**Review Article**

# An Overview of *Helicobacter Pylori* and Diagnostic Methods

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**Received:** 22 July 2019;

**Accepted:** 28 August 2019;

**Published:** 30 August 2019

**Abstract**

*Helicobacter pylori* are a gram negative, spiral, micro-aerophilic and slow-growing organism with a length of 3 and a diameter of about half a micrometer. The bacterium also produces some enzymes, the most important of which are oxidase, catalase and urease. More than half of the world's people are infected with this bacterium.

The bacteria are the main cause of diseases such as gastric ulcer and gastric and intestinal complaints. The incidence of infections with this bacterium in the countries of Europe and North America are 10 times higher than in other countries. *Helicobacter pylori* are basically a spiral bacterium, but it can also be transformed into a spherical shape that can also be survived and pathogenic and connects to the gastric mucosa.

Several laboratory methods are available including invasive and non-invasive methods. Each test has its own characteristics, but is not complete on its own. Usually, several tests are used together, but this depends on our goal of testing. Invasive method is endoscopic biopsy of gastric mucosa and rapid urease test on biopsy sample. Noninvasive methods are included, serologic tests (IgM, IgG & IgA), breath urease test and examination of H. pylori antigens in the stool. In specialized laboratories PCR is also used to identify bacteria.

Regarding the prevalence of gastrointestinal diseases and the lack of adequate knowledge in today's societies about this bacterium, this study was conducted with the aim of reviewing *Helicobacter pylori* and different diagnostic methods.

**Keywords:** *Helicobacter pylori*; Diagnosis methods

**Citation**

Rahimkhani M, Mordadi A. An Overview of *Helicobacter Pylori* and Diagnostic Methods. International Journal of Applied Biology and Pharmaceutical Technology 10 (2019): 022-034.

## Introduction

The first of those who discovered the mysterious bacteria in the stomach was a scientist named Bizzozero from Italy who, in 1893, as a pathologist, identified the bacterium in the gastric mucosa of dogs and humans (Spirilli) [1]. Lieber, who once worked in New York about the role of alcohol in developing liver disease, wrote in a 1985 article about ammonia in the gastric juice that ammonia, after treatment with tetracycline, significantly decreased and he concluded that ammonia in the stomach must have a bacterial origin [2]. Another physician named Lykoudis in Athens, due to himself being peptic ulcer hemorrhage several times in 1985, he began to take antibiotics and then he noticed that all his stomach ulcers had disappeared completely. He performed this treatment in several patients at his office, and he also saw this improvement [3]. In Oxford, Steer observed a bacterial relationship with gastric mucosa in an accurate examination of the images obtained with electron microscopy and published an article in the 1975 in Journal of Pathology [4]. The bacterium was re-discovered in 1979 by an Australian pathologist, Warren Robin. With Marshall Barry, he has been doing more research since 1981, he has done more research and these microorganisms isolated from gastric mucosal specimens and successfully cultured for the first time [5]. In the original essay presented by these scientists, it was claimed that most gastric and gastric ulcers, by contrast, were caused by infection with this bacterium, not by stress and spices [6,7]. At that time, members of the World Medical Committee ignored the role of this bacterium in causing ulcers and gastritis, Because they believed that no organism could survive for a long time in the acidic environment of the stomach, but after further studies in this area, the presence of this bacterium was confirmed in the stomach [8']. In 2005, Marshall and Warren jointly won the Nobel Prize in Medicine for their extensive studies on H. pylori [9,11]. *Helicobacter pylori* compared with other Helicobacter species is a gram-negative, spore-free, spiral-shaped (s-shaped) or bent-shaped bacterium that has a length of about and 1/5 μm in diameter and has 6-4 polar flagella. The tips of the flagella are button-like and are actively animated. This specific morphology and motility are essential for its colonization in gastric mucosa and gastrointestinal mucosa [14].

This micro-anaphylactic bacterium has aerobic metabolism and, if there is high moisture content, grows at temperature 37 ℃ and in standard CO2 atmosphere. This bacterium will grow at a pH of 7.5 to 8 [15].

The flagellum of this bacterium is approximately 30 nm in diameter and has a filamentary content of about 12 to 15 nm. H. Pylori have a glycocalyx-rich or polysaccharide-rich outer layer that appears to be thicker in vivo than invitro [16]. This bacterium is the same as the germ cell wall structure. The bacteria have an external membrane protein content of 48-67 KDa, which has the ability to create a pore that is specific to each specific substrate. The lipopolysaccharides of *Helicobacter pylori* also have an unusual structure [17]. This bacterium has a hydrogenase , catalase, oxidase, and urease enzymes, which makes the diagnostic test positive for them. This bacterium has the ability to form biofilm and also convert from spiral to cocoid form, both of which have the potential to survive more in the environment and are considered as effective factors in epidemiology. Cocoidal form is found mostly in the aquatic environment and in vivo conditions at the level of gastric epithelium cells but not cultivated [18].

