year old. Most spontaneous infections are thought to be acquired in childhood. The individual's age

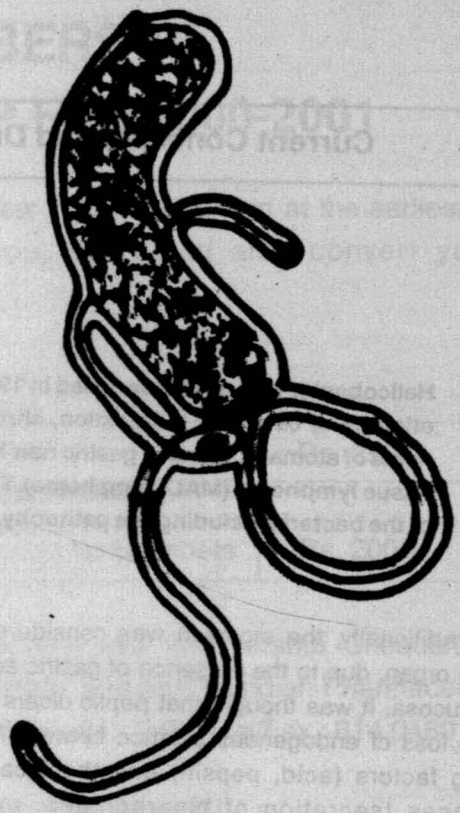

Fig. 1 : Electron micrograph of *H. pylori*.

at acquisition of infection may also influence the pattern of infection in the stomach and the pattern of disease. Infection during early childhood may lead to gastric ulcers and gastric carcinoma while infection later in childhood may lead to antral predominant gastritis and duodenal ulcers[5]. Because of the long time required for superficial gastritis to progress to atrophic gastritis, a significant delay in the acquisition of infection might allow the individuals to live out their life span before the development of cancer[19].

Other risk factors for infection include low income, poor hygiene, low education level and overcrowding in childhood. Habits of the host may also influence the infection. While smoking does not show any significant effect on infection, alcohol has been shown to decrease and coffee to increase the infection[20].

Some bacterial strains are more likely to cause duodenal ulcer disease while other to promote the develop-

ment of gastric cancer. Differences in *H. pylori* related diseases prevalent in different geographical areas might be caused by differences in predominant *H. pylori* strain circulating in that region[19].

TRANSMISSION

Various modes of transmission for *H. pylori* have been postulated

1. Feco-oral: mainly responsible in developing countries[21].
2. Oral-oral: pylori has been detected in dental plaques, saliva and gastric juices of infected subjects[22]. Dental plaques may lead to reinfection after eradication from gut because the bacteria may not be eradicated from plaques after antibiotic treatment.

Other oral-oral routes include kissing or feeding the children with pre-masticated food at the time of weaning[23]. Communal eating habits and use of chopsticks are also sometimes responsible[24].

3. Gastro-oral transmission : It can be through vomiting or regurgitation[25]. Occupational transmission to endoscopist and nurses can also be responsible.
4. Intrafamilial spread through intrafamilial contact or through spouse[26,27].
5. Between institutionalized patients because of overcrowding and/or poor hygiene or patient to patient through contaminated endoscopes.
6. Environmental : Spread of infection through contaminated food and water[28].

MAIN LINES EVIDENCE FOR CAUSAL ROLE OF *H. pylori*

Certain facts that prove *H. pylori* to be involved in the etiology of above-mentioned disease.

1. Presence of infection is a risk factor for development of ulcers.
2. Ulcers do not develop in the absence of infection except in cases with other known etiological factors such as treatment with NSAIDS.
3. Cure of infection results in a dramatic drop in the rates of ulcer relapse (80 to 15%) in one year and lower rates thereafter.
4. Experimental infection of gerbils and mice causes gastroduodenal injury[5].

PATHOPHYSIOLOGY

H. pylori infection is the main etiopathogenic agent responsible for inflammatory and ulcerative changes in gastroduodenal mucosa and the basis for both the intestinal and diffuse type of gastric carcinoma. Various theories have been put forward to explain the pathogenesis of peptic ulcers. According to one theory, it causes stepped pathogenesis of peptic ulcer.

