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REVIEW ON STIMULATIONS OF THE SPREAD OF HELICOBACTER PYLORI INFECTION IN IRAQ

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Abstract

Helicobacter pylori (H. pylori) bacteria are microaerophilic Gram-negative bacteria that inhabit the human stomach and duodenum. If left untreated, it can lead to prolonged infection in human life. Numerous studies have shown that H. pylori infection can cause several important gastrointestinal diseases, ranging from chronic active gastritis without clinical symptoms to peptic ulcer, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma. Most modern publications are devoted to the pathogenic properties of microorganisms in the development of chronic gastritis, gastric ulcers, and gastric cancer, and methods for their eradication. Helicobacter pylori was the first officially recognized bacterial carcinogen and one of the most successful human pathogens, as more than half of the world's population is colonized by this Gram-negative bacterium. The disease course is the result of complex interactions between the host and bacteria. Host immunity gene polymorphisms and gastric acid secretion largely determine the ability of bacteria to colonize specific gastric niches. Bacterial virulence factors such as the cytotoxin-related gene pathogenicity island-encoded protein CagA and the vacuolar cytotoxin VacA support this colonization of the gastric mucosa and subsequently appear to regulate the host's immune system. This study focuses on the microbiological, clinical, and immunological aspects of H. pylori pathogenesis; it also reveals the prevalence of H. pylori in some Iraqi governorates.

Keywords: Helicobacter Pylori, Gastrointestinal Diseases, Stimulations, Spread Of Infection.

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Introduction

Lush microbial flora important to the health and well-being of the host inhabit the human gastrointestinal tract. The gut microbiota contributes to a variety of functions, including energy production and storage from food, development and regulation of the gut-associated mucosal immune system, regulation of the central nervous system, detoxification of foreign substances and carcinogens, and prevention of pathogen colonization. The gut microbiota is acquired early after birth and is influenced by a variety of factors including diet, genetic background, and the environment.

Its composition and complexity can be altered by physiological changes such as aging and pregnancy. Fluctuations in the gut microbiota can also be caused by antibiotic treatment, metabolism, immunity or infectious diseases. In particular, chronic infectious and non-infectious diseases can cause long-term changes in the gut microbiota, which can have a major impact on gut homeostasis and contribute to the development of other diseases. Analysis of the gut microbiota and its variation is emerging as a medical approach for preventing or treating disease. The major pathogen associated with humans for over 60,000 years has been *Helicobacter pylori* (*H. pylori*). It is estimated that more than half of the world's population is infected with *H. pylori*. However, in most cases, *H. pylori* infection remains asymptomatic. This study examines *H. pylori* and the reasons for its prevalence in Iraq [1].

Genus Description and Phylogeny

Helicobacter pylori belong to the ε branch of the Proteobacteria, Campylobacter, and *H. pylori* families. The family also includes the genera Wolinella, Flexispira, Sulfurimonas, Thiomicrospira and Thiovulum. To date, the *Helicobacter* genus consists of more than 20 recognized species, many of which are awaiting official recognition [2]. Members of the genus Helicobacter are microaerophilic organisms, catalase and oxidase positive in most cases, and many but not all species are also urease positive. *H. pylori* can be subdivided into two main lineages, gastric *H. pylori* and enterohepatic (non-gastric) *H. pylori*. Both groups exhibited a high level of organ specificity, and thus gastric *H. pylori* were generally not able to colonize and recognize in the gut or liver [3]. Here, we briefly discuss those Helicobacter species associated with human disease or have relevance for animal models of human Helicobacter infection.

Helicobacter pylori

Helicobacter stomach has adapted to the unsuitable environment of the gastric mucosal surface, and it is currently believed that the stomach of all mammals can be colonized by members of the Helicobacter genus. All known gastric *H. pylori* species are urease-positive and highly motile due to flagella [4]. Urease is thought to allow short-term survival in the highly acidic gastric lumen, while exercise is thought to allow rapid movement to the more neutral pH of the gastric mucosa [5]; this may explain why both factors are prerequisites for gastric mucosal colonization. Upon entry, gastric *H. pylori* exhibited urea- and bicarbonate-mediated chemotactic movements to the mucus layer. The helical morphology and flagellar movement then facilitate penetration into the viscous mucus layer, where more neutral pH conditions allow gastric *H. pylori* species to grow.