## Pollution sources and infection transmission routes

The presence of H. pylori in drinking water has been proven and the bacteria can survive in water for up to four days. Boiling water also has no effect on the prevention of transmission. Therefore, water is an important focal point for the propagation of *Helicobacter pylori*, and water contaminated with feces, especially in developing countries, and in areas that do not have adequate drinking water, is one of the major ways of transmitting bacteria [19].

One of the first reports of drinking water as a source of H. pylori contamination was published in 1991 by Klein et al. [20]. Food is also contaminated with contaminated water. The bacteria can enter the stomach through the mouth with contaminated food [21]. In people who have gastroesophageal reflux, the bacteria from the mouth enter the stomach through the transfer of gastric mucus to the esophagus [22]. Many studies have been conducted on the development of oral *Helicobacter pylori*. *Helicobacter pylori* are also found in the tooth plaque [23].

A potential source of infection is hospital. Transmission of bacteria through endoscopic tubes can be done from one person to another and using appropriate disinfection techniques, the risk of transmission of this bacterium by endoscopy is minimized. Specialist physicians and other people working in health care centers who are in contact with the upper gastrointestinal tract and do not use gloves are at risk for *Helicobacter pylori* infection [24]. The hosts of this bacterium include humans and animals such as pigs, cows, dogs, cats, birds and rodents. Communication with these animals can be a source of transmission of contamination. Oral- fecal and oral- oral routes are the main ways of transmission of infection from infectious sources to a healthy person [25,26].

## Epidemiology

*Helicobacter pylori* is one of the most common human pathogens in the world, accounting for more than 50 percent of the world's population, infected with this bacterium, especially in developing countries, which may over 90 percent of the people in these countries become chronic in chronic infection [27]. The prevalence of *H. pylori* infection is widely affected by factors such as geographical area, age, race and socioeconomic status [28]. In a study on 98 children in one of the villages in Lingu County, Shandong Province, China, it was shown that nearly 70% of of children with five to six years old are infected with *Helicobacter pylori* [29]. The highest infection rates occur in early childhood, and it seems to decrease with increasing hygiene [30].

A study was conducted in 2008 and on immigrant children in Australia. The results showed that H. pylori infection in these migrant children was 82% and the chance of infection was significantly increased with age, but the prevalence of infection in Australian children was low [31]. Most of the adults in the developing and underdeveloped countries are infected and estimated inaccurate of *Helicobacter pylori* infection in adults in both developed and developing countries. The prevalence of this bacterium in developing countries is 70% and in the United States and other industrialized countries is up to 40% [32,33].

*Helicobacter pylori* should penetrate the mucous membrane of the stomach, making it colonized in gastric epithelial cells, which have the main role [34]. This bacterium is a flagella moving organism that penetrates the stomach mucus by its spiral movement

and its special structure. Non-flagellate mutated helicobacter is non-pathogenic in animal models [35]. Flagella is composed of two types of flagelin: the more abundant protein called Fla A, which is a major part of the filament, and a larger protein that appears to be located exclusively in the proximal area of the ligament's hook [36]. Both of these proteins are essential for completing the structure of the flagellum and its function to rapidly push the bacteria from an undesired acidic environment into the gastric mucosal layer of the gastric mucosal cells. The mutated species of this bacterium that are deficient in the protein-fiber of the flagellum have less pathogenic [37].

## Important enzymes in *Helicobacter pylori* virulence

### Collagenase

*Helicobacter pylori*, after entering the gastric mucosal layer, change its environment to allow comfortable movement on the surface of the epithelial cells. This bacterium has a collagenase enzyme. The gene encoding this enzyme in the Helico is Hpo169 Pylori bacteria [38]. After production of this enzyme by bacteria, it is actively transferred to the surface of the bacterial cells and remains there, or it secretes into the outer space of the cell and digest collagen until the bacterium has a more free movement. Collagenase secreted from *H. pylori* by collagen digestion causes delay in wound healing and gastritis, resulting in ulcers and chronic gastritis. In addition, some components of the host immune response, such as IgA antibodies, may be degraded by bacterial collagenase and help the pathogenicity of H. pylori in the digestive tract [39].

### Urease

Urease enzyme generates ammonia and alkaline bicarbonate from urea, thereby neutralizing the acidic environment surrounding the bacteria [40].

The enzyme is bound to the outer membrane of the bacterium and is part of the external enzyme of the cell. Its molecular weight is about 580 kDa [41]. Which consists of three UreA subunits and a UreB subunit and UreC [42]. The researchers have shown that the presence of urease and ammonia production is effective in the survival and colonization of *Helicobacter pylori* at lower gastric pH and is necessary for the pathogenicity of *Helicobacter pylori* [43,44].

