

## Review

### ***Helicobacter pylori* infection: past, present and future**

**Abiodun Christopher Jemilohun<sup>1,&</sup>, Jesse Abiodun Otegbayo<sup>2</sup>**

<sup>1</sup>Department of Medicine, Ladoke Akintola University of Technology/LAUTECH Teaching Hospital, Ogbomoso, Oyo State, Nigeria, <sup>2</sup>Department of Medicine, College of Medicine, University of Ibadan/University College Hospital, Ibadan, Nigeria

<sup>&</sup>Corresponding author: Abiodun Christopher Jemilohun, Department of Medicine, LAUTECH Teaching Hospital, P.M.B. 4007, Ogbomoso, Oyo State, Nigeria

Key words: *Helicobacter pylori*, historical perspectives, dyspepsia, pathogenesis, diagnosis, treatment

Received: 14/01/2016 - Accepted: 15/01/2016 - Published: 22/04/2016

#### **Abstract**

The discovery of *Helicobacter pylori* (*H. pylori*) by Warren and Marshall in 1982 was preceded by nearly a hundred year of inconspicuous publications in regard to spiral bacteria, achlorhydria, gastritis, gastric urease, and antimicrobial therapy for peptic ulcers. The infection has now been implicated in the etiopathogenesis of chronic gastritis, peptic ulcer disease (PUD), gastric carcinoma, and gastric mucosal associated lymphoid tissue (MALT) lymphoma. The understanding of the etiopathogenesis of dyspepsia and the approach to its management continues to evolve such that PUD and MALT lymphoma are now being considered as infectious diseases in which elimination of the causative agent cures the conditions. Various diagnostic tests with good diagnostic accuracies have been developed and effective multiple antimicrobial therapies are now available for the eradication of the infection. Despite the substantial progress made, there still exists a considerable gap to be filled. A significant number of information generated from studying the bacterial characteristics and host response to the infection has not yet been translated into clinical practice. A major challenge is the absence of a specific antibiotic monotherapy for effective treatment of the infection.

**Pan African Medical Journal. 2016; 23:216 doi:10.11604/pamj.2016.23.216.8852**

This article is available online at: <http://www.panafrican-med-journal.com/content/article/23/216/full/>

© Abiodun Christopher Jemilohun et al. The Pan African Medical Journal - ISSN 1937-8688. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## Introduction

---

It has been abundantly demonstrated that *H. pylori* plays a major role in several upper gastrointestinal diseases which present as dyspepsia since its discovery in the early 1980s by Warren and Marshall [1,2]. *Helicobacter pylori* is usually found under the mucus layer in the gastric pits in close apposition to gastric epithelial cells where it causes chronic active gastritis [3]. It is a major etiological factor for PUD, gastric carcinoma, and gastric MALT lymphoma [1,4]. This discovery has generated significant interest in research on the organism with attendant continual evolution of the approach to investigation and treatment of patients with dyspepsia. Prior to the discovery of *H. pylori* the hyperacidity theory held sway in the pathogenesis of PUD. Various diagnostic tests with good diagnostic accuracies have been developed and effective multiple antimicrobial therapies are now available for the eradication of the infection [2,5]. Peptic ulcer disease and MALT lymphoma are now being considered as infectious diseases in which elimination of the causative agent cures the conditions because of the current level of understanding of the organism [3].

Despite the substantial progress made, there still exists a considerable gap to be filled. Some important information generated from studying the bacterial characteristics and host response have not yet been translated into clinical practice. A major challenge is the absence of a specific antibiotic monotherapy for effective treatment of the infection. This review appraises *H. pylori* in regard to the historical perspective, the current scientific understanding of the organism on its various aspects that are relevant to the clinician and the future direction in treatment.

## Methods

---

Relevant literatures on the subjects in texts books and scientific journals were reviewed. Extensive internet literature search for both original research and review articles in biomedical databases was made through Google scholar, PubMed, HINARI and Ovid. Keywords employed were "*Helicobacter pylori*", "Dyspepsia", "Peptic ulcer disease" "Gastric cancer", "Epidemiology" and "Management". The bibliographies of the articles on hand were used to find other references. An electronic reference manager (Mendeley) was used to store the articles. Of the 230 articles reviewed, 82 were cited in the final draft of the article.

## Current status of knowledge

---

### Historical perspective (Historical timeline)

The discovery of *H. pylori*, by Warren and Marshall, was preceded by nearly a hundred year of inconspicuous publications relating to spiral bacteria, achlorhydria, gastritis, gastric urease, and antimicrobial therapy for ulcers [6]. Investigation of gastric bacteria properly began in the latter half of the 19th century when microscope resolution had sufficiently advanced [7].

Bottcher and Letulle firstly hypothesized that bacteria caused ulcer disease in 1875, after they discovered bacteria in the floor and margins of gastric ulcers. In 1889 Walery Jaworski described spiral organisms (*Vibrio rugula*) in gastric washings. He suggested that these organisms might be implicated in causation of gastric disease. Similar spiral organisms were found in stomach of humans and other species by several scientists between then and the 20th century. For instance, in 1893 Bizzozero noted spirochetes in the gastric mucosa of dogs, which were named *H. bizzozeroni*. Kasai and Kobayashi in 1920 isolated spirochetes in cats and transmitted them to rabbits to produce ulcers [7].

Warren in 1979 identified *Campylobacter pylori* as the putative causative agent of human gastritis [7,8]. Culture of the organism (*H. pylori*) was elusive until 1982 when it was obtained by Barry Marshall [6]. Earlier attempts to culture the organism proved abortive because incubation was usually limited to 48 hours. Success at culture was incidental, as one of them spanned a holiday period and hence, lasted for 5 days, thereby yielding a growth. History was then made in April 1982 at the Royal Perth Hospital in Australia where *H. pylori* was cultured. Examination of the plate showed a pure growth of 1mm transparent colonies. Gram stains of the colonies showed slightly curved organisms and not spiral as in the smear of the specimen, which made Marshall to doubt whether it was the organism in question that was grown. Armstrong and Wee produced electron micrograph scans from the culture obtained, which showed that the bacterium was a spiral organism with five flagella.

Further studies on the organism and its RNA sequence in ribosomes helped correct the earlier misconception that the organism belonged to the *Campylobacter* family (initially called *Campylobacter pyloridis*). At the 5th International *Campylobacter* workshop in

Mexico in February, 1989 the *Campylobacter* taxonomy committee agreed that *H. pylori* should no longer be included in the *Campylobacter* group. There was initial difficulty in nomenclature before Steward Goodwin who was head of the Microbiology Department at Royal Perth Hospital at that time reportedly suggested '*Helicobacter*', and this was published in 1989.

The World Health Organization classified *H. pylori* as a class 1 (definite) carcinogen implicated in the etiopathogenesis of gastric malignancies in 1994 [9]. Parsonnet et al. also describe an association between *H. pylori* and gastric lymphomas [10]. Tomb *et al.* completed the sequencing of the entire 1,667,867 base pairs of the *H. pylori* genome in 1997 [11]. And in 2005 Warren and Marshall were awarded the Nobel Prize in Physiology or Medicine for their work on *H. pylori* and PUD [12].

