Isolation and Identification of *H. pylori* among Iraq patients with chronic gastric inflammation

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Abstract:

Background: H. pylori is one of the gastrointestinal organisms in which more than half the world's population was infected. The aim of this study was to Isolation, identity, and determine antibiotic susceptibility of H. pylori from samples of the patient (biopsies) using various procedures.

Patients and methods: A total of 90(58 males, 32females) patients with different age groups from both genders are involved in this study suffering from dyspeptic symptoms They underwent diagnostic 'upper' gastrointestinal (G.I.) endoscopy at "Endoscopy unit of AL- Sadder Teaching Hospital in Baghdad "during the period November 2021 to March 2022.

Results: The results showed a relationship between H. pylori infection occurrence and endoscopic ally diagnosed dyspeptic patients; it was recorded that H. pylori was isolated in 30 cases. Antibiotic resistance test for H. pylori isolates showing susceptibility to levofloxacin, clarithromycin, ciprofloxacin, and amoxicillin. Whereas all the tested isolates were appearing resistant to metronidazole, and tetracycline.

Conclusion: The results showed a relationship between H. pylori infection occurrence and endoscopic ally diagnosed dyspeptic patients. Culture and antibiotic sensitivity assay for detection of H. pylori are more reliable in biopsies than urease tests. H. pylori isolates were sensitive to levofloxacin, clarithromycin, ciprofloxacin, and amoxicillin.

Keywords: Helicobacter pylori, chronic gastric, biopsy urease test, Antibiotic resistance, Iraq patients

Introduction:

Infection with Helicobacter pylori (H. pylori) is one of the most frequent infections in humans (1). This spiral Gram-negative microaerophilic bacterium enters the stomach and penetrates the mucus gastric layer (2) but does not cross the epithelial barrier making it non-invasive bacteria. Although the majority of H. pylori organisms live free in the mucus layer, some attach to the apical surface of gastric epithelial cells (3) and a small number have been shown to invade epithelial cells (4).

Antibiotic resistance discerns in bacteria recently emerged as a major issue in the treatment of human infectious diseases. This problem also affects the treatment of Helicobacter pylori (H. pylori) infection, which is still the leading cause of duodenal and peptic ulcers, as well as a danger factor for stomach cancer. Extra-gastric illnesses have been linked to this infection in recent years (5,6).

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bacterium in the past. The European Helicobacter Study Group's recommendations, which to the Antibiotics such as –lactams, macrolides, and tetracycline have been used to eradicate this Polish Society of Gastroenterologist guidelines, recommend treating patients with two or three antibiotics (amoxicillin, clarithromycin, or metronidazole). When H. pylori resistance to metronidazole resistance is less than 40% clarithromycin is less than amoxicillin with metronidazole clarithromycin or clarithromycin can be used (7,8). Detection of H. pylori infection can be made with both invasive and non-invasive tests. Invasive tests include histology, culture, and rapid urease test, which require endoscopy to obtain biopsies of the gastric mucosa which is expensive and inconvenient. Noninvasive tests, which are based on analysis of samples of breath, blood, or stools' have been developed. (9). Detecting bacterial antigens in stool offer, an alternative noninvasive diagnostic test. Its performance in patients has been tested in some developed countries, showing sensitivity and specificity above 90% (10). The aim of this research: is that Isolation and distinguish H. pylori from samples of the patient (biopsies) using various procedures' biochemical tests culture and antibiotic susceptibility of H. pylori.

Material and Methods:

A total of 90 patients (58 males, and 32females) of different age groups from both genders were represented suffering from dyspeptic symptoms.

They underwent diagnostic upper' gastrointestinal'' (G.I.) endoscopy at the Endoscopy Unit of AL-Sadder Teaching Hospital in Baghdad during the period November 2021 to March 2022. Several gastric biopsy samples were taken from the stomach cavities. They are subjected to various mucosal, which have been also bacteriological examinations for patients.

