

Factors affecting infection with *Helicobacter pylori* and measuring their sensitivity to antibiotics

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ABSTRACT

Helicobacter pylori infection is a global health problem that leads to gastric ulcers and cancer. The current study aimed to identify some of the factors affecting infection with *H. pylori* and to study its sensitivity to some antibiotics. This study was conducted in the Endoscopy Unit in the Gastrointestinal Center of Baghdad Teaching Hospital for the period from 25/1/2021 to 4/3/2022. 55 isolates of *H. pylori* were detected. Factors affecting infection with *H. pylori* that were studied included age, as it reached the highest rate of infection in the age group (11-20 years), with a rate of 33%, as well as gender had an effect on infection, as females showed a higher infection rate than male, it reached 71% and 29%, respectively. Smoking was also associated with infection with *H. pylori*, by 24%, and the type of food was also associated with infection, by 36%. There was also a relationship between the infection and the place of residence, as the infection appeared in villages higher than in cities, by 58% and 42%, respectively, some immune diseases associated with *H. pylori* infection were also studied, as it was found that there is a relationship between *H. pylori* infection and diabetes, with a rate of 41%. The sensitivity test using the Kirby-Bauer disk diffusion method showed that most of the *H. pylori* isolates are sensitive to the antibiotics under study and gave the highest sensitivity to Rifabutin (99%) and lowest sensitivity to Penicillin (8%). Therefore, early detection and knowledge of the factors affecting the increase in infection with this bacterium, as well as regular antibiotic sensitivity test, helps to limit its spread and improve control of the outbreak of the disease.

Keywords: *Helicobacter pylori*, Infection factors, Antibiotics, Gastric ulcer.

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INTRODUCTION

Helicobacter pylori is a Gram-negative, helical, multi-flagellum, microaerophilic, inhabiting the mucous layer above human gastric epithelial cells [1,2], infecting nearly half of the world's population [3], isolated for the first time by scientist Warren and Marshall in 1983 [4]. It is one of the common pathogenic bacteria in humans, as it is considered one of the main factors causing infectious diseases such as gastritis and peptic ulcers, as well as being described as one of the dangerous factors that cause cancer stomach [5]. It is also strongly associated with the development of gastric mucosa-associated lymphoid tissue (MALT) lymphoma and is classified by the World Health Organization as a first-class carcinogen based on the results of epidemiological studies [6]. As the eradication of *H. pylori* infection leads to a reduction in the recurrence of ulcers and a reduction in the risk of developing stomach cancer among patients [7]. The methods of transmission from one person to another are either by the oral-oral pattern, which is the most prevalent pattern in developed countries due to overcrowding, which has led to a decline in living conditions and the spread of unhealthy habits or by the fecal-oral pattern, which is the most common pattern in developing countries due to poor hygiene and lack of good awareness [8]. The health status of the family plays a major role in the infection of this bacteria. These bacteria possess many virulence factors that are responsible for the pathogenesis of bacteria, including the cytotoxic associated gene (*cagA*) which is the most dangerous factor, which is responsible for the development of stomach cancer, and the vesicle-forming gene (vacuolating cytotoxin gene (*vacA*)), which is responsible for the formation of vesicles in the epithelial cells, which destroys epithelial cells, and thus is the factor responsible for the occurrence of stomach ulcers, and among other factors is the duodenal ulcer promoting gene (*dupA*) and the enzyme urease, which is responsible for neutralizing the acidity in the stomach cavity, and the flagella responsible for movement [9]. The gastric mucosa is immune to bacterial infections due to the high acidity in the stomach cavity, but the *H. pylori* bacteria came and violated this rule and was able to colonize the gastric mucosal layer because it possessed several unique characteristics that enabled it to enter the mucous layer, such as movement and taking a spatial space in the mucous layer, adhesion to epithelial cells, escape from the immune response, all this helped *H. pylori* in its ability to colonize and

transmigrate [10]. Thus, *Helicobacter pylori* are the main cause of gastritis, gastric and duodenal ulcers, and is associated with cases of gastric mucosa-associated lymphoid tissue (MALT) lymphoma and the development of gastric cancer [11].