First consequence of *H. pylori* infection is acute neutrophillic gastritis which is short lived (7-10 days) with mild clinical symptoms and is usually overlooked by the patient. Following ingestion, the bacteria penetrates the gastric mucosa, multiplies gets entry into lamina propria through endocytosis, provokes an intense polymorph response, causes epithelial degeneration including mucin depletion and cellular exfoliation eventually causing ulcerogenesis[29]. Degenerative changes are mediated by both direct (bacterial toxin) and indirect (inflammatory responses) mechanisms.

Direct toxicity : *H. pylori* produces vacolating cytotoxin[30], ammonia and ammonium products[31], phospholipase[32], chemotoxins and PAF[33], which act on epithelial cell surface and damage the defense system. Pathological importance of vacolating cytotoxin is controversial[34]. That some persons develop an overt disease whereas others do not, may be due to bacterial factors, host factors (including age) and environment.

Bacterial factors i.e. a cytotoxin, vacA and a high molecular weight protein cag A are important. The cytotoxin causes vacolation of epithelial cells and gastric damage in mice. Only about 50% of strains of *H. pylori* have cytotoxic activity detectable *in vitro*, although essentially all strains possess the gene vacA encoding the toxin. Allelic variants of gene associated with different levels of cytotoxic activity have been determined. Patients with ulcers are more likely to be infected with strains with detectable cytotoxin activity and the sla allele of vacA is an even better marker of ulcer disease. The gene encoding cagA protein cagA is one of the 20 genes making up the cag virulence island. Another gene important in the induction of inflammation is pic B which is believed to permit the induction of cytokines[5].

The urease property of *H. pylori* leads to formation of ammonia and different ammonium products as mono-N-chloramine, because of the interaction of ammonia with hypochlorous acid, which act as a potent cellular toxin[31]. *H. pylori* occasionally found in the duodenum, residing in

the islands of gastric mataplasia, may be acquired as a consequence of increased acidity in the duodenum or as a congenital tissue rest[35]. Normally *H. pylori* is inhibited by bile, therefore it should not grow well in the duodenum. But bile acids are precipitated at low pH. This low pH may be because of hypergastrinemia (genetically determined or acquired through stress or smoking) and to increased responsivity of the parietal cells mass to gastrin. The number of antral G cells producing the gastrin is unchanged, but the number of D cells producing somatogstatin is decreased. The reduction of feedback by somatostatin leads to increased gastric acidity which gives rise to duodenal gastric mataplasia, which gets infected with *H. pylori*[5]. The bacteria then split urea, which facilitates back diffusion of hydrogen ion thereby producing inflammation and eventually focal ulceration (Fig. 2)[36].

Antral gastritis is because of increase in plasma gastrin, increased mucosal cell proliferation because of increased expression of EGF and TGFα. Eradication of *H. pylori* leads to decrease in plasma gastrin but not EGF and TGFα suggesting that they may be involved in ulcer healing[37].

Urease also stimulates dose-dependently the production of IL-1β, IL-6, IL-8, TGFα peptides and mRNA. This local production of cytokines by urease stimulates mononuclear phagocytosis and this may play a central role in the development of gastroduodenal ulceration.

Phospholipase C and phospholipase A_2 produced by *H. pylori* disrupt the normal phospholipid layer, alter the integrity of epithelial cell and by releasing arachidonic acid also forms leukotrienes and other eicosanoids that alter the permeability of membrane and mucus discharge and exerts their pro-inflammatory effect[32]. *H. pylori* also produces PAF, which leads to ischemia that may affect the integrity of epithelial cells[33].

Indirect inflammatory response : The immune response to *H. pylori* infection includes both, the production of antibody and a cell mediated response, which is ineffective in clearing the infection but leads to inflammation in stomach (pangastritis, which is linked with gastric ulceration and gastric carcinoma) and duodenal bulb (antral predominant, closely linked with duodenal ulceration). Over the years, inflammation may progress to atrophy, intestinal metaplasia, dysplasia and then to carcinoma. Atrophic gastritis may lead to vitamin B_{12} deficiency will associated hematological and neurologic sequel[5].

