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Helicobacter pylori: A Pathogen of Ample Risk to Health

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Abstract

Helicobacter pylori is considered a pathogen of global interest because it is a microorganism of very easy contagion between the hosts or host. *Helicobacter pylori* infection is now recognized as a problem that causes chronic gastritis, peptic ulcer disease, and lymphoproliferative disorders and is a major risk factor for gastric cancer. The diagnostic methods to detect *H. pylori* are classified such as direct or invasive, when the identification is directly, the bacterium obtained from gastric mucosa biopsy by endoscopy histology with various staining, culture and PCR techniques, while indirect or noninvasive or serological tests such as the breath test with urea marked with ^{13}C .

Keywords: *H. pylori*, pathogen, risk to human

1. Introduction

Helicobacter pylori is considered a pathogen of global interest because it is a microorganism of very easy contagion between hosts or susceptible hosts. The first isolation of *H. pylori* was in 1982 by Marshall and Warren who ushered us into a new era of gastric microbiology [1].

The number of infected by *H. pylori* has been increased considerably, since a third of the world population has it, while the rest do not know if they have it or not; in developing countries there is an infection rate that goes from 60% and 90% of the population, which is not the case in developed countries ranging from 20–40% [2]. Gastroduodenal ulcer diseases are a major factor in the development of gastric adenocarcinoma and lymphoma [3].

According to studies conducted, many of the pathogenic species of *Helicobacter* are of fecal origin. The transmission to human seems to be associated with the consumption of water and raw or undercooked foods [4, 5]. In Ecuador, according to the statistics, the poverty quintiles reached up to 2015 are 35% according to INEC data, which are closely related to the lack of basic services such as drinking water and sanitary services, a common factor in the population being contamination by water and food [6].

The different methods used for diagnosis range from antigenic screening (Ag) to molecular techniques. Antigenic screening techniques have been associated with a high sensitivity of a detection limit of the *Helicobacter pylori* test with a 95% concordance in specificity compared to the ELISA test [7]. In plate culture it is usually

considered a difficult and tedious technique; the diagnostic method has the advantage of typifying the organism and determining its sensitivity to antibacterial agents. The methods such as endoscopy to obtain a sample through a biopsy are very used nowadays; it is a traumatic and invasive procedure that can cause complications such as infections, perforations, aspiration, bleeding, and incarceration of the endoscope [8].

2. Theoretical framework

2.1 *Helicobacter pylori*

Helicobacter pylori (*H. pylori*) is a spiral bacterium that does not form spores and is Gram-negative, which colonizes the human stomach and is prevalent throughout the world [9]. It has been associated with peptic ulcer disease, gastric adenocarcinoma, and lower grade B-associated lymphoma associated with the mucosa. In addition, it is thought that the organism is involved in other human diseases such as hematological and autoimmune disorders, insulin resistance, and metabolic syndrome [10]. Although almost 50% of the population is infected with *H. pylori* worldwide, the prevalence, incidence, age distribution, and sequelae of infection are significantly different in developed and developing countries.

Helicobacter pylori (previously known as *Campylobacter pylori* or *pyloridis*) was first isolated from humans in 1982 [11]. Since 1994, *H. pylori* has been considered carcinogenic to humans, and it has even been associated with other diseases, such as cerebrovascular accidents, autoimmune thyroiditis, and diabetes mellitus, among others [12]. This bacterium resides in the stomach of most humans and is usually found in the deeper portions of the mucus gel that lines the gastric mucosa or between the mucus layer and the gastric epithelium [13].

The bacterium is one of the most important findings for gastroenterologists, who for years sought answers to multiple intestinal problems. This is how the gastroenterologist Walery Jaworski in 1899 after analyzing samples of human gastric expirations isolated spiral elongated bacteria and called them *Vibrio reguła*, and the said results were published in the manual of gastric diseases; however, these findings were not given the importance they deserved to be written in Polish and not in English [14].

So, it took 79 years for the bacteria to be rediscovered by the Australian doctors Barry Marshall and Robin Warren, who managed to make the first isolation through a pure culture in 1979. This rediscovery allowed them to be Nobel Laureates in 2005 [15].

