Chapter 4

Functional Dyspepsia and *Helicobacter pylori* Infection

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Additional information is available at the end of the chapter

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1. Introduction

Helicobacter pylori (*H. pylori*) was first observed over 100 years ago yet its association with clinical diseases was not fully understanding until 1982 when Marshall and Warren identified and subsequently cultured the gastric bacterium. At their first attempt to culture the bacteria was not successful. Colonies finally grew when they accidentally left some culture plates over the Easter holiday. Dr. Barry Marshall subsequently inoculated himself with culture broth containing more than 1 billion organism to prove that this bacterium would cause peptic ulcers supporting Koch's postulate. He developed acute gastritis 1 week after the inoculation. *H. pylori* is a microaerophilic, spiral shaped, gram negative bacterium measuring about 3.5 microns in length and 0.5 microns in width. In vitro, this bacterium is a gradually growing organism that can be cultured on blood agar incubated at 37ºC in a microaerophilic condition (5% oxygen) for 4-7 days. The colony of this bacteria is tiny, uniformly sized and translucence (fig 2A).

H. pylori is a Gram-negative, spiral shaped, bacterium about 3.5 microns long and 0.5 microns wide. (fig 2B). This bacterium uses its 2-7 unipolar flagella to escape the harsh luminal acidity by burrowing into the mucus layer that covers the gastric mucosa and so reside in close proximity to the more neutral pH of the epithelial cell surface of the gastric mucosa. It can convert from a highly motile, helical (spiral) shape to a more dormant coccoidal form, perhaps a survival benefit depending upon its local environment. Being microaerophilic, *H. pylori* requires oxygen. *H. pylori* is biochemically characterized as positive for catalase, oxidase, and urease. The urease enzyme, which has been located on the surface of the bacteria, is important and likely to be vital for bacterial survival and colonization in the highly acidity milieu of the stomach. Urease breaks down the luminal urea normally produced by the gastric mucosa, yielding carbon dioxide and ammonia.

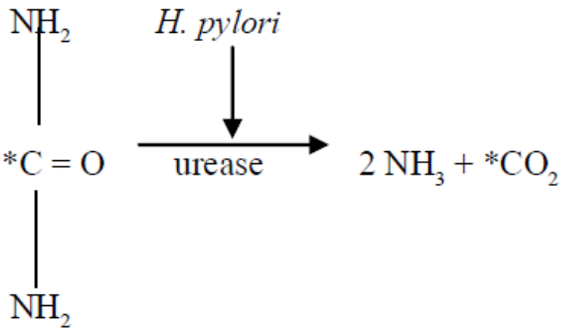

Figure 1. or 14C-urea is hydrolyzed by the *H pylori* urease enzyme and can be detected by CO2 in breath samples