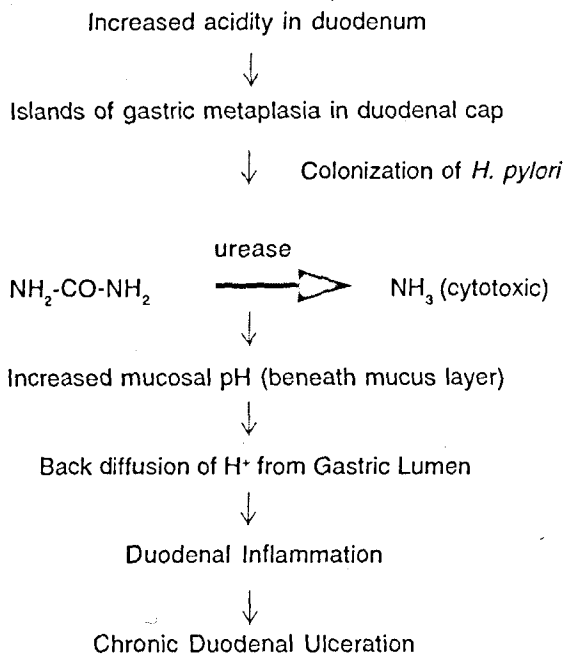

Fig. 2 : Pathogenesis of duodenal ulceration. Hypergastrinemia, increased responsivity of parietal cell mass to gastrin and decrease in the number of D cells producing somatostatin leads to increased gastric acidity in the duodenum, which gives rise to gastric metaplasia that gets infected with *H. Pylori.* The bacteria then splits urea, which facilitates back diffusion of hydrogen ion thereby producing inflammation and eventually focal ulceration.

In fact, when *H. pylori* attaches to the epithelium, it causes the production of various antigens such as lipopolysaccharides (LPS)-endotoxin, 961 kDa heat shock protein, cytotoxin and urease[38]. These antigens are taken up by monocytes present in lamina propria which produces IL-1β, IL-6 and TNFα.

TNFα facilitates the adhesion of leukocytes to endothelium[39]. With IL-1β and IL-6, it stimulates T-helper cells (CD_4) and produces various leukotrienes that include, IL-4, IL-5, IL-6, IL-8, INFγ[34]. IL-4, IL-5, IL-6 along with TNFα stimulate B-cells and produce antibodies IgG, IgA, IgM which recognize the microbial antigen on the epithelial surface and react with them but the increased production of antibodies lead to autodestruction of epithelial cells by antibodies which leads to damage of epithelium.

IL-8, produced by T-cells is a potent activator of neutrophills. Activated neutrophills secrete polymorph protease, superoxide ions, oxygen metabolites[40,41] and leukotrienes, which damage the mucosa and lead to