### **Epidemiology and transmission**

*Helicobacter pylori* infection occurs globally, but the prevalence differs among countries and population clusters within the same country [1]. The prevalence of the infection is associated with age, socioeconomic class, and country of origin [13]. Prevalence rates ranging from 20-50% are reported in the adult populations of the developed world but the prevalence is much more in the developing countries with prevalence as high as 90% in some countries [1,4]. Higher prevalence exists in regions of low socioeconomic and poor sanitary conditions, and in rural as contrasted to urban areas. The socioeconomic status of the family during childhood appears to be the major marker of infection [14]. There is an age-related increase in prevalence in the developed countries which is a reflection of birth cohort effect [14]. The general belief is that infection takes place mostly in childhood and the rate of acquisition has reduced with improved sanitary condition and probably antibiotic use among children in the developed world. Genetic susceptibility to infection has been demonstrated by studies that showed a higher concordance rate of the infection among monozygotic twins reared apart or together than in age-matched dizygotic twins [15].

*H. pylori* has been found in water; stomach of animals like cat and sheep; and milk of goat, sheep and cow [16]. Despite these, available evidence suggests that humans are the primary reservoir and that *H. pylori* in animals represents an anthroponosis [17]. *Helicobacter pylori* has been found in saliva and dental plaques of humans [18,19]. Some studies have suggested that dental plaques serve as reservoir for both person to person transmission of

the organism and re-infection after successful eradication of organism [19]. The primary mode of transmission is person to person and clusters are found in families. This can be through oral-oral, feco-oral, and gastro-oral (through gastric secretions, vomitus, and improperly disinfected endoscopes). The infection could also be transmitted from water [20].

### **Bacteriology and pathogenesis**

*Helicobacter pylori* is a microaerophilic slow-growing gram negative spiral organism. It is 0.5 to 8.0µm wide and has multiple ensheathed flagella at one end [21]. It has the capability of dual existence in bacillary and coccoid forms [22]. The bacillary form is motile while the coccoid form is non-motile. The *genome* of the organism codes for about 1,500 proteins [1]. Bacterial etiology of dyspepsia has been disputed in the past because of the knowledge that the stomach is unfavorable to bacterial growth due to its high acid content but *H. pylori* is able to overcome this by means of virulence factors which enhance colonization of the gastric epithelium and induction of tissue damage.

The actual outcome of *H. pylori* infection (gastritis, PUD, gastric MALT lymphoma or gastric cancer) is determined by a complex interplay between bacterial, host and environmental factors.

### **Bacterial factors**

These include factors that enhance mucosal colonization and factors that mediate tissue injury.

### **Colonization factors**

**Flagella:** The possession of spiral shaped, unipolar, sheathed flagella [23] allows the organism to move rapidly from the lumen of the stomach, where the *pH* is low, through the mucus layer to an area where *pH* is near neutral to permit optimal growth.

**Urease:** *H. pylori* has a great capacity for urease production, probably more than almost all other bacterial species. Urease hydrolyses urea to produce ammonia (NH<sub>3</sub>) and carbon dioxide (CO<sub>2</sub>). The presence of NH<sub>3</sub> reduces the acidity of the stomach; which may be necessary for providing a congenial environment for *H. pylori*. *Ure1*, a *pH*-gated channel helps to regulate the production of urea [1]. Possession of urease however, may not be

absolutely essential for colonization because urease negative *H. pylori* have been cultured from patients with duodenal ulcer.

**Adherence factors:** *H. pylori* has tissue tropism for the gastric epithelium. It possesses fibrillar adhesins, located on its surface which binds closely to the carbohydrate receptors on the mucosal cell leading to the formation of an adherence pedestal [1]. The best-characterized of these adhesins is BabA, which is a 78-kD outer-membrane protein that binds to the fucosylated Lewis B blood group antigen. BabA is relevant in *H. pylori* associated disease and may influence disease severity, although the results of several studies are contradictory [1]. This property prevents the organism from being shed during cell and mucus turnover.

### Factors mediating tissue injury

**Lipopolysaccharides (LPS):** They are glycolipids found in the cell envelope of gram negative bacteria of which *H. pylori* is one. Lipopolysaccharides are *endotoxins* which stimulate the release of cytokines through their lipid A component. They also interfere with gastric epithelial cell–laminin interaction, which may lead to loss of mucosal integrity; inhibit mucin synthesis, and stimulate pepsinogen secretion [24].

**Leukocyte recruitment and activating factors:** These are soluble surface proteins with chemotactic properties produced by the organism. They help to recruit monocytes and neutrophils to the lamina propria and to activate these inflammatory cells. These include *H. pylori* neutrophil-activating protein, expressed by the *napA* gene, and the immunologically active porins [25].

**Vacuolating Cytotoxin (VacA):** It is a protein that induces vacuole formation in eukaryotic cells. It is encoded by the *vacA* gene [1]. All strains of *H. pylori* possess the *vacA* gene, but only about 50% express the mature protein. Antibodies to VacA can be used to detect VacA-producing *H. pylori* strains. VacA is an exotoxin, which inserts itself into the epithelial cell membrane to form a hexameric anion-selective, voltage-dependent channel through which bicarbonate and organic anions can be released [26]. It also acts on the host mitochondrial membrane to induce apoptosis leading to release of cytochrome c from the intermembrane space [27].

**Cytotoxin-Associated Antigen (CagA):** It is a highly antigenic protein encoded by the *cagA* gene that is part of the *cag*

pathogenicity island (*Cag* PaI). The presence of *Cag* PaI is associated with a more prominent tissue inflammatory response than is seen with strains lacking it. This increase in inflammation is associated with an increased risk of developing a symptomatic outcome of the infection, especially PUD and gastric adenocarcinoma [28]. Antibodies to CagA can be used to detect CagA-producing *H. pylori* strains. The *cag* PaI encodes a type IV secretory apparatus that injects CagA into mammalian cells [29] where it triggers cytokine production. *Cag* PaI-positive *H. pylori* also induces apoptosis via the mitochondrial pathway [27]. Apoptosis of epithelial cells compromises epithelial barrier which protect the epithelium against luminal acid and pepsin.

**Outer Membrane Inflammatory Protein(OipA):** It is possessed by most strains with CagA. It acts synergistically with CagA to produce a more intense inflammatory response than would have otherwise occurred in either [30].

**Heat shock proteins:** These are highly antigenic heat shock proteins known as HspA and HspB. Their role in the pathogenesis of the infection is still not fully known. It has been observed that, even in the absence of VacA and CagA, *H. pylori* can sensitize human gastric epithelial cells and enhance susceptibility to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mediated apoptosis [31]. The infection chronicity is sustained through suppression of *H. pylori* specific memory CD4+ T-cell responses by antigen specific CD4+ CD25 high regulatory T cells [32].

### Host response

*H. pylori* does not invade the gastric mucosa but its attachment to the surface mucosal epithelial cells sets off a remarkable cellular and humoral host immune responses. It provokes a chronic inflammatory response involving recruitment of neutrophils, followed by T and B lymphocytes, plasma cells and macrophages, which altogether cause gastric epithelial damage by their activities [33]. Human antibody response to *H. pylori* lipopolysaccharide (LPS) is majorly IgG antibodies directed against a highly immunogenic antigenic-epitope in the polysaccharide chain of *H. pylori* LPS. The dominant subclass is IgG2. There are also IgA and IgM responses, but they are less specific. *H. pylori* infection also enhances the release of interleukin-1 $\beta$  (IL1 $\beta$ ), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor (TNF) [34].

As Part of the host immune response to invading microorganisms T cell subgroups are produced which assist in protecting the mucosa and distinguishing pathogenic bacteria from commensals [35]. Immature T helper (Th) 0 cells expressing CD4 have the ability to differentiate into two functional subtypes: Th 1 cells which are induced mostly in response to intracellular antigen and secrete interleukin-2 (IL-2) and interferon- $\gamma$  (IF- $\gamma$ ); Th 2 cells which are induced in response to extracellular pathogens and secrete interleukin-4 (IL-4), interleukin-5 (IL-5) and interleukin-10 (IL-10).