Morphology

H. pylori is a Gram-negative bacterium, 2 to 4 µm long and 0.5 to 1 µm wide. While the bacterium is usually spiral-shaped, it can take the shape of rods and spheroids after prolonged in vitro culture or antibiotic treatment. These spheroids cannot be cultured in

vitro and are thought to represent dead cells, although it has been suggested that the spheroid form may represent a viable, unculturable state. The organism has 2 to 6 unipolar, sheathed flagella about 3 µm long, usually ending in a prominent nodule. Flagella impart motility and allow rapid movement in viscous solutions, such as the mucus layer covering gastric epithelial cells [6]. Compared to many other pathogens of the gastrointestinal tract, it lacks piladhesin.

H. pylori proteases and lipases degrade gastric mucus and disrupt the phospholipid-rich layer on the surface of apical epithelial cells, leading to reverse diffusion of gastric acid and cell damage. This cellular damage may lead to cell death, believed to be the result of induction of apoptosis.

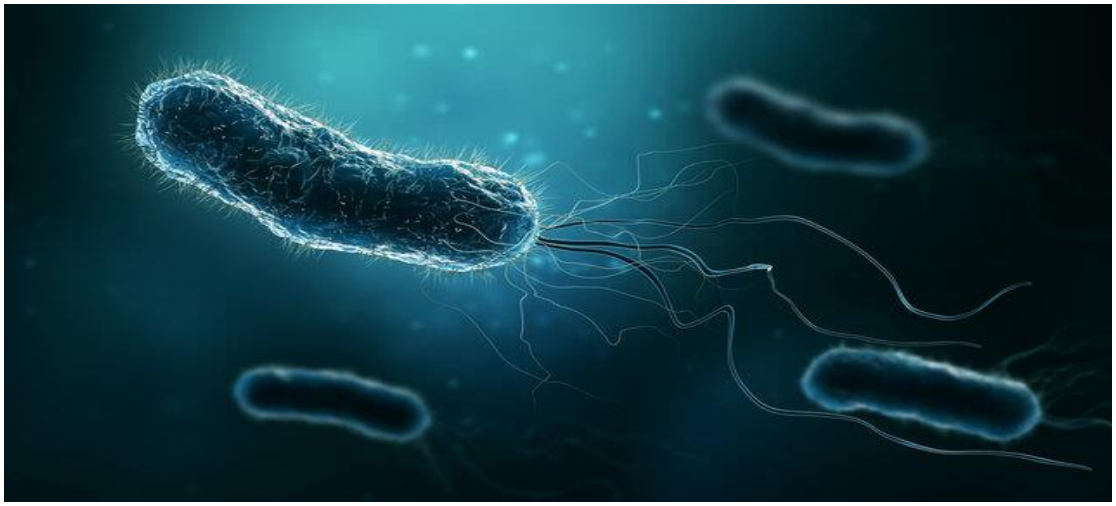


Figure 1: *Helicobacter pylori* (gastric *H. pylori*)

There are sufficient data to suggest that *H. pylori* significantly contribute to the gastric mucosal damage associated with this infection through a direct pathogenic mechanism and may enhance the susceptibility of gastric epithelial cells to oncogenic transformation. The mucosal damage associated with this infection may enhance the susceptibility of gastric epithelial cells to oncogenic transformation.

Growth requirements

A key feature of *H. pylori* is its microaerophilicity, with optimal growth at 2% to 5% O2 concentration with the additional requirements of 5% to 10% CO2 and high humidity. H2 is not necessary, although it is not detrimental to growth.

Many laboratories use standard microaerophilic conditions of 85% N2, 10% CO2, and 5% O2 for *H. pylori* cultures. Growth occurs at 34 to 40°C with an optimum temperature of 37°C. Although its natural habitat is the acidic lining of the stomach, *H. pylori* is considered neutrophilic. Bacteria survive brief exposures to pH <4, but only grow in a relatively narrow pH range of 5.5 to 8.0, growing best at neutral pH [7,8].

H. pylori is a finicky microorganism that requires complex growth media. Usually these media are supplemented with blood or serum. These supplements can act as an additional source of nutrients and also prevent the toxic effects of long-chain fatty acids [9]. Common solid media for routine isolation and culture of *H. pylori* consist of Columbia or Brucella agar supplemented with (lysed) horse or sheep blood, or neonatal or fetal bovine serum. Selective antibiotic mixtures can be used for primary isolation as well as for routine culture, but these are not required by themselves.

