

## Molecular detection of *Helicobacter pylori* isolated from biopsies of gastritis patients based on the presence of *16srRNA* and *VacA S1/S2-UreA* virulence genes

<sup>1</sup>Ahood Abdul Hussein Habeeb, <sup>2</sup>Halal abdukhaliq Awadh, <sup>3</sup>Firas Faris Rija

<sup>1</sup>Salah Al-Din Health Department - Balad General Hospital

<sup>2,3</sup>Department of Biology, College of Sciences, Tikrit University, Tikrit,

**Keywords:** VacS1/S2, gastric cancer, urease gene and chronic gastric.

### ARTICLE INFO.

#### Article history:

-Received: 18 Nov. 2023

-Received in revised form: 24 Jan. 2023

-Accepted: 25 Jan. 2023

-Final Proofreading: 24 Feb. 2024

-Available online: 25 Feb. 2024

#### Corresponding Author\*:

[ahoodalrubaye@gmail.com](mailto:ahoodalrubaye@gmail.com)

©2024 THIS IS AN OPEN ACCESS ARTICLE UNDER THE CC BY LICENSE  
<http://creativecommons.org/licenses/by/4.0/>



### ABSTRACT

**Background:** Although most infections with *Helicobacter pylori* are asymptomatic, some people with this bacterium end up with chronic gastritis, peptic ulcers, or even stomach cancer. The study's objectives was to detect the prevalence of *Helicobacter pylori* by identifying *16srRNA* and to determine the virulence genes (*ureA* and *vacAS1/S2*) in biopsies of patients with gastritis.

**Method:** The study was conducted between September 2022 and June 2023 at the public hospital and several private clinics in Balad City, Iraq. A total of 50 samples were taken, spanning from 15-85 years of age (50 patients). Balad General Hospital and a private upper surgical endoscopy clinic. The specialist doctor (gastroenterologist) took the gastric biopsy samples from the stomach's antrum and corpus during normal endoscopy. The gastric biopsies were used for PCR.

**Results:** The current study includes 50 samples suffering from gastritis. Patients according to age groups 26-34 and 45-54 had the highest frequency, while, 55-64 had the lowest frequency. *Helicobacter Pylori* was detected by *16SrRNA*. According to the result *16SrRNA* by, the research found that positive gastric patients was 42 (84%) and 8 (16%) negative. In addition, this research found that the *UreA* gene was 39 (78%) positive and 11 (22%) negative and the *VacS1/S2* gene was 22 (44%) positive and 28 (56%) negative.

## الكشف الجزيئي عن بكتريا الملوية البوابية المعزولة من خزعات مرضى التهاب المعدة

بناء على وجود *16srRNA* وجينات الضرواة *UreA, VacAs1/S2*

أ. عهود عبد الحسين حبيب، أ. هاله عبد الخالق عوض، أ. فراس فارس رجا

دائرة صحة صلاح الدين - مستشفى بلد العام

قسم الأحياء، كلية العلوم، جامعة تكريت، تكريت، العراق

## المخلص

**الخلفية:** على الرغم من أن معظم حالات العدوى بالبكتيريا الحلزونية البوابية لا تظهر بأعراض، فإن بعض الأشخاص المصابين بالبكتيريا الحلزونية البوابية يصابون في النهاية بالتهاب المعدة المزمن، ومرض القرحة الهضمية، وحتى سرطان المعدة. كان الهدف من الدراسة هو الكشف عن انتشار بكتريا الحلزونية البوابية عن طريق تحديد *16sRNA* وتحديد جينات الفوعة (*ureA*، و *vacAS1 / S2*) في خزعات مرضى التهاب المعدة.

**الطرق:** أجريت الدراسة في الفترة من أيلول (سبتمبر) ٢٠٢٢ إلى نيسان (أبريل) ٢٠٢٣ في مستشفى عام وعدة عيادات خاصة في مدينة بلد، العراق. تم جمع (٥٠) عينة تراوحت أعمارها من ١٥ إلى ٨٥ سنة (٥٠ مريضا). في مستشفى بلد العام وعيادة الناظور العلوي الجراحيه الخاصة قام أخصائي (أخصائي أمراض الجهاز الهضمي) بأخذ عينات من غار المعدة والجسم اثناء التنظير الروتيني. تم استخدام خزعة المعدة من أجل تفاعل البوليميراز المتسلسل pcr.

**النتيجة:** الدراسة الحالية شملت ٥٠ عينة مصابة بالتهاب المعدة. أعلى معدل تكرار حدث في المرضى الذين تتراوح أعمارهم بين ٢٦-٣٤ إلى ٤٥-٥٤ سنة، وأدنى معدل في المرضى الذين تتراوح أعمارهم بين ٥٥-٦٤ سنة. تم الكشف عن هيليكوباكتر بيلوري بواسطة *16sRNA* وفقاً لاكتشاف *16sRNA* بواسطة PCR وجد البحث أن نتيجة مرضى المعدة الموجبة كانت ٤٢ (٨٤٪) و ٨ (١٦٪) سلبية. بالإضافة إلى ذلك، وجد هذا البحث أن جين *UreA* كان ٣٩ (٧٨٪) إيجابياً و ١١ (٢٢٪) سلبياً وأن الجين *VacS1 / S2* كان ٢٢ (٤٤٪) إيجابياً و ٢٨ (٥٦٪) سلبياً.