Material and Methods

Sample collection: Samples were collected from the Endoscopy Unit in the Gastrointestinal Center of Baghdad Teaching Hospital for the period from 25/1/2021 to 4/3/2022, from both sexes for age groups (16-70) years. 55 pathological samples (Gastric biopsy, stool, and blood) were collected. Pathological samples were collected from patients with gastric inflammation or ulcers, and duodenal inflammation or ulcers.

Stool samples: A stool sample was taken from each patient and placed in a sterile container. Then a part of the sample was taken to detect the presence of *H. pylori* fecal antigen by Cassette- *H. pylori* Ag Rapid test. [12].

Blood samples: collected from each patient (3-5) ml of blood after completing the endoscopic examination, the blood was separated by a centrifuge at 3000 rpm for 10 minutes to obtain the serum, and then the serum was used to detect the antibodies (IgG) formed against *H. pylori* by the test of the immune strip for the rapid initial detection, and the remaining part of the serum is kept at a temperature of - 20 ° C until use for the ELISA test [13].

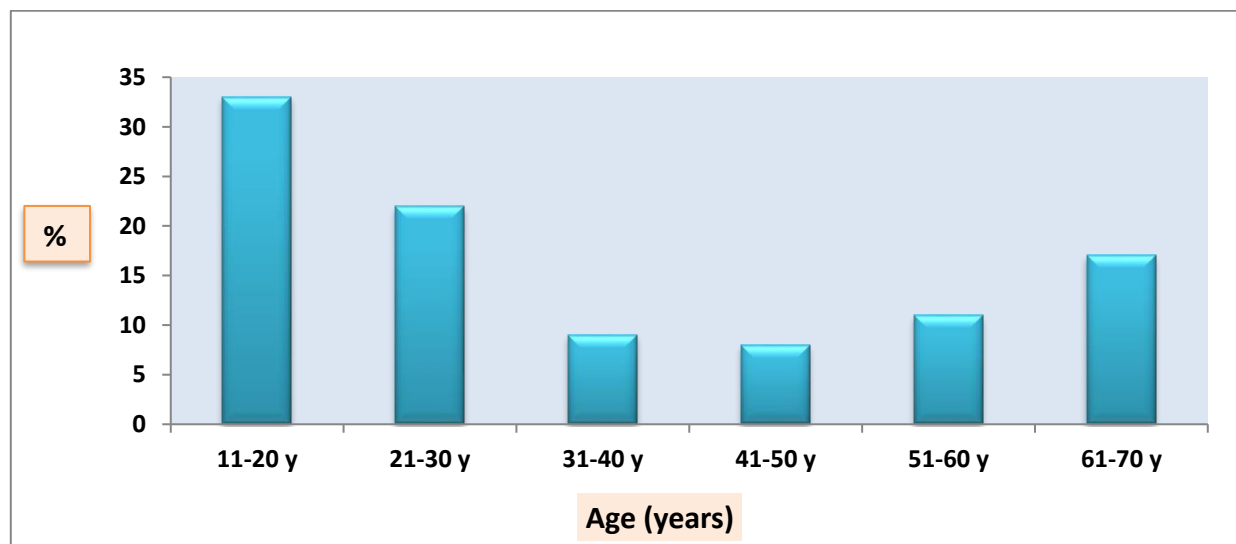
Gastric biopsy samples: The biopsies were taken for *H. pylori* culture. Each specimen was cultured on chocolate agar and pylori agar and incubated for up to 7 days in a microaerophilic atmosphere at 37°C. The isolates were diagnosed as *H. pylori* based on standard laboratory tests such as colony morphology and helical shape on gram stain and biochemical tests which showed positive results of oxidase, catalase, and urease tests [14, 15]

Antimicrobial susceptibility test: was performed in 55 isolates of *H.pylori*, according to the availability of antibiotic discs using the Kirby-Bauer method according to Clinical Laboratory Standard Institute[16].

