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A review on *Helicobacter pylori* Infection

Manish Kumar Maity[1, 2], Mamta Naagar[1]

[1]*Department of Pharmacy Practice, MM College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana-133207, Ambala, India*

[2]***Corresponding Author's Email:*** *manishkumarmaity@gmail.com*

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Abstract

Helicobacter pylori (*H. pylori*) was first identified in 1982 by the Australian physicians Barry Marshall and Robin Warren. *Helicobacter pylori* is also known as *Campylobacter pylori*. It is a gram-negative, micro-aerophilic, spiral (helical) bacterium usually found in the stomach. *H. pylori* infection is one of the most common and chronic bacterial infection, affecting approximately half of the world's population. Peptic ulcer disease, gastric ulcers, mucosa-associated lymphoid tissue lymphoma and gastric cancer all are linked to *H. pylori.* There are various diagnostic procedures to detect *H. pylori* infection and the choice of one approach over another is based on a number of considerations including accessibility, benefits and drawbacks of each method, cost, and the age of the patients. When *H. pylori* infection is diagnosed, doctor determines the therapy on the basis of patient's clinical status. Generally, eradication of *H. pylori* is recommended for treatment and prevention of the infection. In most of the cases *H. pylori* infections are cured with triple therapy. Moreover, quadruple therapies, sequential therapies and concomitant therapies have been developed as major alternative options to treat *H. pylori* infection. In this review pathophysiology, prevalence, transmission, clinical sign & symptoms, risk factors, and diagnostic techniques used to detect *H. pylori* infection as well as *H. pylori* eradication therapy regimens are discussed.

Keywords: Diagnosis, *Helicobacter pylori*, Treatment, Hybrid therapy, Concomitant therapy, Sequential therapy

❖ Introduction

Helicobacter pylori (*H. pylori*) infection is the most frequent and persistent bacterial infection. This bacteria is adapted to live in the harsh and acidic environment of the stomach. In stomach *H. pylori* changes the environment by reducing the acidity, which creates a favourable condition to survive. According to a study, in 2015, over 50% of the world's population had *H. pylori* in their upper gastrointestinal tracts [1]. In developing countries almost 90% of people and in developed countries (excluding Japan) less than 40% of the populations are infected with this infection [2]. *H. pylori* can be transmitted from one person to another through direct contact with infected person's saliva, vomit or feces. Risk factors for *H. pylori* infection are related to living in crowded environment, living without a reliable supply of clean water, living with *H. pylori* infected person.

Diagnostic methods of *H. pylori* infection depends on several factors like availability of diagnostic tests, cost, accessibility, advantages and disadvantages of each method, age of patients etc. Triple therapy is the standard treatment option for *H. pylori* infection. Other treatment options like quadruple therapies (which involve diverse combinations of medicines), sequential therapies and concomitant therapies have been proposed as successful alternatives for *H. pylori* treatment, despite the fact that triple therapy delivers satisfactory cure rates.

❖ Pathophysiology

- **Adaptation to the stomach**

H. pylori uses its flagella to burrow into the mucus lining of the stomach to reach the epithelial cells underneath, where it is less acidic, in order to avoid the acidic environment of the stomach (lumen) [3]. *H. pylori* can detect the pH gradient in the mucus and then move towards the less acidic area (chemotaxis). This also keeps the bacteria from being swept away into the lumen with the bacteria's mucus environment, which is constantly moving from its site of creation at the epithelium to its dissolution at the lumen interface [4]. *H. pylori* is found in the mucus, on the inner surface of the epithelium, and sometimes inside the epithelial cells [5]. It adheres to the epithelial cells by producing adhesins, which bind to lipids and carbohydrates in the membrane of the epithelial cell. BabA is an adhesin that binds with Lewis b antigen, which is present on the surface of stomach epithelial cells [6]. BabA mediated *H. pylori* adhesion is acid sensitive and can be completely reversed by decreased pH. According to one theory, BabA's acid responsiveness promotes adherence which also facilitates an effective escape from an unfavourable environment at pH that is harmful for the organism [7]. SabA is an adhesin which binds to increased quantities of sialyl-Lewis x antigen expressed on gastric mucosa [8]. *H. pylori* not only uses chemotaxis to avoid low pH regions, but it also neutralises the acid in its surroundings by producing large amount of urease, which converts the urea present in the stomach to carbon dioxide and ammonia. These interact with the strong acids for creating a favourableneutralised area around *H. pylori* [9]. Mutants lacking urease are unable to colonise. Urease expression is necessary for both initial colonisation and the maintenance of chronic infection [10].

- **Adaptation of *H. pylori* to high acidity of stomach**

H. pylori produces huge amount of urease, to produce ammonia to counteract stomach acidity. *H. pylori* arginase, a bimetallic enzyme binuclear Mn2-metalloenzyme arginase, crucial for pathogenesis of the bacterium in human stomach, a member of the ureohydrolase family, catalyzes the conversion of L-arginine to L-ornithine and urea, where ornithine is further converted into polyamines, which are necessary for several critical metabolic processes [11]. This provides acid resistance, which is necessary for the bacteria to colonise in the gastric epithelial cells. Arginase of *H. pylori* also plays a role in evasion of the pathogen from host immune system. Arginase competes with host-inducible nitric oxide (NO) synthase for the same substrate, L-arginine, which lowers the production of NO, a vital part of innate immunity and a powerful antimicrobial agent that can directly kill the pathogens [11]. Dysregulation of the host immunological response to *H. pylori* infection is mainly attributed to variations in the availability of L-arginine and its conversion into polyamines [11].

- **Inflammation, gastritis and ulcer**

There are various ways that *H. pylori* damage the linings of the duodenum and stomach. The biochemicals generated by *H. pylori*, such as proteases, vacuolating cytotoxin A (VacA), which harms epithelial cells, destroys tight

junctions, and induces apoptosis, and certain phospholipases, are toxic to epithelial cells and the ammonia produced to control pH [12]. CagA is a gene related with cytotoxins, has the potential to be carcinogenic and can also lead to inflammation [13]. Colonization of the stomach by *H. pylori* can lead to chronic gastritis (an inflammation of the stomach lining), at the site of infection. *Helicobacter* cysteine-rich proteins (Hcp), particularly HcpA (hp0211), are known to provoke an inflammatory immunological response [14]. *H. pylori* can increase the levels of COX-2 in *H. pylori* positive gastritis [15]. Chronic gastritis is likely to underlie *H. pylori* related diseases [16]. When the effects of inflammation allow stomach acid and the digesting enzyme pepsin to surpass the defences that protect the stomach and duodenal mucous membranes, ulcers develop in the stomach and duodenum. The location of *H. pylori*colonisation, which affects the where the ulcer develops, is influenced by the stomach's acidity [17]. In people producing large amounts of acid, *H. pylori* colonizes near the pyloric antrum (exit to the duodenum) to avoid the acid-secreting parietal cells at the fundus (near the entrance to the stomach). *H. pylori* can also colonise in the stomach for persons who produce normal or less acid. The inflammatory response caused by bacteria colonizing near the pyloric antrum induces G cells in the antrum to secrete the hormone gastrin, which travels through the bloodstream to parietal cells in the fundus [18]. In addition to increasing the amount of parietal cells over time, gastrin induces the parietal cells to produce more acid into the stomach lumen [19]. The increased acid load damages the duodenum, which can cause duodenal ulcer. When *H. pylori* colonize other areas of the stomach, the inflammatory reaction can result in atrophy of the stomach lining and ulcer formation in the stomach. This can increase the risk of stomach cancer.

- **Cag Pathogenicity Island**

About 50 - 70% of *H. pylori* strains in Western countries possess the cag pathogenicity island genes, which may increase the pathogenicity of *H. pylori* [20]. The risk of developing peptic ulcers or stomach cancer is higher in the people of Western countries, who infected with strains carrying the cag PAI than those infected with strains lacking the island. Following attachment of *H. pylori* to stomach epithelial cells, the type IV secretion system expressed by the cag PAI "injects" the inflammation-inducing agent, peptidoglycan, from their own cell walls into the epithelial cells. The immunological sensor Nod1 in the cytoplasm recognizes the injected peptidoglycan, which stimulates the release of cytokines that cause inflammation [21]. The cag PAI-encoded protein CagA is also delivered through the type-IV secretion system into the stomach's epithelial cells, where it interferes with the cytoskeleton, cell adhesion, intracellular signaling, cell polarity, and other cellular processes [22]. A host cell membrane-associated tyrosine kinase (TK) phosphorylates the CagA protein on tyrosine residues after it has entered the cell. Then, protein tyrosine phosphatase or protooncogene Shp2 is allosterically activated by CagA [23]. Pathogenic strains of *H. pylori* activate the membrane protein with a tyrosine kinase (TK) domain known as the epidermal growth factor receptor (EGFR). The pathogenesis of *H. pylori* is associated with the activation of the EGFR, which may be facilitated by altered signal transduction and gene expression in host epithelial cells. It has also been proposed that the CagA protein's C-terminal domain (amino acids 873 – 1002) functions independently of protein tyrosine phosphorylation to control host cell gene transcription [24, 25].

- **Cancer**

Researchers are looking into two connected processes through which *H. pylori* could

encourage cancer. One method entails an increased rate of host cell mutation and greater free radical generation close to *H. pylori*. The other hypothesised process, known as a "perigenetic pathway", entails improving the phenotypic of the transformed host cell through changes to cell proteins, such as adhesion proteins [26]. It has been proposed that *H. pylori* causes inflammation and elevated levels of TNF-α and / or interleukin-6 (IL-6). According to the proposed perigenetic mechanism, inflammation-associated signalling molecules, such as TNF-α, can alter gastric epithelial cell adhesion and result in the dispersion and migration of mutant epithelial cells without the requirement of additional mutations in tumour suppressor genes, such as genes encoding cell adhesion proteins [27]. The strain of *H. pylori* to which an individual is exposed may have an impact on their chance of getting stomach cancer. The cytotoxin-associated gene A (CagA) and the vacuolating toxin A (VacA), two proteins produced at high levels by *H. pylori* strains, appear to cause greater tissue damage than those that produce lower levels or that lack those genes completely [28]. These proteins are toxic to the cells lining the stomach and signal strongly to the immune system that an invasion is occurring. As a result of the bacterial presence, neutrophils and macrophages set up residence in the tissue to fight the bacteria assault [29]. A significant contributor to cancer mortality globally is *H. pylori* [30]. In general, 1 to 3 % of people with *Helicobacter pylori* infection acquire stomach cancer in their lifetime compared to 0.13 % of people without *H. pylori* infection; however statistics differs by country [31]. Gastritis occurs in around 75% of people with *H. pylori* infection [32]. Consequences of *H. pylori* infection often include chronic and asymptomatic gastritis [33]. Due to the typical absence of symptoms, stomach cancer is sometimes well advanced when it is finally diagnosed. When they get their first diagnosis, more than half of patients with stomach cancer have lymph node metastases [34]. By infiltrating neutrophils and macrophages into the gastric epithelium, inflammation brought on by *H. pylori*-caused gastritis promotes the buildup of pro-inflammatory cytokines and reactive oxygen species / reactive nitrogen species (ROS/RNS) [35]. DNA damage, particularly 8-oxo-2'-deoxyguanosine (8-OHdG), is caused by a substantial presence of ROS/RNS [35]. If the infecting *H. pylori* carry the cytotoxic cagA gene (present in about 60% of Western isolates and a higher percentage of Asian isolates), they can increase the level of 8-OHdG in gastric cells by 8-fold, while if the *H. pylori* do not carry the cagA gene, the increase in 8-OHdG is about 4-fold [36]. Infection with *H. pylori* also results in DNA double-strand breaks and other distinctive DNA damages, such as the oxidative DNA damage 8-OHdG [37]. Numerous epigenetic changes brought on by *H. pylori* are also connected to the development of cancer [38, 39]. These epigenetic changes are brought on by *H. pylori*-induced CpG site methylation in gene promoters and *H. pylori*-induced altered expression of multiple microRNAs [38, 39]. *H. pylori* infection is linked to epigenetically decreased DNA repair machinery efficiency, which promotes the accumulation of mutations and genomic instability as well as gastric carcinogenesis, according to a review by Santos and Ribeiro [40]. Raza et al. [41] shown in particular that expression of two DNA repair proteins, ERCC1 and PMS2, was significantly decreased once *H. pylori* infection had progressed to cause dyspepsia. 20 % of infected people get dyspepsia [42]. The human gastric infection with *H. pylori* also results in epigenetically decreased protein expression of the DNA repair proteins MLH1, MGMT, and MRE11, according to a review by Raza et al. Reduced DNA repair in the presence of increased DNA damage boosts carcinogenic mutations and is probably a major factor in the development of *H. pylori* carcinogenesis.

- **Survival of *Helicobacter pylori***

H. pylori's pathogenesis depends on its capacity to endure in the harsh gastric environment, which is characterized by acidity, peristalsis, and phagocyte assault accompanied by release of reactive oxygen species [43]. During host colonisation, *H. pylori* causes an oxidative stress reaction. The *H. pylori* genome develops potentially fatal and carcinogenic oxidative DNA adducts as a result of this oxidative stress response [44]. Many bacterial pathogens, such as *Neisseria gonorrhoeae, Hemophilus influenzae, Streptococcus pneumoniae, S. mutans, and H. pylori* are susceptible to oxidative stress and oxidative DNA damage [45]. Each of these pathogens appears to rely on transformation-mediated recombinational repair to survive the DNA damage brought on by oxidative stress. Therefore, it would seem that transformation and recombinational repair help an infection succeed. DNA transfer between bacterial cells via an intermediary medium, known as transformation, appears to be a component of an adaptation for DNA repair. *H. pylori* is naturally competent for transformation. While many organisms are competent only under certain environmental conditions, such as starvation, *H. pylori* is competent throughout logarithmic growth [46]. All living organisms have genetic code for responses to stressful situations including those that result in DNA damage [46]. In *H. pylori* to fix DNA double-strand breaks (DSBs), homologous recombination is necessary. RecA is loaded onto single-strand DNA (ssDNA) by the AddAB helicase-nuclease complex, which resects DSBs and promotes strand exchange that results in homologous recombination and repair. RecA and AddAB are necessary for effective gastric colonisation, which implies that *H. pylori* either experiences double-strand DNA damage in the stomach that must be repaired or has to undergo another recombination-mediated process. In particular, natural transformation is increased by DNA damage in *H. pylori*, and there is a link between the DNA damage response and DNA uptake in *H. pylori* [46]. This suggests that natural competence aids in the persistence of *H. pylori* in its human host and explains why most clinical isolates still retain competence. Since it resolves Holliday junctions, an intermediary in the recombinational repair process, RuvCprotein is crucial. Reduced macrophage survival, increased vulnerability to oxidative stress and DNA-damaging agents, and an inability to establish successful infection in a mouse model are all characteristics of *H. pylori* mutants that are defective in RuvC [47]. Similar to this, RecN protein is crucial for DSB repair in *H. pylori* [48]. The decreased capacity of an *H. pylori*recN mutant to colonise mice stomachs emphasises the significance of recombinational DNA repair in *H. pylori* survival inside its host [48].

