

Original Article

Investigating the Potential Association of *Helicobacter pylori* *cagA*, *vacA s1/s2*, *iceA1*, *iceA2*, *babA2*, *sabA*, and *oipA* genotypes with gastric disease severity

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Article Info

Abstract



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Helicobacter pylori is a pathogen that infects the gastric mucosa of the stomach and can be asymptomatic or lead to disorders ranging from gastric inflammation to gastric adenocarcinoma. This study aimed to find the association of *H. pylori* virulence factors and their combinations with the severity of the disease caused by the bacterium. This cross-sectional study involved 203 patients admitted to the gastroenterology units of the Rizgary Hospital, Erbil, Iraq from July 2021 to May 2022. Biopsy samples were taken, cultured and identified as *H. pylori* using biochemical and molecular approaches. PCR was employed to amplify the virulence genes *cagA*, *vacA s1/s2*, *iceA1*, *iceA2*, *babA2*, *sabA*, and *oipA*. The most common allelic combination found among the isolates was *s2/m1* detected in 33 (36.26%), followed by *s1/m1* which was detected in 17 (18.68%). Other genotypes *s2/m2* and *s1/m2* were recorded in 15 (16.48%) and 12 (13.18%) of the total samples respectively. While the *cagA* gene was present in 55/91 (60.43%), *iceA1* and *iceA2* were found in 70 (76.92%) and 54 (59.34%) of the tested isolates respectively. Furthermore, the results showed that only four isolates were positive for all virulence factor genes (4.39%). In conclusion, data produced from this study confirmed that the rate of the isolates with all virulence factors was very low. The presence of different virulence factors combination could be used to identify patients who are at high risk for the disease caused by the pathogen and its severity.

Keywords: *Helicobacter pylori*, Gastritis, Peptic ulcer, Genotypes, Virulence factor.

1. Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative microaerophilic bacterium, spiral-shaped and a highly motile pathogen, that infects the gastric mucosa of the stomach and causes several disorders that colonises more than half of the global population. Gastric cancer is the most universally fatal malignancy, with around 738,000 deaths each year[1-4]. The infections caused by this pathogen usually occur in childhood and last for many years or even a lifetime and can be asymptomatic or lead to disorders ranging from gastric inflammation and gastroduodenal ulceration to mucosa-associated lymphoid tissue B-cell lymphoma (MALT lymphoma) and gastric adenocarcinoma[1, 5]. This bacterium is a Group I carcinogen, according to reports from the World Health Organization. Studies suggest that eliminating *H. pylori* could potentially lower the risk of developing gastric cancer[1, 6]. Studies emphasize that geographic variations and individual differences influence the rate of infection and disease development associated with the bacterium. These variations are attributed to factors such as host genetics, dietary habits, and environmental conditions[7, 8]. Furthermore, genetic differences affecting the host's inflammatory responses influence the

severity of the inflammatory process, which in turn impacts the clinical outcomes of *H. pylori* infection[7, 8].

The pathogen has a variety of virulence factors that play a crucial role in its ability to adapt, colonize, and invade the epithelium. These factors include flagellin, urease, adhesin, vacuolating cytotoxin A (VacA), Cytotoxin-associated gene A (*cagA*), genes induced by contact with gastric epithelium (*iceA*), and outer membrane proteins (OMPs)[9]. The bacterium survives the acidic conditions of the gastric mucosa by producing urease, which neutralizes stomach acid. The ability of the pathogen to survive in these conditions is by the production of urease enzymes to neutralize the acidic condition[5, 10]. In addition to that, the high motility of the pathogen, its ability to penetrate gastric mucosa membrane and bind to the host cell receptors enhances invasion and establish a persistent infection[5, 10, 11]. Eventually, *H. pylori* release several effector proteins and toxins, such as VacA and CagA, which contribute to host tissue damage[5, 12]. The gastric epithelium also secretes chemokines that transactivate neutrophils and promote innate immunity. Eventually, clinical disorders such as ulcerative colitis develop gastritis. CagA has been considered a major pathogenic factor for