Therefore, it can be concluded that with no ammonia production, Helicobacter pylori cannot withstand gastric ulcer and its acidic pH, and proliferation and pathogenesis largely cease [45].

Urease in in vitro acidic medium induces self-destruction of H. pylori. Urease is strongly immunogenic to phagocytes [46,47]. Urease enzyme activity is strongly controlled by a pH dependent urea channel. This canal is open at low pH levels and is closed at neutral pH levels and allows the bacterium to control the pH of its environment very accurately [48].

### Superoxide Dismutase and Catalase

Superoxide dismutase breaks down the superoxide is produced in the PMN and macrophages, thereby preventing the bacteria is killed by these compounds [49]. Inflammation in the gastric mucosal layer increases the toxic oxygen metabolites, such as superoxide anions. These compounds are converted by superoxide dismutase to $H_2O_2$, and subsequently hydrogen peroxide is converted to water and oxygen by the catalase enzyme.

Urease and catalase are secreted by the bacteria to the environment and this pathogen is protected from the effect of the hemorrhagic immune response [46].

Lipopolysaccharide in cell wall of *Helicobacter pylori* has a much lower toxicity than other gram-negative pathogens such as *E.coli* and Salmonella. Lipopolysaccharide is a very weak inducer for Tumor Necrosing Factor (TNF) and other cytokines; this causes mild and chronic inflammation in chronic gastritis due to *Helicobacter pylori* [50,51].

The similarity of the products of the polysaccharide layer to human gastric mucosal antigens can lead to a weak immune response against the lipopolysaccharide of the bacterium. Acidic pH of the mucosal gastric layer is likely to cause the loss and destruction of surface proteins of *H. pylori* Similar to the incubation of the *Helicobacter pylori* at low pH and in vitro, the low pH causes rapid loss of many surface proteins without the other lipopolysaccharide molecules being decomposed or released [52].

## *Helicobacter pylori* pathogenicity factors

The island of cag is about 40 kb and consists of 31-27 genes. One of the important genes of the Cag Island is CagA gene, which produces an immune system stimulant called the related cytotoxin A or cytotoxin-associated gene A antigen which is briefly called CagA. Different strains of H. pylori are divided into two main groups based on the ability to produce this protein: Strains that have CagA or CagA positive gene and strains that lack this gene or CagA negative [54]. Infection with CagA positive strains of H.pylori leads to more severe inflammation of the gastric mucosal layer compared with the infection by CagA negative strains and is associated with atrophic gastritis and subsequent gastric adenocarcinoma. ; Therefore, patients infected with CagA positive strains are at increased risk for peptic ulcer and gastric cancer [55]. The CagA protein is also transmitted to the host epithelial cells by the type I V secretion system [56]. This protein is one of the major proteins of Helicobacter pylori, which, in several ways not yet fully understood, activates the transcription factor NF-kB and produces IL-8. Interleukin 8 is an inflammatory cytokine that promotes inflammation and, since most cancers start with inflammation, CagA proteins are effective in the development of cancer [57,58] also, after entering the epithelial cells, phosphorylation of tyrosine is carried out on the protein, which allows tyrosine phosphorylation to remove intercellular signals from the control. Exit of intercellular signals from the control directly and indirectly helps to cause gastric carcinoma [59].

## Outer Membrane Proteins (OMP)

It is estimated that 4% of the H. pylori genome contains outer membrane proteins (OMP) [60]. This bacterium has an enormous amount of outer membrane proteins that is significantly higher than other bacterial species [61]. The role of these proteins in the process of infection is not well defined. *Helicobacter pylori* outer membrane protein (HOP) and HOP – related protein ( Hor) groups form are a large family of outer membrane proteins, including 33 members. Some OMPs, such as AlpA, AlpB, BabA, SabA and HopZ, are considered as bacterial enterobacteria and cause bacterial adhesion to gastric epithelial cells and facilitate colonization of this bacterium [62]. The oipA pathogen factor is a member of the Omp. Recently, this new pathogenic agent, called oipA (external inflammatory protein), has been identified which encodes one of the extra-membrane proteins and an inflammation-related gene located at the 100 kb of cag pAI on the *Helicobacter pylori* genome [63]. It has been gradually clarified that one of the

functions of OipA induces inflammation with phosphorylation of multiple signal paths [64,65].

OipA proteins increase the production of IL-8 from the stomach cancer cells. OipA, like cag PAI, induces the secretion of IL-8 by epithelial cells [66]. The function of OipA has been reported as an adhesion factor that interferes with the binding of *Helicobacter pylori* to gastric envelope cells in vitro [65] but the receptor has not yet been identified [67]. In a number of different cancers, including gastric cancer, AKT (also known as Protein Kinase B) is increased and is uncontrolledly activated. It is suggested that one of the functions of OipA is phosphorylation of AKT, which is an intracellular signaling regulator in regulating a number of functions involved in gastric carcinogenesis. Another OipA suggested function is to disable GSK3 after activating AKT. GSK3 inactivation is associated with cell proliferation, metabolism, inflammation, apoptosis, and the progression of various cancers through the AKT signaling pathway activity [65].