- **Prevalence of *Helicobacter pylori* Infection**

In Europe, Northern countries have a lower frequency of *H. pylori* infection than in Southern and Eastern countries. In Netherlands, the existence of antibodies against *H. pylori* and the CagA antigen was examined in a randomly chosen sample of 1550 blood donors from four different areas [49]. In this study, non-European immigrants were not examined; only native Dutch respondents were tested. According to this study, the prevalence of *H. pylori* infection was 32%, with 28% of participants who tested positive for the infection carrying CagA-positive strain. Due to a birth cohort effect, the seroprevalence of *H. pylori* decreased from 48% in persons born between 1935-1946, and 16% in those born between 1977-1987. Additionally, in the same age cohorts, the percentage of CagA-positive participants dropped from 38% to 14 %. According to these findings, it is possible to anticipate a further decline in *H. pylori* prevalence in Netherlands in future decades.

A population-based prospective analysis of a cohort of more than 6500 pregnant women from the Netherlands was also published [50]. According to this study, 24 % of Dutch women have *H. pylori*. The most significant conclusion was that non-Dutch women had a much greater prevalence of *H. pylori*, with 64% of them being seropositive. However, in the latter group, first-generation immigrants who were infected and born abroad had a higher risk of *H. pylori* infection than second-generation immigrants. Therefore, in this study, ethnicity was a significant predictor of *H. pylori.*

Portugal reported to have a greater frequency of *H. pylori* infection than Northern European countries, with an infection rate of 84.2 %, with 61.7 % of strains also positive for CagA [51]. Another study, based on a proportion of included participants, defined an incidence rate of infection of 3.6/100 person-years, confirming that Portugal's *H. pylori* infection rate remains the highest in Europe.

Similarly, Eastern Europe also reported to have high infection rates. More than 4600 individuals were examined across the country, as part of a population-based cross-sectional survey in Turkey, resulting in a weighted overall infection prevalence of 82.5 % [52]. It's interesting to note that the incidence was lowest among residents of the southern region of the country, where people usually consume a diet rich in citrus fruits. However, vitamin C is successful in preventing the most of the infections, but few researches shown that it may also be involved in the *H. pylori* infection.

The prevalence of *H. pylori* in North America appears to be similar as comparable to North Europe. Further evidence was reported from a Canadian study where presence of *H. pylori* infection was evaluated in 203 indigenous individuals with dyspepsia who were referred for gastroscopy. *H. pylori* infection was found in 37.9% of individuals [53].

A research from Mexico reported that high frequency of *H. pylori* infections are mostly seen in Latin America [54, 55]. According to a study, it is reported that 343 pregnant women residing in rural areas in Mexico had a seroprevalence of 52.2%.

In Asia *H. pylori* infection rate is very high (approximately 54 % to 76 %) [56 - 62]. Only one research conducted in Saudi Arabia on healthy people revealed a low frequency of *H. pylori* infection of around 28 % [63]. More than 10,000 asymptomatic participants without a history of *H. pylori* eradication were included in a major cross-sectional countrywide multicenter study in Korea [56]. The infection seroprevalence was 54.4 %. However, this prevalence was lower than the previously performed two surveys (in 1998 and 2005 where the prevalence of *H. pylori* was 66.9% and 59.6% respectively) [64, 65]. This decrease was significant for all age groups in the country.

In China, a survey of *H. pylori* infection was carried out on a sample of the general population from areas with high prevalence of gastric cancer [57]. The ^{13}C-urea breath test was done among 5417 healthy people between the ages of 30 to 69 years. 63.4 % of people had *H. pylori* infection. According to reports, India, Kazakhstan and Bhutan also had high levels of *H. pylori* infection. In India the frequency of infection varied from 58 % to 62 % with dyspeptic symptoms [58, 59]. In Kazakhstan, the prevalence of *H. pylori* infection was 76.5 % among symptomatic and asymptomatic patients [60]. This infection was also found Bhutan with a frequency of 73.4 % of cases; however it was lower in the capital city, Thimphu, than in rural areas, mostly due to hygienic conditions [61]. Another research conducted in the same country reported to have a higher incidence rate of 86% [62].

Additionally, new data have been reported from African countries. According to studies, the prevalence of *H. pylori* infection was 75.5

% in Morocco and 65.7 % in Ethiopia respectively. Both studies reported a significant rise in all age groups [66, 67]. Higher infection rates were found in a survey from Nigeria; where the prevalence was 80 % when measured by histology and was even higher, reaching 93.6 %, when serology was used [68].

Few studies reported that *H. pylori* infection is common in children and young people. An investigation was carried out among children and young people in Belgium. It is found that 3.2 % of children born in Belgium and 60 % of children born from foreign parents have *H. pylori* infection [69]. Portuguese children had very high infection prevalence (nearly 66.2%), according to Bastos et al. [70]. More than half of the negative subjects were again tested after a median follow-up of 37 months, which revealed an incidence rate of 4.1/100 person-years. In Brazil, Pacheco et al. reported a high prevalence of *H. pylori* infection (41.1 %) between the ages of 2 - 19 years [71].

In China, the presence of *H. pylori* infection was assessed among 1634 children and adolescents with upper gastrointestinal symptoms who underwent gastroscopy and gastric biopsies [72]. The histologic analysis of gastric biopsies showed 32.1% rate of *H. pylori* infection. In Iran, a greater *H. pylori* infection rate in children was recorded. Ghasemi-Kebria et al. reported a seroprevalence of 50.5 %, with 61.7 % of children had CagA in Iran [73].

❖ Transmission of *Helicobacter pylori* Infection

It is still unclear that how *H. pylori* infection is transmitted. According to several studies, *H. pylori* can be transmitted through drinking contaminated water.

H. pylori can also be transmitted through parental transmission. Didelot et al. sequenced the genomes of 97 *H. pylori* isolates from 52 individuals of two families residing in rural areas of South Africa [74]. *H. pylori* transmission occurred more frequently between the members of the same house and close relatives.

In order to do a multilocus sequence type DNA analysis, Osaki et al. used the stools of parents from three different families with children who are tested positive for *H. pylori* infection [75]. According to this study, all of the chosen families experienced intrafamilial transmission (i.e, from a mother to child transmission occurs in these families).

Urita et al. investigated the intrafamilial transmission of *H. pylori* infection by examining 838 children and their relatives from a small village in Japan [76]. Later it was reported that, grandmother to child transmission and mother to child transmission is a significant mechanism for the spread of *H. pylori* infection. Mothers could transmit the infection through using common spoons or tasting their children's food. While mothers are at work, grandmothers take care their grandchildren, which increase the risk of transmission.

❖ Signs and symptoms of *Helicobacter pylori* Infection

In acute stage, up to 90% of *H. pylori* infected patients never experience any symptoms of illness or any other problems [77]. But *H. pylori* infection can increases the chance of developing peptic ulcers by 10% to 20% [78, 79]. Acute infection might manifest as acute gastritis with stomachache and nausea. In chronic gastritis, the symptoms of non-ulcer dyspepsia can be seen like stomach aches, nausea, bloating, belching, and sometimes vomiting [80]. Pain usually occurs when the stomach is empty, between meals or in the early morning time. Black stools are the signs of gastrointestinal bleeding, which can also happen. Prolonged bleeding can cause anemia, which makes the patient feel weakness and fatigue. Hematemesis, hematochezia, or

melena may also happen in case of severe bleeding. Duodenal ulcers are more likely to develop from inflammation of the pyloric antrum, whereas gastric ulcers are more likely to develop from the inflammation of the corpus (i.e, stomach's body) [81, 82]. *H. pylori* infection can cause gastric polyps or colorectal polyps [83, 84]. Gastric polyps can cause gastric outlet obstruction and dyspepsia, heartburn, bleeding from the upper gastro-intestinal tract, and constipation [84]. While colorectal polyps can cause rectal bleeding, anemia, constipation, diarrhea, weight loss, and abdominal pain [85]. Patients with chronic *H. pylori* infection are more likely to develop a cancer that is directly linked to this infection [86]. These cancers include stomach adenocarcinoma, less commonly diffuse large B-cell lymphoma of the stomach, extranodal marginal zone B-cell lymphomas of the stomach etc [87, 88].

❖ Risk Factors for *Helicobacter pylori* Infection

According to several studies, age and gender usually don't have any link with an increased risk of *H. pylori* infection. In fact, most of the studies reported no difference of *H. pylori* infection between men and women in both adults and children [59]. In the adult population, no significant correlation was found between infection and age [50, 54]. The birth cohort effect appears to be responsible for the age-specific gradient in *H. pylori* prevalence [72].

H. pylori infection has been linked with certain socioeconomic factors. Particularly, people with a low socioeconomic status were more likely to carry *H. pylori* infection [50, 63]. Risk factors for *H. pylori* infection are associated with residing in rural areas [56, 61, 63], crowded households [62, 63, 70], and having contaminated sources of drinking water [52]. Few researches claimed that smoking and drinking alcohol is the main risk factors for this infection [52, 63]. On the other hand, one research shows that frequent alcohol use was found to be protective against *H. pylori* infection [52].

❖ Diagnosis of *Helicobacter pylori* Infection

Several techniques are available for detecting the presence of *H. pylori*, each with its own set of benefits, drawbacks, and limits. The simplest method for classifying these procedures is to determine whether or not an endoscopy is necessary. Histological assessment, culture, polymerase chain reaction (PCR), and the rapid urease test (RUT) are examples of biopsy-based tests that are performed on tissue obtained during endoscopy. Non-invasive methods such as the urea breath test (UBT), serology, and stool antigen test (SAT) are also available. Another approach to categorise these tests is whether they are performed before or after *H. pylori* eradication therapy.

❖ Diagnostic tests before treatment of *Helicobacter pylori* Infection

- **Invasive methods to detect *Helicobacter pylori* Infection**

➢ **Histology**

Histological examination is the gold standard method to diagnose *H. pylori* infection because it offers crucial information about the mucosa (e.g., presence and severity of inflammation, intestinal metaplasia, glandular atrophy, dysplasia, and neoplasia). Biopsies of the antrum and corpus have been advised in several studies [89 - 92]. The gold standard for gastric biopsy collection is the updated Sydney classification system, which recommends sampling from 5 biopsy sites. One sample each should be obtained from the lesser curvature of the corpus about 4 cm proximal to the angulus (I), from the lesser (II) and larger curvature of the antrum (III), all within 2 to 3 cm of the pylorus, from the central region of the greater

curvature of the corpus, approximately 8 cm from the cardia (IV), and from the incisura angularis (V) [93]. Despite the recommendations, due to the huge number of samples required, this strategy of biopsy collection is rarely employed in regular practice. Endoscopy is a time-consuming and uncomfortable procedure. However, the analysis of fewer biopsy samples than recommended might result in an underestimation of the presence of *H. pylori* infection, as well as sampling error and false negatives.

A routine hematoxylin and eosin (HE) stain is enough to detect *H. pylori* in biopsy samples. Special stains, such as Warthin-Starry, Giemsa, Toluidine blue, Acridine orange, McMullen, Genta, Dieterle, and Romanouski stains, or immunochemical procedures can be used if the results from hematoxylin and eosin (HE) stain are inconclusive. According to present guidelines, on biopsied tissue, at least two separate stain methods should be used: HE to evaluate inflammatory cells, and Giemsa or Genta stain to detect *H. pylori*. Genta stain is technically complex. Genta stain is able see inflammatory cells and *H. pylori* by combining a silver stain, HE, and Alcian blue. On the other hand, Giemsa stain is technically simple, very sensitive, and affordable. Thus, in clinical practice, Giemsa stain is mostly preferred. All other techniques are reserved for research proposals [94 - 97].

There are certain limitations in histology. The tissue changes are appraised subjectively, resulting in score differences amongst observers for the investigated parameters. In order to obtain tissue samples for histology, an endoscope is also required. Because of the patchy distribution of *H. pylori* in the gastric mucosa, tissue specimens should be obtained from different areas of the stomach. Histology's sensitivity and specificity for *H. pylori* diagnosis range from 53% to 90%, depending on the pathologist's experience and colonization density. The sensitivity of histology can be improved by increasing the number of biopsies and using specialized stains [98].

In histological sections *H. pylori* appear as a curved or spiral bacillus on the epithelial surface, in the mucus layer, and within stomach glands. Other species like *Helicobacter heilmanii* (*H. heilmanii*) also found in human stomachs. *H. heilmanii* is a zoonotic infection in humans that can come from cats or dogs and can cause chronic gastritis, is found in roughly 0.1 % of stomach biopsies. *H. heilmanii* is straight and much longer than *H. pylori.* For this reason, two species can be easily distinguished [99].

