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Research Article

Evaluating Different Methods of Helicobacter pylori Detection and Antibiotics Resistance

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I N F O

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A B S T R A C T

Background: Around half of the world's population is infected with Helicobacter pylori (H. pylori) and it is the most common chronic infection in human. H. pylori has been linked to certain diseases in the gastrointestinal system as well as extra- gastric disorders.

Aim of this study: to evaluate different methods used for the diagnosis of H. pylori and determine the H.pylori resistance to commonly used antibiotics.

Methods: This is a prospective study including 125 patients (60 men and 65 women), their mean age is 41.8±19.4 years. All patients underwent upper gastrointestinal endoscopy during the period from November 2023 to January 2024 for dyspeptic symptoms, a biopsy was taken from the stomach of all patients, and a, rapid urease test and H. pylori culture were culture done on all biopsies, After the growth of H. pylori we did a Gram stain and PCR test.

Results: 76 patients (60.8%) out of 125 a positive H. pylori rapid urease test. Just 40 specimens (32%) had positive of H. pylori on culture. Four antibiotics are selected to test for sensitivity and showed high resistance to metronidazole and high sensitivity to clarithromycin, amoxicillin and tetracycline as 100%, 90% and 80% respectively.

Conclusion: The rapid urease test is a good diagnostic test for H.pylori as it is quick, sensitive and inexpensive, although culture is the gold standard test, but the result of the H.pylori culture should be interpreted with the results of other tests as culture had low sensitivity. Metronidazole had a very high resistance rate to H.pylori that is why it should not be included in the eradication regimen while H.pylori had a high sensitivity to clarithromycin and amoxicillin.

Keyword: Helicobacter Pylori, Rapid Urease Test, Culture

Introduction

Helicobacter pylori (H.pylori) is a spherical or spiral-shaped Gram-negative bacterium. Approximately 50% of the world's population may be infected by this bacteria,[1] 1–10% of infected people develop clinical complications from the infection. H.pylori is usually contracted during childhood or youth and the majority of infected persons remain asymptomatic, it has multiple ways of transmission including spread from one person to another by oral-oral or oral-fecal routes, or because of contaminated water and food. It can causes chronic gastritis that can progresses over the course of a lifetime to intestinal metaplasia, peptic ulcer disease including both gastric and duodenal ulcers, gastric atrophy, and eventually gastric cancer or mucosa-associated lymphoma (MALT lymphoma).[2] H.pylori growth requires special air conditions because it is a microaerophilic bacterium (5% oxygen, 87% nitrogen, 10% carbon dioxide).[3] The virulence that is responsible for the pathogenicity of these bacteria is related to different factors like the gene associated with cell toxicity (cagA), which is the most dangerous factor that is responsible for the development of stomach cancer, VagA (vacuolating cytotoxin gene), Ure A, and other factors that help the bacteria adhere to the stomach tissue.[4]

The diagnosing H. pylori infection is very important, several methods are available and can be used for the diagnosis of this bacteria .These methods are divided into invasive methods and non-invasive methods.[5] Invasive methods, that depend on gastric tissue acquired by endoscopy, include bacterial culture test, the polymerase chain reaction (PCR) test, histopathology and Rapid urease test. The precision of sample collection determines the sensitivity and specificity of these tests. The non-invasive techniques (do not require the use of an endoscope) include urea breath test, stool antigen test, and serology test for H. pylori antibody. The duration of infection and whether the patient is receiving treatment affect the sensitivity and specificity of these tests.[6] Because there is currently no effective vaccine, treatments for chronic H. pylori infections have been developed to stop the its spread throughout the population, induce healing of gastrointestinal lesions and decrease the incidence of stomach cancer.[7] However, treatment of H. pylori infection has proven to be difficult and no single treatment has been able to reach sufficient cure from H. pylori infection.[8] The World Health Organization (WHO Clinical practice has demonstrated that combination of different antibiotics such as metronidazole, clarithromycin, amoxicillin, tetracycline, levofloxacin, and rifabutin are effective in eradicating H. pylori, typically when administered as combination of two or three of these antibiotics. Bismuth and/or acid inhibitor provide extra antibiotic action and shield the mucous membrane from harmful substances. The rapid development of primary antibiotic resistance has been observed as a result of the general population's extensive use of certain antibiotics and the limitation of effective treatments, as well as the adaptability of bacterial species.[9,10] There are three distinct drug resistance patterns that have been thought of in H. pylori. With regard to their molecular mechanisms and clinical implications, single drug resistance (SDR), multidrug resistance (MDR), and heterogeneous resistance (HR) are likely to overlap. It seems that structural alterations in the genetic sequence that impair the cellular activity of antibiotics—either by altering the drug target, preventing intracellular drug activation, or through mutations rather than gene gain or loss—are the primary cause of resistance in H. pylori.