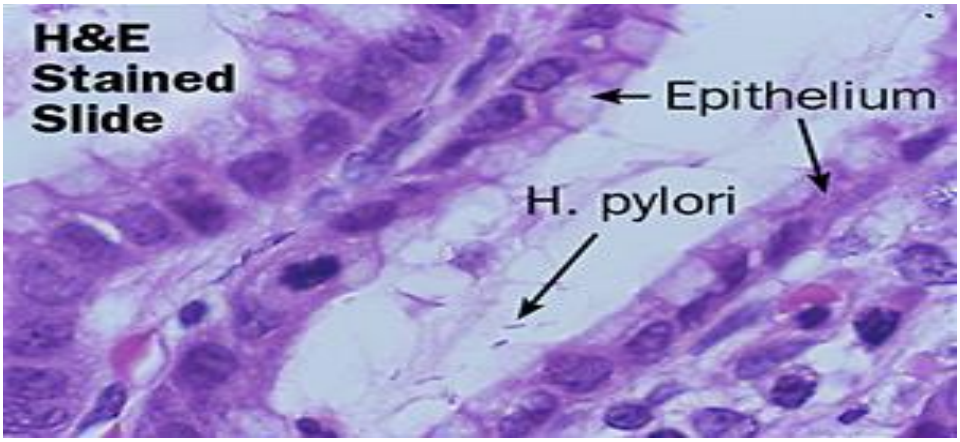
Liquid media typically consist of Brucella, Mueller Hinton, or brain heart infusion broth supplemented with 2 to 10% calf serum or 0.2 to 1.0% beta-cyclodextrin, usually with Dent or Skirrow supplements. Growth of *H. pylori* in chemically defined media has been reported, but these are not suitable for routine growth and isolation of *H. pylori*. Most commercially available synthetic media, such as B. tissue culture medium, do not support the growth of *H. pylori* without the addition of serum, with the possible exception of Ham's F-12 nutrient mix [10].

Isolation of *H. pylori*

Isolation of *H. pylori* from gastric biopsy specimens is difficult and not always successful. Cultures should be examined from day 3 to day 14.

H. pylori forms small (~1 mm), translucent, smooth colonies. After successful subculture, *H. pylori* isolates tend to adapt to the growth conditions used in the laboratory. Thereafter, when using reference strains and laboratory-appropriate *H. pylori* isolates, good growth is usually achieved after 1 to 3 days of incubation. It should be noted that once the culture reaches stationary phase, the growth rate drops rapidly while the morphology becomes spherical. Prolonged incubation does not result in a significant increase in colony size, but rather transitions to an unculturable spheroid state.

H. pylori can be stored long-term at -80 °C in brain-heart infusion or in Brucella broth supplemented with 15% to 20% glycerol or 10% dimethyl sulfoxide, but optimal viability requires use of less than 48 Hour in culture with 90% helical cells [11].