Histopathologic tests are practiced less often in children (i.e. pediatric patients) because of the need to perform an endoscopy. According to a recent study which included an analysis of histopathologic lesions in 96 Brazilian children with *H. pylori* infection showed that in 51.8 % of children have *H. pylori* [100]. Moderate to severe chronic active gastritis may be seen in both the antrum (70.5 %) and corpus (45.2 %). But more severe gastritis is observed in the antrum than in corpus ($P < 0.05$). The topographic distribution of inflammation was pangastritis (61.9%), followed by antral (32.1%) and corpus (5.9%). The antrum had a greater *H. pylori* density than in the corpus.

Fluorescent *in situ* hybridization (FISH) is a new method used in histological preparations. It is used for detecting a specific bacterial component or factor, such as clarithromycin resistance [101, 102]. FISH employs a series of fluorescent protein-labeled oligonucleotide probes to target a specific gene. For example - 16S and 23S ribosomal RNA genes are the commonly used probes. This test takes around 3 hours to complete this assay and probe for both *H. pylori* and clarithromycin resistance. The ability to evaluate both probes in a short time adds value to the *H. pylori* diagnosis. The specific location of the bacteria in the stomach mucosa has been determined using *in situ* hybridization and immunochemical methods

[103]. Despite its benefits, FISH is time-consuming, expensive method. So, it is not used in clinical practice.

- **Culture**

Because no significant amount of commensal bacterial flora is expected, a newly acquired gastric biopsy specimen is the ideal specimen for culturing *H. pylori* (except in patients with reduced gastric acid production, in whom an overabundance of commensal bacteria is possible). Gastric juice sample or the string test is the less invasive alternatives for biopsy collection. Culture may also be done using gastric juice samples or the string test, although the sensitivity is lower than when biopsy specimens are used [104 -106].

When performed under optimal conditions, culturing generally has a sensitivity of greater than 90% and a specificity of 100% [107]. Lower sensitivity values (85.4 %) with proven 100 % specificity and culture sensitivity of 40.0 % have been reported in bleeding patients [108, 109]. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy for culture in patients with atrophy were 96 %, 100 %, 100 %, 80 %, and 97 % respectively [110]. Sensitivity and specificity values of 95.8% and 96.4 % have been reported in a pediatric population [111].

H. pylori is very sensitive organism. So, it must be cultured as soon as possible after being collected. Biopsies can be stored at 4 °C for up to 24 hours in a transport medium (e.g., Stuart's transport medium). *H. pylori* can be kept frozen at -80 °C after isolated, ideally in broth with 15% to 20% glycerol. Various types of medium can be used for *H. pylori* culture like selective agars (e.g., Pylori-agar, Skirrow agar, Wang media), which contain specific antibiotics to suppress commensal bacteria, and nonselective agars (e.g., blood agar, Columbia blood agar). Before discarding cultures as negative, these should be cultured for at least 7 days under microaerobic conditions (85% N_2, 10% CO_2, 5% O_2) at 35 °C to 37 °C. *H. pylori* is identified by morphological traits as well as positive catalase, oxidase, and urease reactions [112].

Culturing is the most specific approach for identifying *H. pylori*. But the results depend on the microbiologist's skill, specimen quality, and the transport media which is used for culture [113]. From a long time, the use of *H. pylori* culture to diagnose infection was limited in research and epidemiology studies. Culturing is mostly used to confirm the antibiotic sensitivity of *H. pylori* after 2 treatment failure in patients. However, few laboratories are performing the culture and susceptibility test before 2 treatment failures and it would be preferable because of the increase in antibiotic resistance rates, particularly to clarithromycin and metronidazole. Because standard therapies are failing at an increasing rate, bacterial culture may become an essential tool for assessing antibiotic resistance in patients and managing the antibiotic failure. According to a recent study, antibiotic resistance is high in the pediatric population of Brazilian children and adolescents [114]. This study described that 77 *H. pylori* clinical isolates obtained from patients without previous eradication treatment for *H. pylori* infection, and 6 strains from patients in whom previous eradication treatment had failed. The study reported a worldwide resistance rate of 49.3 %, with 40% of strains resistant to metronidazole and 19.5 % resistant to clarithromycin. Another research of 61 *H. pylori* strains isolated from Japanese childrendemonstrated 36.1 %, 0 %, and 14.8 % overall resistance to clarithromycin, amoxicillin, and metronidazole respectively [115].

- **Polymerase chain reaction**

In clinical practice, PCR is used to detect *H. pylori* in small samples that have few bacteria present. PCR does not require any specific processing equipment or transportation, and it

may be used on samples collected using both invasive and noninvasive methods. Furthermore, PCR is faster than many other diagnostic procedures, and it may be used to identify a wide range of bacterial genotypes and in epidemiological investigations. A significant disadvantage of PCR is that it can identify DNA segments of dead bacterium in the stomach mucosa of patients after treatment; as a result, it can yield false-positive results [116, 117]. *H. pylori* may be detected by PCR in materials collected using non-invasive or minimally invasive techniques, such as gastric juice, stomach content, saliva, feces etc. So, molecular approaches can be applied to the specimens acquired from string tests or orogastric brushes. Molecular testing may be useful for the materials that cannot be properly cultured due to prolonged transport or in cases where *H. pylori* isolation is impossible due to contamination.

In gastric biopsy specimens, molecular approaches such as PCR have been shown to be effective in identifying infections and testing for clarithromycin resistance, which is caused by changes in the 23S rRNA gene [118]. Because of the increasing frequency of antibiotic resistance in some countries with a high prevalence of *H. pylori*, molecular tests may be useful as *H. pylori* diagnostic options. Efflux pumps play a key role in antibiotic resistance in other Gram-negative bacteria, and treatment options caused by multidrug-resistant bacteria are limited [119].

- **Rapid urease test (RUT)**

The RUT utilizes the ability of *H. pylori* to create significant amounts of urea as the basis criteria for diagnosing infection. Biopsies obtained during endoscopy are put in a solution that contains urea and a pH indicator. If urease is present, then urea will break down into carbon dioxide and ammonia. That causes the raising of medium's pH and colour change in pH indicator. Depending on the amount of bacteria in biopsy sample, the RUT can give results in minutes to 24 hours. The RUT is a low-cost, widely accessible, and highly specific test.

Although few members of the oropharyngeal microbiota produce urease, which is swallowed in the saliva, the stomach's strong acid rapidly denatures this weaker enzyme. However, due to reduced urease activity, which might be induced by a recent intake of antibiotics, bismuth compounds, or proton pump inhibitors (PPIs), there is a substantial risk of false-negative results with RUT [120]. In patients with achlorhydria, a false-negative urease test can be obtained. The number of bacteria in the biopsy affects RUT sensitivity; a positive result requires at least 10000 cells. In the presence of blood, the RUT has been found to have low sensitivity and specificity. By increasing the incubation time, RUT specificity decreases and hence the risk of a false positive rises. When RUT findings from individual gastric antrum and corpus tissue specimens were compared to histology results (as the gold standard), combining the tissues increased *H. pylori* identification from 64 % in separate specimens to 69.2 % [121]. Commercial RUTs have specificities ranging from 95% to 100%; although their sensitivity is slightly lower (approximately 85 % - 95 %). Gel-based (CLOtest, HpFast) and paper-based (PyloriTek, ProntoDryHpOne) RUTs are commercially available [122, 123].

- **Noninvasive methods to detect an infection**

- **Serology**

Several types of tests are available to detect antibodies against *H. pylori.* The enzyme immunoassay (EIA) test is mostly used. The majority of commercial EIA tests work by detecting IgG, with sensitivity and specificity ranging from 60% to 100%. The incidence of infection, changes in location, and features of the study populations are all relevant elements to consider when evaluating the quality of serology tests for the identification of active *H.*

pylori infection. Local validation of a serology test is required, and it is important to make modifications to cut-off levels for particular populations are critical. The maximum sensitivity is shown in tests comprising complicated antigen combinations of multiple strains [124].

When deciding whether a serology test should be used as the method of choice, there are a number of factors to consider. Serology testing should be considered in patients who have recently taken antibiotics or PPIs, have bleeding ulcers, or have gastric atrophy [125]. Whole-blood tests, as well as antibody detection in urine or saliva, do not show the same level of reliability as laboratory-based testing, and they are not recommended for diagnosing of *H. pylori* infection [125].

Serology tests are inexpensive and widely available. In fact, the accessibility of these tests can result in their use by laboratories lacking in expertise in the diagnosis of *H. pylori*, which may lead to incorrect data interpretation. Another disadvantage is the antibodies persist in the host after eradication therapy [126]. As a result, it may take some time after the start of eradication therapy and the confirmation of a significant decline in antibody titers. Due to this circumstance, serology can only be used to establish an infection that has been completely eradicated. On the other hand, serology findings are unaffected by recent antibiotic or PPI therapy. In general, the serology test is very useful alternative to the UBT test for properly identifying individuals with a negative result [127].

Both the full bacterial cell and particular *H. pylori* proteins have been detected by using serology. Not all individuals who infected with *H. pylori* gets develop disease, and the vast range of disease linked to *H. pylori* infection may be due to the heterogeneity of *H. pylori* strains [128]. The presence or lack of virulence factors, some of which have been utilised as serological markers, can cause between-strain variability. The cagA and vacA genes have been associated to enhanced pathogenicity of *H. pylori*.

CagA is a 120-kDa to 140-kDa protein that is encoded by the cagA gene [129]. Strains expressing the CagA protein induce a greater frequency of duodenal ulcer and gastric adenocarcinoma, as well as more severe inflammation and gastric atrophy [130]. Although the vacA gene is found in all *H. pylori* strains, it is only expressed in 50 % to 65 % of the strains. VacA is an 81-kDa to 91-kDa protein that is encoded by the vacA gene, causes vacuole formation in gastric epithelial cells [129]. CagA and VacA are both immunogenic proteins, and serologic assays that have been develop to identify and diagnose *H. pylori* infection and seroprevalence of virulence factor [131 - 133]. Currently, CagA, VacA, GroEL, gGT, HcpC, and UreA are the six highly immunogenic virulence factors, which are expressed in *Escherichia coli*, purified, and immobilised on nitrocellulose membranes to detect serologic immune responses against these virulence factors in the patients. The sensitivity and specificity of this novel assay were 97.6 % and 96.2 %, respectively [134].

❖ Tests used to detect the eradication of *Helicobacter pylori* Infection

➢ Urea breath test (UBT)

The UBT is based on *H. pylori'*s capacity to break down orally absorbed ^{13}C- or ^{14}C-labeled urea into CO_2 and ammonia if it is present in the stomach environment. $^{13}CO_2$ or $^{14}CO_2$ diffuses into the bloodstream, is expelled through the lungs, and can be measured in the exhaled air. The test is simple to carry out and does not need an endoscopy. ^{13}C is a non-radioactive, non-harmful isotope that may be safely used in children and pregnant women. ^{13}C is often measured in breath samples using an isotope ratio mass spectrometer, although the instrument is expensive [135]. On the other hand, ^{14}C-urea is affordable, but it must be used in a nuclear medicine department that is

permitted to store and dispose of radioactive reagents [136, 137].

Majority of the studies show that the UBT's sensitivity and specificity are more than 90% [138]. The UBT's high sensitivity, especially after eradication therapy, may be explained by the fact that in situations of moderate colonization or patchy *H. pylori* distribution, the UBT is more likely to provide positive results than biopsy-based tests. Other urease-forming pathogens rarely cause false-positive results. In contrast to serology, the UBT can provide false-negative findings if it is conducted after the use of medications such as PPIs and antibiotics. The UBT may have limited value if the patient has recently taken PPIs, antibiotics, or bismuth compounds [137]. False-negative ^{13}C-UBT results can be caused by corpus-predominant gastritis. The misdiagnosis of corpus-predominant gastritis may result in a significant misassessment of patients who require endoscopy and eradication therapy [139].

Post-treatment UBT is usually performed in 4 to 6 weeks after eradication therapy [140]. According to a meta-analysis, the ^{13}C-UBT test was found to be accurate for all ages. The authors achieved 95.9% sensitivity, 95.7 % specificity, likelihood-ratio (LR) + 17.4, LR - 0.06 and diagnostic odds ratio (OR) of 424.9. The ^{13}C-UBT demonstrated a high sensitivity of 96.6 %, specificity of 97.7%, LR+ 42.6, LR- 0.04, and diagnostic odds ratio of 1042.7 in children older than 6 years. Children less than 6 years old showed greater variability in accuracy in this study, with the test showing a sensitivity of 95%, specificity of 93.5 %, LR+ 11.7, LR- 0.12, and diagnostic odds ratio of 224.8 [141].

- **Stool antigen test (SAT)**

To identify antigens against *H. pylori* in stool samples, the SAT employs an enzyme immunoassay. It is a reliable method for diagnosing an active infection and confirming that the infection has been well treated. Stool samples can be kept for 24 hours at room temperature or 72 hours at 4 °C. Without refrigeration, the SAT's sensitivity drops significantly in 2 to 3 days [142]. Results of the SAT may be influenced by the disorders of digestive problems, PPI therapy, or the presence of a bleeding ulcer.

SAT's diagnostic accuracy in identifying *H. pylori* infection eradication has been evaluated. A recent research compared the ability of 2 SAT approaches to identify *H. pylori* after eradication therapy in dyspeptic patients: the enzyme immunoassay (Premier Platinum HpSA) and the immunochromatographic method (ImmunoCardHpSA STAT). For ImmunoCardHpSA STAT, the sensitivity, specificity, PPV, NPV, and accuracy were 100 %, 91.0 %, 84.6 %, 100 %, and 94.0 % respectively. On the other hand, for Premier Platinum HpSA, the sensitivity, specificity, PPV, NPV, and accuracy were 84.9 %, 92.5 %, 84.8 %, 92.5 %, and 90.0 % respectively. In another investigation, the capacity of 5 SATs, including 2 monoclonal EIAs and 3 rapid immunochromatographic assay tests, to identify *H. pylori* infection in adult patients exhibiting dyspeptic symptoms prior to eradication therapy was assessed. The sensitivity and specificity for the two EIAs were 92.2 % and 94.4 % and sensitivity and specificity for the Premier Platinum HpSA Plus test was 48.9 % and 88.9 % for the *H. pylori* antigen test. The One Step HpSA test had a sensitivity and specificity of 86.7 % and 88.9 % respectively. The ImmunoCard STAT HpSA test had a sensitivity and specificity of 68.9 % and 92.6 % respectively. In *H. pylori* fecal antigen test had a sensitivity and specificity of 78.9 % and 87 % respectively. In adult dyspeptic patients, the Premier Platinum HpSAPlus EIA test was the best reliable test for diagnosing *H. pylori* infection [143].

