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A Review Article in:

Helicobacter pylori infection and antibiotic resistance

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Abstract

Gastric cancer (GC) represents the second leading cause of cancer-related mortality worldwide.

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that inhabits the gastric environment of more than half of the world population. It is the main cause of gastroduodenal diseases. In addition, it is accused as a major risk factor for gastric cancer (GC). This discovery has been considered a major breakthrough in gastroenterology and dramatically change the management of these diseases (which now are considered as an infectious diseases and treated with antibiotics). A number of trials have demonstrated the possibility of cancer prevention through HP screening and eradication transmission. Many treatment modalities have been introduced to treat *H. pylori* and medications have evolved sequentially. However, the risk of resistance is high, since *H. pylori* employs a complex mechanisms of resistance. Thus, treatment by triple or quadruple therapeutic regimens now is applied.

Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that inhabits the gastric environment of more than half of the world population. Studies have demonstrated that the prevalence of *H. pylori*-positive status varies according to different factors such as age, geographical area, living condition and socioeconomic status (1). Oral-oral transmission seems to be the main route of *H. pylori* transmission. This explains the common occurrence of the infection among members of the same family, such as parents and children (2). In this way, the sharing of utensils during feeding seems to be important for infection establishment. Fecal-oral transmission is another form of infection that occurs through ingestion of contaminated water mainly due to

unsatisfactory basic sanitation conditions. Therefore, it is important to highlight that increasing socioeconomic status and the improvement of living conditions are factors that greatly influence the reduction in *H. pylori* infection prevalence (3).

Until Warren and Marshall's discovery of *H. pylori* infection in gastric mucosa, it was believed that the gastric environment was sterile because of its high acidity (4). Aiming for successful colonization under such hostile conditions, the bacterium uses a wide range of mechanisms that provide improved mobility, robust adherence to epithelial cells and an enzymatic apparatus that allows the establishment of an appropriate microenvironment for infection perpetuation. In addition, the potential of pathogenicity of this infection is provided by certain virulence factors such as cytotoxin associated antigen A (CagA), vacuolating cytotoxin (VacA), duodenal ulcer promoting gene A protein (DupA), outer inflammatory protein (OipA) and gamma-glutamyl transpeptidase (GGT) (5).

In this review, we discuss the epidemiology, pathogenesis, clinical manifestations, the main approaches of diagnosis, treatment and mechanisms of antibiotic resistance of *H. pylori*.