**الكلمات المفتاحية:** *VacAS1/S2*-سرطان المعدة- جين اليوريز- التهاب المعدة المزمن

## Introduction

*Helicobacter pylori* is a gram-negative bacteria that has the form of a spiral and colonises the mucoid lining of the human stomach [1]it exhibits a characteristic known as polymorphism and may take both the coccoid and the bacillary shape[2]. Increasingly than half of the world's population is affected by this form of bacteria, which is considered to be extremely harmful [3]., the virulence genes (*Ure A* and *VacS1/S2* genes) that are carried by distinct genetic patterns of *H. pylori* are the most significant virulence genes that are associated with stomach and bowel sickness[4]. This bacterium's prevalence is owing to the fact that these virulence genes are carried by *H. pylori*. It is difficult to develop and is regarded as a slow microorganism., it is proposed that these bacteria be diagnosed directly from clinical models by using molecular techniques such as polymerase chain Reaction, which is exceptional in its sensitivity and high specificity in diagnosis, as well as its accuracy in determining both the presence of infection and the genotype of these bacteria [5]. Because of the enormous surface area of the stomach, inflammation caused by chronic gastritis is not confined to the gastric mucosa alone; rather, it has the potential to produce inflammation throughout the body as a result of a rise in pro-inflammatory cytokine levels in the serum[6]. The virulence factors of *H. pylori* have been shown to contribute to the development of gastric cancer when combined with the genetic information of host cells that line the stomach (gastric epithelial cells). The bacteria contains numerous virulence factors , including cytotoxin A (*CagA*), the cellular toxin that causes vacuolization (*VacS1/S2*), and its synthesis of the urease enzyme, which analyzes the urea into the ammonia that has antacid effect anthe stomach lining [7]

**Aim of this study:** The aim of this study was to detect the prevalence of *Helicobacter pylori* by determining *16sRNA* and identifying virulence genes (*ureA- vacAS1/S2*) in gastric biopsies using Polymerase Chain Reaction (PCR) Technique.

## Material and Method

The study was conducted between September 2022 and June 2023 at t the Balad City, Iraq. A total of 50 samples were taken spanning 15-85 years of age.from Balad General Hospital,and private upper surgical endoscopy clinic. The specialist doctor (gastroenterologist) took the gastric biopsy samples from the stomach's antrum and corpus during normal endoscopy. When transporting biopsies, we always use sterile tubes with a medium of brain heart infusion (BHI) broth (Oxoid, UK) and 5% foetal bovine serum. PCR was performed on gastric biopsies. Samples were collected biopsy individuals suffer from different complaints such as( loss of appetite, weight loss, diarrhea, vomiting, dyspepsia[8]).

**Molecular technique:** DNA was extracted from biopsy samples using a method described by [9]Specific primers are provided in table 1 and were used for the detection of *H. pylori*, *16SrRNA*,*ure A*, and *vacS1/S2* genes.

**Table1:** Primers used in PCR to 16SrRNA and virulence factors genes

Target gene	Primer pair (5'-3')	Product Size(bp)	References
<i>16srRNA</i>	5'- CTGGAGAGACTAAGCCCTCC- 3' 5'-ATTACTGACGCTGATTGTGC - 3	110	[11]
<i>UreA</i>	5'-TGATGGGACCAACTCGTAACCGT-3' 5'-CGCAATGTCTAAGCGTTTGCCGAA – 3'	244	[12]
<i>Vac S1/S2</i>	5'- ATGGAAATACAACAACACAC- 3' 5'- CTGCTTGAATGCGCCAAAC- 3'	259/286	[13]

**Table:** Mixture of the specific interaction for diagnosis gene

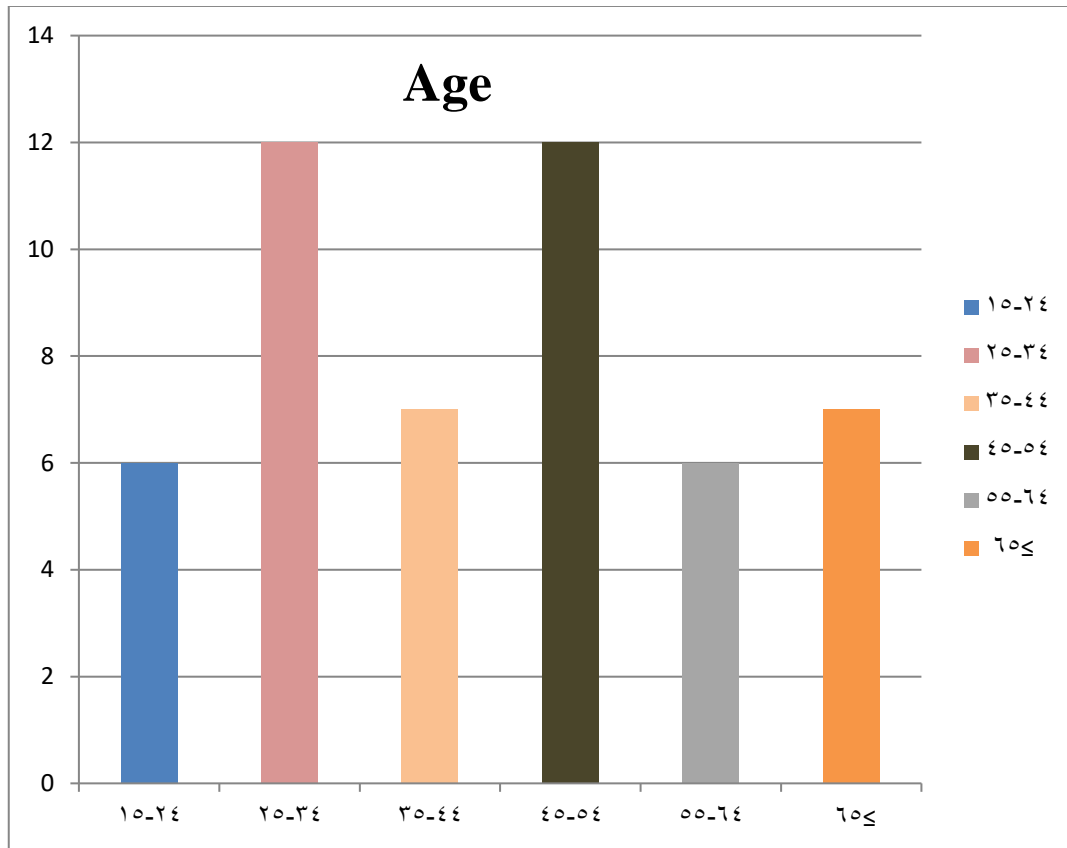
Components	Concentration
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl ( 1 µl )
Reverse primer	10 picomols/µl ( 1 µl )
DNA	1.5µl
Distill water	16.5 µl
Final volume	25µ