Results

1-The effect of age on *H. pylori* infection

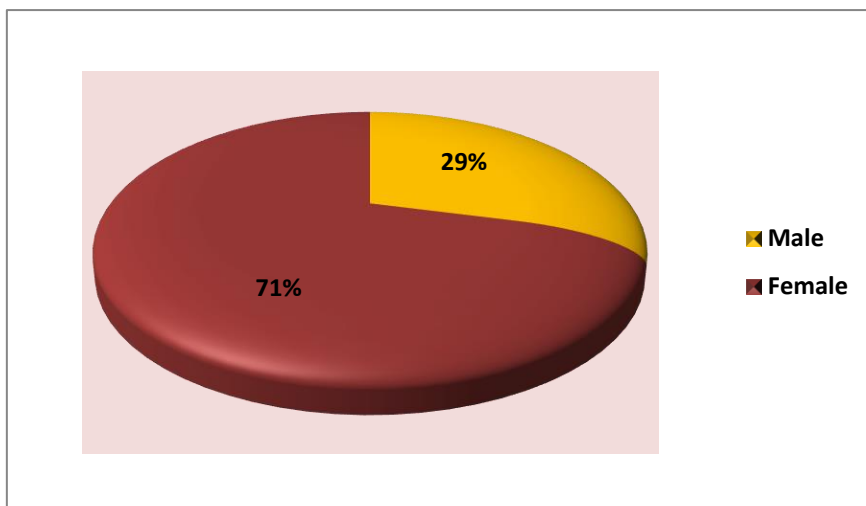
The results of our current study showed that the age group 11-20 years was the most age group for *H. pylori* infection with a percentage of 33%, followed by the age group from 30-21 years up to 22%, and the age group 40 -31 years is about 9%, and the age group is from 50-41 years is about 8%, and the age group is from 60-51 years is around 11%, and finally, the age group is from 70-61 years is about 17%. Figure (1).



Figure(1): Percentages of *Helicobacter pylori* infection by age groups

2-The effect of gender on *H. pylori* infection

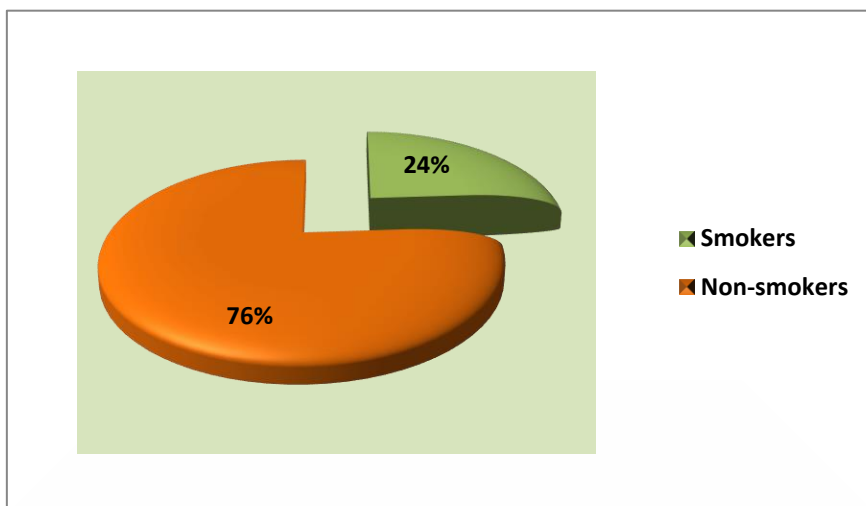
It was noted from the results of the current study that the rate of *H. pylori* infection in females is more than in males, as the percentage of infection reached 71% and 29%, respectively Figure (2).



