Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.13.03.016

New recording genes and genetic mutations of H. pylori genome in gastroduodenal disease patients in Basrah province-Iraq

Ahmed W. Al-Hilfi[1], Ihsan E. Alsaimary[1*], Ali D. Al-Hilfi[2]
[1]Department of Microbiology, College of Medicine, University of Basrah
[2]Consultant GIT, HBP, Laparo-Endoscopic & Bariatric Surgeon Al-Sader Teaching Hospital
***Corresponding author:** Ihsan E. Alsaimary, Department of Microbiology, College of Medicine, University of Basrah, Email: ihsanalsaimary@gmail.com

Submitted: 15 November 2022; Accepted: 12 December 2022; Published: 30 January 2023

ABSTRACT

The aim of this study was to investigate the molecular finding of the most important H. pylori virulence factors cagA, vacA genotypes in gastric biopsies of patients with gastroduodenal diseases infected with Helicobacter pylori in Basrah-Iraq by using conventional PCR and sequencing technologies, to give acknowledge about the immunopathogenesis of the studied virulence factors in patients with gastroduodenal disorder. A case-control study included 112 confirmed gastroduodenal patients and 112 healthy individuals as a control group. Data about age, gender, smoking, alcohol abuse, family history, occupation, residence, and clinical findings for all study populations were collected. The study show a significant effect of these virulence factors with an increased risk of gastroduodenal disorder.

Keywords: *gastroduodenal disorder, Helicobacter pylori, cagA, vacA, and new genetic mutations.*

INTRODUCTION

It was first reported in 1984 that gastroduodenal diseases in humans are caused by a bacterial infection (1). In 1892, Giulio Bizzozero discovered the spiral-shaped microorganism Helicobacter pylori for the first time in the stomachs of dogs (2). Robin J. Warren and Barry J. Marshall in Australia 1983 observed spiral, campylobacter-like bacteria in close opposition to the gastric mucosal tissues within multiple reported cases of gastritis and duodenal ulcers. At first, they were originally known as Campylobacter pyloridis due to their habitat and some characteristics in common with Campylobacter jejuni, then Campylobacter pylori which were confirmed to be different from other Campylobacter species by Goodwin et al. in 1989 (3), and recently redesigned as Helicobacter and Helicobacter pylori became a pioneer of the new species (4). Warren and Marshall received the 2005 Nobel Prize in Physiology and Medicine for this patent. Thus, H. pylori were confirmed as the primary cause of gastritis and the more severe gastroduodenal diseases (5). Helicobacter pylori can cause many gastroduodenal and intestinal disorders including peptic ulcer diseases (PUDs), duodenal ulcers (DUs), and low-grade B-cell mucosa-associated lymphoid tissue (MALT) gastric lymphoma (6). Since 1994, the WHO International Agency for Research on Cancer (WHO/IARC) has first formally classified H. pylori as a class I carcinogen, a definite cause of cancer in humans, and that the eradication of H. pylori can reduce the risk of gastric cancer (7).

MATERIAL AND METHOD

A Case-control study was conducted between November 2021 to November 2022 carried for patients with gastroduodenal disorder according to a minimum sampling size equation that depends on the disease ratio. The total number of gastroduodenal disorder patients involved in this study are (112) individuals were taken from Al-Sadder Teaching Hospital in Basrah province, the age of patients range from 15-66 years, and (112) individuals considered as control group after they were checked and confirmed to be free from any clinical problems. During the collection process, data about each individual were reported in a questionnaire paper for each one, which included age, gender, family history, smoking, alcohol drinking, occupation, residence, and clinical findings of the disease which we have highlighted in the current study. The endoscopic examinations were done and recorded under the supervision of a gastroenterologist. Each patient and control group were subjected to a biopsy urease test and urea breath test for a definitive diagnosis of H. pylori infection. Bacterial DNA was extracted from g astric biopsies of patients infected with H. pylori to evaluate the genetic changes that may occur in Iraq population

Exclusion criteria

Patients on recent antibiotics.

Proton pump inhibitors (PPIs) consuming patients, two weeks before endoscopy.

Immunocompromised patients.

Pregnant women

were excluded from this case-control study.

Inclusion criteria

Any patient suffering from gastroduodenal disorder (epigastric pain, dyspepsia, abdominal pain, and heartburn) associated with H. pylori infection which is diagnosed by urea breath test and biopsy urease test and under the supervision of a specialist GIT physician was included in this case-control study.

