DOI: https://doi.org/10.53350/pjmhs20221612748

ORIGINAL ARTICLE

Immunological Study of Gastroduodenal Disorders Patients Infected with H. Pylori 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, Iraq ihsanalsaimary@gmail.com*
[2]*Consultant GIT, HBP, Laparo-Endoscopic & Bariatric Surgeon Al-Sader Teaching Hospital*
Corresponding author: Ihsan E. Alsaimary, Email: ihsanalsaimary@gmail.com, Cell: +964 7801410838

ABSTRACT

The purpose of the current study was to investigate the immunological finding of the most important Helicobacter pylori virulence factors CagA, VacA, DupA, HopQ, and IceA genotypes in gastric biopsies of gastroduodenal diseases patients infected with H. pylori, and to determine the concentrations of CagA, VacA, DupA, HopQ, and IceA in the sera of the patients infected with H. pylori in Iraq-Basrah, by using ELISA technique, to give acknowledge about the role of these virulence factors in patients with the 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. Blood samples have been gathered from each patient as well as the control group. Each individual in this study was examined under the supervision of a gastroenterologist and was subjected to a urea breath test (UBT) for definitive confirmation of H. pylori infection. The finding of this study show a significant effect of these factors with an increased risk of gastroduodenal disorder.

Conclusion: The study show a significant effect of the studied virulence factors (CagA, VacA, DupA, HopQ, and IceA) with an increased risk of gastroduodenal disorder of Iraqi population in Basrah province.

Keywords: Immunological factors, gastroduodenal disorder, Helicobacter pylori, ELISA, CagA, VacA, DupA, HopQ, and IceA.

INTRODUCTION

Helicobacter pylori (Hp), the spiral shape Gram-negative microaerophilic organism can chronically reside in the harsh environment of the stomach, associated with a formidable array of gastroduodenal and intestinal disorders including gastritis, peptic ulcer diseases (PUD), duodenal ulcers (DU), and low-grade B-cell mucosa-associated lymphoid tissue (MALT) gastric lymphoma (1). Helicobacter pylori is the most common transmissible human gastric pathogen worldwide, infecting an estimated 50% of the global population (2,3) equivalent to approximately 4.4 billion people (4), with most cases being asymptomatic (5). Several studies have shown that the prevalence of H. pylori is relatively high in most countries. In Iraq, Erbil, Kurdistan region, the prevalence of H. pylori infection was 39.4% (6), and up to (58%) in people who suffer from abdominal pain and stomach discomfort in Basrah (7). During childhood, Helicobacter pylori infection is usually acquired early in life and establishes lifelong chronic progressive gastric inflammation leading to clinical complications in 1-10% of infected individuals (8). Since 1994, the WHO International Agency for Research on Cancer (WHO/IARC) has first formally categorized H. pylori as a class I carcinogen, a confirmed cause of human gastric tumors, and recently classified by the WHO as a high-priority antibiotic-resistant bacterium that represents a great problem for public health and that the eradication of H. pylori can reduce the risk of gastric cancer (9).

Significant of the study and research gap was to investigate the immunopathogenesis of the most important virulence factors among Iraqi population infected with H. pylori in Basrah province

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) individual 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.

Exclusion criteria:

1. Patients on recent antibiotics.
2. Proton pump inhibitors (PPIs) consuming patients, two weeks before endoscopy.
3. Immunocompromised patients.
4. 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: From each individual, 5ml of fresh venous blood was drawn into a non-pyrogenic, non-endotoxin vacutainers gel tube from gastroduodenal diseases patients and the control group. The samples were separated by centrifugation. The sera were kept in deep freeze under -40℃ and later utilized to determine CagA, VacA, DupA, HopQ, and IceA concentrations in the study group by ELISA technique.

Statistical analysis: Statistical analysis was performed with SPSS statistical program version 23 and Microsoft Excel 2021. Numerical data were defined according to the mean, and standard deviation of the mean, for comparison between different groups, linear regression was used. The lowest accepted difference in statistical importance is 0.05 or less.

RESULTS

Virulence factors concentrations (ng/L) among the studied group: Table (1-1) show that the mean concentration of virulence factors was higher among gastroduodenal patients in comparison to the control group. The mean concentration of CagA was (6.40±6.44), VacA was (13.58±10.53), DupA was (5.48±3.54), HopQ was (2.68±2.30), and IceA was (2.24±2.53) in the patients' group and the mean concentration of CagA was (1.04±0.87), VacA was (1.02±1.09), DupA was (1.01±1.00), HopQ was (0.95±1.08), and IceA was (0.36±0.39) in the control group. Statistically, these differences were significantly higher (P-value=0.001).