Figure(2):Percentage of *H. pylori* infection according to gender

3-The effect of smoking on *H. pylori* infection:

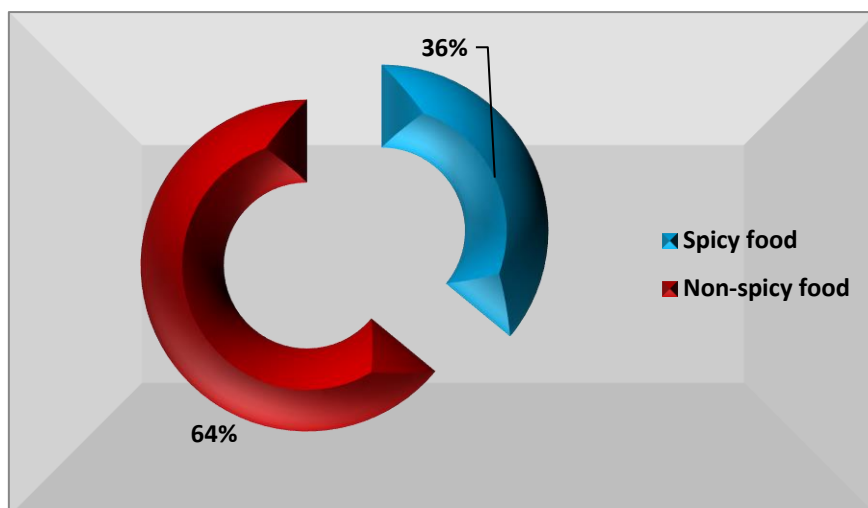
The results of the current study showed that the infection rate of *H. pylori* infection reached 24% for smokers, while it reached 76% for non-smokers. Figure (3).



Figure(3):Percentage of *H. pylori* infection according to smoking

4-The effect of food type on *H. pylori* infection

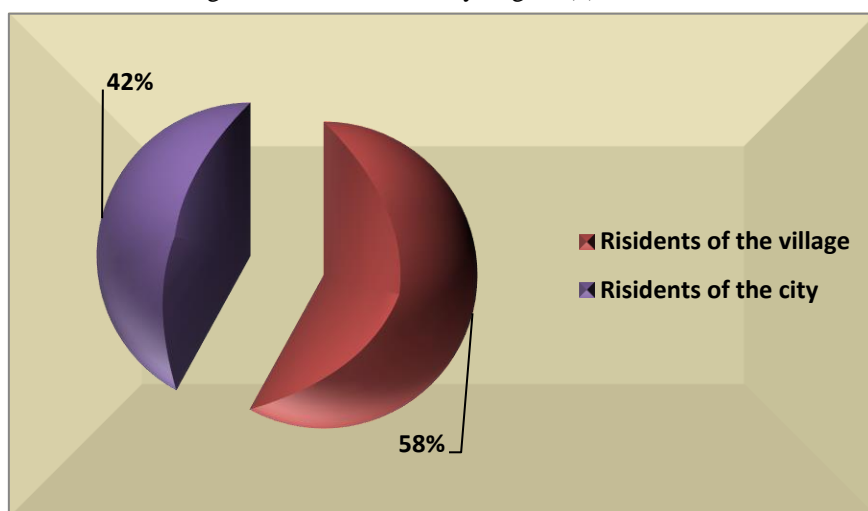
The results obtained in our current study showed that there is a correlation between the rate of *H. pylori* infection, type of food, and dietary habits, as the infection reached among individuals who eat spicy food (- Spicy) reached 36%, while it reached 64% in the people who do not eat spicy food (Nonspicy) Figure (4).