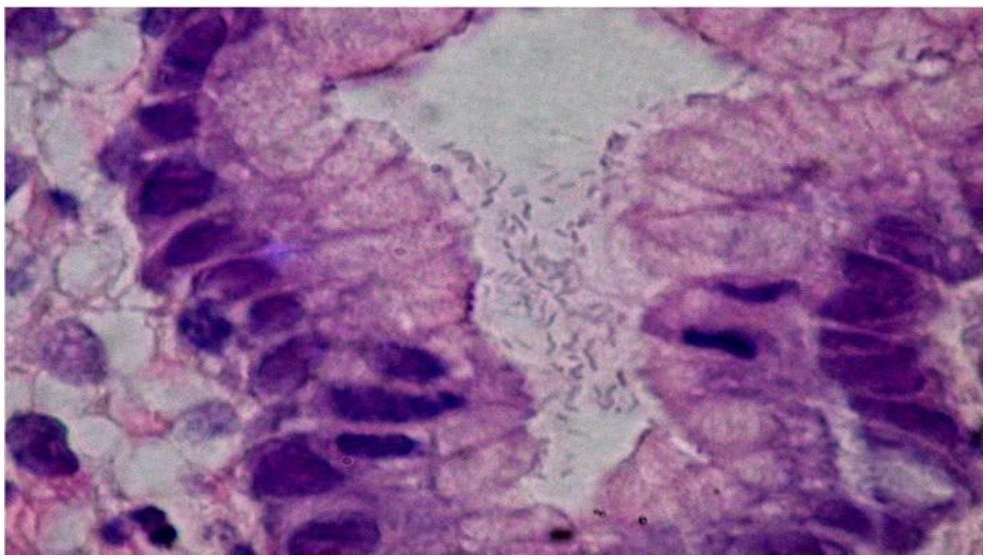


Figure 2: Demonstration of *H. pylori* by H&E stain x1000 (High power view, immersion). Gastric pits show numerous spiral-shaped and coccoid forms of *H. pylori*.

H. pylori virulence factors and its role in systemic diseases

In order to survive in the unfavorable, overly acidic conditions of the stomach, *Helicobacter pylori* synthesizes many virulence factors that can both improve important conditions of activity in an acidic environment and have a damaging effect on the gastric mucosa. After entering the stomach of the host, *Helicobacter pylori* use urease activity to neutralize hydrochloric acid, which is one of the protective factors and has a strong antibacterial effect. The regulation of urease synthesis is encoded by a group of genes called the urease gene cluster. This group of genes includes the catalytic part (urea A/B), acid-gated urea channel (ureI), and accessory assembly proteins (ure E-H).

Interestingly, urease synthesis depends on the pH of the bacteria: the urea channel is tightly closed at pH 7.0 and fully opened at pH 5.0. That is, when external conditions change, that is, when the level of gastric acid increases, *Helicobacter pylori* releases urease, which hydrolyzes urea into carbon dioxide and ammonia (NH3), which in turn combines with water to form a Stabilized ammonium hydroxide. This series of biochemical reactions results in moderate alkalization. This mechanism of overcoming the acid barrier is extremely important for the survival of bacteria, and has a helical shape, smooth cell wall, and helical motion. Another important virulence factor that promotes the spread of bacteria to the gastric epithelium is its motility, due to the presence of 4-7 motile enveloped flagella. Flagella are a complex organ composed of several protein subunits, consisting of a basal body, hooks, and filaments. There are different types of flagella-driven movements:

Swimming, dispersion and shoaling. In addition to urease activity, flagellar motility has been shown to be an important factor in gastric mucosal colonization. There are also studies showing the importance of flagella in the formation of microbial biofilms on the gastric mucosal surface.

Bacterial adhesion is a key stage of colonization and determines the series of processes by which *H. pylori* persists in the stomach. Adhesion molecules (outer membrane proteins) such as pylori outer membrane protein and LacDiNAc-binding adhesin have been described in the literature.

Among the key factors for colonization, virulence factors that have a direct damaging effect on the gastric mucosal epithelium can be singled out; they are cagA, γ-glutaminyltransferase, hyperthermia requirement A and vacuolated cytotoxin A (vacA) [12].

CagA is a highly antigenic protein with a molecular weight of 120-145 kDa. The location of the gene responsible for the synthesis of cagA and the type IV secretion system (T4SS) is called the cag pathogenicity island. CagA acts intracellularly through epithelial cells via T4SS: the latter forms a syringe-like pili structure through which cagA molecules enter host epithelial cells. After transport into cells, cagA is phosphorylated on the inner side of the cytoplasmic membrane of epithelial cells, thereby acquiring biochemical activity. The main pathogenic effect of cagA is to enhance the mitotic activity of gastric epithelial cells, which can lead to malignancy if the microbe persists for a long time. This is achieved by reducing the activation of T lymphocytes in the lamina propria and disrupting the process of autophagy [12].

Immune response to *Helicobacter pylori* invasion

After entering the human body, *H. pylori* is always under the control of the immune system. Inflammatory responses are known to be hallmarks of evolved immune responses. In *H. pylori* persistence, inflammatory foci primarily affect gastric epithelial cells; in this case, the inflammatory response involves neutrophils, lymphocytes, macrophages, and

dendritic cells (DCs), which migrate through the systemic circulation to site of infection. Contact of DCs with *H. pylori* epitopes results in autocrine activation of the immature CD4+ T cell pool, which subsequently differentiate into T helper (Th) 1 lymphocytes through the production of interleukin (IL)-12.