From the foregoing, it is expected that *H. pylori* would provoke inflammatory response via Th 2 cells, being an extracellular antigen. The contrary is however true, as Th 1 response has been observed to be predominant [36]. This distorted response results in an imbalance production of the pro- and anti-inflammatory cytokines regulated by the Th cells and may subsequently result in tissue damage similar to what is seen in autoimmune diseases [37]. Class II MHC molecules on the host cell act as receptors for *H. pylori* to this effect [36,37].

*H. pylori*-stimulated gastric epithelial cells produce monocyte chemo-attractant protein I (MCP-I) which cause cyclooxygenase 2 (cox-2) induction and activation of T-cells [38]. Additional mechanisms by which *H. pylori* may cause epithelial cell injury include: activated neutrophil-mediated production of reactive oxygen and nitrogen spp., enhanced epithelial cell turnover and apoptosis due to chronic inflammation which may be as a result of the combined effect of direct Fas-mediated contacts between epithelial and Th1 cells and interferon- $\gamma$  [1].

Pro-inflammatory polymorphisms of the interleukin-1 $\beta$  gene favor the development of stomach body predominant gastritis that is associated with hypochlorhydria, gastric atrophy, and gastric adenocarcinoma. Absence of these pro-inflammatory polymorphisms leads to development of *H. pylori* associated antral predominant gastritis which is associated with a normal to high level of acid secretion [1].

**Disease spectrum** It has been demonstrated that *H. pylori* is involved in the pathogenesis of several diseases [1,39]. These include:

**Gastritis:** This could occur in form of either acute or chronic gastritis. The inflammatory mechanisms described above are some of the ways by which *H. pylori* causes gastritis. Acute infection is

typically manifested as a transient mild illness characterized by epigastric pain, nausea, histological finding of neutrophilic gastritis and a transient hypochlorhydria [4]. It is not known how often acute infection clears spontaneously. However, studies in children suggest that spontaneous loss of infection may be common [15]. Acute *H. pylori* infection is diagnosed by the presence of a positive UBT and negative IgG anti-*H. pylori* antibodies [4]. Chronic gastritis often presents in form of chronic active, non-atrophic superficial antral gastritis, with a picture of focal epithelial cell damage [4]. This is usually asymptomatic, although it may be associated with PUD. Chronic atrophic gastritis resulting from progression of the non-atrophic chronic gastritis may also occur in a smaller percentage of patients with gastritis.

**Peptic ulcer disease:** An individual infected with *H. pylori* has an estimated lifetime risk of about 10-20% for the development of PUD. This is at least 3-4 folds higher than in non-infected subjects. *H. pylori* infection can be diagnosed in 90-100% of duodenal ulcer (DU) patients and in 60-100% of gastric ulcer (GU) patients [40]. The gastritis induced by *H. pylori* can progress to ulceration of the mucosa. Apoptosis of epithelial cells and subsequent compromise in the mucosal protective barrier exposes gastric mucosa to the direct assault of luminal acid and pepsin. Occlusion of mucosal end-arteries due to impaired fibrinolytic activity may contribute to the focal nature of PUD [41]. NSAID ingestion also increases the chances of developing GU.

Patients with DU have been known, before the discovery of *H. pylori*, to secrete about twice as much acid as controls because they have twice as many parietal cells [42]. Acid hypersecretion in DU is virtually always due to *H. pylori* infection because secretion returns to normal after the infection is eradicated. The predominantly antral gastritis in DU diminishes the number of somatostatin-producing cells in the antrum. This leads to a reduction in production of somatostatin, reduced somatostatin-mediated inhibition of gastrin release from the G cells, and eventual development of hypergastrinemia [40]. The increased acid secretion may, on its own, increase the risk of duodenal ulceration or may induce gastric metaplasia in the duodenum, which becomes colonized by *H. pylori*, then inflamed (duodenitis), and finally ulcerated [42]. Successful eradication of *H. pylori* leads to PUD healing and less frequent recurrence of the ulcer [43].

**Gastric adenocarcinoma:** *H. pylori* has been implicated as the strongest risk factor in the pathogenesis of gastric adenocarcinoma,

especially the distal type [1,44] and has thus, been classified as a class I (or definite) carcinogen by the WHO [9]. The pathogenesis of gastric cancer includes a sequence of events that begins with *H. pylori*-induced chronic superficial gastritis, progressing towards atrophic gastritis, intestinal metaplasia, dysplasia and eventually gastric cancer. This sequence takes decades to complete [9,44]. Several aspects of the inflammatory milieu that have been implicated as carcinogens include: increased oxidative stress and the formation of oxygen-free radicals leading to DNA damage, increased pro-inflammatory cytokine production such as IL-1 $\beta$  and TNF which stimulate greater cell turnover and reduced apoptosis, and potential for faulty or incomplete DNA repair [44].

Bacterial virulence has been shown to be an important factor in carcinogenesis. For instance, patients infected with CagA-positive strains have been shown to have a higher risk of developing gastric carcinoma than those infected with CagA-negative *H. pylori* strains [9,44].

*H. pylori*-infected cells also express some factors similar to those commonly implicated in carcinogenesis e.g. matrix metalloproteinase-7 (MMP-7) which has been found to be up-regulated in colorectal cancer [45]. MMP-7 is important in the normal and pathological remodelling of epithelial-matrix interactions and is up-regulated in gastric cancer too. It plays an important role in promoting tissue invasion and metastasis of cancer cells. This up-regulation is also dependent on the *cag-PaI* [45].

Although *H. pylori* is now thought to account for 80% or more of gastric cancers, it is noteworthy that only 3% of infected patients progress to gastric cancer [9]. This suggests that *H. pylori* infection only on its own is generally not sufficient to cause gastric carcinoma [46]. A combination of bacterial factors, environmental insults, the host immune response and other genetic factors is responsible.

**MALT lymphoma:** The molecular pathogenesis of MALT lymphoma is incompletely understood but seems to also involve strain-specific *H. pylori* factors, as well as host genetic factors, such as polymorphisms in the promoters of inflammatory cytokines such as IL-1B and TNF. It is believed that *H. pylori* infection leads to the formation of *H. pylori*-reactive T cells, which then cause polyclonal B-cell proliferations [47]. In time, a monoclonal B-cell tumour emerges in the proliferating B cells, probably as a result of accumulation of mutations in growth-regulatory genes. Some studies have implicated CagA in the development MALT lymphoma

via impairment of p53-dependent apoptosis [48]. In keeping with this, eradication of *H. pylori* "cures" the lymphoma by removing antigenic stimulus for T cells [3].

**Functional dyspepsia:** The prevalence of *H. pylori* is generally high in patients with dyspepsia irrespective of the subgroup. The implication of *H. pylori* in the pathogenesis of ulcer dyspepsia is well established but there are dissenting views on the role it plays in the pathogenesis of functional dyspepsia. While some studies showed association between *H. pylori* infection and the clinical diagnosis of functional dyspepsia [49], others did not show any association [50].

**Extra-gastrointestinal diseases:** *H. pylori* has also been suggested to be causally related to several extra-gastrointestinal diseases [51]. These associations are generally weak because they were not obtained from randomized controlled studies [52]. A list of such diseases is shown in Table 1.