. Initial denaturation at 94degrees Celsius for 5 minutes, followed by 35 cycles at 94 degrees Celsius for 20 seconds, 45 seconds, and 35 seconds; 54 degrees Celsius, 60 degrees Celsius, and 54 degrees Celsius for 20 seconds, 45 seconds, and 35 seconds; 72 degrees Celsius, one final extension cycle for 7 minutes. After staining with RedSafe Nucleic Acid Staining (1 mg/L) and visualising under UV light, 5 µL of ampl icon was electrophoresed in 1.5% agarose gels for (45 min) at 70 V in 1X TBE buffer [10].

## Results and Discussion

### Demographic study

The current study includes 50 patients suffer from gastritis, further analysis in the general Balad City's hospital and some private clinics in Balad City's, Iraq. According to the results of the study, patients were placed into six groups based on their age ranges. According to figure (4-1) the distribution of gastric patients according to age groups from 26-34 and 45-54 year had the highest frequency, followed by those aged 35-44, ≥65, and those aged 15-24 and 55-64, who had the lowest, which were 12, 12, 7, 7, 6, 6, respectively

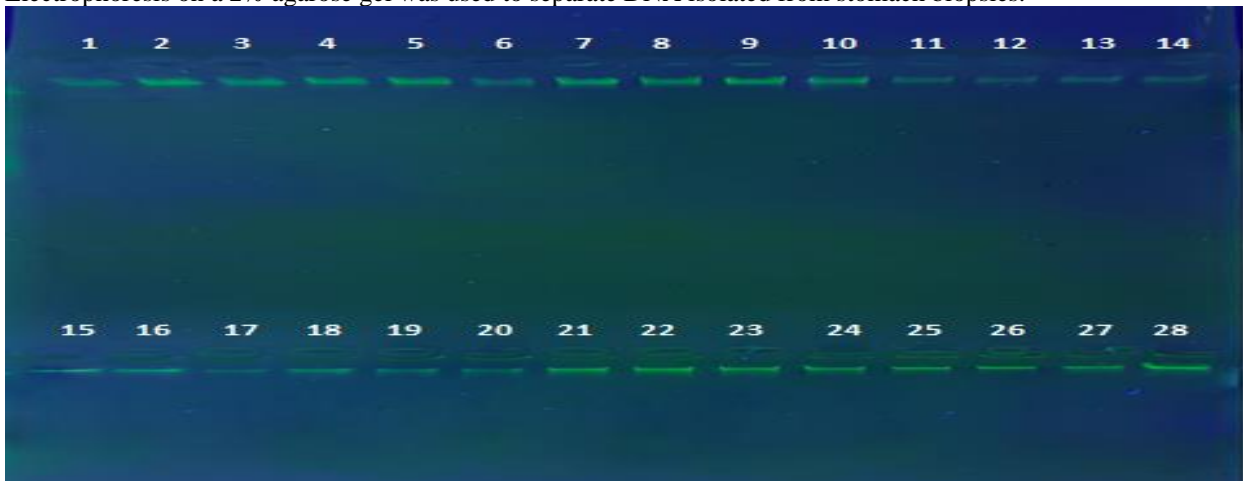


**Figure (1):-** Distribution of the patients according to the age group

A Study in Iraq indicated that high Spread of *H.pylori* occurs in patient with age group 35- 60 (43.9 %) years old compared to younger age (30.89 %) (Salim *et al.* 2020), As, another study showed that the rate infections went up with age, from 42.8% in people than 20 to 60% in people than 50[14] [15]Dida study in 2020 and he found that people between the ages of 31 and 40 were most likely to have *H. pylori* (42.3%), followed by people over the age of 70 (43.6%), and people between the ages of 11 and 20 were least likely to have *H. pylori* (27.3%). [17] Depending on an individual's age,socioeconomic status, and place of origin *H. pylori* infection rates can vary. Children in developing nations often become infected by the age of 10, whereas in wealthier nations, the frequency rises with age[18]