The SAT's sensitivity and specificity depends on the different clinical environment and whether the test is done before or after treatment [144, 145]. The SAT is equivalent to

the UBT in detecting *H. pylori* infection in untreated individuals. However, the UBT is superior to the SAT in post eradication, particularly in populations with low *H. pylori* prevalence.

The polyclonal SAT was the first introduced SAT (Premier Platinum HpSA, Meridian Bioscience Inc., OH, United States). This test was followed by a monoclonal test (Femtolab*H. pylori*, Connex, Germany), which was found to be better than the polyclonal test in both testing untreated patients and monitoring with treated patients [146 - 148].

The monoclonal SAT has been shown to be superior to the polyclonal test for both early diagnosis of infection and confirmation of *H. pylori* eradication [144]. The monoclonal test and the UBT are the only 2 noninvasive tests approved by European Guidelines for determining the effectiveness or failure of eradication therapy.

The use of a monoclonal enzyme-linked immunosorbent assay to detect *H. pylori* antigen in feces is one of the most effective non-invasive techniques for diagnosing infection in children [149]. The *H. pylori* SAT seems to work effectively in children, independent of their age [150 - 152]. The sensitivity, specificity, PPV, and NPV of 20 investigations of the SAT in a total of 2789 patients before they received therapy for *H. pylori* were 90 %, 96 %, 93 %, and 93 % respectively. The confirmation of *H. pylori* eradication after therapy with the SAT yielded good findings in 8 investigations examining a total of 307 children, with a sensitivity of 97 %, specificity of 97 %, PPV of 88 %, and NPV of 99 % [153].

❖ Treatment options for *Helicobacter pylori* Infection

H. pylori causes serious infections in human body [154]. *H. pylori*'s involvement in active chronic gastritis is related to gastroduodenal ulcers, which plays a risk factor for stomach cancer development [155].

There are a variety of treatment options available for *H. pylori* infection; however, there is not a single antibiotic treatment that can completely cure the infection. Historically, the infection was treated with a combination of antibiotics like clarithromycin, amoxicillin, metronidazole, tetracycline, fluoroquinolones, tinidazole etc. These antibiotics are frequently used in conjunction with antisecretory drugs like PPIs or bismuth salts. Different combinations of these drugs have been demonstrated to be successful, with varying eradication rates and tolerability.

The fast rise of antibiotic-resistant strains of *H. pylori*, as well as poor patient adherence to therapy, has harmed the efficiency of the most routinely used medicines. In several geographic locations, these variables have lowered treatment efficacy to unsatisfactory levels ($\leq 80\%$). As a result, alternative treatment procedures have just been validated and are already being employed in place of the standard triple therapy. These methods have been employed in locations where there is a high level of clarithromycin resistance, which is a key risk factor for failure of treatment regimen [156]. Amoxicillin resistance has remained relatively stable, but metronidazole and clarithromycin resistance rates have been progressively increasing [157 - 161]. The prevalence of antibiotic resistance varies greatly by area and it is linked with the use of antibiotics such as clarithromycin and metronidazole, for respiratory or gastrointestinal infections.

The test-and-treat technique was one of the first strategies to be established for the treatment of *H. pylori* infection. This treatment technique is mainly recommended for the patients younger than 45 years old with chronic dyspepsia, peptic ulcer disease, low-grade MALT, and atrophic gastritis. The test-and-treat technique is based on detecting the existence of *H. pylori* and eradicating it once it is found. In dyspeptic patients who reside in populations with a moderate-to-high

prevalence of *H. pylori* infection (≥ 10% to 20%), an alternative to the test-and-treat technique is needed, whereas the empirical PPI strategy may be preferred in populations with a low incidence of *H. pylori* infection [162 - 164]. Because diagnostic tests are less accurate in populations with a low prevalence of *H. pylori*, the test-and-treat technique must be carefully used in these populations [165]. Only a small percentage of patients with functional dyspepsia experience long-term relief from their symptoms once *H. pylori* is eradicated [166 - 168].

It is advised to divide first-line empiric therapy into two large groups: populations with low clarithromycin resistance and populations with high clarithromycin resistance. The acceptable resistance values are set as < 15% to 20% [164]. The following information summarizes current advice and treatment options for *H. pylori* eradication. According to clarithromycin resistance, they are listed as first line, second line, and third line treatments.

- ✓ **First-line treatment in areas with low clarithromycin resistance**

Triple treatment is the most commonly used approach. In this therapy PPI (lansoprazole 30 mg/12 h, omeprazole 20 mg/12 h, pantoprazole 40 mg/12 h, rabeprazole 20 mg/12 h, or esomeprazole 40 mg/24 h), clarithromycin (500 mg/12 h), and amoxicillin (1 g/12 h) are used for 7 to 14 days. The duration of treatment is controversial, while a meta-analysis found that 14 days yields eradication rates that are 5% greater than 7 days. In case of penicillin allergy, metronidazole can be used instead of amoxicillin because it is equally effective and regarded equivalent [169].

There are several factors involved for why clarithromycin susceptibility decreases the success rate of therapy. These factors include the patient's poor adherence to the drug regimen, stomach acidity, bacterial strain concentration, bacterial mutations, and clarithromycin resistance. There is also a lot of variation in these figures. For example, in the Netherlands, where clarithromycin resistance is not prevalent, it is estimated to be between 1% and 5% [164].

The efficacy rates of triple therapy has been demonstrated to be dependent on PPIs; as a result, several strategies have been tried to increase the success of triple therapy, such as increasing the dose of PPIs and increasing the period of treatment. When compared with standard PPI dosages, the eradication rate increased from 6% to 10%, according to a meta-analysis. A double dosage of esomeprazole exhibited a larger positive impact, according to a study. Because PPI activity is dependent on the cytochrome (CYP) 450 2C19 and MDR polymorphisms, the existence of certain polymorphisms in the host's metabolism can alter the effectiveness of PPIs. According to a recent meta-analysis, hosts who are extensive PPI metabolizers (depending on their CYP2C19 status) had lower cure rates. Furthermore, as compared to the T/C and C/C genotypes, the MDR T/T genotype had a lower cure rate [170].

Adjuvant therapy is sometimes used in conjunction with the standard treatment for *H. pylori* infection. For example, lactoferrin has been used as adjuvant therapy. Two meta-analyses that examined at the use of lactoferrin found that it reduce the adverse effects of standard treatment, while the latest consensus of Maastricht (IV) states that more evidence and better-designed research are needed before final conclusions can be drawn [171, 172]. *Saccharomyces boulardii* is another adjuvant that has been used, with positive outcomes in some studies [173].

- ✓ **First-line treatment in areas with high clarithromycin resistance**
  - ➢ **Quadruple therapy**

A quadruple therapy can be used in the areas where clarithromycin resistance is high. In this therapy, for 10 to 14 days, patients are given a combination of a PPI, bismuth subsalicylate

(525 mg × 4 times daily), and 2 antibiotics, metronidazole (250 mg × 4 times daily) and tetracycline (500 mg × 4 times daily). This regimen is well tolerated, and patients tend to adhere to the schedule. But this therapy is not available in all places. Sometimes, it is recommended that doctors have alternatives in mind, such as sequential therapy or quadruple therapy without bismuth [164].

- **Sequential therapy**

A group of Italian researchers proposed the sequential treatment. It involves a 5-day course of a PPI and amoxicillin (1g × 2 times daily), followed by a 5-day course of a PPI and tinidazole clarithromycin / metronidazole (500 mg × 2 times daily). Sequential therapy and bismuth-based quadruple therapy have demonstrated to be equally effective in first-line therapy in most studies [169]. Sequential therapy was studied in a pediatric population with iron deficiency [174]. Children aged 12 to 15 years with active *H. pylori* infection were evaluated for serum ferritin, and then were randomly assigned into 2 groups to receive either standard or sequential eradication therapy. The UBT confirmed eradication after six weeks when the treatment was completed, and serum ferritin levels were assessed. *H. pylori* eradication rates differed between sequential and standard therapy, however serum ferritin levels did not differ substantially between the two therapy groups or between the same group before and after treatment.

H. pylori eradication rates in children after sequential therapy compared to triple therapy were evaluated in a recent meta-analysis. This study included 857 children between the ages of 3 to 18 who matched the inclusion criteria. 318 (78 %, 95 % CI: 73 % - 82 %) of the 409 patients who got sequential therapy were free of infection, compared to 314 (71 %, 95 % CI: 66 % -75 %) of the 444 patients who received standard triple therapy (RR = 1.14, 95 % CI: 1.06 - 1.23). Sequential therapy is preferable to 7 day standard triple therapy, but not substantially better than 10 or 14 day triple therapy. Furthermore, no significant variations in the risk of adverse effects were detected between groups who are receiving various therapies.

- **Concomitant therapy**

In places where clarithromycin resistance is more than 20% and bismuth-based quadruple treatment is not avaible, concurrent therapy is used instead of sequential therapy. Concomitant treatment involves the simultaneous administrationof 3 antibiotics (metronidazole, clarithromycin, and amoxicillin) and a PPI for 10 days. In comparison to conventional triple treatment, this concomitant therapy is more effective and well tolerated [175].

In Greece, a recent controlled trial compared concomitant therapy with triple therapy. There high resistance to clarithromycin (25%) and metronidazole (40%) is mostly seen. Concurrent therapy yielded a 90 % eradication rate, but triple therapy only had a 73.8 % eradication rate [176]. Unfortunately, concurrent treatment had a significant rate of adverse effects, with 30.9 % of individuals reporting at least one adverse effect. On the other hand, the adverse effects were minor, and patients were able to complete their therapy despite them [177]. Another research looked at a combination of PPI, amoxicillin, rifabutin, and ciprofloxacin, and found *H. pylori* eradication rates of 95.2 %. Amoxicillin was substituted by bismuth in patients with a penicillin allergy, with no significant effect on the eradication rate (94.2 %) [178].

A clinical study compared concomitant and sequential therapy and showed no difference in *H. pylori* eradication rates after treatment with these therapies. *H. pylori* infected patients from 11 Spanish hospitals took part in this study. Patients were randomly assigned to undergo either sequential or concurrent therapy. In sequential therapy Omeprazole (20 mg / 12 h) and amoxicillin (1 g / 12 h) were

given for 5 days, followed by 5 days of omeprazole (20 mg / 12 h), clarithromycin (500 mg / 12 h), and metronidazole (500 mg / 12 h). Same medicines were used in concomitant therapy. But in concomitant therapy these medicines were given for 10 days. Four weeks after therapy ceased, *H.pylori* eradication was confirmed with ^{13}C-UBT or histology. The concomitant and sequential eradication rates were 87% and 81%, respectively, by intention-to-treat (P = 0.15) and 91% and 86%, respectively, per protocol (P = 0.131). They came to the conclusion that concomitant therapy had no substantial advantages over sequential therapy [179].

A second study, which compared the sequential and concurrent therapies included 164 *H. pylori* infected patients. Patients were given either sequential (n = 86) or concurrent (n = 78) treatments for 14 days. In the sequential treatment group, patients were given rabeprazole (20 mg) and amoxicillin (1 g) in the first week, followed by rabeprazole (20 mg), clarithromycin (500 mg), and metronidazole (500 mg) in the second week. Rabeprazole (20 mg), amoxicillin (1 g), clarithromycin (500 mg), and metronidazole (500 mg) were given to patients in the concurrent treatment group for 2 weeks. Four weeks after the completetion of therapy, ^{13}C-UBT confirmed the *H.pylori* eradication. The intention-to-treat and per protocol eradication rates were 75.6% (95% CI: 66.3% - 84.9%) and 76.8% (95% CI: 67.1% - 85.5%) in the sequential therapy group, and 80.8% (95% CI: 71.8% - 88.5%) and 81.3% (95% CI: 71.6% - 90.7%) in the concomitant therapy group, respectively. The researchers came to the conclusion that the 2-week concurrent and sequential therapies showed suboptimal efficacy. Furthermore, there were no significant differences in eradication rates, compliance, or adverse effects between the two therapies in this study.

- **Hybrid therapy**

A recently described treatment called hybrid therapy involves two steps: a PPI and amoxicillin (1 g / 12 h) for 7 days, followed by a PPI and three antibiotics - amoxicillin (1 g / 12 h), metronidazole (500 mg / 12 h), and clarithromycin (500 mg / 12 h) for 7 days. The eradication rates in a research contrasting hybrid and sequential therapy were 89.5% and 76.7% (P = 0.001) respectively. Both therapy groups' patients experienced similar serious adverse effects. Particularly, 3.8% of patients receiving sequential therapy and 2.4% of patients receiving hybrid therapy both reported adverse effects [180].

Patients with non-ulcer dyspepsia who were infected with *H. pylori* were included in a recent trial that examined concurrent, sequential, and hybrid therapies [181]. The patients were randomly assigned to one of three treatments:

1) Concomitant therapy with omeprazole (20 mg), amoxicillin (1 g), clarithromycin (500 mg), and tinidazole (500 mg) for 5 days;

2) Sequential therapy with omeprazole (20 mg) and amoxicillin (1 g) for 5 days, followed by omeprazole (20 mg), clarithromycin (500 mg), and tinidazole (500 mg) for 5 days;

3) hybrid therapy with omeprazole (20 mg) and amoxicillin (1 g) for 7 days, followed by omeprazole (20 mg), amoxicillin (1 g), clarithromycin (500 mg), and tinidazole (500 mg) for 7 days.