## Diagnosis

In view of the importance of *H. pylori* in the etiopathogenesis of major gastrointestinal and extra-gastrointestinal diseases, it is pertinent to look into cost-effective and reliable diagnostic tests for early detection and therapy. *Helicobacter pylori* diagnostic tests can be broadly classified into invasive and non-invasive tests [2,5]. Invasive tests require endoscopic gastro-duodenal biopsy samples while the non-invasive tests do not [2,5].

Despite the good sensitivity and specificity of most of the commonly used tests, determination of gold standard has been difficult because none of them is perfect. A combination of at least two tests is commonly used as gold standard, though some researchers now use UBT as their gold standard [53]. It is not uncommon to use at least one invasive test whenever two tests are used as gold standard [54].

## Invasive tests

**Histology:** Biopsies are obtained from the gastric antrum and corpus [55]. Multiple levels of each biopsy are routinely stained with haematoxylin and eosin (H&E) and with a special stain such as Warthin-Starry silver, Giemsa, or Cresyl-fast violet. The standard H&E stain is used to determine histological chronic or chronic active inflammation (gastritis) but could also demonstrate *H. pylori* if a large number of the organism is present. Atrophy and intestinal

metaplasia can also be assessed. Small numbers of bacteria are better detected by the special stains. An important advantage of histology is that in addition to the historical record provided, sections from biopsies (or even additional sections) can be examined in the future [54]. The drawbacks of the test include high observer-dependency, relatively long waiting time for result, requirement of specialized skills for performance and relatively high cost.

**Rapid urease test (RUT):** The urease enzyme which is produced by *H. pylori* is utilized in performing this test. Gastric biopsy is placed in a medium that contains urea and a *pH* indicator. The urease breaks down the urea to produce ammonia that increases the *pH* of the medium which leads to a color change. The specificity and sensitivity of the test are greater than 90%, but false-positive results do occur [4]. It can be performed and read within 1 to 24 hour depending on the make. Its comparative advantage to histology lies in its rapidity, simplicity and inexpensiveness; but it cannot be used to evaluate gastritis.

**Culture:** This is done under stringent conditions. Endoscopy biopsy must be transported to the laboratory at 4°C within 24 hrs. or at -70°C for a longer period. Fresh selective and non-selective media are needed to culture the organism. After introduction of the specimen into the culture medium, the plates are inspected for about 10 days. Due to the focal nature of inflammatory lesions produced by *H. pylori*, multiple biopsies are usually taken from the gastric antrum and corpus to increase the yield of the test. The specificity of the test is 100% while the sensitivity is slightly less [56]. A major advantage of the test is that, pure growth of the organism can be obtained for proper identification and detailed studies e.g. antibiotics sensitivity when there is failure of the second line drugs, strain typing, genetic studies etc. The disadvantages of the test include longer duration for result availability, high cost and the stringent condition needed for transportation to the laboratory.

**Polymerase chain reaction (PCR):** This is a molecular technique which amplifies fragment of a gene specific for the *H. pylori* e.g. *vacA* and *cagA* gene sequences, 16S rRNA, 23S rRNA, and ureC are targeted [56]. The biopsy is lysed to liberate the DNA. Specifically designed primers and polymerase enzyme are used to amplify gene. Amplification is done in 30-40 cycles at different temperatures with each cycle. This allows for denaturation, annealing and elongation. The amplified products are thereafter identified by electrophoresis.

As a result of the development of string-capsule test device, gastric sample for PCR can now be obtained without having to biopsy the stomach [56]. PCR has a sensitivity and specificity that are well above 90%. It can be used to analyze bacterial genotypes, study pattern of antibiotic resistance and *H. pylori* transmission within families and the community. The main disadvantages are that it is expensive, and the procedure requires technical expertise to perform.

**DNA-Enzyme immunoassay:** This is a form of PCR where the PCR amplicons are detected by calorimetric method. It is ELISA-based and involves the use of coated microwells. This method is more rapid than the standard PCR and result can be obtained within a few hours [57].

**Fluorescent in situ hybridization (FISH):** This is another molecular test for diagnosing *H. pylori* that is particularly useful in detection of *H. pylori* clarithromycin resistance/sensitivity [58].

#### **Non-invasive tests**

**Serology:** chronic *H. pylori* infection elicits a circulating antibody response that can be quantitatively measured by serological assay technique like enzyme-linked immunosorbent assay (ELISA) [59]. Though tests for IgG, IgA and IgM antibodies can be done, only IgG antibody test is reliable. It involves the use of serum or plasma, and lately tests on whole blood (in office test) [57]. Microwells coated with *H. pylori* antigen are exposed to *H. pylori* antibody (in serum, plasma or whole blood) in the presence of an indicator. The color change resulting from the antigen-antibody reaction is read visually or with the use of a spectrophotometer. Other serological methods that can be used include immunoblot, flow microsphere immunofluorescence and chromatographic tests.

Because of its easy availability, affordability, and simplicity, it is commonly used in prevalence studies of *H. pylori*. Its major drawback is its poor discriminatory power between current infection and previous exposure, since it may still be positive several months after *H. pylori* eradication. It is therefore generally not useful in confirming cure after antimicrobial therapy but it is useful for the initial diagnosis of *H. pylori* infection and epidemiological surveys [59].

**Urea breath test (UBT):** This is an indirect method of detecting the presence of *H. pylori* in the stomach premised on the ability of *H. pylori* to produce the urease enzyme. Urea labeled with either <sup>13</sup>C or <sup>14</sup>C is ingested by the patient [60]. If urease is present in the stomach as a result of *H. pylori* infection, labeled CO<sup>2</sup> will be split off and absorbed into the circulation, where its presence can be determined by analysis of expired breath by means of a spectrometer. The result is expressed in delta/mil. <sup>13</sup>C UBT is preferred to <sup>14</sup>C UBT especially in children and pregnant women because it is stable and non-radioactive [61].

UBT is now being considered as the gold standard by some researchers [61]. It is the non-invasive test with the highest sensitivity and specificity (>95%) and is the preferred means of evaluating the success of antimicrobial therapy in clinical practice. It is not as expensive as endoscopy. Portable and cheaper spectrometers are now available thereby eliminating the need to send collected air samples to a central spectrometer. There is the possibility of false positive results when there is bacterial overgrowth of urease-producing organisms. Recent use of antibiotics, bismuth preparations or acid suppression therapy, due to their effect on the colony size of *H. pylori*, can produce false negative results.

**Stool antigen test (SAT):** The test is based on the detection of *H. pylori* antigen in the stool. *Helicobacter pylori* adhering to gastric epithelium in infected persons appear in their stool as a consequence of the normal shedding of the epithelium. This means that the test is a direct test of active infection which gives it an advantage over serology. It is an enzyme immunoassay test which is available in both polyclonal and monoclonal forms. The monoclonal immunoassay is newer and more sensitive and specific than the polyclonal assay [58] and may be considered as an alternative to UBT in the initial diagnosis of patients with dyspepsia who do not require immediate endoscopy [62]. SAT is simple and relatively cheap. It can be carried out in most routine laboratories. It is slightly less reliable when used soon after the end of *H. pylori* eradication therapy. It is now generally recommended to wait for about 12 weeks to reliably confirm eradication [58]. Its diagnostic accuracy is impaired by PPIs and gastrointestinal bleeding [63]. A major drawback is related to the inconvenience of stool handling. Additional non-invasive tests that are yet to be recommended for routine clinical diagnosis of *H. pylori* include stool PCR, Urine antibody test and Saliva antibody test [9,64]. The

accuracy of some of the commonly used *Helicobacter pylori* diagnostic methods is depicted in Table 2.