Th1 lymphocytes are the main inflammatory effector cells invaded by *H. pylori* [12]. The initial immune response of Th1 cells is designed to completely eradicate the infectious agent. However, experiments showed that vacA was able to exert an immunosuppressive effect on cells of the immune system by inhibiting the production of IL-23 by DCs. The interaction of *Helicobacter pylori* with Th2 lymphocytes was not obvious. Due to the activation of the cellular Th1 component of the immune system.

Immunoglobulin G (IgG) levels have been shown to be a reliable indicator of the persistence of *H. pylori*. In infected individuals, a Th2 response induces IgG1 production, and a Th1 response leads to a marked increase in total IgG2 levels through the production of IL-2 and IFNγ. IgG2 titers are higher than IgG1 titers in *H. pylori*-infected patients, especially in patients with peptic ulcer disease. Thus, the persistence of *H. pylori* in humans is accompanied by a pronounced immune response to bacterial invasion, which is subsequently replaced by immune tolerance. This fact may suggest that the relationship between *H. pylori* and the host is symbiotic [12].

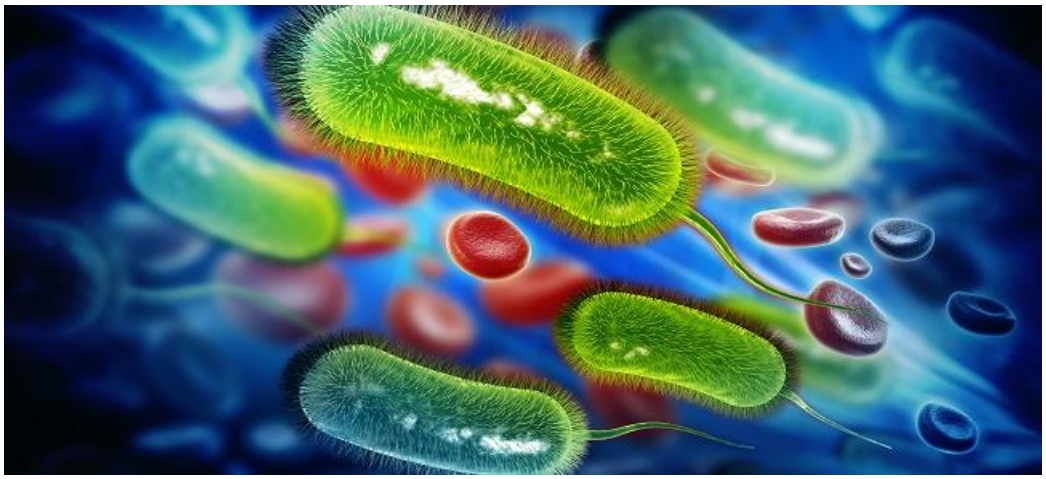


Figure 3: *H. pylori*

Genome, plasmids, and strain diversity

The two sequenced *H. pylori* genomes were approximately 1.7 Mbp in size with 35-40% G+C content. The genome of *H. pylori* strain 26695 includes 1,587 genes, whereas the genome of strain J99 includes only 1,491 genes. Both genomes contain two copies of the 16S, 23S and 5S rRNA genes. Many strains carry one or more cryptic plasmids that do not appear to carry antibiotic resistance genes or virulence genes. Some of these plasmids form the basis of *H. pylori*. Unlike other highly clonal bacterial pathogens such as *Shigella* and *Mycobacterium tuberculosis*, *H. pylori* are genetically heterogeneous, suggesting a lack of clonality.

These results in each *H. pylori*-positive subject carrying a specific strain, although the differences between relatives may be small. Genetic heterogeneity may be an adaptation of *Helicobacter pylori* to its host gastric conditions [11].

Diagnosis

Various tests have been developed to detect *H. pylori*, each with its own specific advantages and disadvantages. Available tests are generally divided into invasive tests based

on gastric samples for histology, culture or other methods, and non-invasive tests for antibodies based on peripheral samples such as blood, breath samples, stool, urine or saliva. Bacterial antigen or urease activity. The choice of a specific test for an individual patient depends on local experience and the clinical setting. During hospitalization, many patients undergo endoscopy followed by invasive testing for *H. pylori.* Otherwise, a breath test and serology are usually used [11].