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H. pylori in these diseases[12]. Thus, CagA is an EPIYA-rich, high-molecular-weight protein encoded by the *cagPAI* pathogenicity island, which is a powerful immunogen. Once internalized, the toxin acts on gastric epithelial cells through direct translocation of the toxin with the type IV secretion system of the bacterium and binds to cellular effectors that in turn stimulate the activation of cell signaling pathways that induce pro-inflammatory and mitogenic responses[11, 12]. The role of CagA in the development of gastropathies appears to vary by geographical region. The prevalence of *cagA*-positive *H. pylori* strains in Turkey, Iran, and Iraq is 78%, 76%, and 71%, respectively, significantly influencing the incidence of peptic ulcer disease in these countries[8]. The *vacA* gene is found in the majority of strains and consists of three polymorphic regions: signal region *s1* and *s2*; intermediate region (*i1* and *i2*); and middle region (*m1* and *m2*)[9]. Allelic combinations of the *s1/s2* and *m1/m2* regions show considerable variation in the vacuolating activity of the different *H. pylori* strains[13, 14]. It has different actions such as cytoplasmic vacuole formation, mitochondrial damage, apoptosis, and modulation of immune signal transduction. This suggests that VacA production plays an important role in the colonization and persistence of the microorganisms in the human stomach[5, 13, 14]. Another factor related to the pathogenicity of the bacteria is the existence of the *iceA* gene, which turns on upon contact with the epithelium. This gene consists of two major allelic kinds: *iceA1* and *iceA2*. The *iceA1* gene is activated when *H. pylori* comes into contact with human epithelial cells, and its activation may be linked to the development of duodenal ulcer disease and an increase in acute neutrophilic infiltration, while the *iceA2* gene has no pathogenic effect[15, 16]. The *iceA1* allele is common in countries such as Saudi Arabia, Turkey, China, Japan, Vietnam, and Korea, while *iceA2* is predominant in Europe, Colombia, and America, suggesting a geographical distribution of *H. pylori* strains[16-19].

Moreover, outer inflammatory protein A (OipA), an inflammatory membrane protein belonging to the OMP family, is another recognized pathogenicity factor[11, 14, 20]. A strong association has been observed between a functioning *oipA* gene in *H. pylori* with gastric cancer, duodenal ulcers, and raised neutrophil infiltration [9, 11, 14, 21]. Numerous studies have investigated the relationship between geographic regions and *oipA* gene presence in clinical *H. pylori* isolates. For example, the presence of the *oipA* gene is associated with an increased risk of gastric cancer in populations from Colombia, China, and Japan, but its impact is lower in Iran[22]. In addition to that, the adhesive genes blood group antigen-binding adhesion (*babA*) and sialic acid-binding adherence (*sabA*) play an important role in the early stage of the infection and inflammation caused by *H. pylori*. These genes allow the bacteria to bind to the stomach mucosa membrane, essentially initiating the invasion process. Studies reported that these genes are not only involved in an early stage of colonization but also enable them to play an important role in the development of severe disease through the simulation of the immune response[5, 23]. Very few researches have been performed on the combination of the pathogen's virulence factors and their impact on the disease severity caused by the bacteria. Therefore, the purpose of this research is to examine the different combinations of these virulence factors on the severity of the infections caused by

the bacterium in this geographic area. Understanding the combinations of virulence factors in *H. pylori* is critical for identifying high-risk populations and tailoring treatment strategies, particularly in regions like Erbil where the prevalence of certain strains is higher.

2. Materials and methods

2.1. Collection of samples

A cross-sectional study was conducted on 203 patients admitted to the gastroenterology units of the Rizgary Hospital in Erbil City, Iraq during the period from July 2021 to May 2022. The sample size was determined using a predicted *H. pylori* prevalence of 60%, a 95% confidence level, and a 5% margin of error, applying the method (Cochran's). Samples were obtained from patients aged 17-71 years. In this study, the patients who received proton-pump inhibitors (PPI) or H2-receptor antagonists, bismuth compounds, and antimicrobial agents in the two weeks prior to the endoscopy and history of certain gastrointestinal surgeries were excluded. Biopsy samples of gastric tissue were obtained from the corpus and antrum of the patient's stomach and were transported immediately to the laboratory at 4°C using sterile phosphate-buffered saline.