## Treatment

It is appropriate for all patients with dyspepsia who are positive for *H. pylori* to undergo *H. pylori* eradication therapy because of the strong association between *H. pylori* and the diseases manifesting clinically as dyspepsia [1–3]. This is particularly important because *H. pylori* eradication has been associated with significant reduction in the rate of recurrence of PUD and cure of MALT lymphoma. The specific recommendations for treatment include patients with PUD and MALT lymphoma; patients with atrophic gastritis; first degree relatives of patients with gastric cancer; patients with unexplained iron deficiency anemia; and patients with chronic idiopathic thrombocytopenic purpura [2,5].

The fact that no single agent is sufficient in eradicating the *H. pylori* is a major challenge [1,2,4,5]. Combinations of antibiotics together with non-antibiotic adjunctive agents are required for eradication of the organism. Drugs are given in combinations of 3 (triple) or 4 (quadruple). Each regimen consists of at least 2 antibiotics. Antibiotics that are traditionally used include amoxicillin, nitroimidazole (metronidazole or tinidazole), clarithromycin, tetracycline and bismuth. Adjunctive agents include Histamine-2 receptor blockers (H2RB), proton pump inhibitors (PPI), and ranitidine-bismuth citrate (RBC).

Triple therapy consists of two antibiotics and one adjunctive agent while quadruple therapy is made up of two antibiotics and two adjunctive agents. The use of dual therapy is discouraged because of wide spread antibiotic resistance. Regional antibiotic sensitivity pattern needs to be properly considered in the choice of antibiotics. The Maastricht III -2007 consensus report and the American College of Gastroenterology Association (ACG) suggested two lines of therapy [2,5]: **first-line therapy:** this can be a combination of a PPI with amoxicillin and clarithromycin; a PPI with amoxicillin and metronidazole, or a bismuth containing quadruple therapy; **Second-line therapy:** a quadruple therapy is often used after failure of first line therapy. Examples include a combination of a PPI with bismuth, metronidazole and tetracycline or a combination of ranitidine with bismuth, metronidazole and tetracycline. If bismuth is not available, a PPI-based triple therapy could be used.



Because failure rate is above 20% in most commonly used first-line therapies [65], there is often the need for a second line therapy. The choice of a second-line treatment is contingent upon the initial treatment. Most authorities agree that culture is not required after a first line eradication failure [2,66]. If a metronidazole-based treatment was used a clarithromycin-based regimen (or at least a metronidazole-free regimen) should be used thereafter, and vice versa [67]. This recommendation is grounded on the observation that acquired bacterial resistance to metronidazole or clarithromycin is often a result of previous treatment failure [68].

Another challenge in the treatment of *H. pylori* infection is the determination of the optimal duration of therapy. While there is controversy as to whether 7, 10, or 14 days is optimal [69,70]; it has been generally observed that longer [71] durations provide better results than a 7 day duration [72]. The current European and United States' recommendations support a 14 day duration therapy [2,5]. Notwithstanding the foregoing, available evidence suggests that a 7-day treatment duration is sufficient when quadruple therapy is used as a second line treatment [73].

### **Sequential therapy**

An ideal *H. pylori* therapy should have a short duration and ought to lead to an eradication rate of the organism greater than 90%, as was the case initially when the triple-therapy regimens were approved [74]. A decreasing efficacy of common regimens has been observed with failure rates now surpassing 20% [5,75,76]. This development most likely result from increased bacterial resistance to some of the antibiotics in the regimens, especially clarithromycin that is often used [76]. The regimen comprises of a PPI and amoxicillin for 5 days followed by a PPI, clarithromycin, and a 5-nitroimidazole (tinidazole or metronidazole) for another 5 days [74]. Sequential therapy originated from Italy and the original therapy utilized tinidazole [74,76]. Several studies from Italy and other parts of the world have reported eradication rates exceeding 90% which is superior to the clarithromycin-based triple therapy [5,74,76]. The tolerability is good in children, adults and the elderly [5]. The basis for this more complex therapy is the belief that amoxicillin could weaken bacterial cell walls in the initial phase of treatment, thereby preventing the development of drug efflux channels that inhibit drugs like clarithromycin from binding to ribosomes [74]. This therapy has a potential to become the standard first-line treatment for *H. pylori* infection because of this observed advantage.

### **Third line and 'Rescue' therapies**

Treatment of patients who have already undergone first- and second-line therapies is a challenge because of the risk of development of double antibiotic resistance [77]. Though, various therapeutic protocols have been tested by different groups, a standard third-line therapy is currently lacking [2,78]. Endoscopic biopsy culture and antibiotic sensitivity testing is the most suitable option for patients with two eradication therapy failures [2,78]. The third-line therapy should avoid metronidazole and clarithromycin and other antibiotics that are likely to have contributed to development of the resistance.

Other classes of antibiotics that have emerged in the treatment of *H. pylori*, especially for 'rescue' therapies include: fluoroquinolones like levofloxacin (gatifloxacin and moxifloxacin); rifabutin (rifamycin); and furazolidone (a nitrofurantoin) [2,4,78,79]. They can be used to replace clarithromycin and metronidazole in rescue therapies and in first or second line regimens [2,78-80]. They are particularly important because the use of quadruple therapy as the optimal second-line therapy is plagued with problems of a relatively high incidence of adverse effects and regimen complexity [79]. These lead to poor compliance, failure of eradication in about 20 to 30% of patients and subsequent drug resistance [79].

### **Conclusion**

---

Substantial progress has been made in the understanding of the pathogenicity and treatment of *H. pylori* but there still exists a considerable gap to be filled. Information generated from studying the virulence factors of *H. pylori* are yet to be translated to clinical practice. Associations between bacterial characteristics and disease risks have not been defined sufficiently to guide the clinician in treatment decisions [1]. A major challenge exists in developing specific antibiotic monotherapy for effective treatment of the infection. Since the *H. pylori* genome has now been sequenced, this provides an opportunity both to identify specific targets for drug therapy, and to facilitate the identification and production of antigens that may be helpful in manufacturing vaccines [81]. Encouraging results from animal models have been obtained in the areas of both therapeutic and prophylactic vaccination; however, translation to human vaccine remains difficult probably because the

immunology of the human stomach is still poorly understood [82]. All these developments and more will be necessary to adequately treat and prevent *H. pylori* infection in the future, especially in regions of the world with high prevalence.

#### What is known about this topic

- *H. pylori* plays a major role in the pathogenesis of several upper gastrointestinal diseases that present as dyspepsia;
- Reasonably sensitive and specific tests are available for diagnosing *H. pylori*;
- Combination therapy for *H. pylori* eradication are available.

#### What this study adds

- The review is a summary of a myriad of materials already written on the topic presented in a concise and easy to read manner;
- The sections on historical perspective and future direction on the topic were written to help stimulate the interest of the reader in further research.

#### Competing interests

---

The Authors declare no competing interest.

#### Authors' contributions

---

Jemilohun AC: conception and design of the study, literature search and review, and manuscript write up. Otegbayo JA: design of study, took oversight of all the stages of the work, critical review of manuscript content. All authors read and agreed to final manuscript.