Figure(4): Percentages of *H. pylori* infection according to the type of food eaten

5-The effect of the place of residence on the infection with *H. pylori*

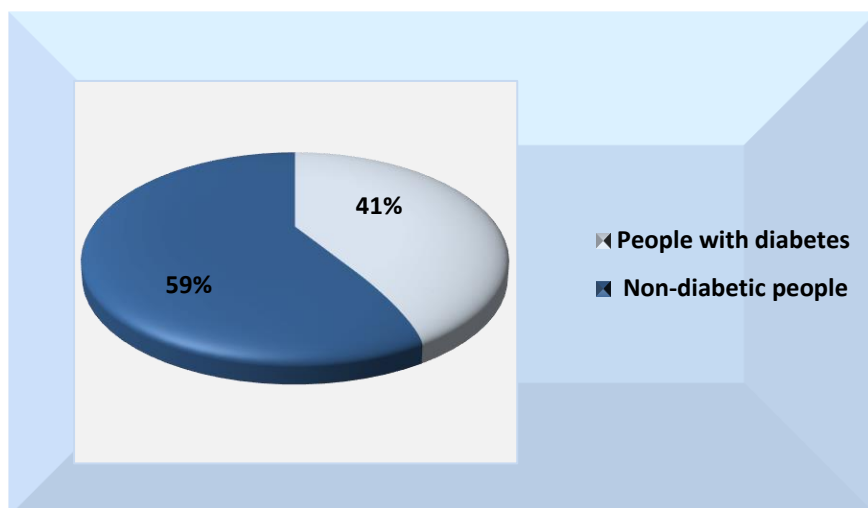
The results of our current study showed that the infection rate of *H. pylori* bacteria reached 58% of the residents of the village, while it reached 42% among the residents of the city, Figure (5).



Figure(5): Percentages of *H. pylori* infection according to the place of residence

6-The relationship between diabetes and *H. pylori* infection

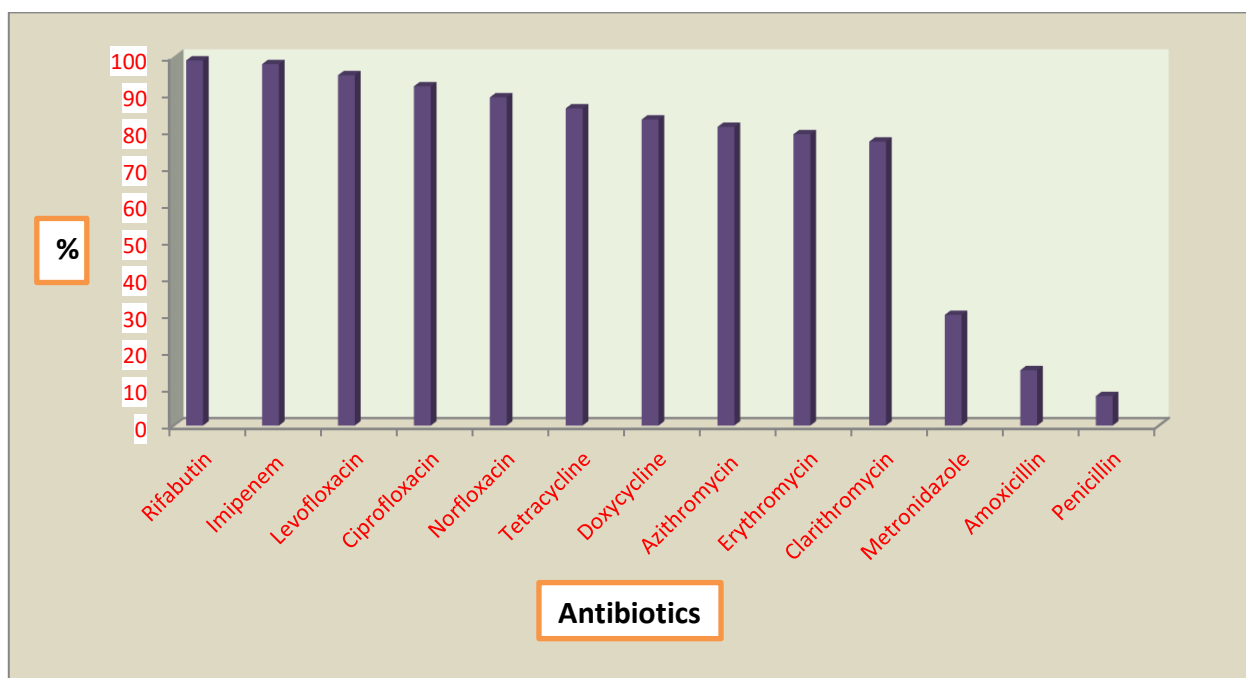
Through the results of our current study, a relationship was observed between *H. pylori* infection and people with diabetes, as it was noted that the percentage of diabetes among people infected with *H. pylori* bacteria reached 41%, while the percentage of infected people who do not suffer from diabetes reached 59% Figure(6).