Comparison of virulence factors concentrations among study population according to sex: Table (1-2) documents that the mean levels of CagA among males (3.32±2.28) and females (8.54±7.48) in the patients' group were higher than males (1.16±0.90) and females (0.95±0.84) in the control group, and the

mean levels of HopQ among males (2.02±2.14) and females (3.14±2.31) in the patients' group were higher than males (1.13±1.42) and females (0.80±0.69) in the control group. Statistically, these differences were highly significant with p-value= 0.046 and 0.029 respectively.

Comparison of virulence factors concentration among the study population according to the residence: Table (1-3) document the concentration of each virulence factor in relation to the residence of the studied population. The mean values of genes concentration were relatively close. Statistically, this difference was non-significant.

Comparison of virulence factors concentration among the study population according to marital status: Table (1-4) document the concentration of each virulence factor in relation to the marital status of the studied population. The mean values of genes concentration were relatively close. Statistically, this difference was non-significant.

Comparison of virulence factors concentration among the study population according to smoking: Table (1-5) document that the mean levels of CagA (4.02±2.98), VacA (17.36±13.31), DupA (4.79±2.65), and IceA (2.72±3.26) were higher among smokers' group than non-smokers group. Statistically, these differences were highly significant with p-value= 0.001.

Table 1: Virulence factors concentrations among the studied group.

Category		CagA (ng/L)	VacA (ng/L)	DupA (ng/L)	HopQ (ng/L)	IceA (ng/L)
Patients	No.	112	112	112	112	112
	Mean	6.40	13.58	5.48	2.68	2.24
	SD	6.44	10.53	3.54	2.30	2.53
Control	No.	112	112	112	112	112
	Mean	1.04	1.02	1.01	0.95	0.36
	SD	0.87	1.09	1.00	1.08	0.39
P-value		0.001	0.001	0.001	0.001	0.001

* Mann-Whitney U Test

Table 2: Comparison of virulence factors concentrations among study population according to sex.

Category			CagA (ng/L)	VacA (ng/L)	DupA (ng/L)	HopQ (ng/L)	IceA (ng/L)
Patients	Male	No.	46	46	46	46	46
		Mean	3.32	13.88	4.93	2.02	2.55
		SD	2.28	12.17	2.50	2.14	2.94
	Female	No.	66	66	66	66	66
		Mean	8.54	13.37	5.87	3.14	2.02
		SD	7.48	9.31	4.09	2.31	2.19
Control	Male	No.	49	49	49	49	49
		Mean	1.16	1.46	1.12	1.13	0.35
		SD	0.90	1.38	1.29	1.42	0.49
	Female	No.	63	63	63	63	63
		Mean	0.95	0.68	0.93	0.80	0.37
		SD	0.84	0.64	0.69	0.69	0.29
P-value			0.046	0.427	0.657	0.029	0.234

* Mann-Whitney U Test

Table 3: The study population's comparison of virulence factors concentration according to the residence.

Residence		CagA (ng/L)	VacA (ng/L)	DupA (ng/L)	HopQ (ng/L)	IceA (ng/L)
Rural	No.	133	133	133	133	133
	Mean	3.67	7.70	3.22	1.86	1.39
	SD	5.20	9.87	3.41	1.96	2.07
Urban	No.	91	91	91	91	91
	Mean	3.80	6.72	3.29	1.75	1.17
	SD	5.50	9.62	3.47	2.04	1.99
P-value		0.929	0.264	0.784	0.293	0.120

* Mann-Whitney U Test

Table 4: The study population's comparison of virulence factors concentration according to marital status.

Marital status		CagA (ng/L)	VacA (ng/L)	DupA (ng/L)	HopQ (ng/L)	IceA (ng/L)
Single	No.	27	27	27	27	27
	Mean	3.06	6.67	2.66	1.37	0.91
	SD	4.15	10.63	2.20	1.18	1.12
Married	No.	158	158	158	158	158
	Mean	3.39	7.26	3.22	1.83	1.29
	SD	4.48	9.70	3.56	2.03	2.13
Widow	No.	20	20	20	20	20
	Mean	5.23	5.72	3.26	1.85	1.62
	SD	8.75	8.32	3.25	1.83	2.05
Divorced	No.	19	19	19	19	19
	Mean	5.79	10.19	4.33	2.25	1.61
	SD	7.78	10.53	3.86	2.66	2.23
P-value		0.448	0.396	0.568	0.776	0.395

* Mann-Whitney U Test

Table 5: Comparison of virulence factors concentration among the study population according to smoking.