H. pylori eradication was detected by ^{13}C-UBT six weeks after treatment. In this study, the intention-to-treat and per protocol analyses revealed eradication rates of 85.5% and 91.6%, respectively, with the concomitant therapy regimen; 91.1% and 92.1%, respectively, with the sequential therapy; and 80% and 85.7%, respectively, with the hybrid therapy regimen.

- ✓ **Second-line treatment in areas that have a low clarithromycin resistance**

Bismuth-based quadruple treatment and regimens combining a PPI and levofloxacin /

amoxicillin are available in places where clarithromycin resistance is low [173, 182]. However, due to a rise in levofloxacin resistance, the usage of levofloxacin has been questioned [183]. As a result, susceptibility studies should be done prior to commencing therapy.

✓ **Second-line treatment in areas that have high clarithromycin resistance**

If bismuth-based quadruple therapy fails, a triple therapy comprising a PPI, levofloxacin, and amoxicillin is recommended. The rise in levofloxacin resistance should be considered once more [164].

✓ **Third-line treatment**

It is not recommended to provide further antibiotic treatments after 2 unsuccessful treatments in areas with low or high clarithromycin resistance. Biopsy specimens should be acquired whenever feasible to culture and test for susceptibility [168]. In some circumstances, "rescue" or "salvage" therapy have shown positive effects. For 14 days, rifabutin (150 mg × 2 times daily), amoxicillin (1 g × 2 times daily), and ciprofloxacin (500 mg × 2 times daily) are used as a rescue treatment. Despite the fact that this therapy produces outstanding results, serious adverse effects have been reported [184]. In addition to a base therapy of furazolidone (200 mg × 2 daily), bismuth subcitrate (120 mg × 4 daily), and tetracycline (500 mg × 4 daily), other rescue therapies include a double dose of PPIs plus azithromycin (500 mg / day for 3 days), followed by a double dose of PPIs plus furazolidone (200 mg × 3 times daily) for 10 days. The risk of *H. pylori* infection recurrence after effective eradication has been calculated at 11.5 % for this therapy [177].

✓ **Eradication of *H. pylori* in pregnancy**

If a patient develops a peptic ulcer while pregnant or breastfeeding, the illness should be managed only with acid suppression. Eradication of *H. pylori* should be accomplished after childbirth. In pregnancy bismuth, quinolones, and tetracyclines are contraindicated and metronidazole should be avoided [176].

Only one published report has examined *H. pylori* eradication in pregnant women, especially in pregnant women with iron deficiency anemia [185]. The researchers conducted a randomised placebo-controlled experiment on 40 women aged between 14 to 30 weeks who had *H. pylori* infection as identified by the SAT. Women were randomly assigned into two groups: group I (n = 20) was treated with amoxicillin, clarithromycin, and omeprazole for two weeks, whereas group II (n = 20) was treated with placebo. Iron and folic acid were given to both groups at therapeutic dosages. The rise in haemoglobin, packed cell volume, serum iron, and percentage of transferrin saturation was substantially larger ($P < 0.05$) in the group receiving *H. pylori* eradication medication than the placebo group after 6 weeks of iron and folic acid supplementation therapy. The researchers found that *H. pylori* infection is common in pregnant women with iron deficiency anaemia, and that eradication therapy improved the response to oral iron supplementation in *H. pylori* infected pregnant women with iron deficiency anaemia.

✓ **Use of probiotics in treating *H. pylori* infection**

Probiotics may compete directly with *H. pylori* by interfering with *H. pylori* adherence or by generating antimicrobial compounds, according to certain theories. *Lactobacillus reuteri* (*L. reuteri*) has been studied for its effectiveness in *H. pylori* eradication treatment. Gastric histology and ^{13}C-UBT were used to identify *H. pylori* infection in this investigation. For 8 weeks, intervention consisted of 10^8 colony-forming units of *L. reuteri* (DSM 17938) with pantoprazole (20 mg × 2 times daily). Patients were assessed 4

to 6 weeks after receiving ^{13}C-UBT treatment for *H. pylori* eradication. They revealed that *L. reuteri* with pantoprazole twice daily cured 13.6% (3/22; 95% CI: 2.9% - 34.9%) of patients with *H. pylori* infection by intention-to-treat analysis and 14.2% (3/21; 95% CI: 3.0% -36%) by per protocol analysis. If the cure rate can be increased by changing the dosage, dosing interval, or duration of therapy, researchers believe *L. reuteri* might play a role in *H. pylori* eradication therapy [164].

A recent meta-analysis looked into whether a probiotic product comprising *Lactobacillus* and *Bifidobacterium* may increase *H. pylori* eradication rates while reducing adverse effects [186]. The study comprised 1469 patients in 10 clinical trials, including 708 patients in the probiotic supplementation group and 761 patients in the control group. Pooled ORs by intention-to-treat and per-protocol analyses in the probiotic supplementation group vs the control group were 2.066 (95% CI: 1.398 - 3.055) and 2.321 (95% CI: 1.715 - 3.142) respectively. The pooled OR of overall side effect incidence was considerably lower in the probiotics supplementation group (OR = 0.305; 95 % CI: 0.117 - 0.793). They concluded that adding a probiotic comprising *Lactobacillus* and *Bifidobacterium* with initial *H. pylori* eradication medication in adults may improve eradication rates and reduce overall side effects.

Another study aimed to determine whether adding probiotics to a standard anti-*H. pylori* regimen could minimize the prevalence of gastrointestinal side effects and improve the eradication rate. In a double blind, randomized, placebo-controlled study, 66 *H. pylori*-positive children were diagnosed by RUT or histology then treated with a triple medication treatment protocol (omeprazole, amoxicillin, and furazolidon) and randomly assigned to receive either a probiotic or a placebo [187]. Esophagogastroduodendoscopy was performed on all of the patients. The SAT was used to determine *H. pylori* status in 4 to 8 weeks after therapy was completed. The group that got probiotics had a considerably greater percentage of *H. pylori* eradication (P = 0.04). Furthermore, the probiotic-supplemented children had a lower rate of nausea / vomiting (P = 0.02) and diarrhoea (P = 0.039) throughout therapy than the placebo-treated children. Probiotics have a good effect on the eradication of *H. pylori* infection and the negative effects of *H. pylori* therapy, according to the authors.

❖ Conclusion

H. pylori infection is still the most common and long-lasting bacterial infection on the planet, thus accurate diagnosis is critical. There are numerous options for diagnosing infection and detecting eradication of *H. pylori* infection therapy. In addition, there are a variety of therapeutic options. The diagnostic technique and treatments to employ for each patient are determined by a variety of criteria, including the patient's clinical condition, the prevalence of infection, and the prevalence of clarithromycin resistance.

❖ References

[1]. Hooi JK, Lai WY, Ng WK, Suen MM, Underwood FE, Tanyingoh D, et al. (August 2017). "Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis". Gastroenterology. 153 (2): 420–429. doi:10.1053/j.gastro.2017.04.022.

[2]. Tonkic A, Tonkic M, Lehours P, Mégraud F. Epidemiology and diagnosis of Helicobacter pylori infection. Helicobacter. 2012;**17Suppl 1**:1–8.

[3]. Amieva MR, El-Omar EM (January 2008). "Host-bacterial interactions in Helicobacter pylori infection". Gastroenterology. 134 (1): 306–23. doi:10.1053/j.gastro.2007.11.009

[4]. Schreiber S, Konradt M, Groll C, Scheid P, Hanauer G, Werling HO, et al. (April 2004). "The spatial orientation of Helicobacter pylori in the gastric mucus". Proceedings of the

National Academy of Sciences of the United States of America. 101 (14): 5024–9. Bibcode:2004PNAS..101.5024S. doi:10.1073/pnas.0308386101.

[5]. Petersen AM, Krogfelt KA (May 2003). "Helicobacter pylori: an invading microorganism? A review". FEMS Immunology and Medical Microbiology (Review). 36 (3): 117–26. doi:10.1016/S0928-8244(03)00020-8

[6]. Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, et al. (January 1998). "Helicobacter pylori adhesin binding fucosylatedhisto-blood group antigens revealed by retagging". Science. 279 (5349): 373-7. Bibcode:1998Sci...279..373I. doi:10.1126/science.279.5349.373

[7]. Bugaytsova JA, Björnham O, Chernov YA, Gideonsson P, Henriksson S, Mendez M, et al. (March 2017). "Helicobacter pylori Adapts to Chronic Infection and Gastric Disease via pH-Responsive BabA-Mediated Adherence". Cell Host & Microbe. 21 (3): 376–389. doi:10.1016/j.chom.2017.02.013

[8]. Mahdavi J, Sondén B, Hurtig M, Olfat FO, Forsberg L, Roche N, et al. (July 2002). "Helicobacter pylori SabA adhesin in persistent infection and chronic inflammation". Science. 297 (5581): 573–8. Bibcode:2002Sci...297..573M. doi:10.1126/science.1069076

[9]. Mobley HL (1 January 2001). "Urease". In Mobley HL, Mendz GL, Hazell SL (eds.). Helicobacter pylori: Physiology and Genetics. Washington, DC: ASM Press. ISBN 978-1-55581-213-3.

[10]. Debowski AW, Walton SM, Chua EG, Tay AC, Liao T, Lamichhane B, et al. (June 2017). "Helicobacter pylori gene silencing in vivo demonstrates urease is essential for chronic infection". PLOS Pathogens. 13 (6): e1006464. doi:10.1371/journal.ppat.1006464

[11]. George G, Kombrabail M, Raninga N, Sau AK (March 2017). "Arginase of Helicobacter Gastric Pathogens Uses a Unique Set of Non-catalytic Residues for Catalysis". Biophysical Journal. 112 (6): 1120–1134. Bibcode:2017BpJ...112.1120G. doi:10.1016/j.bpj.2017.02.009

[12]. Smoot DT (December 1997). "How does Helicobacter pylori cause mucosal damage? Direct mechanisms". Gastroenterology. 113 (6 Suppl): S31-4, discussion S50. doi:10.1016/S0016-5085(97)80008-X

[13]. Hatakeyama M, Higashi H (December 2005). "Helicobacter pylori CagA: a new paradigm for bacterial carcinogenesis". Cancer Science. 96 (12): 835–43. doi:10.1111/j.1349-7006.2005.00130.x

[14]. Dumrese C, Slomianka L, Ziegler U, Choi SS, Kalia A, Fulurija A, et al. (May 2009). "The secreted Helicobacter cysteine-rich protein A causes adherence of human monocytes and differentiation into a macrophage-like phenotype". FEBS Letters. 583 (10): 1637–43. doi:10.1016/j.febslet.2009.04.027

[15]. Sajib S, Zahra FT, Lionakis MS, German NA, Mikelis CM (February 2018). "Mechanisms of angiogenesis in microbe-regulated inflammatory and neoplastic conditions". *Angiogenesis*. **21** (1): 1–14. doi:10.1007/s10456-017-9583-4

[16]. Shiotani A, Graham DY (November 2002). "Pathogenesis and therapy of gastric and duodenal ulcer disease". The Medical Clinics of North America. 86 (6): 1447–66, viii. CiteSeerX 10.1.1.550.8580. doi:10.1016/S0025-7125(02)00083-4

[17]. Dixon MF (February 2000). "Patterns of inflammation linked to ulcer disease". Bailliere's Best Practice & Research. Clinical Gastroenterology. 14 (1): 27–40. doi:10.1053/bega.1999.0057

[18]. Blaser MJ, Atherton JC (February 2004). "Helicobacter pylori persistence: biology and disease". The Journal of Clinical Investigation. 113 (3): 321–33. doi:10.1172/JCI20925

[19]. Schubert ML, Peura DA (June 2008). "Control of gastric acid secretion in health and disease". Gastroenterology. 134 (7): 1842–60. doi:10.1053/j.gastro.2008.05.021

[20]. Peek RM, Crabtree JE (January 2006). "Helicobacter infection and gastric neoplasia". The Journal of Pathology. 208 (2): 233–48. doi:10.1002/path.1868

[21]. Viala J, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP, et al. (November 2004). "Nod1 responds to peptidoglycan delivered by the Helicobacter pylori cag pathogenicity island". Nature Immunology. 5 (11): 1166–74. doi:10.1038/ni1131

[22]. Backert S, Selbach M (August 2008). "Role of type IV secretion in Helicobacter pylori pathogenesis". Cellular Microbiology. 10 (8): 1573–81. doi:10.1111/j.1462-5822.2008.01156.x

[23]. Hatakeyama M (September 2004). "Oncogenic mechanisms of the Helicobacter pylori CagA protein". Nature Reviews. Cancer. 4 (9): 688–94. doi:10.1038/nrc1433

[24]. Baldwin DN, Shepherd B, Kraemer P, Hall MK, Sycuro LK, Pinto-Santini DM, Salama NR (February 2007). "Identification of Helicobacter pylori genes that contribute to stomach colonization". Infection and Immunity. 75 (2): 1005–16. doi:10.1128/IAI.01176-06

[25]. Broutet N, Marais A, Lamouliatte H, de Mascarel A, Samoyeau R, Salamon R, Mégraud F (April 2001). "cagA Status and eradication treatment outcome of anti-Helicobacter pylori triple therapies in patients with nonulcer dyspepsia". Journal of Clinical Microbiology. 39 (4): 1319–22. doi:10.1128/JCM.39.4.1319-1322.2001

[26]. Tsuji S, Kawai N, Tsujii M, Kawano S, Hori M (July 2003). "Review article: inflammation-related promotion of gastrointestinal carcinogenesis--a perigenetic pathway". Alimentary Pharmacology & Therapeutics. 18 Suppl 1 (Suppl 1): 82–9. doi:10.1046/j.1365-2036.18.s1.22.x

[27]. Suganuma M, Yamaguchi K, Ono Y, Matsumoto H, Hayashi T, Ogawa T, et al. (July 2008). "TNF-alpha-inducing protein, a carcinogenic factor secreted from H. pylori, enters gastric cancer cells". International Journal of Cancer. 123 (1): 117–22. doi:10.1002/ijc.23484

[28]. Alfarouk KO, Bashir AH, Aljarbou AN, Ramadan AM, Muddathir AK, AlHoufie ST, et al. (22 February 2019). "Helicobacter pylori in Gastric Cancer and Its Management". Frontiers in Oncology. 9: 75. doi:10.3389/fonc.2019.00075

[29]. Kim W, Moss SF (December 2008). "The role of H. pylori in the development of stomach cancer". Oncology Review. 1 (Supp l1): 165–168. Retrieved 25 August 2014.