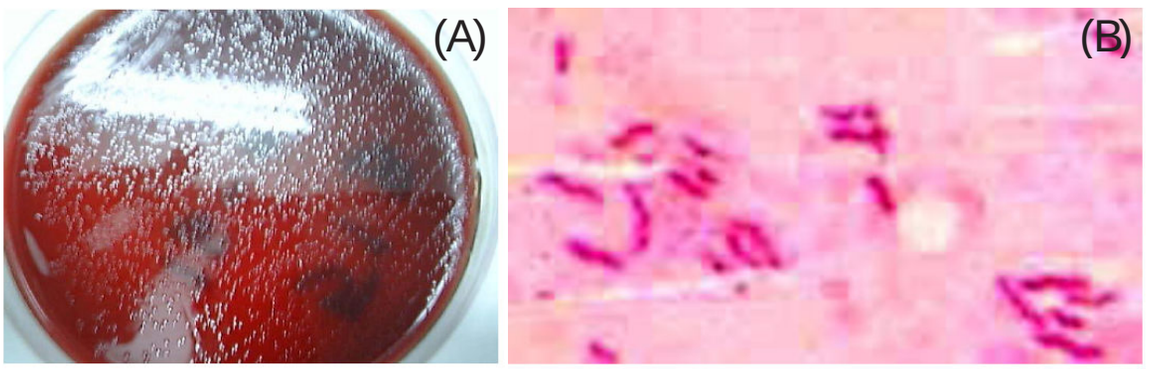

Figure 2. A) *H. pylori* colonies; (B). *H. pylori* detected by gram stain

Ammonia then accepts a proton (H^+), lessening the nearby acidity and forming protective surroundings that allow its survival. Furthermore, urease activity is clinically relevance in the form of several tests to diagnose infection such as rapid urease test and urea breath test. [1-4] The ammonia produced however is toxic to the epithelium, and aided by other products like proteases, vacuolating cytotoxin A (associated with cytotoxin-associated gene A), and certain phospholipases damages the mucosa. *H. pylori* infection also increases gastric acid secretion (suppressing somatostatin to allow increased gastrin), down regulates mucosal defense mechanisms and elicits an inflammatory response.

H. pylori infects the gastric mucosa in 20-80 % of humans throughout the world, making it a very common bacterial infection. In developing countries, the infection tends to be acquired via the fecal-oral or oral-oral route during childhood and subsequently persists through adulthood. In developed countries, childhood infection is no longer common (rare under 10 years of age) though prevalence does increase during adulthood (>50% if over 50 years); the latter cohort likely acquired H. pylori during childhood. This bacteria is the major pathologic agent in the development of gastritis, gastric ulcer, duodenal ulcer, MALT lymphoma and gastric cancer. The International Agency for Research into Cancer has classified *H. pylori* as a class 1 carcinogen which is in the same class as cigarette smoke. [1-3]

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Functional dyspepsia (FD) is one of the most common causes of dyspeptic symptoms. FD is recognized as heterogeneous group of symptoms located in the center of upper abdomen. The prevalence of dyspepsia is variable in different populations and environmental factors. In 2006 the Rome III provides the diagnostic criteria, which are included one or more of the following [5, 6]

1. Bothersome postprandial fullness
2. Early satiation
3. Epigastric pain
4. Epigastric burning

The FD patient must not have any evidence of structural disease to explain the dyspeptic symptoms. Symptom onset should occur at least 6 months prior to diagnosis while the criteria must be fulfilled for the last 3 months. FD also can be divided into two major syndromes: the postprandial distress syndrome and the epigastric pain syndrome. The postprandial distress syndrome type of FD constitutes bothersome postprandial fullness and early satiation, occurring after meal and at least several times per week. Upper abdominal bloating or postprandial nausea or excessive belching might be present. In contrast, the epigastric pain syndrome type of FD mostly suffers with intermittent epigastric pain or burning at least once a week. The pain should not refer to other abdominal or chest regions, and should not be relieved by defecation of passage of flatus. [5]

2. Epidemiology of *H. pylori* infection and FD

H. pylori is a global bacterial infection. Its prevalence varies greatly from 10-80% between countries, being quite elevated in developing countries in Asia, Africa, and South America but rather low in North America and Western Europe. In developed countries, approximately 20% of the population under the age of 40 years and 50% of those over the age of 60 years carry the infection. [6]

The prevalence of *H. pylori* infection also varies depending on age, socioeconomic status, sanitation and ethnic group [4, 7-9]. Typically, the infection is acquired in childhood before the age of 10 and the rate of acquisition is related inversely to household hygiene and the general levels of sanitation; wherever sanitation and standards of living have improved, the incidence of transmission has declined. The low prevalence in middle and upper socioeconomic populations in Western Europe and North America reflect better sanitation and quality of living. In the United States, the prevalence rate is approximately 50% in African Americans, 60% in Mexican Americans, and 26% in whites. [4] In developing countries, the prevalence among adult people is between 50-80%.