Smoking		CagA (ng/L)	VacA (ng/L)	DupA (ng/L)	HopQ (ng/L)	IceA (ng/L)
Yes	No.	33	33	33	33	33
	Mean	4.02	17.36	4.79	2.11	2.72
	SD	2.98	13.31	2.65	2.15	3.26
No	No.	191	191	191	191	191
	Mean	3.67	5.56	2.98	1.76	1.06
	SD	5.62	7.84	3.48	1.96	1.63
P-value		0.001	0.001	0.001	0.217	0.001

* Mann-Whitney U Test

Table 6: Comparison of virulence factors concentration among the study population according to epigastric pain.

Epigastric pain		CagA (ng/L)	VacA (ng/L)	DupA (ng/L)	HopQ (ng/L)	IceA (ng/L)
Yes	No.	13	13	13	13	13
	Mean	5.19	14.38	4.91	2.71	1.26
	SD	3.75	10.38	3.28	1.87	1.02
No	No.	211	211	211	211	211
	Mean	3.63	6.86	3.15	1.76	1.30
	SD	5.39	9.58	3.42	1.99	2.08
P-value		0.004	0.001	0.011	0.022	0.173

* Mann-Whitney U Test

Comparison of virulence factors concentration among the study population according to epigastric pain: As documented in table (1-6) the levels of mean concentrations for CagA (5.19±3.75), VacA (14.38±10.38), DupA (4.91±3.28), and HopQ (2.71±1.87) was higher in patients suffering from epigastric pain than the group not suffering from epigastric pain. Statistically, these differences were highly significant with p-value= 0.004, 0.001, 0.011, and 0.022 respectively.

Comparison of virulence factors concentration among the study population according to gastritis: As documented in table (1-7) the mean concentrations for each virulence factor were higher in patients suffering from gastritis than in the non-gastritis group. Statistically, these differences were highly significant with p-value= 0.001.

Comparison of virulence factors concentration among the study population according to dyspepsia: Table (1-8) document that the level of mean concentrations of CagA (6.87±5.08), VacA (12.75±11.27), DupA (6.13±3.40), and IceA (2.28±2.25) was higher in patients suffering from dyspepsia than people not suffering from dyspepsia. Statistically, these differences were highly significant with p-value= 0.001.

Comparison of virulence factors concentration among the study population according to gastric cancer: Table (1-9) document that the level of mean concentrations of CagA (13.77±6.71), DupA (7.72±1.52), HopQ (6.65±4.64), and IceA (6.48±2.71) was higher in patients suffering from gastric cancer than people not suffering from gastric cancer. Statistically, these

differences were highly significant with p-value= 0.009, 0.020, 0.019, and 0.006 respectively.

Table 7: Comparison of virulence factors concentration among the study population according to gastritis.

Gastritis		CagA (ng/L)	VacA (ng/L)	DupA (ng/L)	HopQ Ng/L)	IceA (ng/L)
Yes	No.	97	97	97	97	97
	Mean	6.31	13.97	5.55	2.48	2.09
	SD	6.78	10.73	3.67	2.12	2.42
No	No.	127	127	127	127	127
	Mean	1.74	2.21	1.49	1.30	0.70
	SD	2.42	4.54	1.84	1.73	1.42
P-value		0.001	0.001	0.001	0.001	0.001

* Mann-Whitney U Test

Table 8: Comparison of virulence factors concentration among the study population according to dyspepsia.

Dyspepsia		CagA (ng/L)	VacA (ng/L)	DupA (ng/L)	HopQ (ng/L)	IceA (ng/L)
Yes	No.	39	39	39	39	39
	Mean	6.87	12.75	6.13	2.29	2.28
	SD	5.08	11.27	3.40	2.14	2.25
No	No.	185	185	185	185	185
	Mean	3.06	6.15	2.64	1.71	1.10
	SD	5.13	9.04	3.12	1.95	1.93
P-value		0.001	0.001	0.001	0.054	0.001

* Mann-Whitney U Test

Table 9: The study population's comparison of virulence factors concentration according to gastric cancer.