Prevalence of *H. pylori* in Iraq

Studies revealed that the prevalence of *H. pylori* in some Iraqi governorates. Results conducted in the province of Babylon showed that the prevalence of *H. pylori* was higher in men (57.7%) than in women (42.5%), and that the highest prevalence of infection was in the elderly (30-40 years old), because Other causes such as alcohol consumption, smoking, and dietary habits may also be part of the family history [13].

Table 1: Age distribution among *H. pylori* infection of patients.

Age group	*H. pylori* patients	Controls
	No.	**No.**
20-30	8	5
30-40	**13**	**8**
40-50	10	4
50-60	5	2
Above 60	4	1
Total	40	20
p-value:0.9, P>0.05		

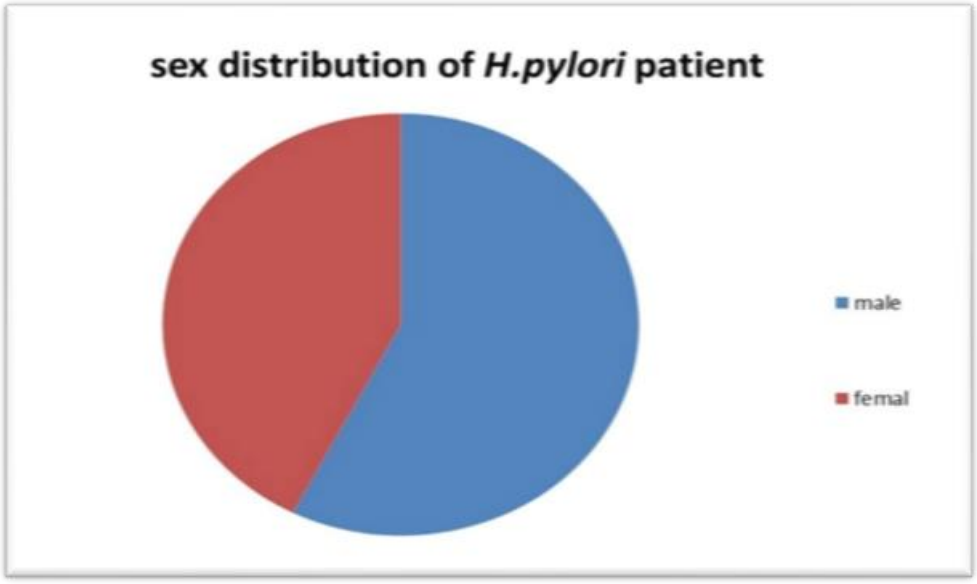


Figure 4: Sex distribution of *H. pylori* patient.

As students were noted that the overall prevalence of *H. pylori* infection in 240 patients with gastroduodenal disease in Erbil city was found to be 128 (53.3%). There was a significant association between *H. pylori* infection and gender (43.75% in men and 59.72% in women). The highest prevalence of *H. pylori* infection is in the age group over 50 years old [14].

Table 2: Seroprevalence of anti-*H. pylori* antibodies in relation to gender

Genders	Test No. (%)		Totals
	Positive	Negative	
Male	42 (43.75)	54 (56.25)	96 (40%)
Female	86 (59.72)	58 (40.28)	144 (60%)
Total	128 (53.3)	112 (46.7)	240 (100%)
The p-value is 0.015106. This result is significant at p < 0.05.			

Culture Method and PCR for the Detection of *Helicobacter pylori* in Drinking Water in Basrah Governorate Iraq this research reported that among 471 water samples, 14 (2.76%) *H. pylori* isolates were isolated from samples collected from 14 districts in Basra Governorate by culture and identified by biochemical tests [15].