**H.pyloridetection by PCR technique**

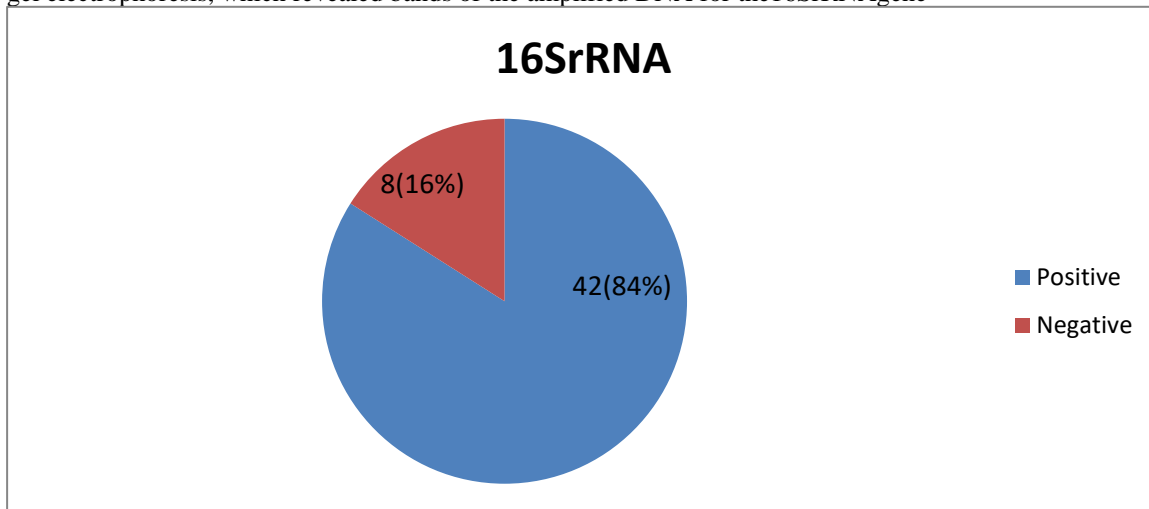
Fifty biopsy samples were utilised to directly extract genomic DNA for PCR analysis of *H. pylori* infection. Electrophoresis on a 2% agarose gel was used to separate DNA isolated from stomach biopsies.



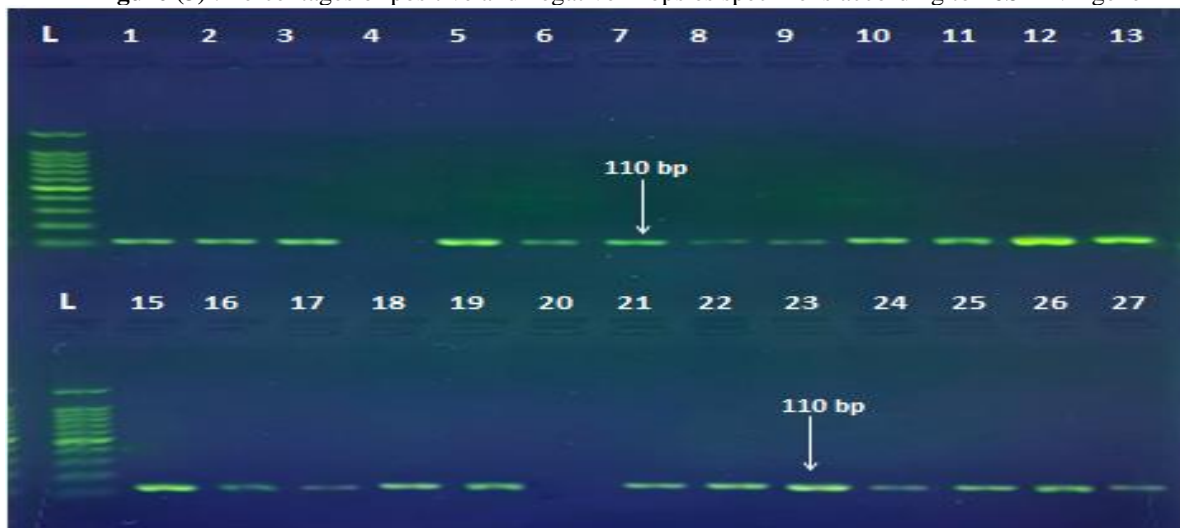
**Figure (2):** 0.7 % Agarose gel electrophoresis at 72 volt for 30 minutes for DNA samples only.

### Determination of 16SrRNA gene

Positive PCR findings for the 110 bp domain of the 16S rRNA gene were found in 42 (84%) of 50 biopsy samples, as shown in figure (3). Figure (4) shows the results of an analysis of the PCR products by 2% agarose gel electrophoresis, which revealed bands of the amplified DNA for the 16SrRNA gene



**Figure (3)** : Percentages of positive and negative Biopsies specimens according to 16SrRNA gene



**Figure (4)** PCR product the band size -110 bp. The product was electrophoresis on 2 % agarose at 5 volt/cm<sup>2</sup>.in x TBE buffer for 1 hour. L: DNA ladder (100).