The current study's objectives were to evaluate methods used to diagnose H.pylori, including rapid urease test and culture of gastric biopsy, as well as to know antibiotic resistance of H.pylori organisms.

Patients and methods

This is a prospective study conducted over aperiod of 3 months (November 2023-January 2024), One hundred twenty-five patients (63 were males (50.4%) and 62 were females (49.6%) were included in this study, all patients were suffering from some dyspeptic symptoms, their ages ranged between (15 and 70 years years, all of them were tested for pylori by rapid urease test (RUT), 76 patients (60.8%) out of 125 patients were positive for H.pylori RUT, 54 patients were males (51.4%) and 51 patients were females (48.6%).

Two biopsies taken from stomach, one the gastric antrum and the other from gastric corpus, we use pyloplus H.pylori test for RUT. RUT is considered the least costly and time-consuming method when compared to other techniques, and has high sensitivity and specificity, this test depends on whether biopsy specimens contain urease which is produced by the H. pylori bacteria. Bacterial urease hydrolyses urea to produce ammonium ions, which raise pH. The phenol red indicator, which turns pink at pH 8.4 and yellow/brown at pH 6.8, detects this pH shift, RUT were allowed to be regularly inspected at room temperature for 1 hour, shift in the cassette's color from yellow to red was regarded as favorable.[11] Other gastric biopsies were taken from 105 patients who had positive RUT and we kept the samples moist, they were submerged in two milliliters of saline solution. The samples were kept in a refrigerated box and then cultured using Heart and brain infusion medium with the antibiotics: vancomycin 10 micrograms, trimethoprim 5 micrograms, polymyxin 2.5 international units, with the addition of 7% human blood and left to cool, then poured into sterile dishes and kept in the refrigerator. When the samples arrive, they are processed within two

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hours, and the samples are planted after they have been homogenised with a blender. The samples are grown by taking a quantity of the tissue extract and using a planting method. The samples are planted and then incubated in an anaerobic container with the gas kit prepared by Oxoid Company to provide the appropriate conditions for growth, including 5% O_2, 10% CO_2, and 85% N_2. The incubation was done at 37°C for (3-7) days. H.pylori were diagnosed by microscopic examination of Gram-stained smears as being Gram-negative, bacillary or spiral-shaped, and the colonies were small, transparent, and positive for urease, oxidase, and catalase tests, and a test for the sensitivity of the isolates to antibiotics.[12] The molecular diagnosis of H.pylori was done by using DNA extraction kit (Geneaid, Singapore) to extract DNA from each sample. Initial denaturation (95°C for 3 min), denaturation (95°C for 15 s), annealing (50°C for 30 s, extension at 72°C for 30 s), and final fixation (10°C for 1 min) were the primers specific for the 16S rRNA virulence factors of H. pylori PCR and its PCR cycle.).[13] Mueller-Hinton was used to cultivate H. pylori isolates. 10% lysed human blood was added to the agar, and a sterile cotton swab was used to aseptically spread the colony across the plate's surface. Using sterile forceps, antibiotic discs were positioned on the agar surface and incubated for 24 to 48 hours at 37°C in a microaerophilic environment. Millimeters were used to measure the inhibition zones' diameter.