### Vaculating cytotoxinA (Vac)

The VacA protein is a secretion exotoxin produced by most strains. VacA consists of two parts of the sequence of signs (s) and the middle section (M). Recently, an intermediate region between s and m has been identified as part I, which has a functional role in the activity of vacuolization. this protein can be directly attached to the cell membrane of the stomach cells and cause the colonization of the bacteria in the stomach [68].

### Detection

Several laboratory methods are available including invasive and non-invasive methods. Each test has its own characteristics, but is not complete on its own. From the experts' point of view, most tests have a good sensitivity and sensitivity; usually, several tests are used together, but this depends on our goal of testing. Accurate identification before and after eradication therapy is important for evaluating different treatment regimens [69].

In an invasive method, using an endoscopic method, a sample is prepared from the patient's stomach area. Several tests, such as rapid urease test, histological examination and microscopic examination and microbial culture can be performed on the sample [70]. In specialized laboratories PCR is also used to identify bacteria. Non-invasive tests include urea breath testing, serological tests and detection of H. pylori antigens in the feces. The most common serological tests are ELISA. The best antigens for use in this test are a mixture of purified antigens that are the easiest immunoglobulin to detect IgG [72]. In the respiratory test, the patient drinks urea containing carbon labeled with C12 and C13, which metabolizes this urea, and produces a markerable $CO_2$ that can be detected in the exhalation [70]. These tests have similar sensitivities, which are generally more than 90%; however, these values are lower for serological testing [73]. Various studies have shown that, when the prevalence of H. pylori infection is low or moderate, serological tests are relatively less expensive than fecal test or urea test [74,75]. It should be noted that H. pylori infection may be symptomatic or lacking symptoms. It is estimated that up to 70% of the infection with this bacterium have not significant symptoms, and about two thirds of the world's population is infected with this bacterium [76].

### Treatment

Many treatments have been proposed for eradication. Single-drug and double-drug regimens, such as a proton pump inhibitor by an antibiotic, have always been

disappointing with the results. A highly effective three-agent diet based on proton pump inhibitors, including omeprazole, tinidazole and clarithromycin, was first reported in 1993. Most treatment regimens used to treat *Helicobacter pylori* infection include two antibiotics plus a proton pump inhibitor or a bismuth compound (or both). The most commonly used first-line therapy is the treatment of three drugs including proton pump inhibitors plus clarithromycin and amoxicillin, which are given twice daily for 7 to 12 days. In patients with penicillin allergy, metronidazole is used instead of amoxicillin [77]. Although the use of probiotic supplements, like lactic acid bacteria, along with this triple therapy can help to eradicate bacteria and reduce its adverse side effects [78]. The first line treatment in areas with high prevalence of clarithromycin-resistant H. pylori infection is the treatment of four drugs including a proton pump inhibitor, tetracycline, metronidazole and a bismuth salt for 11 to 14 days; Of course, bismuth salts are not available in some countries, such as the United States [77]. Researchers in South Korea have shown that green tea consumption contributes to the suppression of H. pylori growth; Green tea prevents the binding of H. pylori to gastric epithelial cells due to acid polysaccharides [79].

## Conclusion

*Helicobacter pylori* are a slow-growing micro-aerophilic gram-negative organism found in the stomach and duodenum and associated with a number of stomach-duodenal diseases. This bacterium is characterized by the high production of urease enzymes, which is a virulence factor and can be used for diagnosis. The bacterium uses a needle-shaped organ to inject Cag A positive at the intersection of two cells in epithelium of stomach. Cag A is a poison that produces cytotoxin associated with gene A. all H. pylori genomes do not carry the Cag A gene, but only those classified as positive Cag A do this. This toxin changes the structure of the stomach cells and makes the bacteria easier to stick to. Long-term exposure to Cag A causes chronic inflammation of the tissue. *Helicobacter pylori* is the cause of most cases of gastric ulcers, leading to some gastrointestinal cancers and the most common cause of gastric cancer and MALT lymphoma. But this germ does not cause cancer in all people; about 15% of patients with long-term infections may develop one or more complications. Estimates say that about 25-50 percent of developed countries and up to 70 to 90 percent of people in developing countries are infected with this bacterium. In developing countries, up to 80% of the population may be infected by the age of 20. At high ages, the person gets even more infected. The factors involved in infection, population density, life in unsanitary conditions, contaminated food or water, and contact with the contents of the stomach can be mentioned. Transmission of this disease is carried out individually, mainly from oral-oral or oral-fecal ways.

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