H. pylori infection may be evident in 20-60% of patients with functional dyspepsia, but the clinical relevance in most instances is confounded by the background frequency of this bacteria in the general population. A large scale nationwide community-based endoscopic

survey of 2,488 adult subjects identified an overall *H. pylori* infection at 40.2% that was no different in dyspeptic subjects compared to asymptomatic persons. Differences amongst geographic regions likely related to differences in socioeconomic status and community hygiene during childhood period. [8] The frequency of functional dyspepsia is common in Asia, varying between 8-23% in most reported studies. [10] In fact, given the common frequency of *H. pylori* infection and challenges in obtaining endoscopy to eliminate organic causes of dyspepsia, it is difficult to discern the extent this microorganism is the basis for dyspepsia in Asia. [10, 11]

There are many FD patients in Asian as well as Western countries. The reported prevalence of *H. pylori* infection in patients with FD varies from 39% to 87%. [14] Several epidemiological studies have shown that *H. pylori* infection occurs more frequently in FD than in matched control populations. A meta-analysis published in 1999 reported a summary odds ratio for *H. pylori* infection in FD of 1.6 (95% CI, 1.4 to 1.8). [15]

3. Pathogenesis of functional dyspepsia associated with *H. pylori* infection

The pathophysiological disturbances generally responsible for the dyspepsia focus on hyperacidity, impaired gastric accommodation (the "stiff fundus") and delayed gastric emptying. FD patients who are infected with *H. pylori* have higher stimulated gastric acid secretion than *H. pylori*-negative healthy volunteers. [16] Impaired accommodation to a meal may be common in functional dyspepsia and early satiety, but is not particularly associated with *H. pylori* positivity or delayed gastric emptying. There is no constituent disturbance of sensory or motor function yet reported in H. pylori-infected persons. Another factor possibly responsible for the dyspepsia associated with H. pylori infection is the gut hormone, ghrelin. Secreted from oxyntic cells, ghrelin normally stimulates gastric motility and food intake. Patients with *H. pylori* may have reduction in ghrelin secretion that might lead to impaired gastric emptying and symptoms of postprandial dyspepsia.

Recent study demonstrated that metronidazole resistant strains of *H. pylori* infection were significantly higher in PDS than those of EPS patients. This study also indicated more specific of *cagA* genotype that presence of *cagA 2a* gene of *H. pylori* infection was significantly higher in metronidazole resistant than those of metronidazole sensitive strains especially in EPS patients. This finding might be helpful to identify metronidazole resistant by using *cagA* genotype in dyspeptic patients. [17]

CagA is a highly immunogenic protein encoded by the *cagA* gene, located at end of the *cag* pathogenicity island (PAI). Infection with *cagA*-positive strains was associated with a greater inflammatory response and an increased risk of adverse clinical outcomes than with *cagA*-negative strains. [7, 18-20] Taneike et al recently reported that the metronidazole resistant rate in *cagA* negative group was significantly higher than in *cagA* positive group and suggested that absence of *cagA* might be a risk factor in development of metronidazole resistance. [21] Unlike many countries such as European countries and United State of America, nearly all of *H. pylori* strains in Thailand possess *cagA*-positive strains. [16] These different results might be

explained by variation in *cagA* between the Asian- and Western-types. *CagA* genotype can be divided into *cagA 1a* and *2a* [17] and *cagA 1a* strain of *H. pylori* demonstrated more virulence and associated with more gastric inflammation due to activation of proinflammatory cytokines such as increased production of IL-1β and IL-8 in the gastric mucosa. [21] Previous meta-analysis study reported that *cagA*-positive strain increases the likelihood of successful eradication. [22] The mechanism for the effect of *cagA* on eradication outcome might be explained by the presence of *cagA* induces secretion of inflammatory cytokine in gastric epithelial cells and increased gastric inflammatory response. [22] Consequently, the increase blood flow may help in the diffusion of antibiotics. [23] Another possibility might be explained by the density of *H. pylori* in gastric mucosa which has been reported to be higher in *cagA*-positive strains than *cagA*-negative strains, thus *cagA*-positive strains might be proliferative faster than *cagA*-negative strains. [24, 25] As antibiotics are more active on rapidly growing bacteria, *cagA*-positive strains would be more susceptible to antibiotic activity [23].