2.2. Culturing of *H. Pylori*

Biopsy samples were mixed and homogenized before inoculating on Brain-Heart-Infusion (BHI) Agar (Oxoid, England) and Columbia agar medium (Sigma- Aldrich, USA) supplemented with 10% defibrinated sheep blood and Skirrow's selective supplement (Sigma-Aldrich, USA) at pH 7.0 - 7.2 and incubated at 37°C with a supply of 5%CO₂, 85%NO₂ and 5% O₂ for 5-7 days. Typical colonies were identified as *H. pylori* by morphology, Gram staining and biochemical tests (urease, oxidase, and catalase tests). Clinical isolates were preserved in cryotubes at -70 °C in Brucella broth (HiMedia, Mumbai, India) supplemented with 20% glycerol.

2.3. DNA extraction of *H. pylori*

DNA extraction was performed from pure colonies using the AddPrep Genomic DNA Extraction Kit (Add-Bio Inc-Korea) following the manufacturer's instructions. Extracted DNA samples were stored at -30 °C until use. DNA quality was determined by electrophoresis on 0.8% agarose gels and the A260/A280 absorbance ratio using a Thermo Scientific® NanoDrop 2000™ spectrophotometer.

2.4. Polymerase chain reaction (PCR)

PCR (GenAmp* PCR System 9700) technique was used for amplification of the 16S rRNA and virulence factors *cagA*, *vacA s1/s2*, *iceA1*, *iceA2*, *babA2*, *sabA*, and *oipA* genes (Table 1). PCR mixtures were prepared with 50 ng of genomic DNA, 100 μmol dNTPs, 2.5 μl of 10X PCR buffer, 1.0 mM MgCl₂, 1 U of Taq DNA, and 30 pmol of each of the primers. The reactions started with a denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing at (48-56) °C for 45 seconds and extension at 72 °C for 45 seconds, and a final extension step at 72 °C for 10 min. The products were analyzed by electrophoresis on 1.5% agarose gels at 80 volts for 50 minutes, and genotypes were recognized according to the PCR products and their sizes. In comparison to the standard DNA ladder as a molecular

Table 1. Sequences of oligonucleotide primers used for genotyping of *H. pylori*.

Genes	Forward Primer (5'-3')	Reverse Primer (5'-3')	Product size (bp)
<i>16S rRNA</i>	AGGGGTAAAATCCGTAGAGAT	CGTTTAGGGCGTGGACTA	133
<i>cagA</i>	GTTGATAACGCTGTGCTTC	GGGTTGTATGATATTTCCATAA	350
<i>vacAs1/s2</i>	ATGGAAATACAACAAACACAC	CTGCTTGAATGCGCCAAAC	259/286
<i>vacAm1/2</i>	CAATCTGTCCAATCAAGCGAG	GCGTCAAATAATTCCAAGG	567/642
<i>BabA2</i>	CCAAACGAAACAAAAGCG	GCTTGTGTA AAAAGCCGTCGT	259
<i>iceA1</i>	GTGTTTTTAACCAAAGTATC	CTATAGCCACTTTCTTTGCA	246
<i>iceA2</i>	GTTGGGTATATCACAATTTAT	TTACCCTATTTTCTAGTAGGT	229
<i>oipA</i>	CAAGCGCTTAACAGATAGGC	AAGGCGTTTTCTGCTGAAGC	433
<i>sabA</i>	TTTTGTGTCAGCTACGCGTTC	ACCGAAGTGATAACGGCTTG	581

weight marker (100 bp).

2.4. Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 27 software was used. The chi-square and Fisher’s exact test were used to compare *H. pylori* virulence genotypes with clinical outcomes and among virulence factors. A value of $p < 0.05$ was considered significant.

3. Result

A total of 203 patients with dyspepsia were enrolled and underwent an endoscopy, from which 137 patients showed *H. pylori* positive by rapid urease test, comprising 90 female patients and 47 male patients with age ranges of 17-71 years (mean age 39.971 ± 14.3) and 21-58 years (mean age 34 ± 11.5), respectively. The results of the endoscopic diagnosis showed that 43 patients had peptic ulcer disease, and 92 patients had chronic gastritis. Only 91 *H. pylori* isolates were successfully cultured from biopsy samples in the present study. Conventional microbial tests were used for identification purposes. The *H. pylori* isolates appeared in a spiral shape under a microscope and were positive for oxidase, catalase and urease, and all of them were further confirmed as positive for *H. pylori* using *16S rRNA* gene amplification by PCR (Fig. 1).