Figure(6): Percentages of *H. pylori* infection according to diabetes mellitus

7- Sensitivity of *H. pylori* to antibiotics

The sensitivity test using the Kirby-Bauer disk diffusion method as in figure(7) showed that most of the *H. pylori* isolates are sensitive to the antibiotics under study and gave the highest sensitivity to Rifabutin(99%) followed by Imipenem (98%), Levofloxacin(95), Ciprofloxacin(92%), Norfloxacin(89%), Tetracycline(86%), Doxycycline(83%), Azithromycin(81%), Erythromycin(79%), Clarithromycin(77%) and lowest sensitivity to Metronidazole(30%) followed by Amoxicillin(15%) and Penicillin(8%).



Figure(7): Sensitivity of *H. pylori* to different antibiotics

DISCUSSION

It was found that some external factors have an effective effect on infection with *H. pylori*, and among these factors is the effect of age, as it was found that the age group (11-20 years) is the most vulnerable group to *H. pylori* infection with a rate of 33%, followed by the age group (21-30) at a rate up to 22%. While the lowest age group was (31-40 years) followed by the age group(41-50years) at a rate of 9% and 8% respectively. The results of our current study agreed with a study conducted by [17], which showed that patients at a young age are more susceptible to infection with *H. pylori*, and the results of our current study also agreed with a study [18], which showed the prevalence of infection in childhood and youth, that the infection rate decreases in the elderly, and this was decided two theories have been proposed to explain this result, either *H. pylori* is present in a low number and activity so low that it cannot be detected. Also, the bacteria may have been present in the past (childhood and youth) but were removed due to an unsuitable

intestinal environment at age (31). Other reports attributed the prevalence of infection in childhood and youth may be related to living and health conditions, and for large family members, and low educational level [19,20].

Gender also affects *H. pylori* infection, as it was found that females are more susceptible to infection than males, as the infection rate reached 71% in females and 29% in males. These results are consistent with the results of both [21,22] who found that the prevalence of infection with *H. pylori* in females is higher than the prevalence of infection in males, and the results of our study also agreed with the study of [23], which indicated that females are more susceptible to infection with *H.pylori*, and these differences have been explained to the difference in lifestyles such as smoking and alcohol consumption. It also explained the reason for the higher incidence in females, which could be related to hormonal differences between the sexes. Among other factors that also studied the effect of smoking on the infection of *H. pylori*, it was not found that 24% of the patients were smokers, and this low percentage is because males in the current study are less than females. For several years, it has been observed that smoking causes an increased risk of developing stomach cancer, and as a result, smokers tend to have a high incidence of *H. pylori* infection that causes gastric ulcers such as gastroduodenitis, gastric reflux, and gastric metamorphosis [24]. The relationship between smoking and infection with *H. pylori* may result from a variety of mechanisms that include increased secretion of acid and pepsin, a change in gastric motility, building up of prostaglandins, blood flow in the gastric mucosa and peritoneum [25].