The effect of *H. pylori* eradication on dyspeptic symptoms in FD patients has revealed inconclusive results in several studies, both in developed countries and in Asia. [26, 27, 28, 29] Dyspeptic patients who are infected with *H. pylori* often have functional dyspepsia rather than peptic ulcer disease, yet the outcome of eradicating *H. pylori* infection may be suboptimal in FD compared with that for established duodenal ulcer disease. [30] Nevertheless, at a population level, a Cochrane systemic review indicated that there was a 10% relative risk reduction of persistent symptoms in the *H. pylori*-eradication group compared to placebo; the number needed to treat to cure one case of dyspepsia was 14. [31] A recent meta-analysis of the Chinese literature showed that dyspepsia symptoms in FD improved after *H. pylori* eradication with an odds ratio of 3.61, suggesting that this infection might have a greater role in Asian than in Western countries. [32] Thus, *H. pylori* eradication overall does improve dyspepsia, particularly in regions with high prevalence.

4. *H. pylori* diagnostic tests in FD

Tests to diagnosis *H pylori* infection are divided into those that are invasive requiring endoscopy versus those that are noninvasive, not requiring endoscopy. The choice of test depends on issues such as cost (variable in each country), availability, clinical situation, prevalence of infection, pretest probability of infection, and presence of confounding factors (eg, the use of PPI and antibiotics) that may influence test results.

a. Noninvasive tests for *H. pylori*

The noninvasive tests available in clinical practice include serologic tests, urea breath tests, and stool antigen tests. The choice of test is important in terms of validity

1. Serological tests

IgM and IgA antibody tests have not proven to be useful clinically, whereas anti–*H pylori* IgG has a better result. anti–*H pylori* IgG usually can be detected by 3-4 weeks after infection. The

three mains methods of commercial kits are ELISA ($90–$95/correct diagnosis),, immunochromatography, and Western blotting.

Most serologic tests carries a high sensitivity (~90 to 100%), but variable specificity (under 85-90%). Their positive and negative predicative values depend upon the background prevalence of *H. pylori* infection in the population at risk. In areas where infection is common, a negative test is likely to be a false negative. Conversely, a positive test amongst those in whom *H pylori* is infrequent is more likely to be a false positive. In developed countries with low prevalence of *H. pylori* infection (<20%), for example, a positive serological test signals active infection only about half the time. Hence, serology should be validated locally. Further, antibody tests can remain positive for years after *H pylori* eradication and have limited value to confirm eradication of *H pylori* infection4.

2. Urea breath test (UBT)

The urea breath test provides a reliable noninvasive method for *H pylori* detection with sensitivity and specificity of 88-95% and 95%-100% respectively. [33] Urea breath testing is not only sensitive and specific but has an important advantage to confirm *H pylori* eradication. Following ingestion of 13C- or 14C-urea, *H pylori*-produced urease enzyme that is resident in the stomach hydrolyzes this labeled urea to $^{14}CO_2$ or $^{13}CO_2$, which can be detected in breath samples [34] (fig. 1). The nonradioactive^{13}C (a stable label) test and the radioactive ^{14}C test have received US Food and Drug Administration (FDA) approval for *H pylori* diagnosis. The dose of radiation in the ^{14}C-urea test however is not approved for use in children and pregnant women [4].