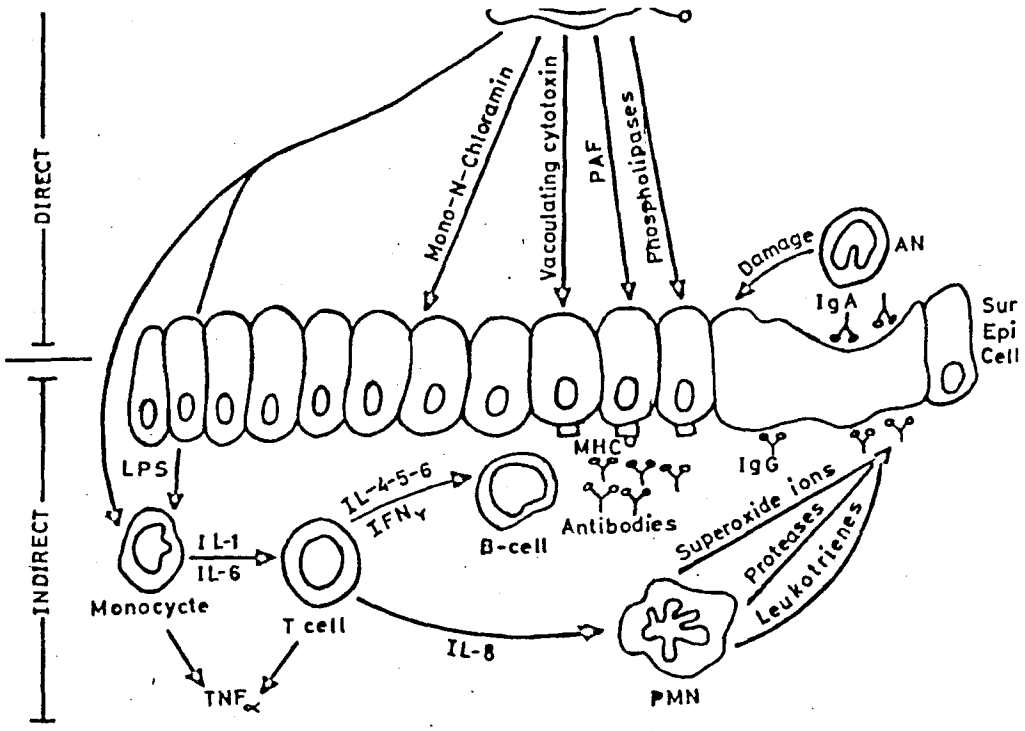

Fig. 3

ulceration. Sulfacone is an effective mucosal protective that inhibits production of vacolating toxins and induction of IL-8 secretion[42]. (Fig. 3).

Another possible mechanism by which *H. pylori* causes gastric carcinoma is that long-term colonization in some patients may lead to the development of chronic atrophic gastritis and hypochlorhydria. Hypochlorhydria may allow bacterial overgrowth and nitrate reduction by the bacteria and the ultimate genesis of nitrosamines, which are, established carcinogens. The WHO has classified *H. pylori* as a group one or definite carcinogen.

In active ulcers, high activity of lipid peroxidation (LPO) and depressed antioxidant defense (AOD) has been found. It is proposed that *H. pylori* may initiate LPO. It is supported by the fact that antihelicobacter therapy decreased LPO hyperactivity and promoted AOD[43]. It is further supported by the observation of negative correlation of *H. pylori* infection with the intake of antioxidants as vitamin C and vitamin E.[44].

It is also postulated that absence of glutathione-S-transferase mu, which impairs detoxification of exogenous carcinogens, predispose some infected individuals to the development of gastric carcinoma[45].

Chronic hepatic encephalopathy is a neuropsychiatric disorder with protean manifestations and with poor understanding of pathogenesis[46]. Raised systemic ammonia concentration has long been implicated in its development. Ammonia produced by *H. pylori* urease in the stomach can be a source of the raised systemic ammonia concentration, frequently seen in patients with liver cirrhosis. Eradication of *H. pylori* is effective in patients with hyperammonemia with diffuse *H. pylori* infection in stomach.

PREVENTION

H. pylori infection is a major public health problem in developing countries and gastric adenocarcinoma is the second leading cause of cancer deaths worldwide. There is certainly a need to prevent the infection. Improvement in living standard[5] and disinfection of water with chlorine can prevent the infection.[47]

Vaccination is another alternative. It acts regardless of antibiotic resistance, prevents the emergence of antibiotic resistant strains (if used in treatment), preserve the value of antibiotics against *H. pylori* with the limita-

tion of high cost and difficulty of patient compliance. Various antigens of *H. pylori* with the ability to confer protection are being studied e.g. urease, cytotoxin vacA, two heat shock proteins HSP A and HSP B and catalase[48,49]. The antigen should be the one, shared by all *H. pylori* isolates without much antigenic variation and should lack intrinsic activity.

Urease is currently the most promising candidate and its importance has been shown by numerous studies in mice, ferrets and non-human primates[50]. In humans, urease, along with heat labile enterotoxin of *E. coli* induced an immune response and a decrease in gastric infection[51].