The findings of this test for identifying *H. pylori* in biopsy samples are shown in Figure 4; out of 50 biopsy specimens, 84% showed a positive result in the 110 bp domain for the 16SrRNA gene, whereas 16% were negative. Positive results for 16S rRNA were obtained in 87% of samples [19] and 100% of samples [20]. 52.38% [21] and 60% ([22]) judged it to be otherwise. Both the presence of infection and assessments of the diversity, pathogenicity, and resistance patterns of these bacteria may be performed with high precision using molecular techniques such as polymerase chain reaction [23]. Although PCR has a number of benefits, including high specificity, rapid findings, and the capacity to type bacteria, its widespread use has been hampered by its expensive price tag and lack of practical application [24].

### Determination of *H.pylori* Virulence Genes by PCR

#### Determination of UreA gene.

All patient samples (50) tested for the presence of *H. pylori* virulence genes. The UreA gene one of the bacterium's primary virulence components, amplification fragments of his gene 244 base pairs was found in 39 patients infected with this bacterium this gene was discovered, with a detection rate of 78%. and 11 gave a negative rate of (22%). As can be seen in Figure 5, the results of the statistical analysis seem to be significant (p0.0001).

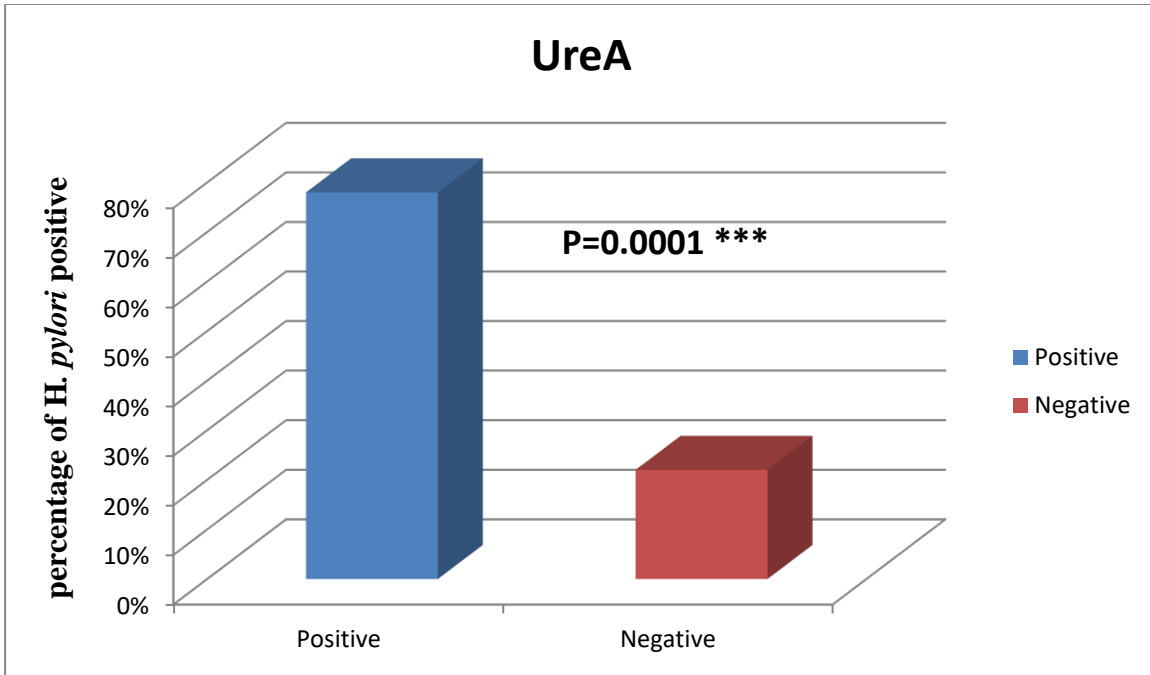


Figure (5): Percentages of positive and negative Biopsies specimens according to UreA gene.

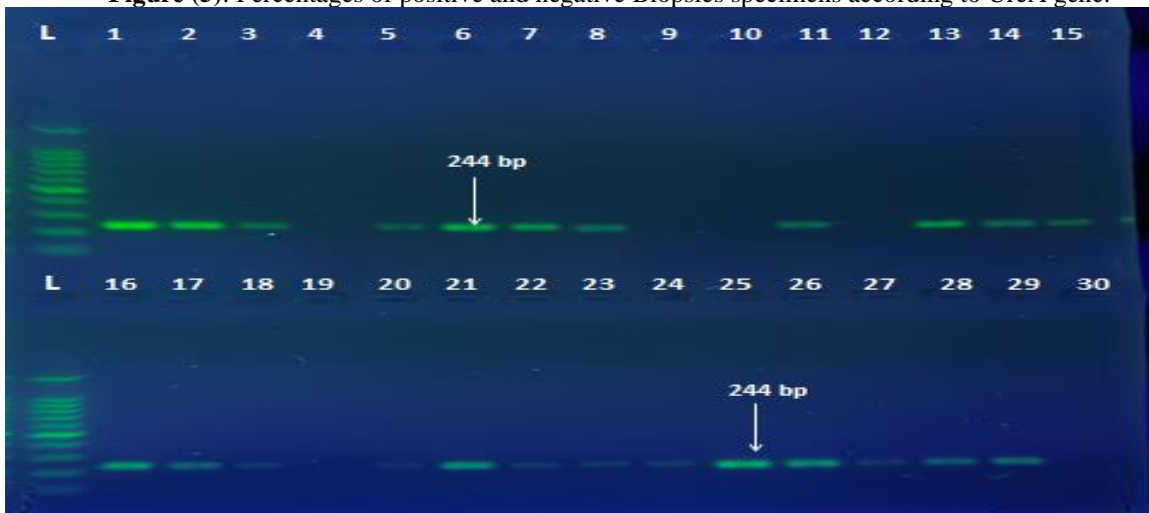


Figure (6) The band size of the PCR result is 244 bp. Electrophoresis on 2% agarose at 5 volt/cm<sup>2</sup> produced the product. 1 hour of TBE buffering. L: number of DNA ladders (100).