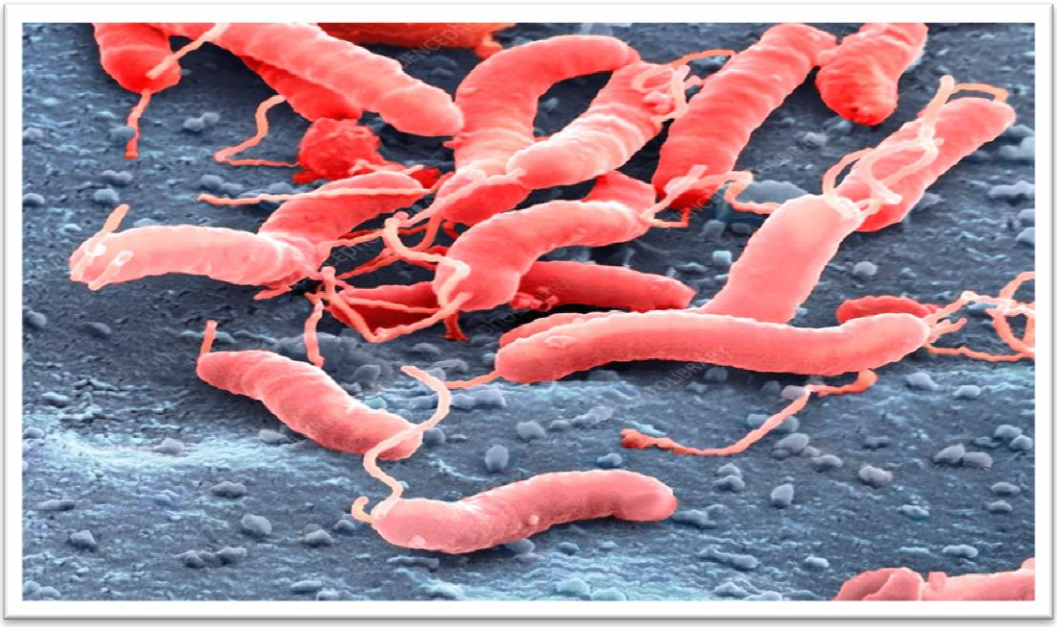

Figure (1): 3D draw of *H. pylori* (30)

Bacteriology

The genus *Helicobacter* belongs to the ε subdivision of *Proteobacteria*, order *Campylobacterales*, family *Helicobacteraceae*. This family also includes the genera *Wolinella*, *Flexispira*, *Sulfurimonas*, *Thiomicrospira*, and *Thiovulum*. To date, the genus *Helicobacter* consists of over 20 recognized species, with many species awaiting formal recognition. Members of the genus *Helicobacter* are all microaerophilic organisms and in most cases are catalase and oxidase positive, and many but not all species are also urease positive (6).

Helicobacter species have adapted to the inhospitable conditions found at the gastric mucosal surface, and it is currently thought that the stomachs of all mammals can be colonized by members of the genus *Helicobacter*. All known gastric *Helicobacter* species are urease positive and highly motile through flagella (7).

Helicobacter pylori is a spiral to curved, rod-shaped bacterium approximately 0.5 micrometer in diameter and **3** to 5 micrometer long. This organism possesses the characteristic ultrastructure of a gram-

negative bacterium. In tissue sections and Gram-stained smears from biopsy specimens, the bacteria usually appear smaller and more curved than cultured organisms, which are longer and less spiral. In older cultures, cells are seen to ball up, form U-shaped structures, and lose their cytoplasmic cylinders and membrane integrity, resulting in the formation of coccoid cells (8).

It has been proposed that this coccoid form is a viable but nonculturable form of the organism, which allows it to survive in hostile environments outside the gastric mucus. Studies provide evidence for the concept that these coccoid forms are degenerative and nonculturable, however, with a significant decrease in the amount and integrity of RNA and DNA and a loss of membrane potential. This concept has been supported by molecular data, but it remains a controversial issue (9).

Helicobacter pylori have 4 to 7 polar sheathed flagella, which enable the bacterium to move freely in viscous environments such as gastric mucus. Several studies have shown that this motility is essential for the bacterial colonization of its host (26). The flagella sheath is a membrane containing proteins and lipopolysaccharides, which probably protects the flagella filaments from the gastric acidity. The flagella filament contains two different flagellin proteins, FlaA and FlaB, both of which have been shown to be necessary for the motility of the organism (9).