Recent studies in mice have shown that immunization with genetically engineered bacteria expressing *H. pylori* antigens can protect against gastric helicobacter infections[51]. In an experiment, oral immunization of mice with urease subunit delivered by an attenuated strain[52] or nasal immunization with live salmonella strain[53] induced a specific immune response and protected mice against *H. pylori* colonization.

RESISTANCE

The increased use of antibiotics against *H. pylori* has led to the emergence of antibiotic resistant strains. Efficacy of therapies rarely exceeds 90% and strains isolated after the treatment failure often show resistance. Resistance to clarithromycin is increasing but that to metronidazole is already high, because it has been available for many years before the discovery of *H. pylori*. This development of resistance may lead to enrichment of human reservoir with resistant strains.

Resistance to nitroimidazoles is because of lack of reduction of NO_2 group, which is required for its activity on bacterial DNA. The genetic background for this is not known. Another reason for resistance to metronidazole is due to mutations in the rdxA gene, which encodes a novel nitroreductase that is responsible for the reductive activation of the drug. It is suggested that metronidazole resistant-rdxA mutations may have been selected by prior use of metronidazole against other infections. Also, the products of metronidazole activation are mutagenic and can be demonstrated to increase both, the mutation frequency and the frequency at which antibiotic resistance arises in *H. pylori*. *H. pylori* itself is an early risk factor for gastric cancer; the possibility that its carcinogenic effects are exacerbated by metronidazole use or the reduction of nitroaromatic compounds to toxic, mutagenic and carcinogenic products, may be significant concern in public health[54,55].

Macrolides such as clarithromycin, to be effective, bind tightly to *H. pylori* ribosomes and inhibit protein synthesis. Resistance is because of lack of binding to ribosomes and to a point mutation in position 2058 or 2059 of the 23s ribosomal r-RNA gene (domain V)[56]. Another report showed that A-C transversion mutations at position 2143 could confer resistance to clarithromycin in *H. pylori*[57].

Since metronidazole resistance leads to a lower eradication rate, a non-metronidazole regimen should preferably be used in areas known to have a high prevalence of pretreatment metronidazole resistance. Various regimens are used. Triple combination of omeprazole, amoxicillin and clarithromycin for 10 days is highly effective and superior to combination of bismuth subcitrate, oxytetracycline and clarithromycin[58].

DIAGNOSIS

Early detection of *H. pylori* is a key to effective eradication therapy required for permanent cure and a better quality of life to the patient. Its presence can be detected by a number of tests. These can be divided into invasive and non-invasive tests. Invasive tests are those, which require upper gastrointestinal endoscopy and analysis of gastric biopsy specimen while non-invasive tests do not involve any such procedure.

Invasive tests

1. Colorimeteric urease test is the most convenient test where two antral biopsy specimens are put into a gel containing urea and an indicator. The presence of *H. pylori* urease with minutes (sometimes hours) split the urea and leads to a color change.
2. Histological examination of the biopsy specimens is accurate; special stains as modified Giesma or silver stain allow optimal visualization of bacteria.
3. Microbiological culture is the most specific diagnostic test but is technically demanding. These cultures can also be used to determine antibiotic sensitivity of bacteria. Identity of bacteria can be confirmed by its typical appearance on Gram's staining, positive reaction in oxidase, catalase and urease test[5].

However the endoscopic biopsies shows only the

current infection and may sometimes show false negative results since infection may be patchy in some individuals[59,60,61].

4. Phenol red test: an endoscopic procedure that uses a pH indicator called Phenol red has recently been developed to access *H. pylori* infected areas of gastric mucosa. In this test, areas infected with *H. pylori* can be seen as colored where phenol red turned from yellow to red[62].

5. An inexpensive method involving hemotoxylin and eosin staining of antral tissue is also widely available[63]. However, even after the clearance from the stomach, *H. pylori* may persist in other reservoirs of the body like dental or cardiac, which may not be detected by endoscopic methods.