Samples collection

Three to Five punch of fresh gastric biopsies were obtained from gastroduodenal disease patients and a control group to perform a biopsy urease test. The biopsies were kept in a transport media to detect virulence factors of H. pylori: cagA, vacA by using specific primers by conventional PCR, and to determine DNA sequencing of PCR products of each virulence factor gene, alignment, identify, and phylogenetic tree in comparison with the preserved gene in NCBI and to determine genetic mutations occur in virulence factor gene in comparison with standard genes in NCBI for possible find a new gene related to Iraqi populations.

J Popul Ther Clin Pharmacol Vol 30(3):e137–e143; 30 January 2023.

RESULTS

DNA extraction

The pure genomic DNA was extracted from gastric biopsies of the study population by using a specialized trusted DNA extraction kit to give a very high quality and quantity of DNA and to confirm H. pylori infection. Horizontal Agarose Gel Electrophoresis was carried out to check extracted DNA, and the bands of total DNA were observed on the gel by using an ultraviolet (UV) imaging system as shown in figure (1).

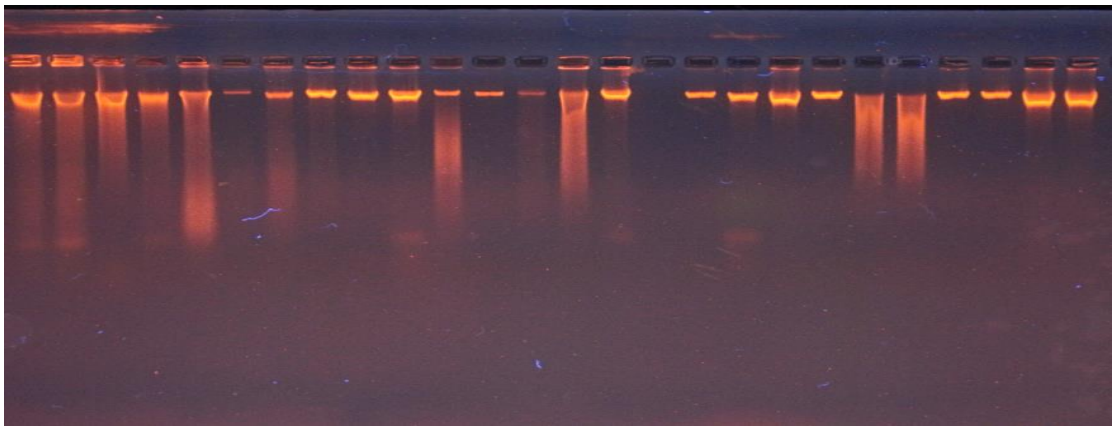

FIGURE 1: DNA products extracted from gastric biopsies running on 1% agarose gel at 100 voltages for 10 min. and then lowered to 70 volts for 60 min.

Cytotoxin-associated gene A (CagA).

PCR amplification results show in figure (2). Positive amplification bands hav been confirmed by Gel Electrophoresis after the successful accurate binding of CagA-specific primer and the target CagA DNA template as illuminating orange bands seen under UV light. The molecular determined size was 350 bp which is specific for CagA primer.