[30]. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, et al. (April 2019). "Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods". International Journal of Cancer. 144 (8): 1941–1953. doi:10.1002/ijc.31937

[31]. Kuipers EJ (March 1999). "Review article: exploring the link between Helicobacter pylori and gastric cancer". Alimentary Pharmacology &Therapeutics. 13 Suppl 1: 3–11. doi:10.1046/j.1365-2036.1999.00002.x

[32]. Prabhu SR, Ranganathan S, Amarapurkar DN (November 1994). "Helicobacter pylori in normal gastric mucosa". The Journal of the Association of Physicians of India. 42 (11): 863–4.

[33]. White JR, Winter JA, Robinson K (2015). "Differential inflammatory response to Helicobacter pylori infection: etiology and clinical outcomes". Journal of Inflammation Research. 8: 137–47. doi:10.2147/JIR.S64888

[34]. Deng JY, Liang H (April 2014). "Clinical significance of lymph node metastasis in gastric cancer". World Journal of Gastroenterology. 20 (14): 3967–75. doi:10.3748/wjg.v20.i14.3967

[35]. Valenzuela MA, Canales J, Corvalán AH, Quest AF (December 2015). "Helicobacter pylori-induced inflammation and epigenetic changes during gastric carcinogenesis". World Journal of Gastroenterology. 21 (45): 12742–56. doi:10.3748/wjg.v21.i45.12742

[36]. Raza Y, Khan A, Farooqui A, Mubarak M, Facista A, Akhtar SS, et al. (October 2014). "Oxidative DNA damage as a potential early biomarker of Helicobacter pylori associated carcinogenesis". Pathology & Oncology Research. 20 (4): 839–46. doi:10.1007/s12253-014-9762-1

[37]. Koeppel M, Garcia-Alcalde F, Glowinski F, Schlaermann P, Meyer TF (June 2015). "Helicobacter pylori Infection Causes Characteristic DNA Damage Patterns in Human Cells". Cell Reports. 11 (11): 1703–13. doi:10.1016/j.celrep.2015.05.030

[38]. Muhammad JS, Eladl MA, Khoder G (February 2019). "Helicobacter pylori-induced DNA Methylation as an Epigenetic Modulator of Gastric Cancer: Recent Outcomes and Future Direction". Pathogens. 8 (1): 23. doi:10.3390/pathogens8010023

[39]. Noto JM, Peek RM (2011). "The role of microRNAs in Helicobacter pylori pathogenesis and gastric carcinogenesis". Frontiers in Cellular and Infection Microbiology. 1: 21. doi:10.3389/fcimb.2011.00021

[40]. Santos JC, Ribeiro ML (August 2015). "Epigenetic regulation of DNA repair machinery in Helicobacter pylori-induced gastric carcinogenesis". World Journal of Gastroenterology. 21 (30): 9021–37. doi:10.3748/wjg.v21.i30.9021

[41]. Raza Y, Ahmed A, Khan A, Chishti AA, Akhter SS, Mubarak M, et al. (May 2020). "Helicobacter pylori severely reduces expression of DNA repair proteins PMS2 and ERCC1 in gastritis and gastric cancer". DNA Repair. 89: 102836. doi:10.1016/j.dnarep.2020.102836

[42]. Dore MP, Pes GM, Bassotti G, Usai-Satta P (2016). "Dyspepsia: When and How to Test for Helicobacter pylori Infection". Gastroenterology Research and Practice. 2016: 8463614. doi:10.1155/2016/8463614

[43]. Olczak AA, Olson JW, Maier RJ (June 2002). "Oxidative-stress resistance mutants of Helicobacter pylori". Journal of Bacteriology. 184 (12): 3186–93. doi:10.1128/JB.184.12.3186-3193.2002

[44]. O'Rourke EJ, Chevalier C, Pinto AV, Thiberge JM, Ielpi L, Labigne A, Radicella JP (March 2003). "Pathogen DNA as target for host-generated oxidative stress: role for repair of bacterial DNA damage in Helicobacter pylori colonization". Proceedings of the National Academy of Sciences of the United States of America. 100 (5): 2789–94. Bibcode:2003PNAS..100.2789O. doi:10.1073/pnas.0337641100

[45]. Michod RE, Bernstein H, Nedelcu AM (May 2008). "Adaptive value of sex in microbial pathogens". Infection, Genetics and Evolution. 8 (3): 267–85. doi:10.1016/j.meegid.2008.01.002

[46]. Dorer MS, Fero J, Salama NR (July 2010). Blanke SR (ed.). "DNA damage triggers genetic exchange in Helicobacter pylori". PLOS Pathogens. 6 (7): e1001026. doi:10.1371/journal.ppat.1001026

[47]. Loughlin MF, Barnard FM, Jenkins D, Sharples GJ, Jenks PJ (April 2003). "Helicobacter pylori mutants defective in RuvC Holliday junction resolvase display reduced macrophage survival and spontaneous clearance from the murine gastric mucosa". Infection and Immunity. 71 (4): 2022–31. doi:10.1128/IAI.71.4.2022-2031.2003

[48]. Wang G, Maier RJ (January 2008). "Critical role of RecN in recombinational DNA repair and survival of Helicobacter pylori". Infection and Immunity. 76 (1): 153–60. doi:10.1128/IAI.00791-07

[49]. van Blankenstein M, van Vuuren AJ, Looman CW, Ouwendijk M, Kuipers

EJ. The prevalence of Helicobacter pylori infection in the Netherlands. Scand J Gastroenterol 2013; 48: 794– 800.

[50]. den Hollander WJ, Holster IL, den Hoed CM, et al. Ethnicity is a strong predictor for Helicobacter pylori infection in young women in a multi-ethnic European city. J GastroenterolHepatol 2013; 28: 1705– 11.

[51]. Bastos J, Peleteiro B, Barros R, et al. Sociodemographic determinants of prevalence and incidence of Helicobacter pylori infection in Portuguese adults. Helicobacter 2013; 18(6): 413– 22.

[52]. Ozaydin N, Turkyilmaz SA, Cali S. Prevalence and risk factors of Helicobacter pylori in Turkey: a nationally-representative, cross-sectional, screening with the (1)(3)C-Urea breath test. BMC Public Health 2013; 13: 1215.

[53]. Sethi A, Chaudhuri M, Kelly L, Hopman W. Prevalence of Helicobacter pylori in a First Nations population in northwestern Ontario. Can Fam Physician 2013; 59: e182– 7.

[54]. Alvarado-Esquivel C. Seroepidemiology of Helicobacter pylori infection in pregnant women in rural durango. Mexico Int J Biomed Sci 2013; 9: 224– 9.

[55]. Porras C, Nodora J, Sexton R, et al. Epidemiology of Helicobacter pylori infection in six Latin American countries (SWOG Trial S0701). Cancer Causes Control 2013; 24(2): 209– 15.

[56]. Lim SH, Kwon JW, Kim N, et al. Prevalence and risk factors of Helicobacter pylori infection in Korea: nationwide multicenter study over 13 years. BMC Gastroenterol 2013; 13: 104.

[57]. Zhu Y, Zhou X, Wu J, Su J, Zhang G. Risk factors and prevalence of Helicobacter pylori infection in persistent high incidence area of gastric carcinoma in Yangzhong city. Gastroenterol Res Pract 2014; 2014: 481365.

[58]. Sodhi JS, Javid G, Zargar SA, et al. Prevalence of Helicobacter pylori infection and the effect of its eradication on symptoms of functional dyspepsia in Kashmir
India. GastroenterolHepatol 2013; 28: 808– 1 3.

[59]. Adlekha S, Chadha T, Krishnan P, Sumangala B. Prevalence of Helicobacter pylori infection among patients undergoing upper gastrointestinal endoscopy in a medical college hospital in kerala, India. Ann Med Health Sci Res 2013; 3: 559– 63.

[60]. Benberin V, Bektayeva R, Karabayeva R, Lebedev A, Akemeyeva K, Paloheimo L, Syrjänen K. Prevalence of H. pylori infection and atrophic gastritis among symptomatic and dyspeptic adults in Kazakhstan. A hospital-based screening study using a panel of serum biomarkers. Anticancer Res 2013; 33: 4595– 602.

[61]. Vilaichone RK, Mahachai V, Shiota S, Uchida T, Ratanachu-ek T, Tshering L, Tung NL, Fujioka T, Moriyama M, Yamaoka Y. Extremely high prevalence of Helicobacter pylori infection in Bhutan. World J Gastroenterol 2013; 19: 2806– 10.

[62]. Dorji D, Dendup T, Malaty HM, Wangchuk K, Yangzom D, Richter JM. Epidemiology of Helicobacter pylori in Bhutan: the role of environment and Geographic
location. Helicobacter 2014; 19: 69– 73.

[63]. Hanafi MI, Mohamed AM. Helicobacter pylori infection: seroprevalence and predictors among healthy individuals in Al Madinah, Saudi Arabia. J Egypt Public Health Assoc 2013; 88: 40– 5.

[64]. Kim JH, Kim HY, Kim NY, et al. Seroepidemiological study of Helicobacter pylori infection in asymptomatic people in South Korea. J GastroenterolHepatol 2001; 16: 969– 75.

[65]. Yim JY, Kim N, Choi SH, et al. Seroprevalence of Helicobacter pylori in South Korea. Helicobacter 2007; 12: 333– 40.

[66]. Benajah DA, Lahbabi M, Alaoui S, El Rhazi K, El Abkari M, Nejjari C, Amarti A, Bennani B, Mahmoud M, Ibrahimi SA. Prevalence of Helicobacter pylori and its recurrence after successful eradication in a developing nation (Morocco). Clin Res HepatolGastroenterol 2013; 37: 519– 26.

[67]. Mathewos B, Moges B, Dagnew M. Seroprevalence and trend of Helicobacter pylori infection in Gondar University Hospital among dyspeptic patients, Gondar, North West Ethiopia. BMC Res Notes 2013; 6: 346.

[68]. Olokoba AB, Gashau W, Bwala S, Adamu A, Salawu FK. Helicobacter pylori infection in nigerians with dyspepsia. Ghana Med J 2013; 47: 79– 81.

[69]. Mana F, Vandebosch S, MiendjeDeyi V, Haentjens P, Urbain D. Prevalence of and risk factors for H. pylori infection in healthy children and young adults in Belgium anno 2010/2011. ActaGastroenterolBelg 2013; 76: 381– 5.

[70]. Bastos J, Peleteiro B, Pinto H, Marinho A, Guimaraes JT, Ramos E, La Vecchia C, Barros H, Lunet N. Prevalence, incidence and risk factors for Helicobacter pylori infection in a cohort of Portuguese adolescents (EpiTeen). Dig Liver Dis 2013; 45: 290– 5.

[71]. Pacheco SL, Ogata SK, Machado RS, Patricio FR, Pardo ML, Kawakami E. Diagnosis of Helicobacter pylori infection by means of reduced-dose (13)C-urea breath test and early sampling of exhaled breath. J PediatrGastroenterolNutr 2013; 57: 607– 11.

[72]. Yu Y, Su L, Wang X, Wang X, Xu C. Association between Helicobacter pylori infection and pathological changes in the gastric mucosa in Chinese children. Intern Med 2014; 53: 83– 8.

[73]. Ghasemi-Kebria F, Ghaemi E, Azadfar S, Roshandel G. Epidemiology of Helicobacter pylori infection among Iranian children. Arab J Gastroenterol 2013; 14: 169– 72.

[74]. Didelot X, Nell S, Yang I, Woltemate S, van der Merwe S, Suerbaum S. Genomic evolution and transmission of *Helicobacter pylori* in two South African families. *ProcNatlAcadSci U S A* 2013; **110**: 13880– 5.

[75]. Osaki T, Okuda M, Ueda J, et al. Multilocus sequence typing of DNA from faecal specimens for the analysis of intra-familial transmission of *Helicobacter pylori*. *J Med Microbiol* 2013; **62**: 761– 5.

[76]. Urita Y, Watanabe T, Kawagoe N, et al. Role of infected grandmothers in transmission of *Helicobacter pylori* to children in a Japanese rural town. *J Paediatr Child Health* 2013; **49**: 394– 8.

[77]. Bytzer P, Dahlerup JF, Eriksen JR, Jarbøl DE, Rosenstock S, Wildt S (April 2011). "Diagnosis and treatment of Helicobacter pylori infection". Danish Medical Bulletin. 58 (4): C4271. PMID 21466771.Archived from the original on 5 January 2014.

[78]. Chang AH, Parsonnet J (October 2010). "Role of bacteria in oncogenesis". Clinical Microbiology Reviews. 23 (4): 837–57. doi:10.1128/CMR.00012-10

[79]. Kusters JG, van Vliet AH, Kuipers EJ (July 2006). "Pathogenesis of Helicobacter pylori infection". Clinical Microbiology Reviews. 19 (3): 449–90. doi:10.1128/CMR.00054-05

[80]. Ryan, Kenneth (2010). Sherris Medical Microbiology.McGraw-Hill.pp. 573, 576. ISBN 978-0-07-160402-4.

[81]. Suerbaum S, Michetti P (October 2002). "Helicobacter pylori infection". The New England Journal of Medicine. 347 (15): 1175–86. CiteSeerX 10.1.1.572.9262. doi:10.1056/NEJMra020542

[82]. Wagner AD, Syn NL, Moehler M, Grothe W, Yong WP, Tai BC, et al. (August 2017). "Chemotherapy for advanced gastric cancer". The Cochrane Database of Systematic

Reviews. 8: CD004064. doi:10.1002/14651858.cd004064.