Diagnostic Tests (Invasive tests): Biopsy Urease Test: The test is not final to say as negative until 24hrs, depending on found of urease enzyme in the gastric biopsy. (RUT) with high sensitivity and specificity. Each patient's first biopsy tissue was placed in a tube containing 1 ml prepared of freshly 10 % urea put in distilled water (10gm of urea in 100ml distilled water and pH modified pH to 6.4), to which 0.002gm of phenol red had been added as a pH indicator. A positive (RUT) result has deep pink within minutes, and a negative result appears yellow color (11, 12).

Direct Biopsy Smear: biopsy samples were 'crushed and smears are prepared and stained by routine gram stain protocols (13).

Biopsy Culturing: biopsy is inoculated in selective (modified Colombia base agar) agar media plates that are used for primary isolation of H. pylori. This medium consisted of 44 g Columbia base agar in 1 liter of D.W, which was then dissolved by boiling and autoclaved at 121°C for 15 minutes 15 pounds/inch2. After autoclaving, the medium was chilled to 55°C and supplemented with 5 mg vancomycin, 10 mg amphotericin, 5 mg trimethoprim, and 2500 units polymyxin B, as well as 10 mg hemin and 20 g urea., All of these were sterilized using a Millipore 0.22 mm filter paper, followed mixed thoroughly, and placed into a petri dish, allowing the medium to cool before storage in the refrigerator The cultures are incubated at 37□C under' (5%O2, 10%CO, 85% N2) micro-aerophilic conditions in an anaerobic jar with a gas generating kit. Plates are tested for positive growth for 'intervals of (3-7) days before rejecting as negative. For a positive colony, must be translucent or gray, tiny' glistering, and covered with entire edges (14, 15).

Biochemical tests
urea ager
Catalase Test

Oxidase Test.
Antibiotic sensitivity test
The method (16) was used to test the sensitivity of 10 isolates of H. pylori from biopsy samples to six types of antibiotics.
The test was performed on a pure H. pylori culture plate. A colony was placed in the sterile saline solution. Blend by vortexing to include that no solid matter of colony is the apparent solution. This process frequently even the turbidity of the saline solution appears to match that of the standard turbidity (O.D: 0.5-0.65) into dense chek plus. A sterile cotton swab was descended into the organism's broth culture, and the swab was cultured into each Petri dish containing Muller Hinton Agar (MHA). The inoculums were spread on the medium in different directions using a sterile cotton swab. Next streaking the plate was left to dry for five min. put Antibiotic discs' on the surface of the agar by using sterilized' forceps. It was incubated for 72 hours at 37° C. After incubation, used a metric ruler to calculate the diameter of the inhibition zone for every antibiotic used. The measurement got from the antibiotics was compared with the standard table to assign the resistance zone according to CLSI

Statistics analysis:
Chi-Square test along with the statistical package (SPSS software version)was used to examine the data (17)

Results:
In the present study a total of 90 samples, 30 out of 90patients were positive for RUT shown in table (1), figure 1.

Table 1: Distribution of biopsy sample according to Urea's rapid test

Group	No	Percentage (%)
Positive	30	33.3
Negative	60	67.7
Total	90	100%
NS: Non-Significant. P > 0.05		