#### Tables

---

**Table 1:** Non-gastrointestinal tract diseases possibly associated with *Helicobacter pylori* infection

**Table 2:** Accuracy of diagnostic methods for *Helicobacter pylori* in %

#### References

---

1. Suerbaum S, Michetti P. *Helicobacter pylori* infection. *N Engl J Med.* 2002; 347(15):1175-86. **PubMed | Google Scholar**
2. Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D et al. Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report. *Gut.* 2007; 56(6):772-81. **PubMed | Google Scholar**
3. Talley NJ, Vakil N. Guidelines for the management of dyspepsia. *Am J Gastroenterol.* 2005;100(10):2324-37. **PubMed | Google Scholar**
4. Graham DY, Sung JY. *Helicobacter pylori*. In: Feldman M, Friedman LS, Brandt LJ, editors. *Sleisenger & Fordtran's Gastrointestinal and Liver Disease; Pathophysiology, Diagnosis, Management.* 8th ed. Philadelphia: Saunders; 2006. p. 1049-66. **Google Scholar**
5. Chey WD, Wong BC. American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection. *Am J Gastroenterol.* 2007; 102(8):1808-25. **PubMed | Google Scholar**
6. Marshall BJ. The *Campylobacter pylori* story. *Scand J Gastroenterol Suppl.* 1988; 146:58-66. **PubMed | Google Scholar**
7. Kidd M, Modlin IM. A century of *Helicobacter pylori*: paradigms lost-paradigms regained. *Digestion.* 1998; 59(1):1-15. **PubMed | Google Scholar**
8. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet.* 1984;1(8390):1311-5.. **PubMed | Google Scholar**
9. Houghton J, Wang TC. *Helicobacter pylori* and gastric cancer: a new paradigm for inflammation-associated epithelial cancers. *Gastroenterology.* 2005;128(6):1567-78. **PubMed | Google Scholar**

10. Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E et al. Helicobacter pylori Infection and Gastric Lymphoma. *N Engl J Med.* 1994;330(18):1267-71. **PubMed | Google Scholar**
11. Tomb JF, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD et al. The complete genome sequence of the gastric pathogen Helicobacter pylori. *Nature.* 1997;388(6642):539-47. **PubMed | Google Scholar**
12. The Nobel Prize in Physiology or Medicine 2005. [cited 2016 Jan 10]. Available from: [http://www.nobelprize.org/nobel\\_prizes/medicine/laureates/2005/index.html](http://www.nobelprize.org/nobel_prizes/medicine/laureates/2005/index.html). **PubMed | Google Scholar**
13. Rowland M, Daly L, Vaughan M, Higgins A, Bourke B, Drumm B. Age-specific incidence of Helicobacter pylori. *Gastroenterology.* 2006; 130(1):65-72. **Google Scholar**
14. Malaty HM, Graham DY. Importance of childhood socioeconomic status on the current prevalence of Helicobacter pylori infection. *Gut.* 1994; 35(6):742-5. **PubMed | Google Scholar**
15. Malaty HM, Graham DY, Isaksson I, Engstrand L, Pedersen NL. Are genetic influences on peptic ulcer dependent or independent of genetic influences for Helicobacter pylori infection?. *Arch Intern Med.* 2000;160(1):105-9. **PubMed | Google Scholar**
16. Quaglia NC, Dambrosio A, Normanno G, Parisi A, Patrono R, Ranieri G et al. High occurrence of Helicobacter pylori in raw goat, sheep and cow milk inferred by glmM gene: a risk of food-borne infection?. *Int J Food Microbiol.* 2008;124(1):43-7. **PubMed | Google Scholar**
17. El-Zaatari FA, Woo JS, Badr A, Osato MS, Serna H, Lichtenberger LM et al. Failure to isolate Helicobacter pylori from stray cats indicates that H pylori in cats may be an anthroponosis-an animal infection with a human pathogen. *J Med Microbiol.* 1997; 46(5):372-6. **PubMed | Google Scholar**
18. Ogunbodede EO, Lawal OO, Lamikanra A, Okeke IN, Rotimi O, Rasheed AA. Helicobacter pylori in the dental plaque and gastric mucosa of dyspeptic Nigerian patients. *Trop Gastroenterol.* 2002 ;23(3):127-33. **PubMed | Google Scholar**
19. Agülo?lu S, Turhano?lu M, Eskimez S, Tacir I. Detection of Helicobacter pylori colonization in human dental plaques and saliva of patients with chronic gastritis. *Biotechnol Biotechnol Eq.* 2006;20:173-8. **PubMed | Google Scholar**
20. Brown LM. Helicobacter pylori: epidemiology and routes of transmission. *Epidemiol Rev.* 2000;22(2):283-97. **PubMed | Google Scholar**
21. Suzuki H, Masaoka T, Nomura S, Hoshino Y, Kurabayashi K, Minegishi Y et al. Current consensus on the diagnosis and treatment of H pylori-associated gastroduodenal disease. *Keio J Med.* 2003; 52(3):163-73. **PubMed | Google Scholar**
22. Saito N, Konishi K, Sato F, Kato M, Takeda H, Sugiyama T et al. Plural transformation-processes from spiral to coccoid Helicobacter pylori and its viability. *J Infect.* 2003;46(1):49-55. **PubMed | Google Scholar**
23. Hofman P, Waidner B, Hofman V, Bereswill S, Brest P, Kist M. Pathogenesis of Helicobacter pylori infection. *Helicobacter.* 2004; 9(Suppl 1):15-22. **PubMed | Google Scholar**
24. Moran AP. Lipopolysaccharide in bacterial chronic infection: insights from Helicobacter pylori lipopolysaccharide and lipid A. *Int J Med Microbiol.* 2007; 297(5):307-19. **PubMed | Google Scholar**
25. Brislert M, Enarsson K, Lundin S, Karlsson A, Kusters JG, Svennerholm AM et al. Helicobacter pylori induce neutrophil transendothelial migration: role of the bacterial HP-NAP. *FEMS Microbiol Lett.* 2005; 249(1):95-103. **PubMed | Google Scholar**
26. Szabo I, Brutsche S, Tombola F, Moschioni M, Satin B, Telford JL et al. Formation of anion-selective channels in the cell plasma membrane by the toxin VacA of Helicobacter pylori is required for its biological activity. *EMBO J.* 1999; 18(20):5517-27. **PubMed | Google Scholar**