2.2 Microbiological aspects of *Helicobacter pylori*

Taxonomically, we can describe *H. pylori* because of its size, shape, color, biochemical function, genus, species, and its relationship with other species. *H. pylori* is a slow-growing, spiral-shaped bacterium. It is a small curved bacillus, microaerophilic, and Gram-negative, and mobile by the presence of flagella. The bacillus has rounded ends. These microorganisms measure 0.5–1.0 µm wide by 2.5–4.0 µm long, since they bear a strong resemblance to members of the *Campylobacter* genus [11].

The multiple genotypic and phenotypic characteristics are different from those of *Helicobacter*, so this new genus was established, including *H. cinaedi* and *H. fennelliae*. The two species of *Helicobacter* that cause diarrheal disease, *H. cinaedi* and *H. fennelliae*, are intestinal microorganisms rather than gastric. As for the clinical manifestations of the disease they generate, these bacteria are more similar to *Campylobacter* than *H. pylori* [13]. The clinical characteristics of the infections caused by these *Helicobacter* are similar to those due to *Campylobacter* species.

2.3 Pathways of contagion or infestation of the guests

Although in general there is no difference between the sexes, in some developed countries, there is a higher prevalence of infection in men than in women [16].

The prevalence of *H. pylori* infection in adults of any age in developed countries ranges between 20 and 40% and reaches figures of 60 to 80% in countries considered third world. The most important difference between countries of high and low prevalence is the intensity with which the infection is transmitted in childhood and early adolescence [17].

Epidemiological and microbiological evidences have several transmission routes that have been proposed in the studies carried out. The gastro-oral, oral-oral, and fecal-oral routes are the most important routes of transmission [12]. Other routes of importance are also breastfeeding and iatrogenic transmission which are also included as alternating forms for the transmission of the pathogen. The possibilities of spreading the pathogen are of three possible vectors that have been suggested to maintain the viable form of the bacteria: water, food, and animals.

2.4 Water transmission

The prevalence of *H. pylori* infection shows a strong correlation with access to water. Numerous epidemiological studies confirm this, and the World Health Organization includes it in its list of potential emerging pathogenic microorganisms whose transmission by water is plausible, although it has not yet been confirmed [18, 19].

Through molecular methods, *H. pylori* DNA has been detected in wastewater, drinking water, and other environmental samples throughout the world, and its survival capacity in water, even chlorinated, has been demonstrated. It has also been detected in the drinking water distribution network [20]. These findings indicate that contaminated water and food play a vital role in the survival and spread of *H. pylori*.

In another study, developed by Moreno et al. [21]; Moreno and Ferrús, [22] *H. pylori* was detected in 46% of more than 100 wastewater samples, 40% were of river water samples and, most strikingly, the 66% were public source.

On the other hand, *H. pylori* is able to survive in biofilms when it grows under high C:N conditions [23]. The biofilms formed protect microorganisms from the action of adverse agents, increase the availability of nutrients for their growth, and also increase the frequency of transfer of genetic material [24]. Gião et al. [25] observed that *H. pylori* formed biofilms after 24 hours of being in an unfavorable environment. The association of *H. pylori* with biofilm communities within a water distribution system could offer the bacterium protection against disinfection and predation by protozoa, and there are studies that demonstrate the survival of *H. pylori* within amoebae of free life [26, 27].

2.5 Foods transmission

Those foods that have a water activity (a_w) >0.97 and a pH between 4.9 and 6.0 theoretically provide the ideal conditions for the survival and development of *H. pylori* [28, 29].

Vegetables are one of the foods with the highest risk of fecal contamination, since they are in contact with soil and contaminated irrigation water, which would mean the spread of *H. pylori* in the environment and its transmission to humans. Atapoor et al. [30] and Yahaghi et al. [31] in Iran managed to detect and isolate *H. pylori* in percentages higher than 10%, in vegetable samples. Also, Bayas et al. [32] have detected the pathogen in vegetables by molecular methods.