This table shows there is no significant between positive and negative urea rapid tests the p-value >=0.05
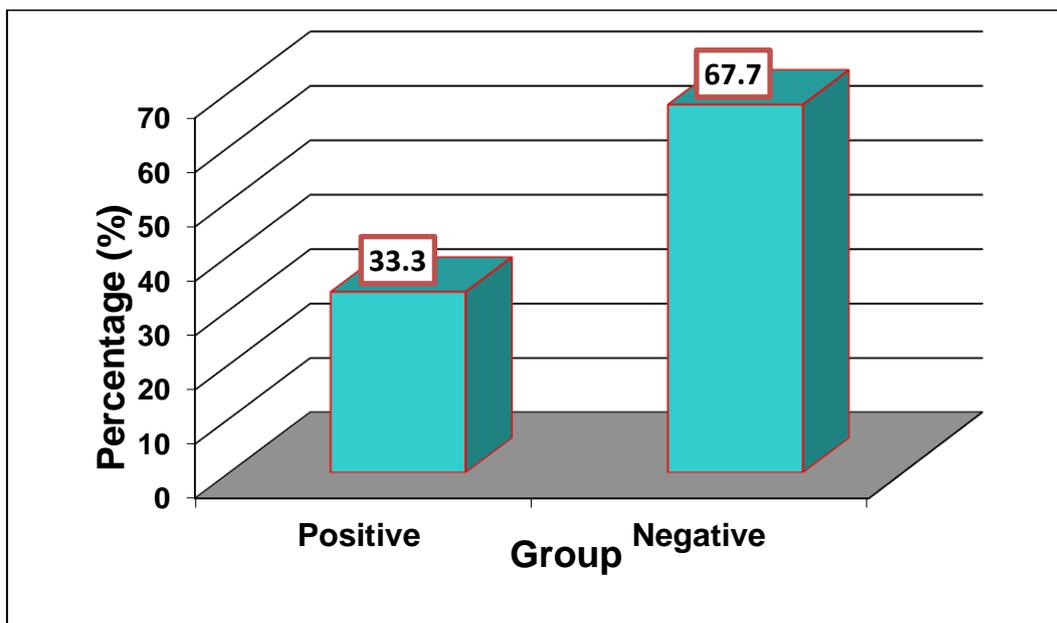
Fig 1: Distribution of sample study according to rapid Ureas test

Table 2: Distribution of biopsy samples according to age group.

Age group	Positive %	Negative	Total
>40	6(28.6)	15(71.4)	21(23.3)
40-50	15(39.6)	31(67.4)	46(51.1)
>50	9(39.1)	14(60.9)	23(25.6)
Total	30(33.3)	60(66.7)	90(100)

P value >0.05

As show in table (2) the age group that were subject to this study ranged from (>40->50) years, the difference no significant P > 0.05 relationship between age and *H. pylori* infection.

Table 3: Distribution of biopsy samples according to gender

Group	Positive %	Negative (%)
Male	12(40)	46(76.6)
Female	18(60)	14(23.3)
Total	30(100)	60(100)

Significant different p <= 0.001

A total number of H. pylori infection cases subject to the examination of the endoscopy and their distribution among males and females, as shown in table (3) 12(40%) males showed positive for H. pylori, while 18(60%) female showed positive for H. pylori. shown in table (3)
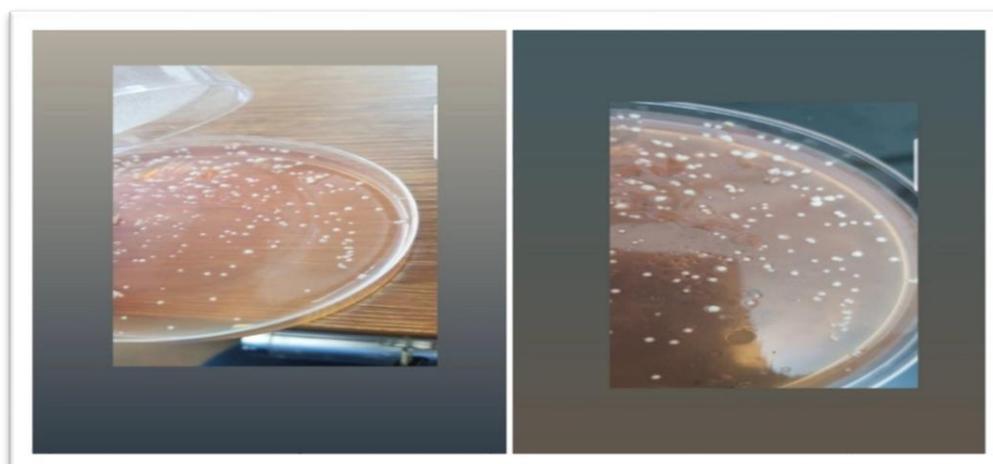