Results produced from this study showed that not all isolates were positive for *vacA* gene, 87.9% and 85.7% had amplified *s* and *m* regions respectively, regarding *s1* and *s2*, they were present in 32.96% and 54.94%, while *m1* and *m2* were present in only 57.14% and 28.57% of the isolates respectively. The most common allelic combination, *s2/m1*, which was detected in 36.26% of the isolates, while *s1/m1* was found in 18.68%; and the other genotypes, *s2/m2* and *s1/m2*, were recorded in 16.48% and 13.18% of the isolates respectively. While the *cagA* gene was detected in 60.43% of isolates, whereas the *iceA* was present in most samples tested, 76.92% and 59.34% tested positive for *iceA1* and *iceA2*, respectively; furthermore,

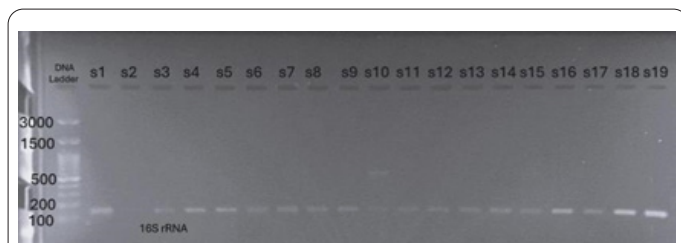


Fig. 1. Amplification of 16S rRNA using PCR and 1.5 agarose gel electrophoresis. compared to represents the DNA ladder as a molecular weight marker (100 bp).

49.45% were double positive for *iceA1/2*. The overall prevalence of the *babA2* genotype was observed in 59.34% of samples. Additionally, the *oipA* and *sabA* genes were detected in 73.62% and 47.25% of samples respectively, while only 4.39% possess all virulence factors. Figure 2 shows an example of the PCR product of the *cagA* 350 bp and *babA2* 259 bp genes. Also Figure 3 shows the example of the PCR product of *oipA* 433bp, *sabA* 581bp, *vacAs1/s2* 259/286bp, *vacAm1/m2* 567/642bp. Moreover Figure 4 show the example of the PCR product of *iceA1/iceA2* 246/229bp.

The present results showed some variances in the percentage distribution of *H. pylori* virulence factors according to gender, in which, *vacAs1*, *iceA1*, *iceA2*, *oipA*, and *sabA* were higher in males while *vacAs2*, *vacAm1*, and *babA2* were higher among females.

Table 2 demonstrates significant differences in the relationship between the clinical status of the patients and different virulence genes. The frequency of *vacAs1* and *vacAs1m1*, virulence factors was significantly higher in patients with peptic ulcer disease who tested positive for *H. pylori* compared to gastritis disease tested positive for *H. pylori* (*vacAs1*: $P < 0.001$.; *vacAs1m1*: $P < 0.001$).

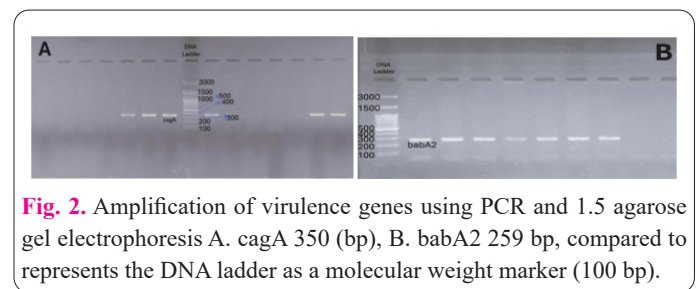


Fig. 2. Amplification of virulence genes using PCR and 1.5 agarose gel electrophoresis A. *cagA* 350 (bp), B. *babA2* 259 bp, compared to represents the DNA ladder as a molecular weight marker (100 bp).