Gastric cancer		CagA (ng/L)	VacA (ng/L)	DupA (ng/L)	HopQ (ng/L)	IceA (ng/L)
Yes	No.	3	3	3	3	3
	Mean	13.77	12.37	7.72	6.65	6.84
	SD	6.71	5.47	1.52	4.64	2.71
No	No.	221	221	221	221	221
	Mean	3.58	7.23	3.19	1.75	1.23
	SD	5.18	9.80	3.41	1.87	1.92
P-value		0.009	0.100	0.020	0.019	0.006

* Mann-Whitney U Test

DISCUSSION

Responding to H. pylori infection, numerous immune cells infiltrate to gastric mucosa and submucosa to clear H. pylori, deceptive H. pylori skew host immune mechanism to avoid eradication and achieve infection, such as cholesterol glycosylation, mimicking the Toll-like receptor (TLR) recognition, endure dendritic cells (DCs), frustrate T cell proliferation, agitate T-reg manipulation, and upregulating PD-L1. Additionally, H. pylori have a variety of strategies to avoid the host immune response, preventing the innate and specific immunity from being sufficient to clear the infection and allowing bacteria to establish and create a chronic infection. For instance, the bacteria can weaken potential antigens that are found on the cell wall, such as the bacterial endotoxin LPS and flagella, and by use of virulence factors, H. pylori control T-cell immunological responses (10).

Based on this study, a significant association were reported between the status of CagA, VacA, DupA, HopQ, and IceA genes and gastrointestinal disorders. All mean values of each studied virulence factor in the patients' group were elevated in comparison with the mean values of the control group with a very high statistically considerable difference (p-value=0.001). As a consequence of these findings, we suggest that the evaluation of virulence gene concentration in the sera of infected patients could be a prediction tool prior to infection.

Our results are in line with many studies, (11) design an immunosensor able to detect a low concentration of VacA antigens in serum of H. pylori infected patients with high sensitivity and stability, suggesting to provide more early reliable clinical diagnosis for H. pylori infection.

The infection with CagA and VacA H. pylori-positive strain may increase many gastrointestinal complaints leading to gastrointestinal inflammation, mucosal barrier collapse, and suppression of IL-1-induced stomach acid secretion. The combined CagA-VacA helicobacter genotype was defined as a highly immunogenic antigen that triggers the production of pro-inflammatory cytokines in the host gastric epithelium leading to an intense inflammatory reaction (12). The results of Costa, et al., (2021) were compatible with our results regarding CagA and VacA.

In another study by Sukri et al., (2022) that demonstrated the critical roles of CD antigens during CagA pathogenesis they conclude that the H. pylori strain harboring CagA upregulated the expression of CD antigen involved in gastric carcinogenesis (13). VacA protoxin amino acid sequence encodes for "vacuolating toxins" that activate the formation of intracellular vacuolation, alteration of a gastric cell's phospholipid bilayer, and generation of pore-forming ion channels (14).

We assume that the type-IV secretion system of H. pylori which resembles a needle-like protrusion can penetrate the host cells to inject the CagA product and lipopolysaccharide into the cytoplasm of host cells. CagA has cytotoxic effects on host cells and LPS is a potent activator of tumor necrosis factor (TNF), LPS can function as a superantigen and significantly activate lymphocytes.

The association between the appearance of DupA and clinical outcome is evident in this study, our finding demonstrates a significant relationship between DupA and the development of gastritis, DupA protein increase the activation of high levels of IL-8, transcription factors nuclear factor kappa light chain enhancer of activated B-cells and act as an ATPase associated efflux pump, which probably confers its virulence (15). DupA activity outcomes are involved in the pathogenesis of H. pylori by enhancing the mitochondrial-apoptotic pathway of the host's cell, which prevents gastric cell growth. This is in line with previous findings of (16). We suggest the probability that DupA mutations might alter the ability of H. pylori to stimulate excessive IL-8 secretion by APCs.

In a study by Silva et al., (2021), they found that infection with dupA-positive H. pylori increases the risk of developing chronic gastritis in women (17).

In our population, we found a significant correlation between the presence of HopQ and the occurrence of gastritis, this result is in agrrement with (18). H. pylori's outer membrane is a bilayer semipermeable membrane, constituting inner phospholipid monolayer and outer bulky glycolipid lipopolysaccharide monolayer which represents porins that possess an active selectivity for antibiotics, nutrients, and enzymes. Porins and proteins act as protective antigens and are involved in host gastric tissue bacterial invasion, H. pylori equipped a large subset of outer membrane proteins that differ from that of other gram-negative bacteria, HopQ is the most important OMPs that act as an adhesin and play a vital role in bacterial binding to the gastric mucosa and promote colonization (19).