Fig 2: Appear colony of *H. pylori* in modified Columbia base agar (MCBA

The H. pylori isolates showed that antibiotic sensitivity test results 'were sensitive to levofloxacin, clarithromycin, amoxicillin, and ciprofloxacin. Whereas all isolates were appearing resistant to metronidazole, and tetracycline. As illustrated in the figure (3), figure (4), and table (4)
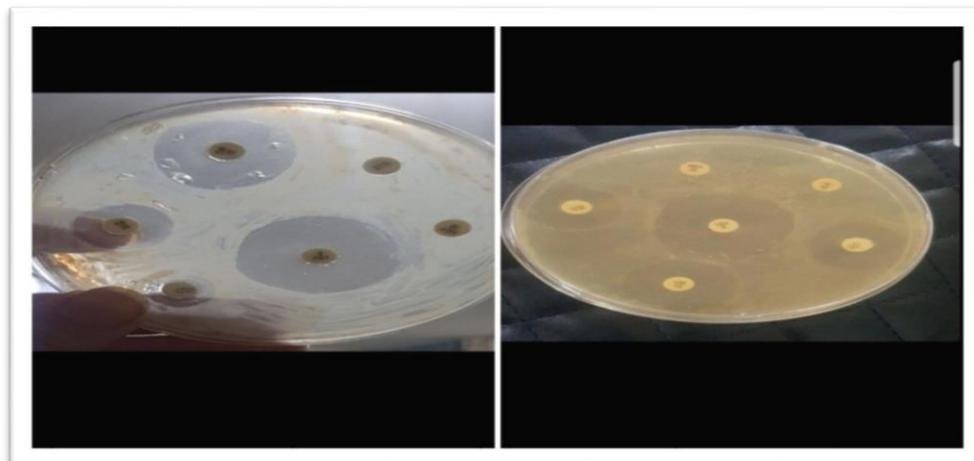

Fig 3: Antibiotic sensitivity test show resistance, the sensitivity of H. pylori to antibiotic.

Table (4): the prevalence of resistance to antimicrobial agents against *H. pylori* isolates

| No. Of Patient	Levofloxacin	Amoxicillin	Metronidazole	Tetracycline	Clarithromycin	Ciprofloxacin
1	Sensitive	Resistant	Resistant	Resistant	Sensitive	Sensitive
2	Sensitive	Intermediate	Resistant	intermediate	Sensitive	intermediate
3	Sensitive	Intermediate	Resistant	intermediate	intermediate	Sensitive
4	Sensitive	Intermediate	Resistant	Resistant	Resistant	Sensitive
5	intermediate	Resistant	Resistant	Resistant	Resistant	Sensitive
6	Sensitive	Sensitive	Resistant	Resistant	intermediate	intermediate
7	Sensitive	Resistant	Resistant	Resistant	Sensitive	Resistant
8	intermediate	Intermediate	Resistant	Resistant	Sensitive	Sensitive
9	Sensitive	Sensitive	Resistant	Resistant	Resistant	Sensitive
10	Sensitive	Resistant	Resistant	Resistant	intermediate	Sensitive
R	0 (0%)	4 (40%)	10 (100%)	8 (80%)	3 (30%)	1 (10%)
S	8 (80%)	2 (20%)	0 (0%)	0 (0%)	4 (40%)	7 (70%)
I	2 (20%)	4 (40%)	0 (0%)	2 (20%)	3 (30%)	2 (20%)
P-	0.0001 **	0.0094 **	0.0001 **	0.0001 **	0.097 NS	0.0001 **