Ethical Approval: we got ethical approval from the ethical committee of college of medicine, university of Mosul, and informed consent was obtained from all precipitants

Results

The study included 125 patients, 63 were males (50.4%) and 62 were females (49.6%), aged between 15-85 years (Mean age 41.8 ±19.38) , 73 patients (58.4 %) were young less than 45 years old and 76 patients (60.8%) had positive RUT as shown in Table (1).

Out of 125 patients included in the study, 76 dyspeptic patients had H.pylori infection (60.8%) and all patients who had H.pylori on culture had positive RUT as shown in Table (2).

Table 1.Age and sex distributions of the study patients

Gender	Frequency of age			Total
	15 – 30	31 – 45	≥45	
Male	23(18.4%)	14(11.2%)	26(20.8%)	63(50.4%)
Female	24(19.2%)	12(9.6%)	26(20.8%)	62(49.6%)
Total	47(37.6%)	26(20.8%)	52(41.6%)	125(100%)

Table 2.Percentages of test results used in diagnosing H.pylori bacteria

	Culture (%)	Rapid Urease Test (%)
Negative	85 68%	49 39.2%
Positive	40 32%	76 60.8%
Total	125	125

As for the bacterial culture test, the results showed a positive culture growth rate in 40 patients (38.09%) out of 105 patients with positive RUT, gastric biopsy was incubated for a period of 5-14 days in conditions with little ventilation, availability of humidity, and 37°C as the temperature, as shown in Figure (1), After the growth of H. pylori was observed, a gram stain was done.