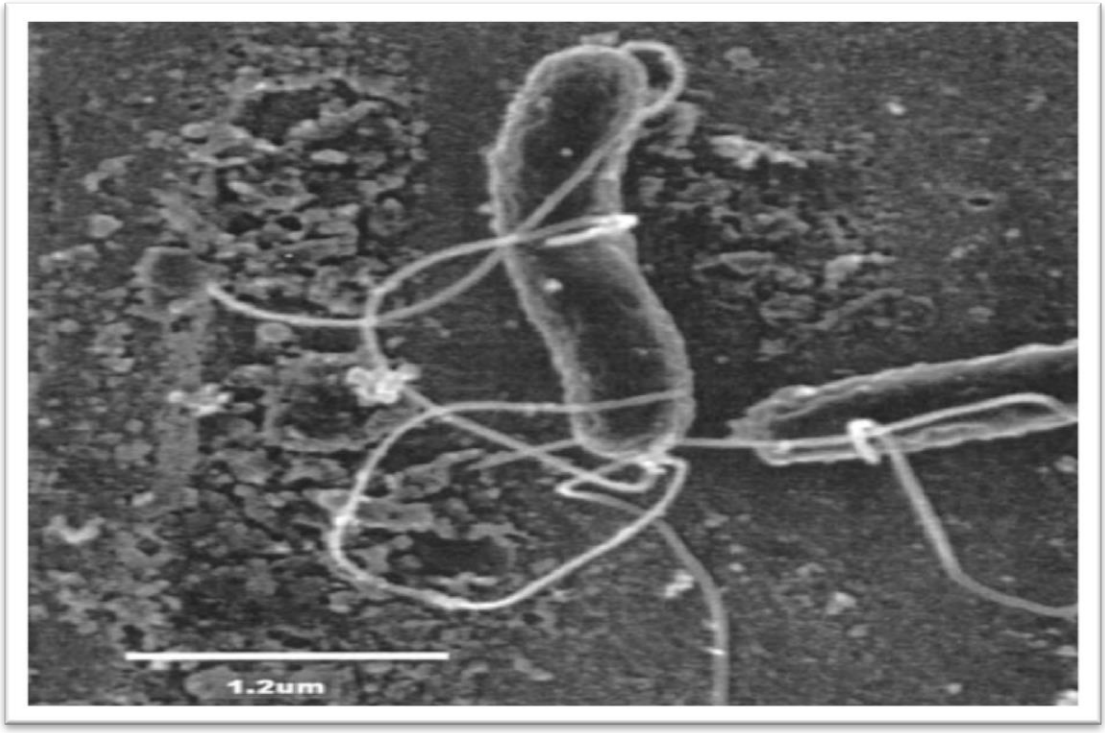

Figure 2. Electronic microscope photo for *H. pylori* (9).

In contrast to other bacterial pathogens that are highly clonal (such as *Shigella dysenteriae* and *Mycobacterium tuberculosis*), *H. pylori* is genetically heterogeneous, suggesting a lack of clonality. This results in every *H. pylori*-positive subject carrying a distinct strain, although differences within relatives may be small. The genetic heterogeneity is possibly an adaptation of *H. pylori* to the gastric conditions of its host, as well as to the distinct patterns of the host-mediated immune response to *H. pylori* infection. Genetic heterogeneity is thought to occur via several methods of DNA rearrangement and the introduction and deletion of foreign sequences (10).

Epidemiology

The epidemiological studies have shown that *H. pylori* prevalence ranges from 20-50% in the adult populations of the developed world but the occurrence is much more in the developing countries with prevalence is high as 90% in some countries. Children usually become infected with

H. pylori at a very young age (<5 years). The socioeconomic status of the family during childhood appears to be the major marker of infection. Higher prevalence exists in regions of low socioeconomic and poor sanitary conditions, and in rural as contrasted to urban areas. Overcrowding is also a risk factor for acquisition of *H. pylori* infection in children (11).

In developed countries, children and adolescents are only infrequently infected, while in adults over 50 years of age the infection ranges from 30–60%. In USA, serologic evidence of *H. pylori* is rarely found before age of 10y but increases to 10% in those between 18–30y of age and to 50% in those > 60y. The rate of infection with this bacterium differs in different countries e.g. in Kuwait 81%, in Jordan 82% and in Turkey 63% (12).

Pathogenesis

The gastric mucosa is well protected against bacterial infections. *H. pylori* is highly adapted to this ecologic niche, with a unique array of features that permit entry into the mucus, swimming and spatial orientation in the mucus, attachment to epithelial cells, evasion of the immune response, and, as a result, persistent colonization and transmission (10). The *H. pylori* genome (1.65 million bp) codes for about 1500 proteins. Among the most remarkable findings of two *H. pylori* genome-sequencing projects were the discovery of a large family of 32 related outer membrane proteins (Hop proteins) that includes most known *H. pylori* adhesins and the discovery of many genes that can be switched on and off by slipped strand mispairing-mediated mutagenesis. Proteins encoded by such phase-variable genes include enzymes that modify the antigenic structure of surface molecules, control the entry of foreign DNA into the bacteria, and influence bacterial motility (10,13).