** ($P \leq 0.01$).
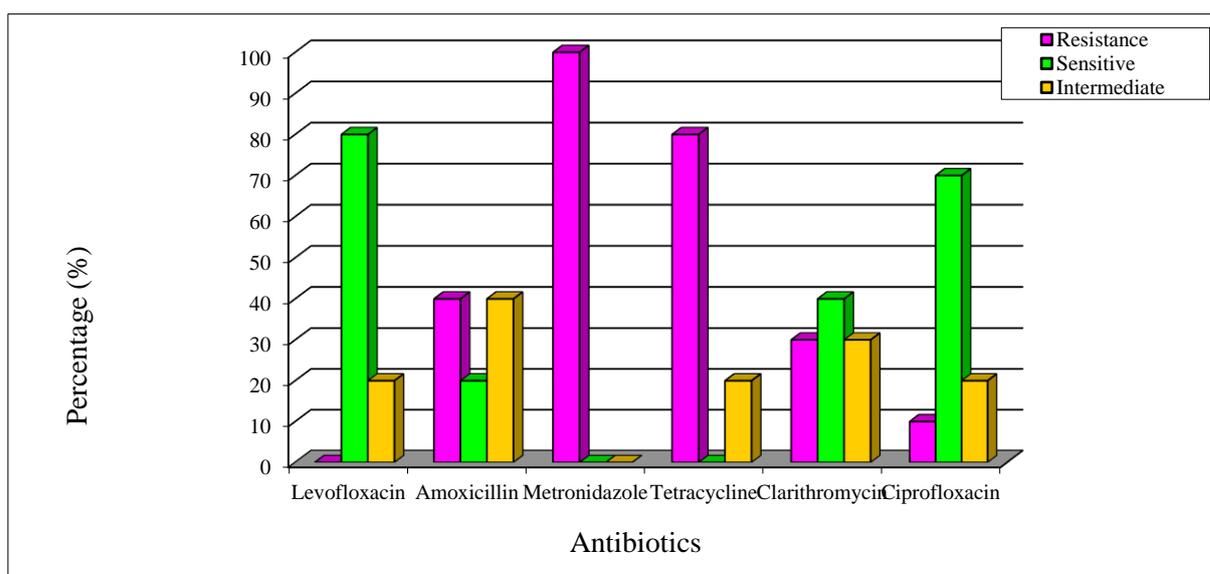
Figure 4: Results of Antibiotics in patients

Discussion:

When a stomach biopsy was taken in the hospital, it was used to diagnose H. pylori infection. Table 1 shows that 30 of the 90 patients tested positive for RUT' giving a positive rate of (33.3%). The reason for a positive RUT for H. pylori is that it secretes urease which converts urea carbon and ammonia to the alkaline indicator phenol red, resulting in a pink colour (18). This result is comparable to that of (19)' who obtained 48 out of 120 patients, resulting in a proportion of positives of 48. Rapid urease tests are simple, cheap, and easy so widely used. The positive bacteria for urease present in the mucosa (staphylococci, streptococci) produce a less amount of urease, which does not react in a short time detection rupture, the method-specific H. pylori (20). In our study, as shown in table 2 patients between 40 to 50 are the most ages group that show higher infection with H. pylori, and this result compared with other studies and agrees with (21) shown results that the spread of H. pylori 'antibodies among human in the age between 41-50 years were (51.2%), then by the human with age more than sixty-one (>61) years (46.5%), then from 31- 40 years (44.4%) after that from 51-60 years 35.6%, and finally, the age between 21 – 30 years which found just 12/44 infected (27.3%). No significant differences in the H. pylori antibodies test between age groups according to positive and negative results (p>0.05). In the present study and shown in table 3 female is more than males in the positive result while the male was 66% in the negative' result when compared with females 24% in a negative result which means more, this result agrees with (22) that showed the highest rate of frequency of H pylori antibodies was found in male 65/170(38.2%) while the all most rate of occurrence was found in female 57/140(40.7%), Statistically' the differences in the H. pylori antibodies test between male and female according to positive and negative examined were not significant (p>0.05). also result was compatible with (23) in Turkey, appear that the male was less exposed to infection with H. pylori, H. pylori stool antigen test' by using monoclonal and the rate of infection in female was 23.8%, compared with the rate of infection in female 76.2 %. Also study by (24) appeared no significant difference between males and females of the asymptomatic group the mean concentration was higher in females rather than in males. Whereas, in symptomatic patients, the mean concentration was significantly (p≤0.002) less in males than in females (25). In fact, the difficult process of isolating H. pylori from biopsy specimens was a due to several factors and the fastidious nature of the bacterium that is difficult to control' causing difficulty with the culturing of the organism, such as patch distribution of the organism on the gastric mucosa, presence of oropharyngeal flora due to contamination of biopsy forceps, loss of oesophageal flora, and loss 'All these