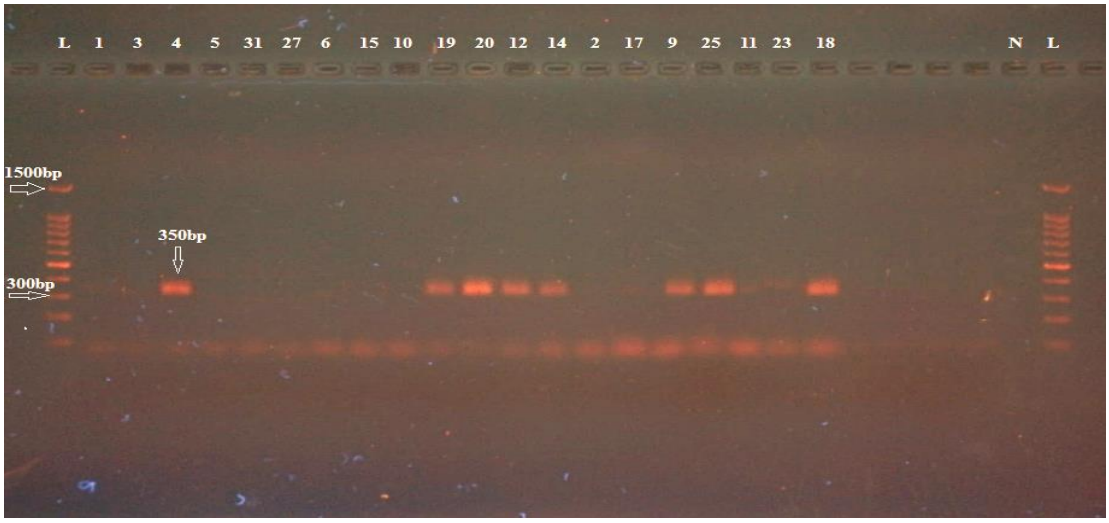

FIGURE 2: Gel Electrophoresis for PCR product of CagA primer which show 350bp at 55°C (Agarose 1%, 10min. at 100 voltages and then lowered to 70 volts for 60min.), then visualized under UV light after staining with ethidium bromide. Lane (L): DNA ladder (1500-100 bp), Lanes (4, 19, 20, 12, 14, 9, 25, and 18) represented positive results, Lanes (1, 3, 5-10, 2, 17, 11, and 23) represented negative result, and Lane (N) represented negative control.

Vacuolating cytotoxic gene A (VacA).

Figure (3) illustrate the positive PCR amplification products. DNA bands have been confirmed by the use of Gel Electrophoresis under the UV imaging system as compact separated orange bands resulting from the accurate special binding between the target VacA DNA template and its specific primer with a molecular size of 259bp or 286bp which is specific for VacA primer.

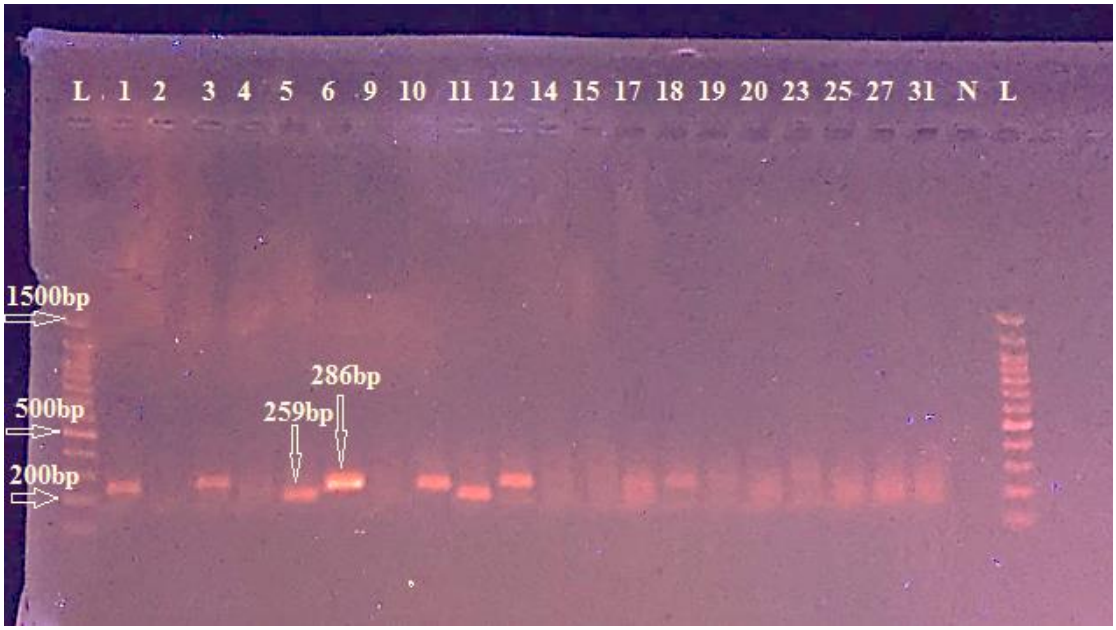

FIGURE 3: Gel Electrophoresis for PCR product of VacA primer which shows either 286bp or 259bp at 53°C, (Agarose 1%, 10min. at 100 voltages and then lowered to 70 volts for 60min.) Visualized under UV light after staining with ethidium bromide. Lane (L): DNA ladder (1500-100bp), Lanes (1, 3, 5, 6, 10, 11, 12, and 17, 18, 20-31) represented positive results, Lanes (2, 4, 9, 14, 15, and 19) represented negative results, and Lane (N) represented negative control.