It was also found that the factor of the type of food had a helpful effect on *H. pylori* infection, with a rate of 36% and this is consistent with a study conducted by [26] who found that infection with *H. pylori* also could be associated with the type of food and dietary habits. [27] also investigated several nutritional factors and found that eating salted fish and fried food was positively associated with infection with *H. pylori*, and this result may be related to the way the food is prepared and the amount of salt and spices used that may cause damage to the mucous membrane and break down the barriers of the mucous membrane. Gastrointestinal, these changes in the gastric mucosal layer may increase the chance of bacteria persisting in infection. Also, among the other factors affecting infection with *H. pylori* is the place of residence, and it was found that village residents are more susceptible to infection than urban residents, as the percentage of infection reached 58% and 42%, respectively. The results of our study agreed with the study of [28], which found a higher prevalence of infection with *H. pylori* in the village population compared with the urban population, and this is consistent with the report of [29], which attributed this difference in the prevalence of infection between villages and cities to factors related to the lack of supply of safe water tanks and hygiene conditions in the villages. It was also found [30] that the low educational level in the villages also affects the high incidence of *H. pylori* infection in villages, as found [31] There is a great diversity in the prevalence of *H. pylori* infection between countries and within the population groups of the same countries, and this diversity may be due to the method used in diagnosis, sample size, and social and economic factors, the reason for these differences.

Finally, among the factors that were studied was the relationship between diabetes and infection with *H. pylori*, as it was found that 41% of people infected with *H.pylori* also had diabetes. The results of our study agreed with the results of the study [32] which found several explanations that depend on the increase in infection with *H.pylori*. in diabetics these clarifications are: First: Diabetes causes a weakness in cellular immunity and humeral, which increases a person's susceptibility to infection with *H.pylori*. Second: Diabetes causes reduced intestinal motility and acid secretion, and this may encourage colonization of the pathogen and increase its rate of infection in the stomach. Third: the change in sugar metabolism may cause chemical changes in the gastric mucosa that encourage colonization of the *H. pylori*. There is also evidence indicating that infection with *H. pylori* may contribute to the development of diabetes by affecting the intestinal hormones regulating insulin [33]. Hence, we find that *H. pylori* are a common infection in diabetic patients [34].

The antibiotic sensitivity test showed that most of the *h. pylori* isolates were sensitive to the antibiotics under study and gave the highest sensitivity percentage to Rifabutin(99%) and the lowest sensitivity percentage to penicillin(8%) and the sensitivity to antibiotics in this study was not 100%, and this indicates the existence of resistant isolates, and the evidence for this is the growth of some isolates within the standard diameter of the inhibition zone. Or it is acquired resistance, and this was confirmed by one study, that the resistance of bacteria to antibiotics is closely related to their previous use in the treatment of other pathological conditions (35). It should be noted that some studies have confirmed the importance of conducting an antibiotic susceptibility test to choose the best antibiotic for the treatment of infection (36). However, other studies have reduced the antibiotic susceptibility test by giving logical and convincing explanations when reading it. These explanations are that *H. pylori* differ in its sensitivity to antibiotics in in-vitro studies from its sensitivity in the area of infection, and this is due to the presence of the bacteria in the deep gastric pits protected by the mucous layer and also its distance from the blood vessels, which prevents the drug from reaching it (37). Also, one of the most convincing explanations is that some antibiotics, despite their effect on bacteria in studies outside the body, do not affect them in the infection area because some of them do not work at low pH (pH=2) as in the stomach, which is the infection area.

CONCLUSIONS

H. pylori infection is considered a serious health problem in the world. Stomach ulcers and cancer are serious side effects of this bacteria. Therefore, early detection and knowledge of the factors affecting the increase in infection with this bacterium, as well as regular antibiotic sensitivity test, helps to limit its spread and improve control of the outbreak of the disease

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