[83]. Wu Q, Yang ZP, Xu P, Gao LC, Fan DM (July 2013). "Association between Helicobacter pylori infection and the risk of colorectal neoplasia: a systematic review and meta-analysis". Colorectal Disease. 15 (7): e352-64. doi:10.1111/codi.12284.

[84]. Markowski AR, Markowska A, Guzinska-Ustymowicz K (October 2016). "Pathophysiological and clinical aspects of gastric hyperplastic polyps". World Journal of Gastroenterology. 22 (40): 8883–8891. doi:10.3748/wjg.v22.i40.8883

[85]. Soetikno RM, Kaltenbach T, Rouse RV, Park W, Maheshwari A, Sato T, et al. (March 2008). "Prevalence of nonpolypoid (flat and depressed) colorectal neoplasms in asymptomatic and symptomatic adults". JAMA. 299 (9): 1027–35. doi:10.1001/jama.299.9.1027

[86]. Kusters JG, van Vliet AH, Kuipers EJ (July 2006). "Pathogenesis of Helicobacter pylori infection". Clinical Microbiology Reviews. 19 (3): 449–90. doi:10.1128/CMR.00054-05

[87]. Guffey Johnson J, Terpak LA, Margo CE, Setoodeh R (April 2016). "Extranodal Marginal Zone B-cell Lymphoma of the Ocular Adnexa". Cancer Control. 23 (2): 140–9. doi:10.1177/107327481602300208

[88]. Saccà SC, Vagge A, Pulliero A, Izzotti A (December 2014). "Helicobacter pylori infection and eye diseases: a systematic review". Medicine. 93 (28): e216. doi:10.1097/md.0000000000000216.

[89]. Genta RM, Graham DY. Comparison of biopsy sites for the histopathologic diagnosis of Helicobacter pylori: a topographic study of H. pylori density and distribution. GastrointestEndosc. 1994;**40**:342–345.

[90]. Satoh K, Kimura K, Taniguchi Y, Kihira K, Takimoto T, Saifuku K, Kawata H, Tokumaru K, Kojima T, Seki M, et al. Biopsy sites suitable for the diagnosis of Helicobacter pylori infection and the assessment of the extent of atrophic gastritis. Am J Gastroenterol. 1998;**93**:569–573.

[91]. van IJzendoorn MC, Laheij RJ, de Boer WA, Jansen JB. The importance of corpus biopsies for the determination of Helicobacter pylori infection. Neth J Med. 2005;**63**:141–145.

[92]. Lan HC, Chen TS, Li AF, Chang FY, Lin HC. Additional corpus biopsy enhances the detection of Helicobacter pylori infection in a background of gastritis with atrophy. BMC Gastroenterol. 2012;**12**:182.

[93]. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System.International Workshop on the Histopathology of Gastritis, Houston 1994. Am J SurgPathol. 1996;**20**:1161–1181.

[94]. Laine L, Lewin DN, Naritoku W, Cohen H. Prospective comparison of H& amp; E, Giemsa, and Genta stains for the diagnosis of Helicobacter pylori. GastrointestEndosc. 1997;**45**:463–467.

[95]. Fallone CA, Loo VG, Lough J, Barkun AN. Hematoxylin and eosin staining of gastric tissue for the detection of Helicobacter pylori. Helicobacter. 1997;**2**:32–35.

[96]. El-Zimaity HM, Segura AM, Genta RM, Graham DY. Histologic assessment of Helicobacter pylori status after therapy: comparison of Giemsa, Diff-Quik, and Genta stains. Mod Pathol. 1998;**11**:288–291.

[97]. Eshun JK, Black DD, Casteel HB, Horn H, Beavers-May T, Jetton CA, Parham DM. Comparison of immunohistochemistry and silver stain for the diagnosis of pediatric Helicobacter pylori infection in urease-negative gastric biopsies. PediatrDevPathol. 2001;**4**:82–88.

[98]. El-Zimaity HM, Graham DY. Evaluation of gastric mucosal biopsy site and number for identification of Helicobacter pylori or intestinal metaplasia: role of the Sydney System. Hum Pathol. 1999;**30**:72–77.

[99]. Morgner A, Lehn N, Andersen LP, Thiede C, Bennedsen M, Trebesius K, Neubauer B, Neubauer A, Stolte M, Bayerdörffer E. Helicobacter heilmannii-associated primary gastric low-grade MALT lymphoma: complete remission after curing the infection. Gastroenterology. 2000;**118**:821–828.

[100]. Carvalho MA, Machado NC, Ortolan EV, Rodrigues MA. Upper gastrointestinal histopathological findings in children and adolescents with nonulcer dyspepsia with Helicobacter pylori infection. J PediatrGastroenterolNutr. 2012;**55**:523–529.

[101]. Trebesius K, Panthel K, Strobel S, Vogt K, Faller G, Kirchner T, Kist M, Heesemann J, Haas R. Rapid and specific detection of Helicobacter pylori macrolide resistance in gastric tissue by fluorescent in situ hybridisation. Gut. 2000;**46**:608–614.

[102]. Rüssmann H, Kempf VA, Koletzko S, Heesemann J, Autenrieth IB. Comparison of fluorescent in situ hybridization and conventional culturing for detection of Helicobacter pylori in gastric biopsy specimens. J ClinMicrobiol. 2001;**39**:304–308.

[103]. Camorlinga-Ponce M, Romo C, González-Valencia G, Muñoz O, Torres J. Topographical localisation of cagA positive and cagA negative Helicobacter pylori strains in the gastric mucosa; an in situhybridisation study. J ClinPathol. 2004;**57**:822–828.

[104]. Windsor HM, Abioye-Kuteyi EA, Marshall BJ. Methodology and transport medium for collection of Helicobacter pylori on a string test in remote locations. Helicobacter. 2005;**10**:630–634.

[105]. Velapatiño B, Balqui J, Gilman RH, Bussalleu A, Quino W, Finger SA, Santivañez L, Herrera P, Piscoya A, Valdivia J, et al. Validation of string test for diagnosis of Helicobacter pylori infections. J ClinMicrobiol. 2006;**44**:976–980.

[106]. Whitmire JM, Merrell DS. Successful culture techniques for Helicobacter species: verification of Helicobacter identity using 16S rRNA gene sequence analysis. Methods Mol Biol. 2012;**921**:37–40.

[107]. Hirschl AM, Makristathis A. Methods to detect Helicobacter pylori: from culture to molecular
biology. Helicobacter. 2007;**12Suppl 2**:6–11.

[108]. Ramis IB, de Moraes EP, Fernandes MS, Mendoza-Sassi R, Rodrigues O, Juliano CR, Scaini CJ, da Silva PE. Evaluation of diagnostic methods for the detection of Helicobacter pylori in gastric biopsy specimens of dyspeptic patients. Braz J Microbiol. 2012;**43**:903–908.

[109]. Choi YJ, Kim N, Lim J, Jo SY, Shin CM, Lee HS, Lee SH, Park YS, Hwang JH, Kim JW, et al. Accuracy of diagnostic tests for Helicobacter pylori in patients with peptic ulcer bleeding. Helicobacter. 2012;**17**:77–85.

[110]. Sudraba A, Daugule I, Rudzite D, Funka K, Tolmanis I, Engstrand L, Janciauskas D, Jonaitis L, Kiudelis G, Kupcinskas L, et al. Performance of routine Helicobacter pylori tests in patients with atrophic gastritis. J Gastrointestin Liver Dis. 2011;**20**:349–354.

[111]. Mendoza-Ibarra SI, Perez-Perez GI, Bosques-Padilla FJ, Urquidi-Rivera M, Rodríguez-Esquivel Z, Garza-González E. Utility of diagnostic tests for detection of Helicobacter pylori in children in northeastern Mexico. Pediatr Int. 2007;**49**:869–874.

[112]. Perez-Perez GI. Accurate diagnosis of Helicobacter pylori.Culture, including transport. GastroenterolClin North Am. 2000;**29**:879–884.

[113]. Ndip RN, MacKay WG, Farthing MJ, Weaver LT. Culturing Helicobacter pylori from clinical specimens: review of microbiologic methods. J PediatrGastroenterolNutr. 2003;**36**:616–622.

[114]. Ogata SK, Godoy AP, da Silva Patricio FR, Kawakami E. High Helicobacter pylori resistance to metronidazole and clarithromycin in Brazilian children and adolescents. J PediatrGastroenterolNutr. 2013;**56**:645–648.

[115]. Kato S, Fujimura S. Primary antimicrobial resistance of Helicobacter pylori in children during the past 9 years. Pediatr Int. 2010;**52**:187–190.
[116]. Rimbara E, Sasatsu M, Graham DY. PCR detection of Helicobacter pylori in clinical samples. Methods Mol Biol. 2013;**943**:279–287.
[117]. Duś I, Dobosz T, Manzin A, Loi G, Serra C, Radwan-Oczko M. Role of PCR in Helicobacter pylori diagnostics and research--new approaches for study of coccoid and spiral forms of the bacteria. PostepyHig Med Dosw (Online) 2013;**67**:261–268.
[118]. Owen RJ. Molecular testing for antibiotic resistance in Helicobacter pylori. Gut. 2002;**50**:285–289.
[119]. Schweizer HP. Understanding efflux in Gram-negative bacteria: opportunities for drug discovery. Expert Opin Drug Discov. 2012;**7**:633–642.
[120]. Lewis JD, Kroser J, Bevan J, Furth EE, Metz DC. Urease-based tests for Helicobacter pylori gastritis.Accurate for diagnosis but poor correlation with disease severity. J ClinGastroenterol. 1997;**25**:415–420.
[121]. Moon SW, Kim TH, Kim HS, Ju JH, Ahn YJ, Jang HJ, Shim SG, Kim HJ, Jung WT, Lee OJ. United Rapid Urease Test Is Superior than Separate Test in Detecting Helicobacter pylori at the Gastric Antrum and Body Specimens. ClinEndosc. 2012;**45**:392–396.
[122]. Monteiro L, de Mascarel A, Sarrasqueta AM, Bergey B, Barberis C, Talby P, Roux D, Shouler L, Goldfain D, Lamouliatte H, et al. Diagnosis of Helicobacter pylori infection: noninvasive methods compared to invasive methods and evaluation of two new tests. Am J Gastroenterol. 2001;**96**:353–358.
[123]. Tseng CA, Wang WM, Wu DC. Comparison of the clinical feasibility of three rapid urease tests in the diagnosis of Helicobacter pylori infection. Dig Dis Sci. 2005;**50**:449–452.
[124]. Harris P, Perez-Perez G, Zylberberg A, Rollán A, Serrano C, Riera F, Einisman H, García D, Viviani P. Relevance of adjusted cut-off values in commercial serological immunoassays for Helicobacter pylori infection in children. Dig Dis Sci. 2005;**50**:2103–2109.
[125]. Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. Gut. 2007;**56**:772–781.
[126]. Hirschl AM, Rotter ML. Serological tests for monitoring Helicobacter pylori eradication treatment. J Gastroenterol. 1996;**31Suppl 9**:33–36.
[127]. Breslin NP, O'Morain CA. Noninvasive diagnosis of Helicobacter pylori infection: a review. Helicobacter. 1997;**2**:111–117.
[128]. Axon AT. Are all helicobacters equal? Mechanisms of gastroduodenal pathology and their clinical implications. Gut. 1999;**45Suppl 1**:I1–I4.
[129]. Blaser MJ. Role of vacA and the cagA locus of Helicobacter pylori in human disease. Aliment PharmacolTher. 1996;**10Suppl 1**:73–77.
[130]. Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. Gut. 1997;**40**:297–301.
[131]. Erzin Y, Altun S, Dobrucali A, Aslan M, Erdamar S, Dirican A, Tuncer M, Kocazeybek B. Analysis of serum antibody profile against H pylori VacA and CagA antigens in Turkish patients with duodenal ulcer. World J Gastroenterol. 2006;**12**:6869–6873.
[132]. Sökücü S, Ozden AT, Süoğlu OD, Elkabes B, Demir F, Cevikbaş U, Gökçe S, Saner G. CagA positivity and its association with gastroduodenal disease in Turkish children undergoing endoscopic investigation. J Gastroenterol. 2006;**41**:533–539.
[133]. Janulaityte-Günther D, Kupcinskas L, Pavilonis A, Valuckas K, Wadström T,

Andersen LP. Combined serum IgG response to Helicobacter pylori VacA and CagA predicts gastric cancer. FEMS Immunol Med Microbiol. 2007;**50**:220–225.

[134]. Formichella L, Romberg L, Bolz C, Vieth M, Geppert M, Göttner G, Nölting C, Walter D, Schepp W, Schneider A, et al. A novel line immunoassay based on recombinant virulence factors enables highly specific and sensitive serologic diagnosis of Helicobacter pylori infection. Clin Vaccine Immunol. 2013;**20**:1703–1710.

[135]. Graham DY, Klein PD, Evans DJ, Evans DG, Alpert LC, Opekun AR, Boutton TW. Campylobacter pylori detected noninvasively by the 13C-urea breath test. Lancet. 1987;**1**:1174–1177.

[136]. Peura DA, Pambianco DJ, Dye KR, Lind C, Frierson HF, Hoffman SR, Combs MJ, Guilfoyle E, Marshall BJ. Microdose 14C-urea breath test offers diagnosis of Helicobacter pylori in 10 minutes. Am J Gastroenterol. 1996;**91**:233–238.

[137]. Goddard AF, Logan RP. Review article: urea breath tests for detecting Helicobacter pylori. Aliment PharmacolTher. 1997;**11**:641–649.

[138]. Gisbert JP, Pajares JM. Review article: 13C-urea breath test in the diagnosis of Helicobacter pylori infection -- a critical review. Aliment PharmacolTher. 2004;**20**:1001–1017.

[139]. Capurso G, Carnuccio A, Lahner E, Panzuto F, Baccini F, DelleFave G, Annibale B. Corpus-predominant gastritis as a risk factor for false-negative 13C-urea breath test results. Aliment PharmacolTher. 2006;**24**:1453–1460.