DISCUSSION

From a molecular standpoint, an infection with H. pylori can cause disorders of the extraintestinal system as well as impact the intestinal microflora biosystem. Early alterations in the microenvironment can modify the gene expression of several immune-related mediators in the host, which subsequently has an impact on the local and systemic immune system. Conventional PCR are the most fundamental techniques for the identification of H. pylori genes directly from gastric biopsies.

Conventional PCR

In this work, the detection of H. pylori gene was estimated by conventional Polymerase Chain Reaction (PCR).

The DNA of H. pylori could be identified by PCR technique directly in human gastric biopsy with excellent sensitivity and specificity (8) .

The part of genetic detection in our study includes DNA extraction from gastric biopsies of the study population by the use of specific primers designed for each gene. Amplification of all DNA samples with agarose gel, 10 min/100 voltages/60 minutes and the results confirmed by gel electrophoresis and the products include: amplicon-size PCR product of 350 bp referred to CagA gene, amplicon-size PCR product of 259 bp referred to VacA gene.

Results of this study show the relationship between the studied virulence genes with predisposition and risks of gastroduodenal diseases. The identification of H. pylori-specific gene region by using molecular techniques such as PCR is faster than culture since H. pylori culturing requires 10-14 days to be confirmed (9). Sulo and Sipkova, (2021) reported that conventional PCR was more reliable than histology to confirm positive results (10).

In a review based on PCR analysis of gastric biopsies, Idowu et al., (2019) revealed a significant association between H. pylori virulence genes (CagA and VacA) and the risk for development of certain gastrointestinal complications (11). Based on data published by El-Khlousy, et al., (2016), from a total of 37 cases detected by PCR technique, out of 17 samples were positive for cagA and 5 samples were positive for VacA in gastric biopsies of H. pylori infected patients (12).

DNA sequencing

The DNA sequencing process through PCR results was performed by Macrogen company/ Korea. The collected data are from Gene Bank and are available at National Center for Biotechnology Information https://www.ncbi.nlm.nih.gov.

The genetic analysis of bacterial genome facilitates the characterization of specific genotypes associated with different diseases. A recent study revealed an association between genetic diversity and the development of gastric cancer and peptic ulcer diseases. H. pylori is one of more than twenty microbial genomic that have been completely sequence (13).

Throughout the world, the DNA sequencing of H. pylori genome shows a great divergence. The current study aims to determine the gene accessibility of virulence factors in the isolated H. pylori strains.

In the present study, the results of DNA sequencing for H. pylori genes CagA and VacA show there is a convergence between the isolated genes and that of the Gene Bank database (NCBI).

Two gastric biopsy samples have been sequenced, the results of CagA sample (1) identity show that the studied CagA gene is present in 45(50.4%) samples from the total cases with 303/309 (98%) identity in forward primer when compared to Gene Bank database (NCBI) with six mutations appeared as L→F; Stop codon→S; N→N; and V→V; I→no functional protein; and S→S, while the reverse primer show 298/303 (98%) identity, when compared to Gene Bank database (NCBI) with five mutations, appeared as no functional protein→Stop codon, N→N; L→L; Stop codon→Stop codon; and S→no functional protein. The forward primer of CagA in sample (2) show two mutations appear as V→V; and I→ no functional protein and the reverse primer of CagA show 295/303(97%) identity and characterized with five mutations appeared as L→L; A→G; S→R; Y→Q; and R→R.

The results of VacA sample (1) identity show that the studied VacA gene appear in 79(88.5%) samples from the total cases and show 179/196 (91%) identity in forward and reverse primer when compared to that of the Gene Bank database (NCBI) with nine mutations appearing as P→P; C→W; V→G; G→G; G→no functional protein; P→P; K→N; P→P; and K→K. The VacA forward primer show 210/291(96%) identity in sample (2) and identified with nine mutations appeared as R→R, S→S, G→G, A→A, S→S, N→N, K→K, R→R, R→G, the reverse primer show 213/219(97%) identity and characterized with six mutations appear as Q→Q, P→P, A→A, K→K, T→T, and F→L.

*The letters represent specific amino acid and all amino acids are illustrated in the table (1).

TABLE 1: Amino acids symbols.