Predominantly, HopQ localized at the outer surface of H. pylori, we hypothesized the potential role of HopQ to facilitate the adherence activity to gastric epithelial cells.

The present study revealed a positive significant correlation between IceA activity and the occurrence of gastritis. This result was compatible with khater & AlFaki, (2022) who reported that the presence of the IceA genes was significantly associated with gastritis, stomach ulcers, and chronic active gastritis, with a p-value of 0.027, about chronic active gastritis, may proceed to gastric dysplasia, metaplasia and can develop to gastric cancer (20). The studies (21,22) linked the association of IceA presence to gastric ulcer and gastritis, while recent study from Iran imply no relation between IceA gene presence and gastritis (23). According to Roesler et al., (2014), the IceA genotype was linked with enhanced mucosal IL-8 expression and acute antral inflammation, in vitro studies demonstrated that adherence to gastric epithelial cells stimulates IceA transcription (24).

REFERENCES

1. Fan, L.; Li, R.; Li, H.; Zhang, J.; and Wang, L. genes in Helicobacter pylori isolated from gastric ulcer patients. 2018, 42(4):155-62. https//:doi.org/10.1515/labmed-2018-0059
2. Yong, X.; Tang, B.; Li, B.; Xie, R.; Hu, C.; Luo, G.: et al., Helicobacter pylori virulence factor CagA promotes tumorigenesis of gastric cancer via multiple signaling pathways. Cell Commun. Signal, 2015, 13(30):1-13.
3. AL-Abdul. A. A. Detection of Helicobacter pylori IgG in diabetic patients and non-diabetic. University of Thi-Qar, J. Sci., 2014, 4(3):53-57. https://doi.org/10.32792/utq/utjsci/vol4/3/17
4. Hooi, JKY.; Lai, WY.; Ng, WK.; Suen, MMY.; Underwood, FE.; Tanyingoh, D. ;et al., The global prevalence of Helicobacter pylori infection: systematicreview and meta-analysis. gastroenterol., 2017, 153(2):420-9. https://doi.org/10.1053/j.gastro.2017.04.022
5. Reshetnyak, VI.; Burmistrov, AI.; and Maev, IV. Helicobacter pylori: Commensal, symbiont, or pathogen? World J. Gastroenterol. ,2021 ,27)7:(545-60. https://doi.org/10.3748/wjg.v27.i7.545
6. AL-Hilfi N.; Mohameed, K.; Al-Hilfi, D.; and Ali, N. Accuracy of serological and stool antigen tests (non-invasive) for detection of H. pylori, J. Uni. Duhok 2021, 24(1):54-59. https://doi.org/10.26682/sjuod.2021.24.1.8
7. Alatbee, A. H. D. High prevalence of Helicobacter pylori in Basra city Southern of Iraq, J. Phys., 2019, 1279(2019):1-7. https://doi.org/10.1088/1742-6596/1279/1/012073
8. Kabamba, T, E.; and Yamaoka, Y. Helicobacter pylori infection and antibiotic resistance- iologyfrom bto clinical implications. Nat. Rev. Gastroenterol. Hepatol. ,2021 ,18)9:(613-29 . https//:doi.org/10.1038/s41575-021-00449- x
9. Ismail, M. B.; Osman, M.; Bou Raad, E.; Achkar, M.; and Hamze, M. Seroprevalence of Helicobacter pylori among dyspeptic patients in northern Lebanon: a 6-year retrospective study in two tertiary hospitals. Access Microbiol., 2022, 4(4):1-7. https://doi.org/10.1099/acmi.0.000337
10. Omid, T; Mobarez, MA; Hassan, MZ; Moazzeni, MS; and Ahmadabad, NH. In Vitro Suppression of Dendritic Cells by Helicobacter Pylori OipA. Helicobacter, 2014, 19:136-43. https://doi.org/10.1111/hel.12107
11. Saxena, K.; Kumar, A.; Chauhan, N.; Khanuja, M.; Malhotra, B. D.; and Jain, U. Electrochemical Immunosensor for Detection of H. pylori Secretory Protein VacA on g-C3N4/ZnO Nanocomposite-Modified Au Electrode. ACS Omega, 2022, 7:32292-32301. https://doi.org/10.1021/acsomega.2c03627