[140]. Shirin H, Levine A, Shevah O, Shabat-Sehayek V, Aeed H, Wardi J, Birkenfeld S, Eliakim R, Avni Y. Eradication of Helicobacter pylori can be accurately confirmed 14 days after termination of triple therapy using a high-dose citric acid-based 13C urea breath test. Digestion. 2005;**71**:208–212.

[141]. Leal YA, Flores LL, Fuentes-Pananá EM, Cedillo-Rivera R, Torres J. 13C-urea breath test for the diagnosis of Helicobacter pylori infection in children: a systematic review and meta-analysis. Helicobacter. 2011;**16**:327–337.

[142]. Malfertheiner P, Mégraud F, O'Morain C, Bell D, Bianchi Porro G, Deltenre M, Forman D, Gasbarrini G, Jaup B, Misiewicz JJ, et al. Current European concepts in the management of Helicobacter pylori infection--the Maastricht Consensus Report. The European Helicobacter Pylori Study Group (EHPSG) Eur J GastroenterolHepatol. 1997;**9**:1–2.

[143]. Korkmaz H, Kesli R, Karabagli P, Terzi Y. Comparison of the diagnostic accuracy of five different stool antigen tests for the diagnosis of Helicobacter pylori infection. Helicobacter. 2013;**18**:384–391.

[144]. Gisbert JP, de la Morena F, Abraira V. Accuracy of monoclonal stool antigen test for the diagnosis of H. pylori infection: a systematic review and meta-analysis. Am J Gastroenterol. 2006;**101**:1921–1930.

[145]. Veijola L, Oksanen A, Löfgren T, Sipponen P, Karvonen AL, Rautelin H. Comparison of three stool antigen tests in confirming Helicobacter pylori eradication in adults. Scand J Gastroenterol. 2005;**40**:395–401.

[146]. Gisbert JP, Pajares JM. Stool antigen test for the diagnosis of Helicobacter pylori infection: a systematic review. Helicobacter. 2004;**9**:347–368.

[147]. Choi J, Kim CH, Kim D, Chung SJ, Song JH, Kang JM, Yang JI, Park MJ, Kim YS, Yim JY, et al. Prospective evaluation of a new stool antigen test for the detection of Helicobacter pylori, in comparison with histology, rapid urease test, (13)C-urea breath test, and serology. J GastroenterolHepatol. 2011;**26**:1053–1059.

[148]. Pourakbari B, Mirsalehian A, Maleknejad P, Mamishi S, Azhdarkosh H, Daryani NE, Najafi M, Kazemi B, Paknejad M,

Mahmoudi S, et al. Evaluation of a new antigen for diagnosis of Helicobacter pylori infection in stool of adult and children. Helicobacter. 2011;**16**:42–46.

[149]. Leal YA, Cedillo-Rivera R, Simón JA, Velázquez JR, Flores LL, Torres J. Utility of stool sample-based tests for the diagnosis of Helicobacter pylori infection in children. J PediatrGastroenterolNutr. 2011;**52**:718–728.

[150]. Kato S, Ozawa K, Okuda M, Fujisawa T, Kagimoto S, Konno M, Maisawa S, Iinuma K. Accuracy of the stool antigen test for the diagnosis of childhood Helicobacter pylori infection: a multicenter Japanese study. Am J Gastroenterol. 2003;**98**:296–300.

[151]. Koletzko S, Konstantopoulos N, Bosman D, Feydt-Schmidt A, van der Ende A, Kalach N, Raymond J, Rüssmann H. Evaluation of a novel monoclonal enzyme immunoassay for detection of Helicobacter pylori antigen in stool from children. Gut. 2003;**52**:804–806.

[152]. Ni YH, Lin JT, Huang SF, Yang JC, Chang MH. Accurate diagnosis of Helicobacter pylori infection by stool antigen test and 6 other currently available tests in children. J Pediatr. 2000;**136**:823–827.

[153]. Guo YY, Zhang ST, Peng XX, Zhan SY. [A systematic review of diagnosis of Helicobacter pylori infection by Helicobacter pylori stool antigen test] Zhonghua Yi XueZazhi. 2005;**85**:1564–1567.

[154]. Sugimoto M, Yamaoka Y. Virulence factor genotypes of Helicobacter pylori affect cure rates of eradication therapy. Arch ImmunolTherExp (Warsz) 2009;**57**:45–56.

[155]. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. N Engl J Med. 2001;**345**:784–789.

[156]. Mégraud F. Current recommendations for Helicobacter pylori therapies in a world of evolving resistance. Gut Microbes. 2013;**4**:Epub ahead of print.

[157]. Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, Andersen LP, Goossens H, Glupczynski Y. Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut. 2013;**62**:34–42.

[158]. Su P, Li Y, Li H, Zhang J, Lin L, Wang Q, Guo F, Ji Z, Mao J, Tang W, et al. Antibiotic resistance of Helicobacter pylori isolated in the Southeast Coastal Region of China. Helicobacter. 2013;**18**:274–279.

[159]. Lee JW, Kim N, Kim JM, Nam RH, Chang H, Kim JY, Shin CM, Park YS, Lee DH, Jung HC. Prevalence of primary and secondary antimicrobial resistance of Helicobacter pylori in Korea from 2003 through 2012. Helicobacter. 2013;**18**:206–214.

[160]. De Francesco V, Margiotta M, Zullo A, Hassan C, Giorgio F, Burattini O, Stoppino G, Cea U, Pace A, Zotti M, et al. Prevalence of primary clarithromycin resistance in Helicobacter pylori strains over a 15 year period in Italy. J AntimicrobChemother. 2007;**59**:783–785.

[161]. Megraud F. Helicobacter pylori and antibiotic resistance. Gut. 2007;**56**:1502.

[162]. Ford AC, Moayyedi P. Should we step-up or step-down in the treatment of new-onset dyspepsia in primary care? Pol Arch Med Wewn. 2009;**119**:391–396.

[163]. Talley NJ. Dyspepsia: management guidelines for the millennium. Gut. 2002;50Suppl **4**:iv72–iv8; discussion iv79.

[164]. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, et al. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. Gut. 2012;**61**:646–664.

[165]. Moayyedi P, Axon AT. The usefulness of the likelihood ratio in the diagnosis of dyspepsia and gastroesophageal reflux disease. Am J Gastroenterol. 1999;**94**:3122–3125.

[166]. Moayyedi P, Soo S, Deeks J, Forman D, Mason J, Innes M, Delaney B. Systematic review and economic evaluation of Helicobacter pylori eradication treatment for non-ulcer dyspepsia. Dyspepsia Review Group. BMJ. 2000;**321**:659–664.

[167]. Laine L, Schoenfeld P, Fennerty MB. Therapy for Helicobacter pylori in patients with nonulcerdyspepsia.A meta-analysis of randomized, controlled trials. Ann Intern Med. 2001;**134**:361–369.

[168]. Malfertheiner P, Mégraud F, O'Morain C, Hungin AP, Jones R, Axon A, Graham DY, Tytgat G. Current concepts in the management of Helicobacter pylori infection--the Maastricht 2-2000 Consensus Report. Aliment PharmacolTher. 2002;**16**:167–180.

[169]. Loyd RA, McClellan DA. Update on the evaluation and management of functional dyspepsia. Am Fam Physician. 2011;**83**:547–552.

[170]. Furuta T, Sugimoto M, Shirai N, Matsushita F, Nakajima H, Kumagai J, Senoo K, Kodaira C, Nishino M, Yamade M, et al. Effect of MDR1 C3435T polymorphism on cure rates of Helicobacter pylori infection by triple therapy with lansoprazole, amoxicillin and clarithromycin in relation to CYP 2C19 genotypes and 23S rRNA genotypes of H. pylori. Aliment PharmacolTher. 2007;**26**:693–703.

[171]. Zou J, Dong J, Yu X. Meta-analysis: Lactobacillus containing quadruple therapy versus standard triple first-line therapy for Helicobacter pylori eradication. Helicobacter. 2009;**14**:97–107.

[172]. Sachdeva A, Nagpal J. Meta-analysis: efficacy of bovine lactoferrin in Helicobacter pylori eradication. Aliment PharmacolTher. 2009;**29**:720–730.

[173]. Szajewska H, Horvath A, Piwowarczyk A. Meta-analysis: the effects of Saccharomyces boulardii supplementation on Helicobacter pylori eradication rates and side effects during treatment. Aliment PharmacolTher. 2010;**32**:1069–1079.

[174]. Ali Habib HS, Murad HA, Amir EM, HalawaTF.Effect of sequential versus standard Helicobacter pylori eradication therapy on the associated iron deficiency anemia in children. Indian J Pharmacol. 2013;**45**:470–473.

[175]. Stenström B, Mendis A, Marshall B. Helicobacter pylori--the latest in diagnosis and treatment. AustFam Physician. 2008;**37**:608–612.

[176]. McColl KE. Clinical practice.Helicobacter pylori infection. N Engl J Med. 2010;**362**:1597–1604.

[177]. De Francesco V, Giorgio F, Hassan C, Manes G, Vannella L, Panella C, Ierardi E, Zullo A. Worldwide H. pylori antibiotic resistance: a systematic review. J Gastrointestin Liver Dis. 2010;**19**:409–414.

[178]. Tay CY, Windsor HM, Thirriot F, Lu W, Conway C, Perkins TT, Marshall BJ. Helicobacter pylori eradication in Western Australia using novel quadruple therapy combinations. Aliment PharmacolTher. 2012;**36**:1076–1083.

[179]. McNicholl AG, Marin AC, Molina-Infante J, Castro M, Barrio J, Ducons J, Calvet X, de la Coba C, Montoro M, Bory F, et al. Randomised clinical trial comparing sequential and concomitant therapies for Helicobacter pylori eradication in routine clinical practice. Gut. 2014;**63**:244–249.

[180]. Sardarian H, Fakheri H, Hosseini V, Taghvaei T, Maleki I, Mokhtare M. Comparison of hybrid and sequential therapies for Helicobacter pylori eradication in Iran: a prospective randomized trial. Helicobacter. 2013;**18**:129–134.

[181]. Zullo A, Scaccianoce G, De Francesco V, Ruggiero V, D'Ambrosio P, Castorani L, Bonfrate L, Vannella L, Hassan C, Portincasa P. Concomitant, sequential, and hybrid therapy for H. pylori eradication: a pilot study. Clin Res HepatolGastroenterol. 2013;**37**:647–650.

[182]. Saad RJ, Schoenfeld P, Kim HM, Chey WD. Levofloxacin-based triple therapy versus bismuth-based quadruple therapy for persistent

Helicobacter pylori infection: a meta-analysis. Am J Gastroenterol. 2006;**101**:488–496.

[183]. Gisbert JP, Morena F. Systematic review and meta-analysis: levofloxacin-based rescue regimens after Helicobacter pylori treatment failure. Aliment PharmacolTher. 2006;**23**:35–44.

[184]. Morgan DR, Torres J, Sexton R, Herrero R, Salazar-Martínez E, Greenberg ER, Bravo LE, Dominguez RL, Ferreccio C, Lazcano-Ponce EC, et al. Risk of recurrent Helicobacter pylori infection 1 year after initial eradication therapy in 7 Latin American communities. JAMA. 2013;**309**:578–586.

[185]. Malik R, Guleria K, Kaur I, Sikka M, Radhakrishnan G. Effect of Helicobacter pylori eradication therapy in iron deficiency anaemia of pregnancy - a pilot study. Indian J Med Res. 2011;**134**:224–231.

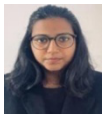
[186]. Wang ZH, Gao QY, Fang JY. Meta-analysis of the efficacy and safety of Lactobacillus-containing and Bifidobacterium-containing probiotic compound preparation in Helicobacter pylori eradication therapy. J ClinGastroenterol. 2013;**47**:25–32.

[187]. Ahmad K, Fatemeh F, Mehri N, Maryam S. Probiotics for the treatment of pediatric helicobacter pylori infection: a randomized double blind clinical trial. Iran J Pediatr. 2013;**23**:79–84.

❖ Author(s) Profile

Mr. Manish Kumar Maity
Department of Pharmacy Practice, Maharishi Markandeshwar (Deemed to be University), Mullana – 133207, Ambala, India.

He is a student of PharmD 4th year, Department of Pharmacy Practice, MM college of Pharmacy, MM (Deemed to be university), Mullana – 133207, Ambala, India. He is a member of International Pharmaceutical Federation, Netherlands. He had attended more than 100 conferences at national and International level.



Dr. Mamta Naagar
Department of Pharmacy Practice, Maharishi Markandeshwar (Deemed to be University), Mullana – 133207, Ambala, India.

She has been completed B Pharm from PDM College of Pharmacy, Bahadurgarh, Haryana and PharmD (PB) from NIMS University, Jaipur, Rajasthan. She had more than 2 years of teaching experience as an Assistant Professor in Department of Pharmacy Practice, MM College of Pharmacy, MM (Deemed to be University), Mullana - 133207, Ambala, India. She had 6months of experience as a Hospital Pharmacist in DevkiIndravati Hospital, New Delhi. She had completed 10 days skill development programme on "Basic and Regulatory Aspects of Pharmacovigilance : Striving for Excellence" organized by Pharmacovigilance Programme of India (PVPI), Indian Pharmacopoeia Commission Ministry of Health & Family Welfare , Govt of India. She had done her PharmD project on the topic of "Assessment of Drug Prescribing Pattern using W.H.O Prescribing Indicators". She is actively involved in research. She had more than 5 Published Research Papers and 2 case study has been published. Currently, 1 PharmD student doing his project under her guidance. She had 2 B Pharm and 2 PharmD students Project guidance to her credit. Major trust areas of her research interests include Drug Related Problem, Medication Error, Anti microbial resistance, Pharmacovigilance and Pharmacoepidemiology. She is GPAT qualified. She has attended more than 250 conferences at national and International Level.