Non-invasive tests

1. Serological test : it involves the assessment of specific IgG levels in the serum[5]. It is the simplest and the cheapest test but has clinical limitations, as it is unable to differentiate between the current and the recently resolved infection. It is not used to study the response of treatment[64].

2. Urea breath test: it involves the ingestion of labeled urea. If *H. pylori* is present, it breaks urea into ammonia and labeled CO_2 that is measured in the exhaled breath. This test can also be used to determine the outcome of treatment, one month after its completion. Unlike invasive tests, non-invasive tests are not subject to sampling error[5].

3. BM (Boehringer Mannheim) test: It can be used for diagnosis on the spot in the physician's office. It gives a visible yes or no result within minutes from a single drop of blood (20 µl)[64] (see Table 1)

Detection of eradication: According to few studies, a decrease in serum level of IgG antibodies to *H. pylori* and gastrin and an increase in pepsinogen I/II ratio can be used an a predictor for the eradication of *H. pylori* in gastric ulcers[65,66].

TREATMENT

At present, *H. pylori* related duodenal and gastric ulceration and the rare low grade B cell MALT lymphoma are the only clear indications for the treatment. *H. pylori* infection should be treated in patients with ulcer disease whether or not the ulcers are currently active, to reduce the likelihood of relapse. Eradication of *H. pylori* is an important factor for prolonged remission of duodenal ulcers. Routine prophylaxis is not recommended because of the expenses involved, induction of morbidity in otherwise healthy people and the risk of widespread antibiotic resistance[5]. Eradication should be measured one month after the cessation of the treatment[67].

H. pylori is susceptible to many antibiotics *in vitro* but has proved difficult to eradicate *in vivo* because the antibiotics are not delivered to the site of infection in effective concentrations and in fully active form. Following agents are generally used.

1. Antibacterial agents

a. aminopenicillins	Amoxicillin
b. macrolides	Clarithromycin, azithromycin, erythromycin
c. tetracyclines	Tetracycline, oxytetracycline
d. nitroimidazoles	Metronidazole, tinidazole, secnidazole.
2. Proton pump inhibitors	Omeprazole, lansoprazole.
3. Bismuth salts	Colloidal bismuth subcitrate (CBS) Tripotassium dicitratobismuthate (TDB)
4. H_2 antagonists	Ranitidine, famotidine, Ranitidine bismuth citrate (RBC)
5. Cytoprotectives	Sucralafate, tetraprenylacetone, plaunotol
6. Active oxygen species inhibitors	Rebamipide

Ideally, the treatment of *H. pylori* should give 90% eradication, the therapy should be simple, of short duration, low cost and effective. Various eradication regimens used include monotherapy, dual therapy, triple therapy and quadruple therapy[68].

Monotherapy

Following an exposure to bismuth preparations, *H. pylori* detaches from the gastric epithelium and lyses with 30-90 min[69]. However, relapse is very common with this alone[70,71]. So, short-term treatment with this is inadequate and the chronic use is avoided because of bismuth toxicity and bismuth encephalopathy[72]. Other agents used in monotherapy are :

TABLE 1 - COMPARISON OF VARIOUS DIAGNOSTIC TESTS

Test	Sensitivity	Specificity	Advantage	Disadvantage
Colorimeteric	85-95%	95-100%	Quick, simple, inexpensive	—
Histology	85-90%	95-100%	Widely available	Skill is required
Hematoxylin & eosin staining	93%	87%	Inexpensive	Inferior to geisma, genta or silver stains
Serology	85-95%	80.95%	Cheap, convenient, precise, quick	Cannot be used for early follow up
Urea breath	—	—	Safe, cheaper than endoscopy	Low dose of ^{13}C required

		Eradication rate
1.	Amoxicillin	20%
2.	Erythromycin	20%
3.	Clarithromycin	54%
4.	Ranitidine-bismuth citrate	2%

Dual therapy

Since single therapy is generally inadequate, use of combination therapy of bismuth with other antibiotics is indicated.