This gene is an important one for the bacteria that make up *H. pylori*; it's responsible for the production of a secondary unit in the synthesis of the urease enzyme, which breaks down urea in the stomach into ammonia. This process is similar to the one that occurs in the stomach's acidic environment, so *H. pylori* may be able to survive there [25]. The reason for the difference in the results in the previous study and percent study is that among pathological cases due to function of this gene found in specimen that were used for diagnosis [26]

**Determination of VacS1/S2 gene**

The size of the VacS1/S2 gene, which is 259 kb, is depicted in Figure 7. As indicated in Figure (8), this gene was discovered in 22 individuals who had *H. pylori* infections, representing a 44% prevalence. However, the statistical analysis did not reach significance at (p0.05).



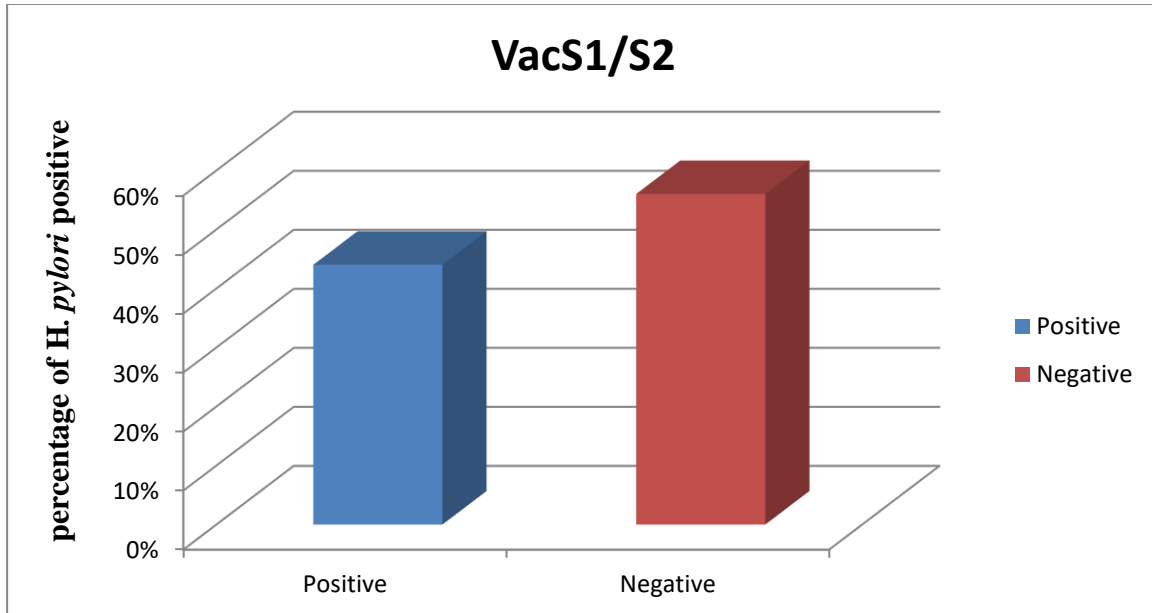


Figure (7) : Percentages of positive and negative Biopsies specimens according to VacS1/S2 gene

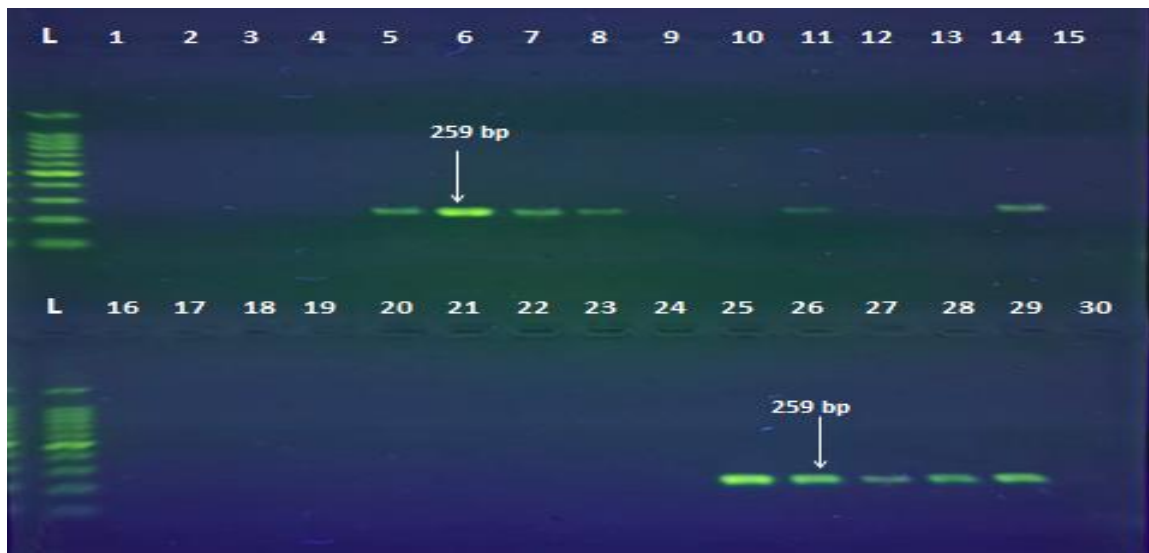


Figure (8) The size of the band made by PCR is 259 bp. Electrophoresis on 2% agarose at 5 volt/cm2 was used to make the product. 1 hour of TBE delayL:DNA ladder (100).