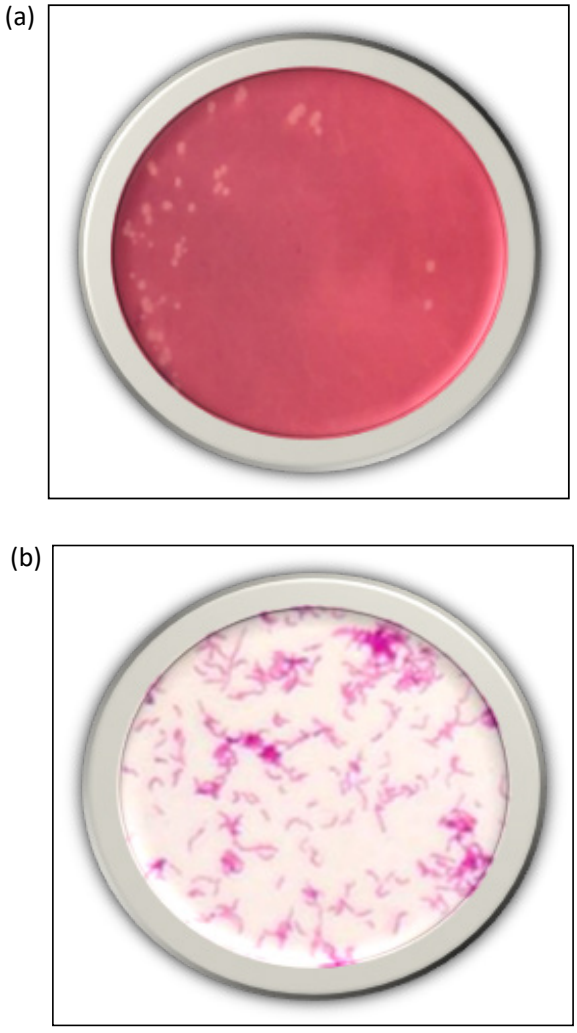

Figure 1.Identification of H. pylori. A: culture, B: Gram staining

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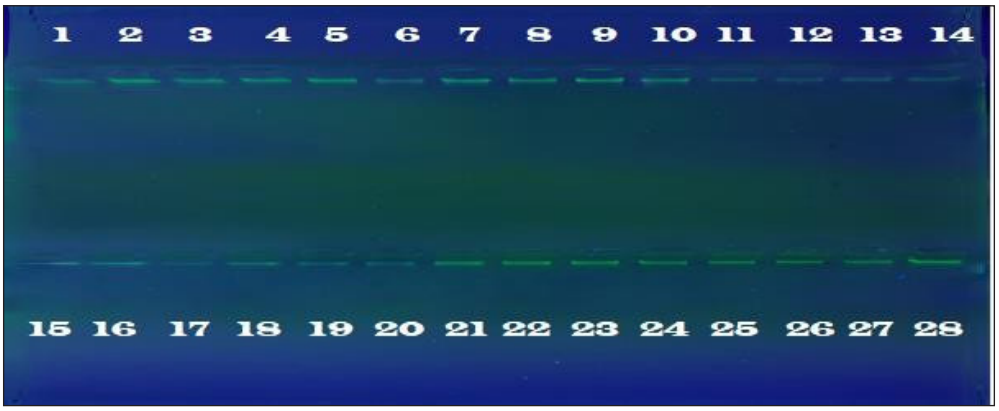
The culture results showed the appearance of small, convex and transparent colonies, as shown in Figure (1). As for the bacterial cells stained with Gram dye, the results showed that they were negative for Gram dye and were helical, and some of them were spherical in shape. The results of the biochemical tests were that they were positive for oxidase and catalase. The isolates also showed antibiotic sensitivity and resistance. We confirm the growth of H.pylori from culture samples by using the double-stranded PCR technique to detect the presence of the 16S rRNA genes. Because because of financial issues regarding the expenses of H.pylori PCR we just test 9 samples. After using biochemical tests to confirm and diagnose the isolates, PRC was positive for the 16S rRNA genes in all 9 culture samples. Accordingly, all these results confirmed that they were primarily positive for H . pylori by PCR, which has a 100% test sensitivity. Every isolate that tested positive for 16S rRNA displayed as shown in Figure (2).

In this study we include 4 antibiotics clarithromycin, metronidazole, amoxicillin and tetracycline to assess the resistance and susceptibility of H.pylori to these antibiotics that are commonly prescribed in our locality. We found that H. pylori isolates were resistant to metronidazole in all samples (100%), to tetracycline in 20% and to amoxicillin in 10% of samples, while all samples (100%) were sensitive to clarithromycin (Figure 3).