		Eradication rate
1.	TDB+Amoxicillin[73]	40%
2.	TDB+metronidazole[74]	80%
3.	Clarithromycin+omeprazole	58-83%
4.	CBS+omeprazole	30-40%
5.	Amoxicillin+omeprazole	72-84%
6.	CBS+erythromycin	40-60%
7.	Ranitidine bismuthcitrate+amoxicillin	40-60%
8.	Ranitidine bismuth citrate+clarithromycin	82-94%

Ranitidine bismuth citrate 400 mg b.d. and clarithromycin 250-500 mg q.i.d both for 2 weeks, or the above regimen followed by ranitidine bismuth citrate 400 mg b.d. for 2 weeks gives *H. pylori* eradication rates equivalent to clarithromycin plus amoxicillin plus pantoprazole combination for 7 days[75], ranitidine bismuth citrate plus clarithromycin plus metronidazole for 7 days[76] and 7 day PPI-triple therapies[77]. Thus, ranitidine bismuth citrate plus clarithromycin is a simple, convenient and well tolerated dual therapy regimen that is effective in eradicating *H. pylori* and healing duodenal ulcer in patients infected with *H. pylori*[78]. The eradication of *H. pylori* in patients with healed ulcers significantly reduced the rate of ulcer relapse. However, the triple regimens have the advantage of being shorter.

Triple therapy

It is still more effective and 96% of patients show eradication of the organism with TDB, metronidazole and tetracycline combination[79]. Amoxicillin can effectively be used in place of tetracycline. This triple therapy still represents a standard reference therapy for many authors, with the advantage of low cost and high efficacy. However, emergence of resistant strains, complexity of treatment (10-12 tabs/day), numerous adverse drug reactions and nonavailability of bismuth salts has led to elaboration of other combinations[68].

		Eradication rate
1.	Omeprazole+clarithromycin+ Amoxicillin[80]	96.6% (12 days)
	(for those resistant to metronidazole)	78.3% (6 days)
2.	Omeprazole+clarithromycin+ tinidazole[80]	82.2% (6 days)
3.	Lansoprazole+amoxicillin+ clarithromycin[81]	92-94%
4.	Pantoprazole+amoxicillin+ metronidazole[82]	85%
5.	Lansoprazole+azithromycin+ tinidazole[83] (ultrashort therapy for 3 days)	80.8%
6.	Lansoprazole+azithromycin+ rebamipide[84]	75%
7.	Omeprazole+amoxicillin+	83.4% (4 weeks)

	plaunotol[85]	100% (3 weeks)
8.	Omeprazole+clarithromycin+ metronidazole	85.3%
9.	Amoxicillin+omeprazole+tinidazole	62.5%
10.	Omeprazole+clarithromycin+TDB	80.6%
11.	Tetracycline+TDB+metronidazole	77.3%
12.	CBS+metronidazole+tetracycline	70%
13.	Omeprazole+azithromycin+ metronidazole	72%

The mechanism by which omeprazole contributes to *H. pylori* eradication is unclear. A direct inhibitory effect is discussed[86]. Another explanation is that it increases the gastric pH towards the pk_a of the given antibiotic[87], which reduces the intragastric MIC of the antibiotic. Antral colonization of *H. pylori* is also reduced. However, according to one study, inhibition of the gastric acid secretion is not necessary to improve the effect of amoxicillin on the cure rates of *H. pylori* infection in patients with duodenal ulcer[88].

It is also proposed that eradication of *H. pylori* leads to decrease in the rise of gastrin during subsequent long-term omeprazole treatment, therefore patient should be rendered *H. pylori* negative prior to commencing a long term treatment with a proton pump inhibitor[89].

Quadruple therapy

It has the advantage of shorter duration of treatment and thus the reduced cost and a lower potential for development of antibiotic resistance[90]. Various regimens are:

		Eradication rate
1.	Omeprazole+CBS+tetracycline+ metronidazole[91]	98%
2.	Omeprazole+amoxicillin+clarithromycin+ metronidazole[90]	96%
3.	Omeprazole+amoxicillin+TDB+ metronidazole (with fewer side effects)	95%

However, compliance problems are sometimes encountered. Triple regimen (for 7 days) is more popular because of high eradication rate, better compliance and shorter therapy.