In a study conducted by a number of researchers [27], the positive specimens of the patients included in the study were diagnosed, and the percentage of positive specimens for *VacA s1/s2* gene was 100%. These results differed from current results, is that diagnosed was 44%. As in another study conducted in Brazil by researchers, the positive specimens that were taken from patients and diagnosed by this gene appeared with a rate 9.2%. Also, this study differed what was diagnosed in current study. The caused or the difference between the studies and percent study may be due to the reduction of specimens taken in these studies from patients with gastritis patients with cancer or peptic ulcers[28] The action of this gene leads to the formation of acidic vacuoles in the cytoplasm of gastric epithelial cells that form pores in the gastric cell's epithelial cell membranes, which allows the anions and urea to be released. This is the reason for a difference in the *vacA* gene ratio among gastric illnesses. In addition, the *vacA* induces the loosening of gastric epithelial cells' tight junctions, which could enable *H. pylori* to absorb nutrients by penetrating the mucosal barrier[29]

## Conclusion

The current study results show that *H. pylori* can be directly detected from biopsy samples confirming that it is possible to diagnose by amplifying the 16S rRNA gene and virulence UreA and VacAS1/S2 obtained from patients with gastritis diseases using (PCR ) polymerase chain reaction .

## References

1. Pegu, S.R.R., Rajkhowa, S., Das, P.J., Sonowal, J., Sengar, G.S., Deb, R., Choudhury, M., Deka, N., Paul, S., Doley, J. and Sarma, D.K., 2023. Incidences of Helicobacter infection in pigs and tracing occupational hazard in pig farmers. *bioRxiv*, pp.2023-03.
2. Canzian, F., Rizzato, C., Obazee, O., Stein, A., Flores-Luna, L., Camorlinga-Ponce, M., Mendez-Tenorio, A., Vivas, J., Trujillo, E., Jang, H. and Chen, W., 2020. Genetic polymorphisms in the cag pathogenicity island of *Helicobacter pylori* and risk of stomach cancer and high-grade premalignant gastric lesions. *International journal of cancer*, 147(9), pp.2437-2445.
3. Faass, L., Stein, S.C., Hauke, M., Gapp, M., Albanese, M. and Josenhans, C., 2021. Contribution of Heptose Metabolites and the cag Pathogenicity Island to the Activation of Monocytes/Macrophages by *Helicobacter pylori*. *Frontiers in Immunology*, 12, p.632154.
4. Bustos-Fraga, S., Salinas-Pinta, M., Vicuña-Almeida, Y., de Oliveira, R.B. and Baldeón-Rojas, L., 2023. Prevalence of *Helicobacter pylori* genotypes: cagA, vacA (m1), vacA (s1), babA2, dupA, iceA1, oipA and their association with gastrointestinal diseases. A cross-sectional study in Quito-Ecuador. *BMC gastroenterology*, 23(1), pp.1-10.
5. Khelaiifia, S., Virginie, P., Belkacemi, S., Tassery, H., Terrer, E. and Aboudharam, G., 2023. Culturing the Human Oral Microbiota, Updating Methodologies and Cultivation Techniques. *Microorganisms*, 11(4), p.836.
6. Săsăran, M.O., Meliț, L.E. and Dobru, E.D., 2021. MicroRNA modulation of host immune response and inflammation triggered by *Helicobacter pylori*. *International Journal of Molecular Sciences*, 22(3), p.1406.
7. Altobelli, A., Bauer, M., Velez, K., Cover, T.L. and Müller, A., 2019. Helicobacter pylori VacA targets myeloid cells in the gastric lamina propria to promote peripherally induced regulatory T-cell differentiation and persistent infection. *MBio*, 10(2), pp.10-1128.
8. Ansari, S. and Yamaoka, Y., 2020. Helicobacter pylori virulence factor cytotoxin-associated gene A (CagA)-mediated gastric pathogenicity. *International journal of molecular sciences*, 21(19), p.7430.
9. Schröder, D., Müller, F., Heesen, G., Hummers, E., Dopfer-Jablonka, A., Vahldiek, K., Klawonn, F., Steffens, S., Mikuteit, M., Niewolik, J. and Heinemann, S., 2023. Feasibility of self-organized blood sample collection in adults for study purposes in a primary care setting. *Plos one*, 18(5), p.e0286014.
10. Hussein, R.A., Al-Ouqaili, M.T. and Majeed, Y.H., 2021. Detection of *Helicobacter Pylori* infection by invasive and non-invasive techniques in patients with gastrointestinal diseases from Iraq: A validation study. *Plos one*, 16(8), p.e0256393.
11. Kagihara, J., Matsuda, B., Young, K.L., Li, X., Lao, X., Deshpande, G.A., Omata, F., Burnett, T., Lynch, C.F., Hernandez, B.Y. and Kuwada, S.K., 2020. Novel association between *Helicobacter pylori* infection and gastrointestinal stromal tumors (GIST) in a multi-ethnic population. *Gastrointest. Stromal Tumor*, 3(1).
12. Abdulrahman, A.T., Hussain, S.S., Ahmed, N.J. and Hassan, A.N., 2022. Detection of *Helicobacter Pylori*'s Virulence Gene (UreA) and its Influence on the Result of Rapid Urease Test (RUT). *Al-Mustansiriyah Journal of Science*, 33(4), pp.42-48.
13. Njenga, P., Njau, A., Molloo, Z., Revathi, G., Tshibangu, E. and Yamaoka, Y., 2023. Pattern and trends of *Helicobacter pylori* genotypes in gastric cancer: A Kenyan 8-year study. *Frontiers in Medicine*, 10, p.1119513
14. Majeed, P.D. and Khoshnaw, K.J.S., 2020. Seroprevalence of *Helicobacter pylori* infection among patients with gastroduodenal disorders in Erbil city. *Diyala Journal of Medicine*, 18(1), pp.91-101.