3. Fecal *H. pylori* detection

H. pylori in the stomach also appears in the stool, allowing the development of fecal assays: *H pylori* culture, DNA detected by polymerase chain reaction (PCR), or *H pylori* antigen testing. Only stool antigen has proven to be clinical useful with sensitivities and specificities of more than 90%. Stool antigen assay is advantageous to confirm eradication. To avoid false negative results, it is generally recommended that post-treatment testing with the UBT, histology, stool antigen test or culture be delayed for 4 weeks and the patients should discontinue proton pump inhibitors (PPI) and antibiotic such as amoxicillin, clarithromycin and quinolone groups to ensure that any remaining organisms can repopulate the stomach [4].

b. Invasive tests

Invasive testing which requires endoscopy should be limited to patients who require endoscopy for diagnostic or therapeutic evaluation. Invasive tests available in clinical practice include: gastric biopsies for culture (fig. 2A), gram stain (fig. 2B), histology (fig. 3A), or rapid urease testing (fig. 3B). Rapid urease test such as CLO test plus upper GI endoscopy usually cost between 276-502 (average 389) US dollars. *H pylori* culture is the absolute gold standard to diagnose *H. pylori* but culture generally is not available in most hospitals. Good quality laboratories are capable to culture *H. pylori* from gastric biopsies in more than 80% of instances and also offer susceptibility testing such as E-test.

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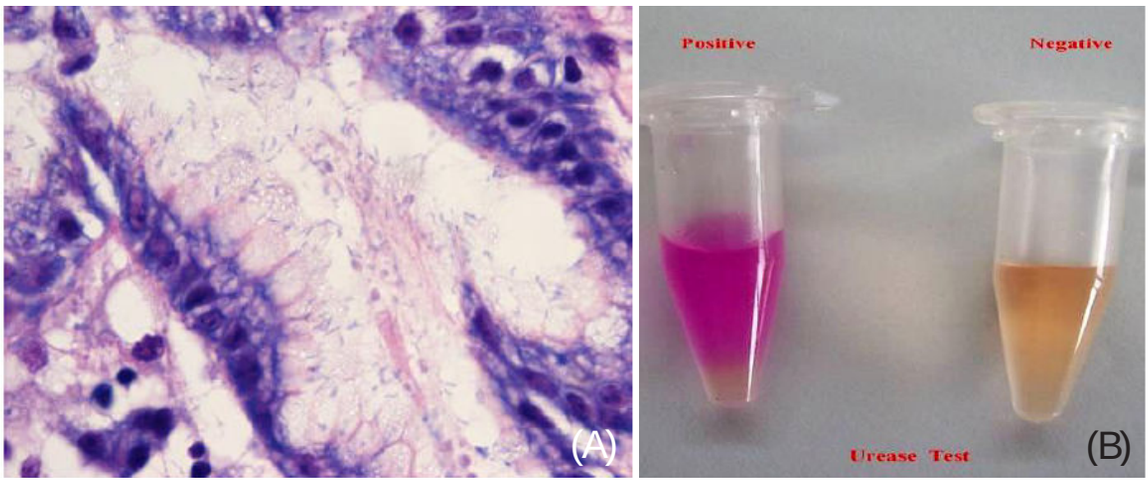

Figure 3. A.) *H pylori* detected by histology; (B.) *H pylori* detected by rapid urease test

Histological examination has an advantage over other diagnostic tests by providing morphological information such as severity of gastritis, and evidence for dysplasia. The accuracy of histological examination however may be variable due to density of *H. pylori* and sampling error, and is dependent upon histopathogical interpretation. The accuracy of histological diagnosis of *H pylori* infection can be improved by adequate biopsies from the antrum and body and by special staining such as a silver staining and the Diff-Quik stain [35].

Rapid urease tests contain a solution or gel with urea and a pH indicator reagent. The presence of urease from *H pylori* results in hydrolysis of neutral urea to alkaline ammonia, which is then visualized by a change in color of the pH indicator. The rapid urease test has a high sensitivity (95%) and specificity (95%), [36] making it an excellent primary diagnostic test.