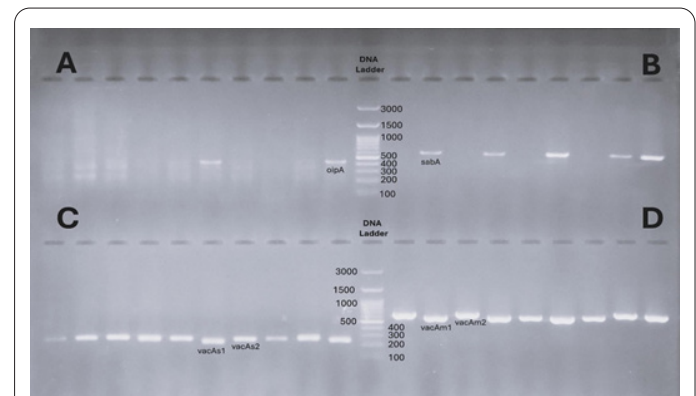


Fig. 3. Amplification of virulence genes using PCR and 1.5 agarose gel electrophoresis A. *oipA* 433bp, B. *sabA* 581bp, C. *vacAs1/s2* 259/286bp, D. *vacAm1/m2* 567/642bp, compared to represents the DNA ladder as a molecular weight marker (100 bp).

Additionally, our data showed a high percentage of *cagA*, *vacAs2m1*, *iceA2*, *oipA*, and *sabA* in gastritis cases compared to peptic ulcer cases (Table 2). However, the frequency of *vacAs2* ($P < 0.001$), *vacAs2m2* ($P < 0.001$), and *iceA1* ($P < 0.001$) virulence factors was significantly higher in *H. pylori*-positive patients with gastritis illness than in *H. pylori*-positive subjects with peptic ulcer illness (Table 2).

The degree of gene-to-gene *cagA* with *babA2*, *iceA1*, *vacA* and subtype *vacAs1/m1* association was evaluated as shown in Table 3. There was no significant correlation between *cagA* with the *iceA1* gene ($P = 1$), *babA2* ($P = 0.08$) and subtype *vacAs1/m1* gene ($P = 0.417$), *babA2* was present in 40.65% while, *babA2* was present in 18.68% of *cagA* negative samples.

Also, the frequency of *cagA/vacA* s1/m1 alleles more in *H. pylori*-positive subjects with peptic ulcers compared

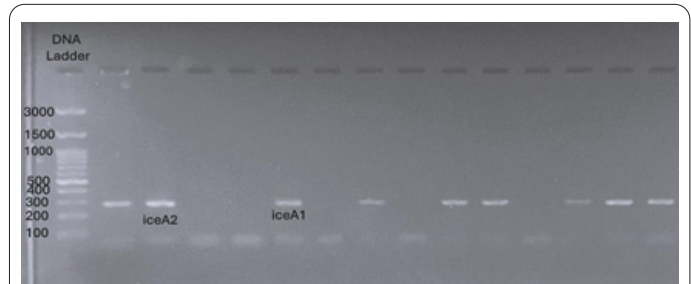


Fig. 4. Amplification of virulence genes using PCR and 1.5 agarose gel electrophoresis *iceA1/iceA2* 246/229bp, compared to represents the DNA ladder as a molecular weight marker (100 bp).

with *H. pylori*-positive subjects with gastritis was statistically significant ($P = 0.01$) (Table 4). The percentage of *cagA/iceA1* alleles with a positive genotype was significantly higher in *H. pylori*-positive patients with gastritis

Table 2. Relationship between the clinical status of *H. pylori* infection and different virulence genes.

Genotype	Total	Gastritis N.= 54		Peptic ulcer N.= 35		P value
		No.	%	No.	%	
<i>cagA</i>	89	35	64.81	19	54.28	0.32
<i>vacAs1</i>	89	10	18.51	20	57.14	<0.001*
<i>vacAs2</i>	89	39	72.22	9	25.71	<0.001*
<i>vacAs1m1</i>	89	4	7.4	13	37.14	<0.001*
<i>vacAs1m2</i>	89	6	11.11	6	17.14	0.41
<i>vacAs2m1</i>	89	24	44.44	9	25.71	0.07
<i>vacAs2m2</i>	89	15	27.77	0	0.0	<0.001*
<i>iceA1</i>	89	48	88.88	20	57.14	<0.001*
<i>iceA2</i>	89	34	62.96	20	57.14	0.58
<i>babA2</i>	89	32	59.25	22	62.85	0.73
<i>oipA</i>	89	41	75.92	24	68.57	0.44
<i>sabA</i>	89	23	42.59	18	51.42	0.41

N: number, %: percentage, * $P < 0.05$

Table 3. Association between *cagA* with *IceA*, *babA2*, *vacA* and *vacAs1/m1* genes.