On the other hand, the ability of *H. pylori* to survive on lettuce leaves forming biofilms has been demonstrated [33].

Milk could also act as a vehicle for *H. pylori*. Several studies have shown that the bacterium is able to survive in inoculated milk stored in refrigeration for more than 6 days or for 3 days at room temperature [34]. In addition, in an investigation developed by Fujimura et al. [35], the presence of the *H. pylori* ureA gene was detected in 13 of 18 samples of raw milk (72.2%) and in 11 of 20 samples of pasteurized milk (55%).

On the other hand, Meng et al. [36] analyzed 11 raw chickens and 18 samples of tuna meat ready for consumption (sushi). *H. pylori* was detected by multiple polymerase chain reaction (m-PCR) in 36% (4/11) of the chickens and 44% (8/18) of the tuna samples.

Studies have also been conducted on the presence of *H. pylori* in shellfish. Fernández et al. [37] detected *H. pylori* DNA in seawater, plankton, and oysters from three different regions of Venezuela. They concluded that mollusks could act as vehicles for *H. pylori* transmission.

2.6 Detection in human samples

The presence of *H. Pylori* was focused on a study developed by Samie [38], on the prevalence of *Campylobacter*, *Helicobacter*, and *Arcobacter*. By molecular methods, in 322 stool samples from HIV-positive and non-HIV-infected patients in South Africa, they found that *A. butzleri* was the third most frequent species (6.2%), after *Helicobacter pylori* (50.6%) and *Campylobacter jejuni* (10.2%).

2.7 Most common pathologies

2.7.1 Gastritis

The term gastritis should be reserved for the histologically demonstrated inflammation of the gastric mucosa. Gastritis is not the mucosal erythema seen during endoscopy, nor is it interchangeable with the term “dyspepsia” [13]. On the other hand, the different etiological factors that cause gastritis are multiple and heterogeneous; to gastritis it has been classified with a chronological base (acute or chronic), such as histological typologies, anatomical distribution or its pathogenic mechanism, clinical correlation, histological data, abdominal pain or dyspepsia, and endoscopic data in gastric mucosal investigation [13].

The pathogenesis of chronic gastritis by *Helicobacter pylori* includes two stages: the first is characterized by the arrival and penetration of the microorganism into the gastric mucus where it sits and multiplies. In the second stage, there is an amplification of the inflammatory response, by the interaction of lymphocytes, neutrophils, macrophages, mastoid cells, and nonimmune cells that, when attracted to the site of the lesion, release a wide variety of chemical mediators such as cytokines, eicosanoids, reactive oxygen metabolites (oxygen free radicals), and the complement system, which perpetuate inflammation [39, 40] (**Figure 1**).

2.7.2 Stomach cancer

It is the uncontrolled growth of stomach cells. Malignant tumors can originate in each of the three layers: mucosa, muscle, and serosa. This is also known as gastric cancer that originates in the stomach [41]. The risk factor is considered any caused that increases the likelihood of having a disease such as cancer, even though several risk components do not mean that the person will have the disease; Some scientists connoted that the risks that take a person to be more prone to suffer stomach cancer are several such as:

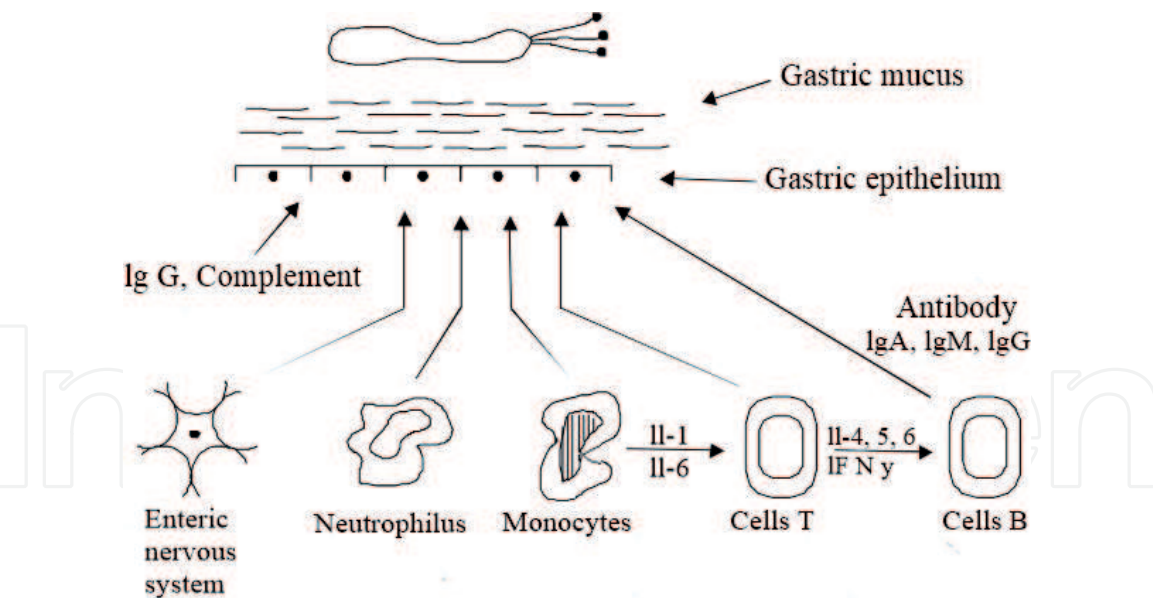


Figure 1.
Second stage of the inflammatory process of the gastric mucosa by *H. pylori*. Grávalos and González [40].

Incidence according to sex: Stomach cancer is more common in men than in women [16].

Age: The rate of stomach cancer in people over 50 years increases sharply [42].

Ethnic origin: In the United States, stomach cancer is more common among Americans of Hispanic origin, black people, and Asians and islanders compared to white people who are not of Hispanic origin [41].

Geography: On a global scale, stomach cancer is more common in Japan, China, Eastern and Southern Europe, as well as Central and South America [41].

2.7.3 Risk factors

Several risk factors for gastric cancer have been described, which play a fundamental role in their genesis, some of them remain under discussion, and others, on the contrary, have been confirmed more and more clearly [43].

2.7.4 Genetic

Within the genetic risk factors [41], we have:

- Families of patients with gastric cancer: incidence 2–3 times higher
- Blood group A.

2.7.5 Environmental

Among the environmental risk factors [41], we have:

- Food (variable in each country): dried and salted fish, very spicy foods, and red meats, among others
- Ingestion of alcohol, hot drinks, and sodium nitrate; chewed tobacco
- Radiation.

2.7.6 Premalignant

Within the premalignant risk factors [41], we have:

- Atrophic gastritis, intestinal metaplasia, and dysplasia.
- Pernicious anemia (20 times more frequent than in normal subjects).
- Gastric polyps: multiple hyperplasia, greater than 2 cm with some degree of dysplasia 0.4–4% of association with gastric cancer [41].

2.7.7 Stomach lymphoma

People who have suffered from a certain type of stomach lymphoma, known as lymphoma of lymphatic tissue associated with the mucosa (MALT), have an increased risk of developing adenocarcinoma of the stomach, probably due to infection with *H. pylori* [41] (**Figure 2**).

2.7.8 *H. pylori* and peptic disorders

The gastric infection produced by *H. pylori* bacteria in most cases of peptic ulcer is also important in the appearance of lymphomas that originate in the lymphoid tissue (MALT) and in gastric adenocarcinoma [13]. The peptic ulcer is an ulcer that affects the lining of the stomach and is the causes of internal bleeding of the upper digestive tract with severe complications that lead to an adenocarcinoma [13, 40].

2.7.9 Diagnostic methods of *H. pylori* infection

The diagnostic methods of *H. pylori* infection have traditionally been classified as direct and indirect; the former is based on the “direct” demonstration of the microorganism by means of the study of samples obtained by gastric biopsy [44]. This technique used is very stressful and uncomfortable for the patient because of the invasive reason.