After entering the host stomach, *H. pylori* utilizes its urease activity to neutralize the hostile acidic condition at the beginning of infection. Flagella-mediated motility is then required for *H. pylori* to move toward host gastric epithelium cells, followed by specific interactions between bacterial adhesins with host cell receptors, which thus leads to successful colonization and persistent infection. Finally, *H. pylori* releases several effector proteins/toxins, including cytotoxin-associated gene A (CagA), and vacuolating cytotoxin A (VacA), causing host tissue damage. In addition, the gastric epithelium layer, which forms the major interface between *H. pylori* and the host, secretes chemokines to initiate innate immunity and activate neutrophils, and further lead to the formation of clinical diseases such as gastritis and ulcer (14).

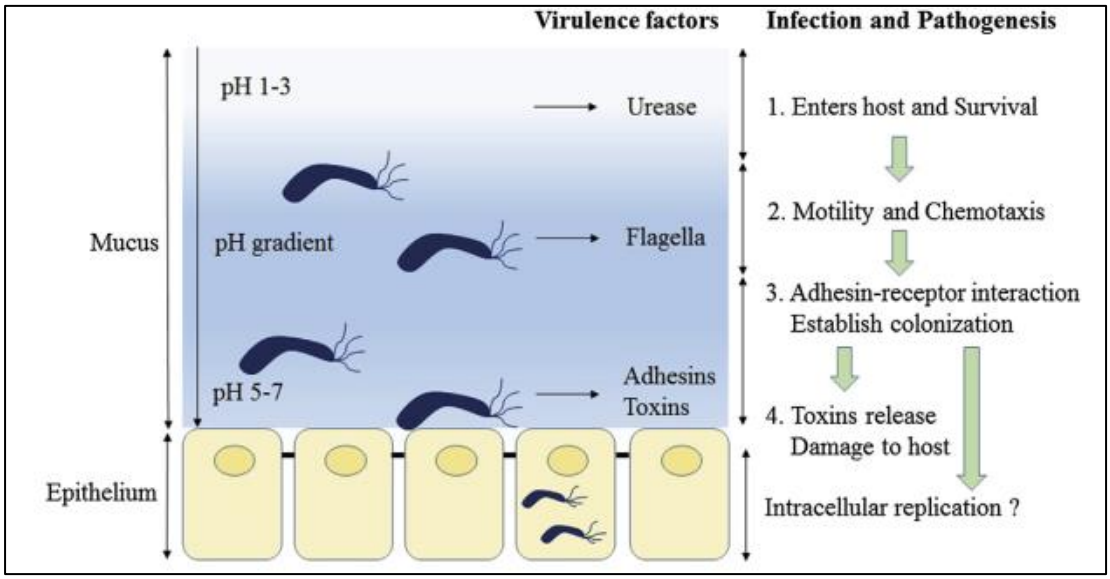

Figure 3. Schematic diagram of *Helicobacter pylori* pathogenesis (10)

Helicobacter pylori and gastrointestinal diseases

Although *H. pylori* typically colonizes the human stomach for many decades without adverse consequences, the presence of *H. pylori* is associated with an increased risk of several diseases, including peptic

ulcers, noncardia gastric adenocarcinoma, and gastric mucosaassociated lymphoid tissue (MALT) lymphoma (15).

The risks of peptic ulcer disease and noncardia gastric adenocarcinoma are determined in part by characteristics of the *H. pylori* strain with which an individual is colonized. Most of the *H. pylori* polymorphisms associated with various disease risk are found in genes that encode bacterial products that interact with host tissue. Numerous studies, particularly in Western countries, have shown that *cag* PAI-positive *H. pylori* strains are associated with a higher risk of peptic ulcer disease, premalignant gastric lesions, and gastric cancer than are strains that
lack the *cag* PAI (16).

Helicobacter pylori strains expressing multiple interaction factors and strains that lack these factors might occupy different niches in the gastric environment, or each could have selective advantages at different times during prolonged colonization. Currently, people in developing countries are predominantly colonized by *cagA* strains, whereas those in many developed countries are colonized by an almost equal proportion of *cagA* and *cagA* strains (17).