	<i>cagA</i> +		<i>cagA</i> -		P value
	N	%	N	%	
<i>IceA1</i> +	42	46.15	28	30.76	1
<i>IceA1</i> -	13	14.28	8	8.79	
<i>babA2</i> +	37	40.65	17	18.68	0.08
<i>babA2</i> -	18	19.78	19	20.87	
<i>vacA</i> +	55	60.43	25	27.47	<0.001*
<i>vacA</i> -	0	0.00	11	12.08	
<i>vacAs1/m1</i> +	12	13.18	5	5.49	0.417
<i>vacAs1/m1</i> -	43	47.25	31	34.06	

N: number, %: percentage, * $P < 0.05$

Table 4. Association between *cagA* with *vacAs1/m1*, *IceA1*, *babA2*, *sabA* and *oipA* genes in different clinical statuses N: number, %: percentage, * $P < 0.05$.

	Gastritis		Peptic ulcer		P value
	N	%	N	%	
<i>vacAs1m1/cagA</i> +	3/35	8.57	8/19	42.1	0.01*
<i>IceA1/cagA</i> +	33/35	94.28	9/19	47.36	<0.001*
<i>babA2/cagA</i> +	22/35	62.85	15/19	78.94	0.35
<i>SabA /cagA</i>	15/35	42.85	10/19	52.63	0.57
<i>oipA /cagA</i>	27/35	77.14	15/19	78.94	1

diseases than with peptic ulcer diseases ($P < 0.001$). The results also showed a high percentage of *cagA*, *cagA/sabA*, and *cagA/oipA* in gastritis cases compared to peptic cases (Table 4).

4. Discussion

In this study, we found a high prevalence of the *vacA* (87.9%) and *cagA* (60%) genotypes, with significant geographic variations in the prevalence of virulence factors. These findings align with some regional studies but also present unique observations regarding the association of specific virulence factors with clinical outcomes.

H. pylori colonizes the mucosal epithelium of the stomach and can cause severe gastric diseases [1]. Several *H. pylori* virulence factors, such as the *vacA*, *cagA*, *iceA*, *oipA*, and *babA2* genes have been reported to be associated with bacterial pathogenicity and severe outcomes[23]. In this study, 67.48 % of the biopsy-positive individuals were detected by the rapid urease test, and 66.42% of the biopsy-positive individuals were grown successfully and detected by colony morphology, gram staining, biochemical tests and PCR assay. The low number of culture-positive results could be due to the differences in biopsies used in the analysis, sampling error occurs when the infection is not evenly dispersed within the mucosa, culturing and environmental conditions. Several factors have an impact on the successful isolation and cultivation of *H. pylori* from gastric biopsy specimens, such as the quality of the clinical specimens, the unequal distribution of the pathogen within the mucosa, (PPI), antibiotics, the period between sampling and culture, and the incubation environment for biopsy samples [24].

The distribution of *H. pylori* infection and virulence factors varies between regions. In Iraq, a variation in *H. pylori* prevalence has been reported at 47.8% in central Iraq [25]. The present study revealed that the *cagA* gene was present in 60% of the subjects, and the frequency of the *cagA* genotype was lower than that reported by Hussein *et al.* [26]. Could this difference be due to factors like sample population, regional strain differences, or other environmental or genetic factors. These findings are similar to a meta-analysis by Šterbenc *et al.*[11] which compared populations from the Middle East and Western countries and reported that *cagA* is detected in nearly 50% to 60% of strains. However, other studies reported different percentages of the *cagA* gene in different regions [6, 27]. The role of *CagA* in the development of gastropathies appears to vary by geographic region, furthermore, Geographical differences in nutrition, human genetic traits, or other environmental factors may also have an impact on the relative prevalence of *cagA* PAI-positive strains within different geographic areas [7, 8].