15. Almashhadany, D. A. (2020). Epidemiology of *Helicobacter Pylori* among human at Erbil Governorate/Kurdistan region/Iraq. *World Journal of Pharmacy and Pharmaceutical Sciences*, 9(11), 435-447
16. Almashhadany, D.A., Mayas, S.M. and Ali, N.L., 2022. Isolation and identification of *Helicobacter pylori* from raw chicken meat in Dhamar Governorate, Yemen. *Italian Journal of Food Safety*, 11(2).
17. Zhu, Y.; Zhou, X.; Wu, J.; Su, J. and Zhang, G. (2014). Risk factors and prevalence of *Helicobacter pylori* infection in persistent high incidence area of gastric carcinoma in Yangzhong city. *Journal Gastroenterology Research and Practice*.
18. Graham, D. Y. (2000). *Helicobacter pylori* infection is the primary cause of gastric cancer. *Journal of gastroenterology*, 35
19. Ahmed, K. S.; Madompoyil, B.; Ghebremedhin, A. A.; Issac, J.; Ahi, J. D.; Khan, A. A.; Tiwari, S. K. 2013. Studying the Importance of *VacA* Gene of *Helicobacter pylori* in Identifying the Pathogenicity of Strains by Comparing It with the Disease Status of the Subjects. *Journal of Cancer Therapy*, 4: 68-74.
20. Ali, S.F. 2013. Detection of *Helicobacter pylori* in saliva from some Iraqi patients in comparison with other methods. M.Sc thesis, College of Science, Baghdad University.
21. Smith, S.M.; O'Morain, C. and Namara, D. M. 2014. Antimicrobial susceptibility testing for *Helicobacter pylori* in times of increasing antibiotic resistance. *World J Gastroenterol*, 20(29): 9912-9921.
22. Mamoun, M.; Elsanousi, S.; Khalid, A.; Abdelmounem E. and Mohamed A. 2015. Molecular Identification of 16s Ribosomal RNA Gene of *Helicobacter pylori* Isolated from Gastric Biopsies in Sudan. *American Journal of Microbiological Research*, 3(2): 50-54.
23. Chiquet, C., Bron, A.M., Lundström, M. and Maurin, M., 2022. Acute postoperative endophthalmitis: Microbiology from the laboratory to the bedside. *Survey of Ophthalmology*.
24. AlObaidi, A., Alquraishi, Z.H.O. and Alsadawi, A.A., 2022. Association among Biofilm Formation and Antibiotic Resistance Genes in *Klebsiella Pneumoniae* Isolates from Urinary Tract Infections. *HIV Nursing*, 22(2), pp.1336-1340.
25. Bouhenni, H., 2023. Study of combined effect of some medicinal plants and probiotics against *Helicobacter pylori* responsible for gastroduodenal diseases (Doctoral dissertation, Faculty of Nature and Life Sciences)
26. Sachs G., Expression of *Helicobacter pylori* urea I gene required for acidic pH activation of cytoplasmic urease, *Infect. Immune*. 68, 470-477 (2000).
27. Bibi, F., Alvi, S.A., Sawan, S.A., Yasir, M., Sawan, A., JimaFatani, A.A. and Azhar, E.I., 2017. Detection and genotyping of *Helicobacter pylori* among gastric ulcer and cancer patients from Saudi Arabia. *Pakistan journal of medical sciences*, 33(2), p.320.
28. Quezado, M., Cavalcante, D. F., Igor, C., Silva, S., Braga-neto, M. B., Bessa, A., Fialho, C., Fialho, A. N., Barbosa, A. M. C., Will, F., Cruz, S., Rocha, G. A., Maria, D., Queiroz, M. De, Libanez, L., & Campelo, B. (2012). *Helicobacter pylori vacA* and *cagA* genotypes in patients from northeastern Brazil with upper gastrointestinal diseases. *Journal of Clinical Microbiology*, 50(6), 1071-1076.
29. Segar R.K. (2007) Pathogenic and molecular study on *Helicobacter pylori* isolated from patients of gastric and duodenal ulcers. Ph.D. Thesis Al-Mustansiriyah University. Iraq