Diagnosis

Various diagnostic methods are developed to detect *H. pylori* infection and diagnostic tests with both high sensitivity and specificity, exceeding 90%, are necessary for accurate diagnosis of *H. pylori* infection in clinical practice. Although many diagnostic tests are available now, each method has its own advantages, disadvantages, and limitations (18). The choice of one method or another could be depended on availability and accessibility of diagnostic tests, level of laboratories,

clinical conditions of patients, and likelihood ratio of positive and negative tests on different clinical circumstances (19). Diagnostic tests are usually divided into invasive (endoscopic-based) and noninvasive methods. Invasive diagnostic tests include endoscopic image, histology, rapid urease test, culture, and molecular methods. Non-invasive diagnostic tests included urea breath test, stool antigen test, serological, and molecular examinations (14).

Treatment

Many treatment modalities have been introduced to treat *H. pylori* and medications have evolved sequentially. Bismuth-based triple eradication therapies for *Helicobacter pylori* infection therapy for 2 weeks was the first eradication treatment to be recommended by a major consensus group. However, proton pump inhibitor (PPI)–based therapies were developed subsequently and 1-week triple therapies, using a PPI combined with amoxicillin and clarithromycin, are now established, based on considerable trial data (20). In the United States, 2 weeks of this treatment is often recommended. More recently, quadruple therapy combining a PPI with bismuth triple therapy has been used as both first- and second-line eradication therapy. With this therapy, the addition of an antisecretory agent may enable the duration of bismuth-containing treatment to be shortened to 1 week and may reduce the adverse impact of metronidazole resistance (MR) on bismuth triple therapy (21).

In the selection of the most appropriate empiric treatment regimen for *H pylori*, previous antibiotic exposure, regional antibiotic-resistance patterns, and eradication rates should be taken into consideration because these factors can impact successful treatment. Successful treatment also relies on host factors such as allergies and adherence (22).

It has also been stated that PPIs, which have been used for a long time in the treatment of *H. pylori* infection, may prevent the absorption of micronutrients as well as their benefits. The United States Food and Drug Administration has suggested that long-term use of PPIs may cause an increased risk of hypomagnesemia and fractures (29).

Antibiotic Resistance

Recently, the WHO published a list of bacteria for which new antibiotics are urgently needed. A total of 12 families are included, grouped according to the priority (critical, high, and medium), and clarithromycin-resistant H. pylori was included in the high priority group (23).

The clarithromycin resistance rate is another key datum as it is part of the first-line triple therapy. Although resistance rates are not as high as those of metronidazole in most countries, very high rates were detected in Spain, Italy, Iran, and the USA (24).

In *H. pylori*, the mechanism of resistance is complex, as at least four possible mechanisms have been described: reduction in the antibiotic uptake and/or an increase in the efflux of the antibiotic though the bacteria wall, reduced activity of the nitroreductases (such as nitroreductase, an oxygen-insensitive nitroreductase), S-nitrosoglutathione reductase (a Flavin oxidoreductase), and ferredoxins-like protein (encodes a ferrodoxin-like protein), so that metronidazole cannot be activated, increase in the activity of the oxygen-radical scavenger system, and increase in the activity of the DNA repair enzymes, such as the one encoded by the bacterial DNA recombination protein gene (25,26).

There are several point mutations described in the domain V of the 23S ribosomal ribonucleic acid (rRNA) gene that decrease the affinity of the ribosome for clarithromycin and, therefore, the bacteria become resistant. A2142G, A2142C, or A2143G are the most frequent mutations found in clinical isolates and less frequently A2144T, T2717C, and C2694A are found (27,28).

Conclusion

H. pylori is a gram negative bacteria which is the first microorganism linked to malignancy. Its potential risk for causing GIT disorders and neoplastic changes should be studied roughly and to develop new eradication methods. To manage *H. pylori* infection, it is important to choose the appropriate regimen considering previous antibiotic exposure, regional antibiotic-resistance patterns, and eradication rates because these factors can impact successful treatment.

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