12. Costa, T. R. D.; Harb, L.; Khara, P.; Zeng, L.; Hu, B.; and Christie, P. J. Type IV secretion systems: Advances in structure, function, and activation. Mol. Microbiol., 2021, 115(3):436-452. https://doi.org/10.1111/mmi.14670
13. Sukri, A; Hanafiah, A; Kosai, NR; Mohammed, M; and Mohamed, R. New insight on the role of Helicobacter pylori cagA in the expression of cell surface antigens with important biological functions in gastric carcinogenesis. Helicobacter, 2022, 27(5). https://doi.org/10.1111/hel.12913
14. Costa, D. M.; Dos Santos Pereira, E.; and Rabenhorst, S. H. B. What exists beyond cagA and vacA? Helicobacter pylori genes in gastric diseases. World J. Gastroenterol., 2015, 21(37):10563-10572. https://doi.org/10.3748/wjg.v21.i37.10563
15. Neto, A. C.; Rasmussen, L. T.; Labio, R. W.; Queiroz, V. F. De; Smith, M. D. A. C.; Viani, G. A.; et al., Gene polymorphism of interleukin 1 and 8 in chronic gastritis patients infected with Helicobacter pylori. J. Venom. Ani. Tox. Tropic. Dis., 2014, 20(17):1-5. https://doi.org/10.1186/1678-9199-20-17
16. Pereira, W. N.; Ferraz, M. A.; Zabaglia, L. M.; de Labio, R. W.; Orcini, W. A.; Bianchi Ximenez, J. P. et al., Association among H. pylori virulence markers DupA, CagA, and VacA in Brazilian patients. J. Ven. Ani. Toxin. Tropi. Dis. 2014, 20(1):2-6. https://doi.org/10.1186/1678-9199-20-1
17. Silva, L. L.; Oliveira, A. K. S.; Gama, A. R.; Ramos, A. F. P. L.; Silva, A. M. T. C.; Blanco, A. J. V.; et al., Helicobacter pylori virulence dupA gene: risk factor or protective factor?. Brazil. J.Microbiol., 2021, 52(4):1921-1927. https://doi.org/10.1007/s42770-021-00553-9
18. Bauwens, E.; Joosten, M.; Taganna, J.; Rossi, M.; Debraekeleer, A.; Tay, A.; et al., In silico proteomic and phylogenetic analysis of the outer membrane protein repertoire of gastric Helicobacter species. Scientific Reports, 2018, September, 1-14. https://doi.org/10.1038/s41598-018-32476-1
19. Matos, R.; Amorim, I.; Magalhaes, A.; Haesebrouck, F.; Gartner, F.; and Reis, C. A. Adhesion of Helicobacter Species to the Human Gastric Mucosa: A Deep Look into Glycans Role. Front. Molec. Biosci., 2021, 8:1-12. https://doi.org/10.3389/fmolb.2021.656439
20. Khater, E. H., and AlFaki, A. Detection of vacA, cagA and iceA genes of H. pylori in dyspeptic patients and their association with clinical data and histopathological abnormalities. Egypt. J. Med. Microbiol., 2022, 31(3):99-107. https://doi.org/10.21608/ejmm.2022.251053
21. Kadi, RH.; Halawani, EM.; and Abdelkader, HS. Prevalence of H. pylori strains harbouring cagA and iceA virulence genes in Saudi patientswith gastritis and peptic ulcer disease. Microbiol. Discov., 2014, 2(1):1-6. https//:doi.org/10.7243/2052-6180-2-2
22. Abu-Taleb, A. M. F.; Abdelattef, R. S.; Abdel-Hady, A. A.; Omran, F. H.; El-Korashi, L. A.; Abdel-Aziz El-Hady, H. et al.,. Prevalence of Helicobacter pylori cagA and iceA Genes and their Association with Gastrointestinal Diseases. Int. J. Microbiol., 2018:1-7. https://doi.org/10.1155/2018/4809093
23. Sedaghat, H.; Moniri, R.; Jamali, R.; Arj, A.; and Zadeh, M. R. Prevalence of Helicobacter pylori vacA, cagA, cagE, iceA, babA2, and oipA genotypes in patients with upper gastrointestinal diseases. Iran J. Microbiol., 2014, 6(1):14-21. https://doi.org/10.1016/j.micpath.2017.02.018
24. Roesler, B. M.; Rabelo-Goncalves, E. M. A.; and Zeitune, J. M. R. Virulence factors of Helicobacter pylori: A review. Gastroenterol., 2014, 7:9-27. https://doi.org/10.4137/cgast.s13760