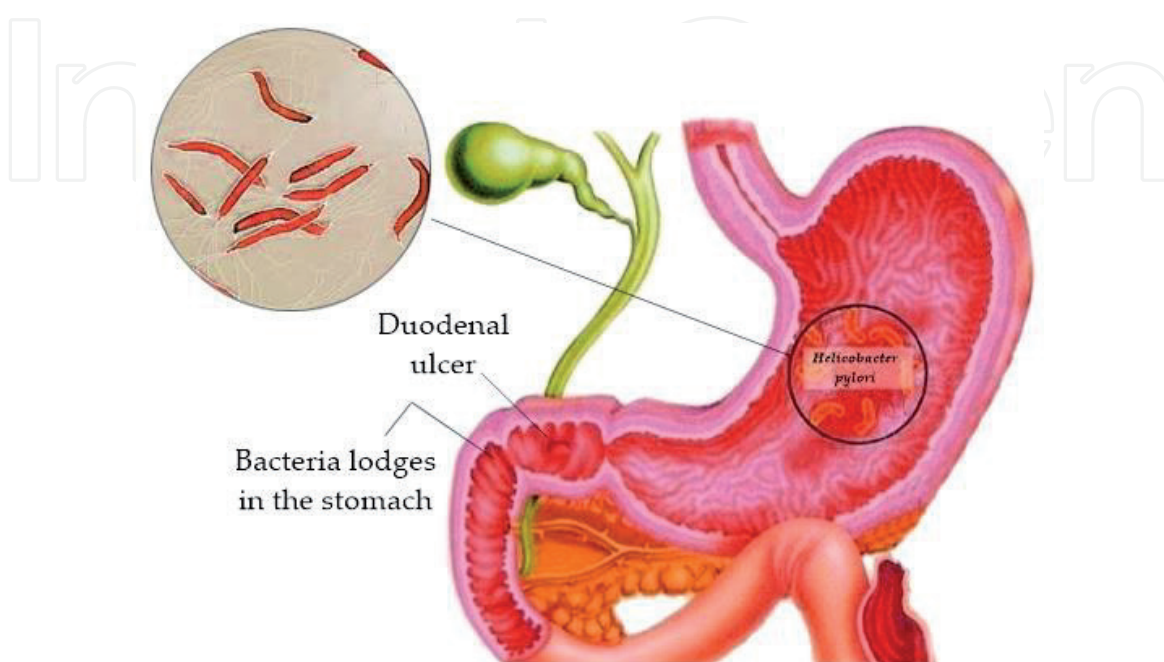


Figure 2.
Entrance and lodging of *H. pylori* in the stomach. Grávalos and González [40].

The other indirect methods are based on the detection of certain characteristics of the bacteria, such as the ability to hydrolyze through urea, and based on the breath test or the response of the immune system through the measurement of specific antibodies. Its primary advantage is its noninvasive nature [44].

2.7.10 Histological techniques

The presence of the germ can be recognized with the usual hematoxylin and eosin stain, although it is more easily demonstrated with other stains such as Giemsa. The histology not only demonstrates the presence of the microorganism but also informs about the morphological changes of the gastric mucosa [44].

2.7.11 Cultivation of *H. pylori*

Under optimal conditions *H. pylori* is extremely difficult to grow, due to its demanding nutritional requirements and its slow growth. The cultivation of *H. pylori* is usually slow, the first colonies usually appear between the fifth and seventh days, and it may take up to 10 days. Being a microaerophilic microorganism requires atmospheres with 5–10% of O₂, 5–10% of CO₂, and 80–90% of N₂ at 35–37°C, with a humidity of 90–95% [45].

The selection and inoculation of the bacteria depend on the number and types of tests to be carried out as well as on the factors, type of bacteria, clinical importance of the isolation, availability of the strain, and reliable method of verification [46]. Plate culture has advantages ranging from typifying the organism to determining its sensitivity to antibacterial agents, so it is important to study it from the epidemiological point of view, because it allows knowing the pattern of resistance to different therapeutic regimens with a specificity of the 100% and a lower sensitivity than other diagnostic techniques [3]. This microorganism is also urease, oxidase, and catalase positive, characteristics that are frequently used in the identification of the microorganism, although its isolation is relatively complex [16].