Amino acids		**Symbol**	**Codons**
Alanine	Ala	A	GCA, GCC, GCG, GCU
Cysteine	Cys	C	UGC, UGU
Phenylalanine	Phe	F	UUC, UUU
Glycine	Gly	G	GGA, GGC, GGG, GGU
Isoleucine	Ile	I	AUA, AUC, AUU
Lysine	Lys	K	AAA, AAG
Leucine	Leu	L	UUA, UUG, CUA, CUC, CUG, CUU
Asparagine	Asn	N	AAC, AAU
Proline	Pro	P	CCA, CCC, CCG, CCU
Glutamine	Gln	Q	CAA, CAG
Arginine	Arg	R	AGA, AGG, CGA, CGC, CGG, CGU
Serine	Ser	S	AGC, AGU, UCA, UCC, UCG, UCU
Threonine	Thr	T	ACA, ACC, ACG, ACU
Valine	Val	V	GUA, GUC, GUG, GUU
Tryptophan	Trp	W	UGG
Tyrosine	Tyr	Y	UAC , UAU

Due to high mutation rates and mismatch homologous recombination frequency, H. pylori strains exhibit very great genetic variations. Most of bacterial resistance cases are related to point mutation during bacterial DNA synthesis (14). Antibiotic-resistant bacteria are one of the most important challenges for the world's health and H. pylori is the most important example, for example, mutations on the gene coding for H. pylori outer membrane proteins such as penicillin binding proteins (PBP-coding gene) resulting in β-lactam resistant genotype (15). Suzuki et al., (2019) reported that from the 17 studied genes, 5 genes were identified with a mutation coding for outer membrane proteins which is potentially accelerate colonization and inflammation (16). Vianna et al., (2018) identified seven mutation in 23s rRNA region related to antibiotic resistance, whereas the results of Jabar & Alfaisal, (2017) documented that no mutations were observed in the codons but the sequencing analysis detect point mutations in exons (17,18). In a study by Dai et al., (2022) which enrolled a total of 133 H. pylori positive patients they reported that no significant link was detected between VacA genotypes patients and antibiotic resistance (19).

Aziz et al., (2022) detect a number of specific virulence proteins in gastric biopsies of gastroduodenal disease H. pylori infected individuals, these proteins were controlled by CagA pathogenicity island (20).

In view of the different mutations that have been observed in this study, we hypothesized that the large number of mutations could lead to increase H. pylori resistance and emergence of disease complication such as gastric cancers in Iraqi population, our results were in line with many studies of (21, 22, 23, 24, and 25).

REFERENCES

1. Haesebrouck, F.; Pasmans, F.; Flahou, B.; Chiers, K.; Baele, M.; Meyns, T.; Decostere, A.; and Ducatelle, R. (2009). Gastric helicobacters in domestic animals and nonhuman primates and their significance for human health. Clin. Microbiol. Rev., 22(2):202-23.
2. Oztekin, M; Birsen, Y.; Duygu, A.; and Raffaele, C. (2021). Overview of Helicobacter pylori infection: clinical features, treatment, and nutritional aspects, Dis., 9(66):1-9.
3. Charitos, I. A.; D'Agostino, D.; Topi, S.; and Bottalico, L. (2021). 40 years of Helicobacter pylori: A revolution in biomedical thought. Gastroenterol. Insights, 12(2):111-135.
4. Kumar S. (2016). Essentials of Microbiology. 1st ed., 299-300.
5. Boren, T. (2014). Helicobacter Pylori; Multitalented Adaptation of Binding Properties. Anticancer Research, 34(10):5840-5841.
6. Fan, L.; Li, R.; Li, H.; Zhang, J.; and Wang, L. (2018). genes in Helicobacter pylori isolated from gastric ulcer patients. 42(4):155-62.
7. Kusters, J. G.; Vliet, A. H.; and Kuipers, E. J. (2006). Pathogenesis of Helicobacter pylori infection. Clin. Microbiol. Rev., 19(3):449-490.
8. Wang, YK.; Kuo, FC.; Liu, CJ.; Wu, MC.; Shih, HY.; Wang, SSW.; Wu, JY.; Kuo, CH.; Huang, YK.; and Wu, DC. (2015). Diagnosis of Helicobacter pylori infection: Current options and developments. World J. Gastroenterol., 21(40):11221-35.
9. Fernandez-Caso, B., Miqueleiz, A., Valdez, V. B., & Alarcon, T. (2022). Are molecular methods helpful for the diagnosis of Helicobacter pylori infection and for the prediction of its antimicrobial resistance?. Front. Microbiol, 13(August), 1-6.
10. Sulo, P; and Sipkova, B. (2021). DNA diagnostics for reliable and universal identification of Helicobacter pylori. World. J. Gastroenterol., 27(41):7100-7112.
11. Idowu, A.; Mzukwa, A.; Harrison, U.; Palamides, P.; Haas, R.; Mbao, M.; Mamdoo, R.; Bolon, J.; Jolaiya, T.; Smith, S.; Ally, R.; Clarke, A.; and Njom, H. (2019). Detection of Helicobacter pylori and its virulence genes (cagA, dupA, and vacA) among patients with gastroduodenal diseases in Chris Hani Baragwanath Academic Hospital, South Africa. Gastroenterol, 19(1):1-10.
12. El-Khlousy, M.; Rahman, E, A.; Mostafa, S.; Bassam, A.; Elgawad, W, A.; Elnasr, E, S.; Mohey, M.; and Ghaith, D, (2016). Study of the clinical relevance of Helicobacter pylori virulence genes to gastric diseases among Egyptian patients, Arab J. Gastroenterol., 17(2):90-94.
13. Wilkinson, D. J.; Dickins, B.; Robinson, K.; Winter J. (2022). Genomic diversity of Helicobacter pylori populations from different regions of the human stomach, Gut Microbes, 14(8):1-19.
14. Marques, A. T.; Vitor, J. M. B.; Santos, A.; Oleastro, M.; and Vale, F. F. (2020). Trends in Helicobacter pylori resistance to clarithromycin: From phenotypic to genomic approaches. Microbial Genomics, 6(3):1-11.