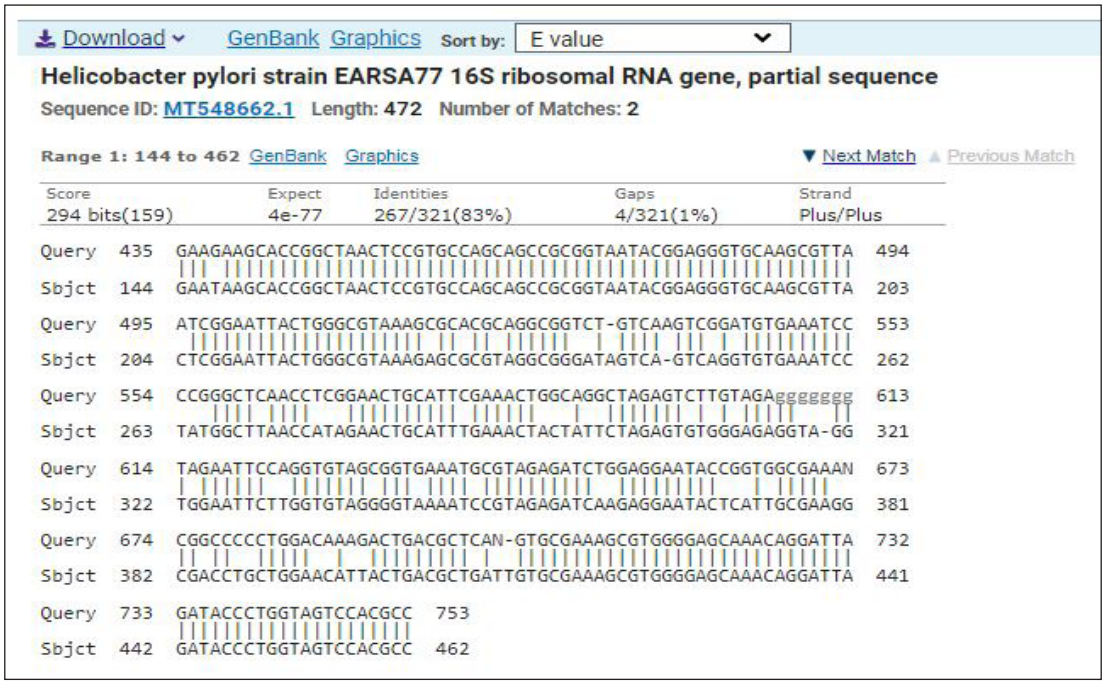


Figure 2.The band size of the PCR product is 1500 bp. Electrophoresis on 2% agarose at 5 volts/cm2

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Figure 3.Antibiotic sensitivity test results

Discussion

H. pylori prefer to live on the stomach mucosa. Due to its unexpected progression and constant contagiousness, it is imperative to treat it as soon as it is diagnosed. It causes mucosal inflammation and damage, ranging from asymptomatic gastritis to severe peptic ulcer disease and malignant lesions. Non-invasive or invasive diagnostic techniques are both possible. These tests are helpful in a range of clinical settings since each one has benefits and drawbacks,[14,15] RUT has a 97–99% specificity and a sensitivity of roughly 80–100%,[16] in this study RUT had a 100% sensitivity and specificity as all cases of positive H.pylori culture as well as positive H.pylori PCR had also positive RUT if we consider H.pylori consider H. pylori culture and PCR as the gold standard tests. The reason why we did not gotget growth on all samples was attributed to H.pylori sensitivity to environmental conditions, the avidity of bacteria or due to recent recent antibiotics usage.

The selection of 16S rRNA genes for the diagnosis of the double-stranded PCR of H. pylori shows that these are essential genes for the life of bacteria (housekeeping genes) and must be present in any strain, and the possible mismatch in each primer pair will be compensated for by the other, and these new strains that appeared with us have been registered in the NBCI.(Helicobacter pylori ZH11 gene for 16S rRNA, partial sequence), 16S rRNA was selected for the detection of H. pylori in this investigation due to its high degree of evolutionary and functional homology among all bacteria and the sequences used for microbial classifications.[17] Out of 125 patients included in the study, 76 dyspeptic patients had H.pylori infection (60.8%) and all patients who had H.pylori on culture had positive RUT this indicate that there is a very high (100%) concordance rate between the RUT and culture as well as these finding suggest findings suggesting that RUT is a good initial test used to diagnose the existence of H.pylori infection, so for RUT as primary diagnostic tests, the sensitivity of several RUT tests is high and has been reported to range between about 80% and 100%, while the specificity ranges from 97% to 99%.[16]

The treatment of H.pylori becomes more challenging due to the steady rise in antibiotic-resistant, different H. pylori strains and their varied resistance profiles, which calls for more researches.[18]

Resistance to Metronidazole in this study was in concordance with other researches these results was similar to other research.[19–21] while in contradictory contradiction to these researches results, still H.pylori had 100% research results, H. pylori still had 100%sensitivity to clarithromycin. We found a comparatively low level of clarithromycin resistance in other studies (26.2%)[22] and (30.0%)[23] These results were consistent with what the researchers reached.[24] Many factors play a role in H.pylori antibiotic resistance like: the use of antibiotics, the genetic transmission of bacteria at sites where the antibiotic binds, inadequate adherence to treatment and the presence of antimicrobial resistance.[25]

Conclusions

RUT test should be considered the preferred test for H. pylori diagnosis when patients undergo endoscopy as it has a high diagnostic yield. Although H.pylori culture is difficult because it needs a suitable environment, the culture is considered the gold standardtest for pylori infection as well as t for knowing the antibiotic sensitivity. Still clarithromycin and amoxicillin are good choices to be include in the H.pylori eradication regimen while metronidazoleshould be excluded from the H.pylori regimen as it had a very high resistance rate.

Conflict of Interest: None

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Authors Contribution: AA: Collecting patients , Writing and editing , MA: Performing H. pylori tests and writing, WA: Collecting patients , Writing and editing.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: None

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