27. Maeda S, Yoshida H, Mitsuno Y, Hirata Y, Ogura K, Shiratori Y et al. Analysis of apoptotic and antiapoptotic signalling pathways induced by *Helicobacter pylori*. *Gut*. 2002;50(6):771-8. **PubMed | Google Scholar**
28. van Doorn LJ, Figueiredo C, Sanna R, Plaisier A, Schneeberger P, de Boer W et al. Clinical relevance of the *cagA*, *vacA*, and *iceA* status of *Helicobacter pylori*. *Gastroenterology*. 1998;115(1):58-66. **PubMed | Google Scholar**
29. Rohde M, Puls J, Buhrdorf R, Fischer W, Haas R. A novel sheathed surface organelle of the *Helicobacter pylori* *cag* type IV secretion system. *Mol Microbiol*. 2003;49(1):219-34. **PubMed | Google Scholar**
30. Yamaoka Y, Kwon DH, Graham DY. A M(r) 34,000 proinflammatory outer membrane protein (*oipA*) of *Helicobacter pylori*. *Proc Natl Acad Sci U S A*. 2000; 97(13):7533-8. **PubMed | Google Scholar**
31. Wu YY, Tsai HF, Lin WC, Chou AH, Chen HT, Yang JC et al. *Helicobacter pylori* enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in human gastric epithelial cells. *World J Gastroenterol*. 2004;10(16):2334-9. **PubMed | Google Scholar**
32. Lundgren A, Suri-Payer E, Enarsson K, Svennerholm AM, Lundin BS. *Helicobacter pylori*-specific CD4<sup>+</sup> CD25<sup>high</sup> regulatory T cells suppress memory T-cell responses to H pylori in infected individuals. *Infect Immun*. 2003;71(4):1755-62. **PubMed | Google Scholar**
33. Goodwin CS, Armstrong JA, Marshall BJ. *Campylobacter pyloridis*, gastritis, and peptic ulceration. *J Clin Pathol*. 1986;39(4):353-65. **PubMed | Google Scholar**
34. Yamaoka Y, Kita M, Kodama T, Sawai N, Kashima K, Imanishi J. Induction of various cytokines and development of severe mucosal inflammation by *cagA* gene positive *Helicobacter pylori* strains. *Gut*. 1997;41(4):442-51. **PubMed | Google Scholar**
35. Ridgway WM, Weiner HL, Fathman CG. Regulation of autoimmune response. *Curr Opin Immunol*. 1994;6(6):946-55. **PubMed | Google Scholar**
36. Fan X, Crowe SE, Behar S, Gunasena H, Ye G, Haeberle H et al. The effect of class II major histocompatibility complex expression on adherence of *Helicobacter pylori* and induction of apoptosis in gastric epithelial cells: a mechanism for T helper cell type 1-mediated damage. *J Exp Med*. 1998;187(10):1659-69. **PubMed | Google Scholar**
37. Fan X, Gunasena H, Cheng Z, Espejo R, Crowe SE, Ernst PB et al. *Helicobacter pylori* urease binds to class II MHC on gastric epithelial cells and induces their apoptosis. *J Immunol*. 2000; 165(4):1918-24. **PubMed | Google Scholar**
38. Futagami S, Hiratsuka T, Tatsuguchi A, Suzuki K, Kusunoki M, Shinji Y et al. Monocyte chemoattractant protein 1 (MCP-1) released from *Helicobacter pylori* stimulated gastric epithelial cells induces cyclooxygenase 2 expression and activation in T cells. *Gut*. 2003; 52(9):1257-64. **PubMed | Google Scholar**
39. Malcolm CA, MacKay WG, Shepherd A, Weaver LT. *Helicobacter pylori* in children is strongly associated with poverty. *Scott Med J*. 2004;49(4):136-8. **PubMed | Google Scholar**
40. Tham TC, Chen L, Dennison N, Johnston CF, Collins JS, Ardill JE et al. Effect of *Helicobacter pylori* eradication on antral somatostatin cell density in humans. *Eur J Gastroenterol Hepatol*. 1998;10(4):289-91. **PubMed | Google Scholar**
41. Adesanya AA, Oluwatowaju IO, Oyediji KS, da Rocha-Afodu JT, Coker AO, Afonja OA. Evaluation of a locally-made urease test for detecting *Helicobacter pylori* infection. *Niger Postgr Med J*. 2002;9(1):43-7. **PubMed | Google Scholar**
42. Calam J, Baron JH. ABC of the upper gastrointestinal tract: Pathophysiology of duodenal and gastric ulcer and gastric cancer. *BMJ*. 2001;323(7319):980-2. **PubMed | Google Scholar**
43. Cryer B, Spechler S. Peptic Ulcer Disease . In: Feldman M, Friedman L, Brandt L, Sleisenger M, editors. *Sleisenger & Fordtran's Gastroenterology and liver disease; Pathophysiology, Diagnosis, Management*. 8th ed. Philadelphia: Saunders; 2006. p. 1089-137. **Google Scholar**

44. Matysiak-Budnik T, Megraud F. Helicobacter pylori infection and gastric cancer. *Eur J Cancer*. 2006 ;42(6):708-16. **PubMed | Google Scholar**
45. Bebb JR, Letley DP, Thomas RJ, Aviles F, Collins HM, Watson SA et al. Helicobacter pylori upregulates matrilysin (MMP-7) in epithelial cells in vivo and in vitro in a Cag dependent manner. *Gut*. 2003;52(10):1408-13. **PubMed | Google Scholar**
46. Ernst PB, Gold BD. The disease spectrum of Helicobacter pylori: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. *Annu Rev Microbiol*. 2000;54:615-40. **Google Scholar**
47. Hussell T, Isaacson PG, Crabtree JE, Spencer J. Helicobacter pylori-specific tumour-infiltrating T cells provide contact dependent help for the growth of malignant B cells in low-grade gastric lymphoma of mucosa-associated lymphoid tissue. *J Pathol*. 1996;178(2):122-7. **PubMed | Google Scholar**
48. Umehara S, Higashi H, Ohnishi N, Asaka M, Hatakeyama M. Effects of Helicobacter pylori CagA protein on the growth and survival of B lymphocytes, the origin of MALT lymphoma. *Oncogene*. 2003;22(51):8337-42. **PubMed | Google Scholar**
49. Moayyedi P, Deeks J, Talley NJ, Delaney B, Forman D. An update of the Cochrane systematic review of Helicobacter pylori eradication therapy in nonulcer dyspepsia: resolving the discrepancy between systematic reviews. *Am J Gastroenterol*. 2003; 98(12):2621-6. **PubMed | Google Scholar**
50. Blum AL, Talley NJ, O'Morain C, van Zanten S V, Labenz J, Stolte M et al. Lack of effect of treating Helicobacter pylori infection in patients with nonulcer dyspepsia - Omeprazole plus Clarithromycin and Amoxicillin Effect One Year after Treatment (OCAY) Study Group. *N Engl J Med*. 1998;339(26):1875-81. **PubMed | Google Scholar**
51. Franceschi F, Roccarina D, Gasbarrini A. Extragastric manifestations of Helicobacter pylori infection. *Minerva Med*. 2006; 97(1):39-45. **PubMed | Google Scholar**
52. Leontiadis GI, Sharma VK, Howden CW. Non-gastrointestinal tract associations of Helicobacter pylori infection. *Arch Intern Med*. 1999;159(9):925-40. **PubMed | Google Scholar**
53. Samarbaf-Zadeh AR, Tajbakhsh S, Moosavian SM, Sadeghi-Zadeh M, Azmi M, Hashemi J et al. Application of fluorescent in situ hybridization (FISH) for the detection of Helicobacter pylori. *Med Sci Monit*. 2006 ;12(10):CR426-30. **PubMed | Google Scholar**
54. Logan RP, Walker MM. ABC of the upper gastrointestinal tract: Epidemiology and diagnosis of Helicobacter pylori infection. *BMJ*. 2001;323(7318):920-2. **PubMed | Google Scholar**
55. Ola SO, Yakubu A, Otegbayo JA, Oluwasola AO, Ogunbiyi JO, Akang EE et al. The most appropriate site for endoscopic biopsy for the detection of H pylori among Nigerians in Ibadan. *West Afr J Med*. 2006;25(4):269-72. **PubMed | Google Scholar**
56. Roth DE, Velapatino B, Gilman RH, Su WW, Berg DE, Cabrera L et al. A comparison of a string test-PCR assay and a stool antigen immunoassay (HpSA) for Helicobacter pylori screening in Peru. *Trans R Soc Trop Med Hyg*. 2001; 95(4):398-9. **PubMed | Google Scholar**
57. Vaira D, Holton J, Menegatti M, Ricci C, Landi F, Ali A et al. New immunological assays for the diagnosis of Helicobacter pylori infection. *Gut*. 1999; 45(Suppl 1):I23-7. **PubMed | Google Scholar**
58. Koletzko S, Konstantopoulos N, Bosman D, Feydt-Schmidt A, van der Ende A, Kalach N et al. Evaluation of a novel monoclonal enzyme immunoassay for detection of Helicobacter pylori antigen in stool from children. *Gut*. 2003;52(6):804-6. **PubMed | Google Scholar**
59. Monteiro L, de Mascarel A, Sarrasqueta AM, Bergey B, Barberis C, Talby P et al. Diagnosis of Helicobacter pylori infection: noninvasive methods compared to invasive methods and evaluation of two new tests. *Am J Gastroenterol*. 2001;96(2):353-8. **PubMed | Google Scholar**
60. Makristathis A, Hirschl AM, Lehours P, Megraud F. Diagnosis of Helicobacter pylori infection. *Helicobacter*. 2004; 9(Suppl 1):7-14. **PubMed | Google Scholar**