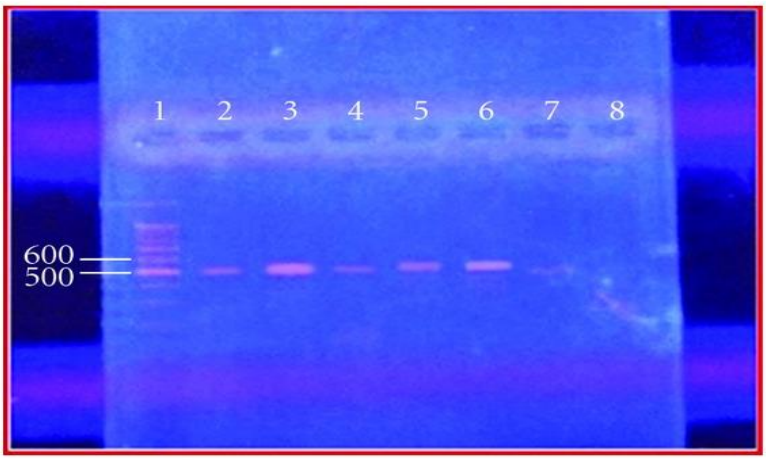


Figure 5: PCR Products for 16SrRNA–based primers gave band on agarose gel corresbonding to a 500 base pair product when compared to the moleculer ladder. Lane 1 moleculer ladder (1500-100) bp, lane(2-6) bands of PCR products for H. pylori 16SrRNA.

A study was also conducted a study of Iraqi college students and showed that among 311 students, 173 (55.8%) were infected with *H. pylori*. *H. pylori* infection was more common among seniors in each college (61.7%) than in other classes (46.6%) [16].

Table 3: Gender and *H. pylori*

Gender	*H. Pylori* test		Total	P*
	Negative	Positive		
Female	75 (41.9%)	104 (58.1%)	179 (57.5%)	0.34
Male	62 (47.3%)	69 (52.7%)	131 (42.3%)	
Total	137 (44.2%)	173 (55.8%)	310 (100%)	

Table 4: *H. pylori* infection in relation to stage of the study

Class of student	*H. Pylori* test		Total	P*
	Negative	Positive		
1 st class	39 (54.4%)	34 (46.6%)	73 (23.2%)	0.21 1
2nd class	27 (45.8%)	32 (54.2%)	59 (18.7%)	
Third class	32 (36.8%)	55 (63.2%)	87 (27.6%)	
Forth class	18 (50.0%)	18 (50.0%)	36 (11.4%)	
Fifth class	23 (38.3%)	37 (61.7%)	60 (19.0%)	
Total	139 (44.1%)	176 (55.9%)	315 (100.0%)	

Treatment

Although *H. pylori* is susceptible to multiple antibiotics in vitro, none of them can be used as monotherapy in vivo. In infected patients, the most effective single agent was clarithromycin, which was administered twice daily for 10 to 14 days, with an eradication rate of about 40%. The lack of efficacy of monotherapy is related to the ecological niche of *H. pylori*, which resides in the lower pH viscous mucus layer. Tetracycline, amoxicillin, imidazole, and some selected macrolides (especially clarithromycin and sometimes azithromycin) are probably the most widely used drugs for *H. pylori* eradication therapy.

More recently, the use of rifabutin and furazolidone has been promoted. However, due to their limited efficacy and the inability of many patients to tolerate furazolidone, the primary use of these two antibiotics is as a second-line rescue treatment for patients with metronidazole-resistant strains.

There have been occasional reports of the use of ciprofloxacin and related fluoroquinolones and other antibiotics such as rifampicin and streptomycin, but these do not appear to have any clear advantages over the aforementioned drugs. The use of these drugs has resulted in effective therapy against *H. pylori* with consistent eradication rates of over 80%. Various treatment durations, doses, and drug combinations have been studied, but none consistently achieve eradication rates greater than 90-95%. Failure is primarily due to insufficient adherence to treatment, often due to side effects and the presence of antibiotic resistance. This resistance is common in patients who have previously received antibiotic therapy, including failure of eradication therapy [11].

Conculation

The prevalence of *H. pylori* infection in Iraq is associated with the following factors:

Age, marital status, education level, drinking water sources, garlic consumption frequency, alcohol consumption, mode of transmission and knowledge of *H. pylori* -related diseases.

The main cause of *H. pylori* is the bacterium, which is spread from person to person through direct contact with saliva, vomit, or feces. *H. pylori* can also be spread through contaminated food or water, also From this study we conclude that

The prevalence of *H. pylori* in some Iraqi governorates. The results conducted the prevalence of *H. Pylori* in high in males individuals more than females, and the highest rate of infected patients were among ages (30-40) years as well as due to Alcohol consumption, Smoking and other resean such as dietary habits also may be to the history of family.

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