Any concomitant use of antibiotics or PPI however will reduces bacterial load, and may lead to false negative tests such as rapid urease tests, urea breath test and histology. [4]

4. Test-and-Treat Strategy for *H. pylori*

Proposed strategies based on the noninvasive diagnosis of H. pylori infection so-called ''test-and-treat'' strategy. This strategy has been proposed for clinical practice in developed countries which has low prevalence of H. pylori infection. Test-and-treat is based on the test of the presence of H. pylori and its subsequent eradication when detected.13 The test-and-scope strategy performing a test to detect H. pylori in all patients and endoscopy only in those who are shown to be infected has been considered useful in clinical practice in some developing countries which has high prevalence of H. pylori infection such as Asia.

5. Management of *H. pylori* infection in FD

Both European (Maastricht IV/ Florence Consensus Report) and Asian consensus reports endorse *H. pylori* testing and eradication as a key management strategy for patients with dyspepsia to produce long-term relief of symptoms. [37,38]

6. Antibiotics use for *H. pylori* eradication

6.1. Amoxicillin

Amoxicillin is a popular antibiotic for treating *H pylori* infection because it is inexpensive and well tolerated, while resistance is rare. [39, 40] Amoxicillin acts by inhibiting the synthesis of the bacterial cell wall and can act locally when delivered into the gastric lumen and systemically once absorbed into the bloodstream. Amoxicillin is pH-dependent; its bactericidal activity increases as the pH rises. As a single agent antibiotic use is not capable of curing *H. pylori* infection, amoxicillin must be combined with other antibiotics such as clarithromycin and metronidazole. [4]

6.2. Clarithromycin

Clarithromycin, a 14-membered ring macrolide antibiotic, is a derivative of erythromycin, sharing a close spectrum and clinical application. Clarithromycin is one of the most acid-stable macrolide with a low minimum inhibitory concentration (MIC) for *H. pylori* treatment. The antimicrobial activity results from its binding to bacterial ribosomes and disrupting bacterial proteinsynthesis. [4] Currently, Clarithromycin resistance is increasing and resulting in a marked reduction in treatment success. [4, 8, 41] Increasing the clarithromycin dosage does not overcome the problem of resistance. This antibiotic frequently causes a bitter taste that causes some patients will stop treatment.

6.3. Metronidazole

Metronidazole is a nitroimidazole group, which is toxic to microaerophilic organisms. Metronidazole is secreted into gastric juice and saliva, and is active after absorption with a half-life of 8 to 12 hours. [4] Metronidazoleis is a pH-independent. [42] After entry into the bacterial cell, metronidazole changes into a toxic form that alters the bacterial enzymes required for transformation. Unlike clarithromycin, metronidazole resistance can be overcome by increasing the dosage. The side effects of short-term use of metronidazole include interactions with alcohol (disulfiram like effect) and gastrointestinal symptoms such as nausea and vomiting. [4]

6.4. Tetracycline

Tetracycline, a derivative of polycyclic naphtacenecarboxamides, is a fine anti–*H. pylori* antimicrobial because it is inexpensive and pH-independent. [4] Tetracycline inhibits bacterial

protein synthesis and seems to act luminal or locally. [43] The site of action of tetracycline is the bacterial ribosome, resulting in the interruption of protein biosynthesis. This antibiotic should not be given to pregnant women or children because it causes permanent staining of developing teeth. [4]

6.5. Fluoroquinolones

Fluoroquinolones have been used more popularly for *H pylori* treatment. These drugs block DNA gyrase and DNA synthesis in the organism. Resistance to fluoroquinolones develops rapidly, so that prior use of these medications is associated with a significant rate of resistance. [4]

6.6. Furazolidone

Furazolidone is a monoamine oxidase inhibitor with widely antibacterial activity based on interference with bacterial enzymes. This antibiotic has proven to be an effective part of triple therapy while the development of resistance is rare. Furazolidone is an underused antimicrobial. [44]