61. Romagnuolo J, Schiller D, Bailey RJ. Using breath tests wisely in a gastroenterology practice: an evidence-based review of indications and pitfalls in interpretation. *Am J Gastroenterol.* 2002;97(5):1113-26. **PubMed | Google Scholar**
62. Andrews J, Marsden B, Brown D, Wong VS, Wood E, Kelsey M. Comparison of three stool antigen tests for *Helicobacter pylori* detection. *J Clin Pathol.* 2003;56(10):769-71. **PubMed | Google Scholar**
63. van Leerdam ME, van der Ende A, ten Kate FJ, Rauws EA, Tytgat GN. Lack of accuracy of the noninvasive *Helicobacter pylori* stool antigen test in patients with gastroduodenal ulcer bleeding. *Am J Gastroenterol.* 2003; 98(4):798-801. **PubMed | Google Scholar**
64. Miwa H, Hirose M, Kikuchi S, Terai T, Iwazaki R, Kobayashi O et al. How useful is the detection kit for antibody to *Helicobacter pylori* in urine (URINELISA) in clinical practice?. *Am J Gastroenterol.* 1999;94(12):3460-3. **PubMed | Google Scholar**
65. Gisbert JP, Pajares R, Pajares JM. Evolution of *Helicobacter pylori* therapy from a meta-analytical perspective. *Helicobacter.* 2007;12(Suppl 2):50-8. **PubMed | Google Scholar**
66. Avidan B, Melzer E, Keller N, Bar-Meir S. The effect of culture results for *Helicobacter pylori* on the choice of treatment following failure of initial eradication. *Isr Med Assoc J.* 2001; 3(3):163-5. **PubMed | Google Scholar**
67. Battaglia G, Di Mario F, Vigneri S, Vianello F, Benvenuti ME, Donisi PM et al. Strategy for the retreatment of failed *Helicobacter pylori* eradication therapy: a case series. *Ital J Gastroenterol Hepatol.* 1998;30(4):370-4. **PubMed | Google Scholar**
68. Peitz U, Hackelsberger A, Malferttheiner P. A practical approach to patients with refractory *Helicobacter pylori* infection, or who are re-infected after standard therapy. *Drugs.* 1999;57(6):905-20. **PubMed | Google Scholar**
69. Calvet X, Villoria A, Vergara M. Could increasing the duration of triple therapy be a clinically useful strategy?. *Ann Intern Med.* 2008; 148(8):624-5. **PubMed | Google Scholar**
70. Fuccio L, Minardi ME, Zagari RM, Grilli D, Magrini N, Bazzoli F. Meta-analysis: duration of first-line proton-pump inhibitor based triple therapy for *Helicobacter pylori* eradication. *Ann Intern Med.* 2007;147(8):553-62. **PubMed | Google Scholar**
71. Calvet X, Ducons J, Bujanda L, Bory F, Montserrat A, Gisbert JP. Seven versus ten days of rabeprazole triple therapy for *Helicobacter pylori* eradication: a multicenter randomized trial. *Am J Gastroenterol.* 2005;100(8):1696-701. **PubMed | Google Scholar**
72. Fischbach LA, Goodman KJ, Feldman M, Aragaki C. Sources of variation of *Helicobacter pylori* treatment success in adults worldwide: a meta-analysis. *Int J Epidemiol.* 2002;31(1):128-39. **PubMed | Google Scholar**
73. De Boer WA, Driessen WM, Potters VP, Tytgat GN. Randomized study comparing 1 with 2 weeks of quadruple therapy for eradicating *Helicobacter pylori*. *Am J Gastroenterol.* 1994; 89(11):1993-7. **PubMed | Google Scholar**
74. Jafri NS, Hornung CA, Howden CW. Meta-analysis: Sequential Therapy Appears Superior to Standard Therapy for *Helicobacter pylori* Infection in Patients Naive to Treatment. *Ann Intern Med.* 2008;148(12):923-31. **Google Scholar**
75. Liou J-M, Chen C-C, Chen M-J, Chen C-C, Chang C-Y, Fang Y-J et al. Sequential versus triple therapy for the first-line treatment of *Helicobacter pylori*: a multicentre, open-label, randomised trial. *Lancet.* 2013;381(9862):205-13. **PubMed | Google Scholar**
76. Vaira D, Zullo A, Hassan C, Vakil N. Sequential therapy for *Helicobacter pylori* eradication: the time is now!. *Ther Adv Gastroenterol.* 2009; 2(6):317-22. **PubMed | Google Scholar**
77. Megraud F. H pylori antibiotic resistance: prevalence, importance, and advances in testing. *Gut.* 2004;53(9):1374-84. **PubMed | Google Scholar**
78. Gisbert JP, Pajares JM. *Helicobacter pylori* "rescue" therapy after failure of two eradication treatments. *Helicobacter.* 2005;10(5):363-72. **PubMed | Google Scholar**

79. Gisbert JP, Pajares JM. Review article: *Helicobacter pylori* "rescue" regimen when proton pump inhibitor-based triple therapies fail. *Aliment Pharmacol Ther.* 2002;16(6):1047-57. **PubMed** | **Google Scholar**

80. Gisbert JP. Second-line rescue therapy of *Helicobacter pylori* infection. *Ther Adv Gastroenterol.* 2009; 2(6):331-56. **PubMed** | **Google Scholar**

81. Axon AT. Treatment of *Helicobacter pylori*: future therapeutic and prophylactic perspectives. *Gut.* 1998; 43(Suppl 1):70-3. **PubMed** | **Google Scholar**

82. Blanchard TG, Czinn SJ. Immunology of *Helicobacter pylori* and prospects for vaccine. *Gastroenterol Clin North Am.* 2000;29(3):671-85. **PubMed** | **Google Scholar**

<b>Table 1:</b> Non-gastrointestinal tract diseases possibly associated with <i>Helicobacter pylori</i> Infection
<b>Iron deficiency anemia</b>
<b>Coronary artery disease</b>
Cerebrovascular disease
Hypertension
Raynaud's phenomenon
Migraine headaches
Vomiting of pregnancy
Immune thrombocytopenic purpura
Hyperammonemia
Sudden infant death syndrome
Growth retardation
Anorexia of aging
Rosacea
Chronic urticarial

<b>Table 2:</b> Accuracy of diagnostic methods for <i>Helicobacter pylori</i> in %				
<b>Method</b>	<b>Sens.</b>	<b>Spec.</b>	<b>PPV</b>	<b>NPV</b>
Warthin-Starry silver stain <sup>+</sup>	93	99	99	89
Rapid Urease Test <sup>+</sup>	90	100	100	84
Serum IgG antibody	91	97	95	85
Urea Breath Test	90	96	98	84

From Cutler AF, Havstad S, Ma CK, *et al*: Accuracy of invasive and noninvasive tests to diagnose *Helicobacter pylori* infection. *Gastroenterology.* 1995; 109(1):136-41. IgG = Immunoglobulin G, NPV = Negative Predictive Value, PPV = Positive Predictive Value; Sens. = Sensitivity, Spec. = Specificity.  
<sup>+</sup>In a gastric mucosal biopsy specimen