This study demonstrated a high prevalence of *vacA* genotypes (87.9%) predominantly of the subtypes *s2/m1*. The frequency of the *vacA* genotype in this study was higher than that reported in Iraq by Al-Ouqaili *et al.*[28]. Similarly, Dabiri *et al.*[27]reported a high prevalence of *vacA*. The frequency of the *vacA* genotype and subtype *s1m2* and *s1m1* genotype was higher than that reported by Xue *et al.* [19] but lower than that found in Iran and Saudi Arabia [17, 21]. Furthermore, the genotype possessing the combination *s1/m1* shows strong vacuolating activity[5]. Studies indicate that *vacA* subtypes associated with clinical outcomes differ across geographic regions [11, 14].

The prevalence of *iceA1*-positive *H. pylori* strains varies from one country to another, as reported by different studies, such as 84% in Korea[6], 58% in Turkey [18], and 42.2% in Saudi Arabia[17], and it is believed that a more severe clinical outcome of the infection is associated with the presence of the *iceA1* gene[12], this study observed that the *iceA1* gene and the *iceA2* gene were present in 76.9% and 59.3% respectively. The predominance of *iceA1* in this study is similar to findings from a study in Indonesia[29]. The prevalence of the *babA2* gene was (59.34%), which is comparable to the prevalence studies in Korea (79%)[6] and Iran (78%)[27]. Despite the *babA2* gene being related to gastric cancer, data from this study could not confirm this relationship similar results were found by Dabiri *et al.*[27]. Regarding other genes, the *oipA* genotype and the *sabA* genotype were found in 73.62% and 47.25%, respectively. Other studies reported variable rates of *oipA* genotypes[20, 21]. The prevalence of *sabA*-positive strains obtained in this study is similar to research conducted in China[30]. These results showed a similar percentage in the distribution of the *H. pylori cagA* and *iceA1* genotypes between males and females, which agrees with data produced from Saudi Arabia[17]. However, the present study observed that gender variations were present in some *vacA s1/2* and *m1/2* subtypes. The data showed a high rate of the less virulent *vacA m1* among females (58.73%), compared to (46.42%) in males. This finding contradicts other reports from Saudi Arabia and Morocco[17, 31].

In gastritis patients, the presence of *cagA* was 66.66%, whereas in peptic ulcer patients, it was 51.42%. The relationship between *cagA* presence and the emergence of gastritis and peptic ulcer is statistically non-significant, supporting *cagA* function as a marker for elevated *H. pylori* virulence. These findings are in good agreement with other studies[32].

Numerous studies have indicated that patients with *cagA*-positive *H. pylori* have an elevated risk of gastric cancer[5]. In this study, *vacA* was detected in high levels of *H. pylori*-positive gastritis and peptic ulcer patients. The frequency of the *vacA s1m1* allele was significantly higher in peptic ulcer patients compared to those with gastritis. These findings align with a meta-analysis by Chang *et al.*[23], who reported that *vacA s1m1* genotypes are associated with an increased risk of peptic ulcer disease (PUD). Conversely, elevated levels of *s2m2* were observed in gastritis patients, similar to findings reported in Turkey[18]. Several studies have recognized the relationship between *vacA* genotypes (*s1m1* and *s1m2*) and severe gastric outcomes, supporting the association of *vacA* with clinical severity[8, 22, 23]. However, no significant differences were observed between *iceA1*, *iceA2*, *sabA*, and *oipA* genes and clinical outcomes. These findings were in good agreement with findings from Iran and Turkey [18, 21], despite the fact that these virulence factors increase the risk of gastritis and ulcers in *H. pylori*-infected patients[1, 5, 19], as they have been identified to play an important role in the pathogenicity of the bacteria.

Moreover, the findings of the present study presented a good correlation between *cagA*-positive samples and *babA2*-positive samples in agreement with another study in Iran [27]. The level of gastritis is greatly influenced by the co-expression of the *cagA* and *babA* genes in addition to the bacterial coccoid form[1]. Our study also revealed that mixed *cagA* and *iceA1* genotypes were present in 46.15%

of the isolates, and both genes can serve as indicators for gastrointestinal diseases. This finding aligns with a study from Egypt [33]. A statistically significant association was observed between *cagA* and *vacA* genes, with the *vacA* gene present in 100% of *cagA*-positive samples. However, there was no significant association between *cagA* and the *vacA* *s1/m1* subtype, consistent with another study in Iran [27].