15. Vital, J. S.; Tanoeiro, L.; Lopes-Oliveira, R.; and Vale, F. F. (2022). Biomarker characterization and prediction of virulence and antibiotic resistance from Helicobacter pylori next generation sequencing data. Biomolecules, 12(5):1-26.
16. Suzuki, R.; Satou, K.; Shiroma, A.; Shimoji, M.; Teruya, K.; Matsumoto, T.; Akada, J.; Hirano, T.; and Yamaoka, Y. (2019). Genome-wide mutation analysis of Helicobacter pylori after inoculation to Mongolian gerbils. Gut Pathogens, 11(1):4-9.
17. Vianna, J. S.; Ramis, I. B.; Ramos, D. F.; Gastal, O. L.; Azevedo, R.; Goncalves, C. V.; and Eduardo, P. (2018). The interplay between mutations in cagA, 23S rRNA, gyrA and drug resistance in Helicobacter pylori, Rev. Inst. Med. Trop. Sao Paulo, 60(25):1-5.
18. Jabar, S. A., & Alfaisal, A. H. M. (2017). The correlation between KRAS mutations and H. pylori in gastric cancer patients. Iraqi J. Biotechnol., 16(3):82-93.
19. Dai, J., Zhao, J., Mao, L., Hu, Y. U. E., & Lv, B. I. N. (2022). Study on the value of antibiotic-resistant gene detection in Helicobacter pylori in China. Experiment. Therap. Med. 23(228):1-10.
20. Aziz, S.; Rasheed, F.; Akhter, T. S.; Zahra, R.; & Konig, S. (2022). Microbial Proteins in Stomach Biopsies Associated with Gastritis, Ulcer, and Gastric Cancer. Molecules, 27(17):1-13.
21. Jin, F.; and Yang, H. (2022). Complete Genome Sequence of Helicobacter pylori Strain 3192, Isolated from a Chinese Patient with Chronic Nonatrophic. Microbiol. Res. Announc., 11(7):0-11.
22. Jia, X.; Huang, Q.; Lin, M.; and Chu, Y. (2022). Revealing the novel effect of Jinghua Weikang capsule against the antibiotic resistance of Helicobacter pylori. Front. Microbiol., 1-15.
23. Mohammed, H. A., & Obaid, Z. H. (2022). Pathogenesis of Helicobacter pylori: An Overview of Bacterial Virulence Agents and the Mechanism of Occurrence Disease. J. Uni. Babyl. Pure Appli. Sci., 3(1):47-53.
24. Saber, F. omer, & Ali, M. K. (2022). Isolation and Identification of H. pylori among Iraq patients with chronic gastric inflammation. J. Faculty. Med. Baghdad, 64(2):102-108.
25. Hamed, A. T.; and Baqer, AA. B. (2022). Review on stimulations of the spread of Helicobacter pylori infection in Iraq. Inter. J. Appli. Sci. Technol., 3(6):146-156.