may be responsible for a negative result value associated with a culture of H. pylori. As shown in the result primary selection media for H. pylori isolates on appear to be tiny, convex round translucent, and smooth colonies. This was similar to (26). H. pylori appeared as a Gram-negative spiral form or curved rods on Gram-stained smears. In ancient cultures, H. pylori lost its spiral structure and became increasingly coccid. It's possible that this is related to a shortage of critical nutrients. According to prior research, these forms are non-cultivable and nonviable, although others claim that some of them appear non-cultivable, forming a bacterium resistant to antibiotics (27). The positive urease enzyme and oxidase catalase tests declare the found of H. pylori on the culture plates. This is similar to the result produced by (28). The findings of this study contradict those of (29). The susceptibility of 10 isolates of H. pylori using six antibiotics. Appear high percentage of antibiotic resistance with the ratio of resistance to amoxicillin 70%, metronidazole 100%, ciprofloxacin 50%, clarithromycin 60%, and tetracycline 80%, while the most effective antibiotic for H. pylori infection is levofloxacin, which has a high (30). In terms of Metronidazole resistance (100%) of clinical isolates were resistant to that antibiotic; similarly, (19). Amoxicillin resistance was very high (70%), which is similar to the previous study the overuse of these two antibiotics in H. pylori infection may interpret these results, also ciprofloxacin and clarithromycin which were also similar to those of (20). The majority of the isolates; on the other hand, had low amoxicillin and tetracycline levels (31). Although levofloxacin remains one of the most widely used second-line antibiotics, bismuth is becoming a more popular option when it is available (32).

Conclusions:

The results showed a relationship between H. pylori infection occurrence and endoscopic ally diagnosed dyspeptic patients. Culture and antibiotic sensitivity assay for detection of H. pylori is more reliable in biopsies than urease tests. H. pylori isolates were sensitive to levofloxacin, clarithromycin, ciprofloxacin, and amoxicillin, whereas all the tested isolates were appearing resistant to metronidazole, tetracycline.

Author contributions:

Both Fatima Omer Saber and Maryam Kareem Ali collaborated equally during the experimental works and discuss the results. All authors have accepted and read the published version of the manuscript.

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عزل وتشخيص لبكتريا المعدة الملوية البوابية بين مرضى العراق المصابين بالتهاب المعدة المزمن

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الخلاصة:

خلفية البحث: تعتبربكتريا المعدة الملوية البوابية أحد الكائنات الحية المعدية المعوية التي أصيب فيها أكثر من نصف سكان العالم ، وكان الهدف من هذه الدراسة هو عزل وتحديد وتحديد حساسية المضادات الحيوية لبكتيريا الملوية البوابية من عينات المريض (الخزعات) باستخدام إجراءات مختلفة.

المواد وطرق العمل: 90 مريضاً (58 رجلاً و 32 أنثى) من مختلف الفئات العمرية من كلا الجنسين يعانون من أعراض عسر الهضم ، وخضعوا للتنظير "العلوي" للجهاز الهضمي في "وحدة التنظير في مستشفى الصدر التعليمي في بغداد. "خلال الفترة من نوفمبر 2021 إلى مارس 2022.

النتائج: أظهرت النتائج وجود علاقة بين حدوث عدوى الحلزونية البوابية ومرضى عسر الهضم المشخصين بالتنظير. وقد تم تسجيل أن الحلزونية البوابية كانت عزل 30 حالة. أظهر اختبار مقاومة المضادات الحيوية لعزلات الحلزونية البوابية القابلية للليفوفلوكساسين والكلاريثروميسين والسيبروفلوكساسين والأموكسيسيلين ، بينما أظهرت جميع العزلات المختبرة مقاومة للميترونيدازول والتتراسيكلين.

الاستنتاجات: اختبار الزرع وفحص الحساسية للمضادات الحيوية للكشف عن بكتريا الحلزونية البوابية أكثر دقة في عينة الخزعة من اختبار اليورياز. كانت جميع العزلات التي تم اختبارها حساسة للليفوفلوكساسين والكلاريثروميسين والسيبروفلوكساسين والأموكسيسيلين بينما وجدت جميع العزلات المختبرة مقاومة لميترونيدازول والتتراسيكلين

مفتاح الكلمات: بكتريا المعدة الحلزونية البوابية "المعدة المزمنة" اختبار اليورياز ، مقاومة المضادات الحيوية. المرضى العراقيين