The current investigation also showed a statistical association between combination virulence factors and the clinical status of the patients. The distribution of *cagA* with genotype *vacAs1/m1* was 42.1% in peptic ulcer cases and 8.57% in gastritis cases. The relationship between the presence of combination *cagA* with *vacAs1/m1* and the development of gastritis and peptic ulcers is statistically significant, consistent with studies from Egypt and Ecuador [34, 35]. Among these combinations, *vacA* genotype *s1m1* is the most virulent combination, while toxin production is moderate in genotype *s1m2* strains. Phosphorylated *cagA* in gastrointestinal epithelial cells includes pro-inflammatory and mitogenic responses that affect multiple cell functions and signaling pathways [23]. Additionally, the combination of *cagA* with *babA*, *oipA*, and *sabA* was higher in peptic ulcer cases than gastritis, co-expression of *babA* with *cagA* results in significant inflammation of the stomach mucosa [36]. Šterbenc *et al.* [11] suggested a significant association between *sabA* gene and both *cagA* virulence factors. Additionally, the *babA*, while *oipA* and *cagA* virulence factors have been considered to have a synergistic effect on the pathogenesis induced by *H. pylori* [5, 37].

The frequency of *cagA*+/*iceA1* combination genotypes was significantly higher in gastritis patients compared to peptic ulcer patients, conversely, other studies found that the *cagA* and *iceA1* alleles were found more frequent in peptic ulcer cases than gastritis cases [38]. The finding by Abu-Taleb *et al.* [33] suggested that the *cagA* gene may be regarded as a predictor for the presence of the *iceA* gene. Limitations to this study were the time for collecting samples due to the study being performed as a part of PhD study, getting samples from the whole country and larger sample sizes will surely end up with more informative and better understanding of the data explanations.

5. Conclusion

The findings of this study confirmed that the rate of the isolates with all virulence factors was very low. Different types of virulence factors and their combination could be used to identify patients who are at high risk for the disease caused by the pathogen and its severity. The most noticed genotype combinations in strains explored in this study were *IceA1/cagA* genotype and they were frequently associated with gastritis compared to peptic ulcer disease, while *babA2/cagA* and *oipA/cagA* genotypes were presented higher with peptic ulcer compared to gastritis. The association between these virulence factors with clinical status, the distribution of *cagA* with genotype *vacAs1/m1* in peptic ulcer cases and gastritis cases. This study suggests that further investigation is necessary using larger sample sizes and in the whole country to better understand the genetic diversity of this pathogen in terms of their virulence genes.

Abbreviation

Helicobacter pylori (*H. pylori*), vacuolating cytotoxin A (VacA), signal regions (*s1* and *s2*), intermediate regions (*i1* and *i2*), and middle regions (*m1* and *m2*). cytotoxin-associated gene A (*cagA*), induced by contact with gastric epithelium (*IceA*), outer inflammatory protein A (*OipA*), blood group antigen-binding adhesion (*BabA*), and sialic acid-binding adhesion (*SabA*). Polymerase Chain Reaction (PCR).

Conflict of interest

The authors declare that they have no conflict of interest
Consent for publications

All authors confirm that they have read and approved the final version of the manuscript for publication. This consent is provided by Salah Balaky and Karwan Othman.

Ethical aspects

All participants agreed to contribute and signed the informed consent form. The research was approved by the Ethics Committee, College of Health Sciences, Hawler Medical University (ERB 1459/2021), following the regulations of the Declaration of Helsinki.

Informed consent

Informed consent was obtained from all patients undergoing stomach biopsy for this study. Participants were thoroughly informed about the study's aims, procedures, and potential risks. They were assured of their right to withdraw at any time without consequences, ensuring transparent and ethical participation.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

Karwan Othman performed the practical part of the study, analyzed data using related software and appropriately investigated the accuracy and integrity of any part of the work. Salah Balaky designed the work idea, drafted the work or reviewed it critically for important intellectual content, guided thoroughly and finally approved the manuscript version to be published.

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