Based on efficacy, proton pump inhibitor (PPI) triple therapy or bismuth triple therapy are recommended as the first line of treatment for *H. pylori* infection and quadruple therapy as the second line of treatment for the eradication of initial failures and in case of metronidazole resistance[92]. Inspite of such efficacious regimens reported, the FDA has approved only 3 regimens[93]

1. Omeprazole +clarithromycin.
2. Ranitidine bismuthcitrate+clarithromycin.
3. Bismuth subsalicylate+metronidazole+ tetracycline+ranitidine.

Also the low-grade gastric MALT lymphoma is associated with *H. pylori* and eradication of *H. pylori* can produce histological regression of lymphoma. Some studies in rats report that methionine can significantly attenuate monochloramine enhanced gastric carcinogenesis[94].

Recent Advances

Rifaximin, a surface antibiotic (not absorbed orally) given with amoxicillin and omeprazole shows a reasonable level of effectiveness in eradication of *H. pylori* but does not differ from amoxycillin-omeprazole combination[95]. Tetraprenylacetone, a cytoprotective has been reported to decrease the reoccurrence of ulcers when given with omeprazole[96]. Emodin decreases the growth of *H. pylori* and damages the DNA of bacteria[97]. It inhibits the arylamine-N-acetyltransferase activity in strains of *H. pylori*[98].

HSP 60 on the cell surface of *H. pylori* is implicated in the requirements for the growth of bacteria. Monoclonal antibody developed against HSP 60 i.e. H20mAb has been found to bactericidal[99]. It is proposed that the urease inhibitors might have a therapeutic potential for Helicobacter infections. Fluorofamide did not show successful results[100]. Acetohydroxamic acid, a potent specific urease inhibitor remarkably inhibits ammonia production and LDH release in a dose dependent manner[101].

Rebamipide protects against activation of neutrophills by *H. pylori* by inhibiting the surface expression of CD_{18} on the neutrophills and by inhibiting the production of active oxygen species from neutrophills[102,103]. With lansoprazole and amoxycillin, it shows eradication rates of 75%.

Recently, it has been proposed that nitrates under acidic conditions can kill *H. pylori in vitro* in a dose dependent manner. Addition of nitrite (1 mM) to acidic solutions (pH 2) results in complete killing of *H. pylori* within 30 minutes of exposure time, whereas acid alone allows the organism to survive. Acidification of nitrate causes

generation of reactive intermediates of nitrogen that are cytotoxic[104].

$$NO_2^- \longrightarrow HNO_2$$

$$2\,HNO_2 \longrightarrow H_2O+N_2O_3$$

$$N_2O_3 \longrightarrow NO+NO_2$$

Even in the presence of urea, nitrites can completely kill *H. pylori* under acidic conditions (pH 2). Nitric oxide inhibits respiratory chain enzymes through inactivation of iron-sulfur complexes and disrupts DNA replication by inhibiting ribonucleotide reductase.

CONCLUSIONS

With the rapid progress in the last two decades in the knowledge of pathophysiology of *H. pylori*, a wide range of new therapeutic regimens have been discovered for the gastropathy caused by this organism. Prevention against infection is the first step that can be taken in the areas with high prevalence of *H. pylori*. Genetic engineering techniques have shown good results in this direction. Early detection of *H. pylori* is a key to effective eradication therapy required for permanent cure. Dual therapy with ranitidine bismuth citrate plus clarithromycin has shown very good eradication rates and ulcer healing profiles, but still triple therapy is preferred being of shorter duration. The indiscriminate use of antibiotics all over the world (especially metronidazole) for various problems and against *H. pylori* has led to the emergence of antibiotic resistant strains. The classic antibiotic therapies are certainly far from obsolete and the marketing of newer drugs in the near future is likely to provide a significant step forward in the treatment of infection. Biotechnological approach lies several years in the future, but the initial studies now being performed are clear signposts for the direction to be followed.

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