6.7. Rifabutin

Rifabutin is a semisynthetic ansamycin antibiotic with low MIC level for *H pylori* infection. This antibiotic is becoming more common and primarily used in combination with PPI and amoxicillin. [4] Rifabutin-based triple therapy for 10 days has been tested as salvage therapy and found to have high eradication rate of over 80%. Ritabutin can have cross-resistance with antimycobacterium. [8]

6.8. Other antimicrobial agent

Bismuth compounds are topically active pH-independent antimicrobial drugs that disrupt the integrity of bacterial cell walls. Bismuth is directly bactericidal, even though its MIC is high for *H pylori.* Bismuth is available in two forms (bismuth subsalicylate and bismuth subcitrate), which have equivalent effect as anti–*H pylori* therapy. *H pylori* resistance has not been reported for this agent. [4]

Regimens available

In recent years, the efficacy of legacy triple therapy for *H. pylori* eradication has declined worldwide to an unacceptable level. The average success rate of triple therapy has also declined to about 70%. [4, 8, 41] Bismuth-based quadruple therapy containing metronidazole is more effective than triple therapy with overall eradication rate of 83% and the eradication rate is higher in metronidazole sensitive group than those of the resistant group. [45] A recent study from Thailand demonstrated that a ten-day sequential therapy is highly effective for *H. pylori* infection with eradication rate of 95% but its efficacy affected by clarithromycin resistance. [41] A study from concomitant therapy evaluated and compared the efficacy of 10-day and 5-day therapy for *H. pylori* eradication using PPI with three antibiotics and found that 10-

day regimen is highly effective with eradication rate of 96% and the 5-day regimen yielded eradication rate of 88%. [46] The available treatment regimens was summarized in table 1. [4]

Legacy therapies
Triple therapy: A PPI plus amoxicillin, 1 g , plus clarithromycin, 500 mg, or metronidazole/tinidazole, 500 mg, twice a day for 14 days
Quadruple therapy: Bismuth, metronidazole, 500 mg, tetracycline, 500 mg, three times a day plus a PPI twice a day for 14 days
Concomitant triple therapies
A PPI plus amoxicillin, 1 g, plus clarithromycin, 500 mg, and metronidazole/tinidazole, 500 mg, twice a day for 14 days
Sequential therapy
A PPI plus 1 g amoxicillin, twice a day for 5 days. On day 6 stop amoxicillin and add clarithromycin, 250 or 500 mg and metronidazole/tinidazole, 500 mg, twice a day to complete the 10-day course.

Table 1. Treatment regimens for *Helicobacter pylori* infections[4]

There are many factors that could influence the eradication rate of *H. pylori*. Compliance is a major concern and how to make the regimen conveniently used by all patients is important. Impact of drug metabolism and CYP2C19 on eradication rate is a new point of concern and needs further research to elucidate this question. The choice of a second-line therapy depends on local antibiotic resistance pattern, previous treatment, drug availability and cost. Second-line salvage therapy after primary therapy failure, levofloxacin based triple therapy resulted in eradication rate of over 80% in patients after failed triple therapy. The accumulation eradication rate after first-line and second-line therapy becomes nearly 90%. This regimen is convenient and well-tolerated but antibiotic resistance to levofloxacin needs to be monitored. Rifabutin-based triple therapy for 10 days has been tested as salvage therapy and found to have high eradication rate of over 80%. Ritabutin can have cross-resistance with antimycobacterium. Furazotidone can also be used as salvage therapy but the use is limited by its availability. The summarized efficacy of *H. pylori* treatment regimens is: [8]

- Triple therapy containing PPI plus amoxicillin and clarithromycin or metronidazole has limited efficacy for *H. pylori* eradication with expected eradication rate of 70%.
- Sequential therapy and concomitant therapy yield high eradication rate of over 90% and could be used as first - line therapy.
- Bismuth based quadruple therapy could be used as alternative first - line therapy with high eradication rate.
- Levofloxacin based- triple therapy and concomitant therapy can be used as a second line salvage therapy after failed first - line therapy.

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