It is usually considered a difficult and tedious technique. However, adopting a series of minimal precautions, most laboratories achieve the growth of the microorganism [44].

2.7.12 Serology

Serological techniques only indicate a previous exposure to the microorganism but do not discriminate between people with active infection and disease in healthy individuals with prior exposure to infection [44]. Rapid tests are methods for the detection of antigens and antibodies in serum, plasma, whole blood, and other fluids, which give results in a few minutes [47]. These serological techniques are widely used today for rapid diagnosis in laboratories.

The enzyme-linked immunosorbent assay (ELISA) is widely used to perform epidemiological studies on a considerable number of individuals [48].

In a work done by Siavoshi et al. [49], for the intracellular detection of *H. pylori* in yeast identified in oral samples of newborns, the authors detected *H. pylori* with immunofluorescence using polyclonal antibodies IgG anti-*H. pylori* in a rabbit labeled with FITC, whose concentration was 5000 mg/ml, with a wavelength of 528 nm.

2.7.13 Antigenic screening

This is a chromatographic immunoassay for the qualitative detection of *H. pylori* antigen in human stool samples, with a relative sensitivity of 94%, a specificity of 95%, and an accuracy of 97.5%, since it is an in vitro technique ad-bio [50].

Methods	Characteristic	Advantage	Disadvantages
Direct			
Histological techniques	Habitual staining of hematoxylin and eosin Giemsa stain	Demonstrates the presence of the microorganism and reports on changes in the mucosa	The technique requires samples obtained from a biopsy Proper selection of stain fixatives
Direct and indirect			
Culture	Cultivation of the microorganism in specific media under microaerobic conditions The optimum temperature of culture is from 35 to 37°C [53]	Isolate the microorganism to study its behavior (in vitro)	It is difficult to isolate, since <i>H. pylori</i> is very sensitive to drying and to the usual atmospheric conditions (it requires the transport of samples in the shortest possible time) [54]. Samples destined for culture remain viable for approximately 5 hours and when stored in saline at 4°C or for more than 24 hours if stored at 4°C in a transport medium specific for <i>H. pylori</i> [55] Another disadvantage is the high contamination of the environment with accompanying biota, which makes it difficult to isolate <i>H. pylori</i> independently
Indirect			
Serological techniques	Methods for the detection of antigens and antibodies (serum, plasma, and whole blood, among others) Enzyme-linked immunosorbent assay (ELISA)	Rapid laboratory tests	It can induce a false-negative result
Antigenic screening	Chromatographic immunoassay for the detection of <i>H. pylori</i> antigens in stool samples	Rapid laboratory tests	Possible false positives due to cross reactions with other organisms [56]
Molecular Methods	DNA amplification of the pathogen	Great versatility as an analysis technique, sequences are amplified from minute amounts of target DNA, even from DNA contained in a single cell	Need to have information on the target DNA sequence Short size of the PCR products The ease with which DNA is amplified requires avoiding the danger of contamination inherent to the multiplier power of the reaction

Table 1.
Comparison of diagnostic methods for H. pylori.

2.7.14 Molecular methods

Molecular methods are the names given to all the laboratory techniques used to isolate DNA or extract it in high purity, visualize it to see its state, cut it and paste it (Iglesias [51]), or amplify a region in a huge amount of molecules: fragment cloning in bacteria or other vectors such as viruses as well as polymerase chain reaction (PCR).

Infectious diseases have become the “spearhead” for the development of molecular diagnostic tests, with more than 50% of the techniques available today. The main explanation for this development is due to the difficulty of detecting a pathogen through classical microbiology [52] (Table 1).

3. Conclusion

H. pylori is a microorganism of global interest, given that, in developing countries, the infection overcomes the 60%. Besides, being microorganisms of difficult isolation, the used techniques to culture are insufficient, so that molecular methods and antigen screening are the most recommended for detection, since these techniques are not invasive to patients.

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