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Genotypic Profiling and Clinical Impact of *Helicobacter pylori* Virulence Genes (*GLM*, *HPU*, *VacA*, *CagA*, and *IceA*) in Gastroduodenal Diseases among Libyan Patients

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Abstract

Helicobacter pylori infection represents a widespread chronic condition with varying prevalence influenced by race, ethnicity, and geography. The severity of *H. pylori*-associated diseases is determined by an array of virulence factors. Although extensive studies have been conducted globally, data on the distribution of *Helicobacter pylori* virulence genes in Libya remain limited, constraining insights into the pathogenicity of local strains and hindering the development of targeted interventions. This study aimed to evaluate the prevalence of *H. pylori* infection, characterize essential virulence genes [*vacA* variants (s1/s2, m1/m2), *cagA*, and *iceA*1], and examine their association with gastroduodenal diseases among Libyan patients. Gastric biopsies from 144 participants were analyzed using polymerase chain reaction (PCR) assays, and risk factor data were collected via questionnaires. *H. pylori* was detected in 63.2% of samples by PCR. The *vacA* gene was present in 84.6% of cases, *cagA* in 58.2%, and *iceA*1 in 29.7%. Among *vacA* variants, s1 allele was most common (53.2%), followed by m1 (42.9%), m2 (37.7%), and s2 (13%) alleles. Significant associations were identified between specific virulence genes and the development of gastroduodenal diseases, highlighting their role in pathogenicity. This investigation is one of Libya's first comprehensive assessments of *H. pylori* virulence factors, addressing a critical epidemiological gap. The high prevalence of virulence genes

suggests their potential as disease biomarkers. These findings contribute to a deeper understanding of *H. pylori* pathogenicity within the Libyan population and establish a basis for future clinical interventions and public health strategies to manage and prevent *H. pylori*-associated diseases in Libya and comparable regions.

Keywords

H. pylori, Virulence Genes, Gastroduodenal Diseases, Gastric Biopsy, PCR

1. Introduction

Helicobacter pylori is a gram-negative, spiral-shaped, microaerophilic bacterium that colonizes the human stomach [1]. Globally, over 50% of the world's population is infected with *H. pylori* [2], yet more than 80% of infected individuals remain asymptomatic [3]. The prevalence exhibits significant geographical variation; infection rates in developing countries can exceed 85%, substantially higher than the approximately 30% - 40% observed in Europe and North America [4]. *H. pylori* is classified as a definite carcinogen due to its association with gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [5]. Additionally, it has been implicated in non-gastrointestinal diseases such as chronic cardiovascular disease and colorectal cancer [6].

H. pylori strains exhibit high phenotypic and genotypic diversity, particularly concerning virulence factors that enhance pathogenicity [7]. This genetic diversity complicates the selection of target genes for detection; even highly conserved sequences like 16S rRNA and *ureA* may fail to detect specific strains. The diversity results from point mutations, substitutions, insertions, and deletions in the genome [8].

The pathogenesis involves several virulence factors facilitating colonization, inflammation, and host cell damage [9]. Key factors include the urease enzyme, flagella, adhesins, cytotoxin-associated gene A (*cagA*), vacuolating cytotoxin A (*vacA*), and the induced by contact with epithelium gene (*iceA*) [10]. Urease neutralizes gastric acid, aiding bacterial survival [11]. Flagella-mediated motility and chemotaxis allow navigation toward the epithelial surface. Adhesion is mediated by outer membrane proteins like blood antigen-binding adhesin (BabA and BabB) and sialic acid-binding adhesin (SabA) [12] [13]. Among key virulence factors, the *vacA* gene exhibits allelic diversity in its signal (s1, s2) and middle (m1, m2) regions [14]. *vacA* induces vacuole formation, disrupts mitochondrial function, and promotes apoptosis (Palframan *et al*., 2012). Strains with *vacA* s1/m1 genotypes are associated with more severe disease outcomes [2]. The *cagA* gene encodes a protein injected into host cells via a type IV secretion system, disrupting cellular signaling pathways [15], and is linked to gastritis, peptic ulcer disease, and increased gastric carcinoma risk [16]. The *iceA* gene is associated with increased interleukin-8 production and acute inflammatory responses [17].

Accurate diagnosis of *H. pylori* infection is essential for effective treatment. Molecular methods like polymerase chain reaction (PCR) offer high sensitivity and specificity, detecting even low bacterial loads and identifying specific virulence genes [18]. Target genes for PCR include housekeeping genes such as 16S rRNA, *glmM*, and *hpu*, as well as virulence genes like *vacA*, *cagA*, and *iceA*. Combining PCR with other diagnostic methods enhances detection accuracy [19].

Despite the high global prevalence of *H. pylori*, there is a lack of comprehensive data on the distribution of its virulence genes in Libya, particularly in Benghazi. Most previous studies have focused on histopathology and serology, with limited molecular characterization of virulence factors. Understanding the prevalence and distribution of virulence genes such as *glmM*, *hpu*, *vacA*, *cagA*, and *iceA* is crucial for assessing the pathogenic potential of circulating strains and tailoring appropriate diagnostic and therapeutic strategies. To date, there have been no published data on the virulence genes of *H. pylori* in Libya, highlighting the need for this research. The primary objective of this study is to detect *Helicobacter pylori* and its virulence genes (*glmM*, *hpu*, *vacA*, *cagA*, and *iceA*) among patients with gastroduodenal diseases in Benghazi Medical Center, Libya, using PCR.

2. Materials and Methods

2.1. Patient Recruitment

One hundred and forty-four subjects suffering from upper gastrointestinal tract symptoms were enrolled in this study. Ten subjects were considered negative control cases [non-ulcer dyspepsia (NUD) patients with normal findings]. The patients were diagnosed by specialist physicians and recruited accordingly. Demographic information—including age, sex, marital status, literacy status, residential status, and socioeconomic status—was obtained by referring to medical records and conducting personal interviews.

Throughout the study period, 73 women and 71 men aged between 18 and 80 years (mean age 51.09 ± 15.39 years) presented with dyspepsia and were referred to the Esophago-Gastroduodenoscopy Unit at Benghazi Medical Center (B.M.C.) for upper endoscopy between March 2020 and February 2022. Based on the endoscopic examination, patients were grouped into six categories: gastritis, peptic ulcer disease (PUD), gastric tumor cancer (GC), mucosa-associated lymphoid tissue (MALT) lymphoma, other gastric diseases (including gastroenteropathy, gastric angiodysplasia, and grade 2 esophagitis), and normal oesophago-gastro-duodenoscopy (OGD) findings. Normal OGD patients were defined as those who had no endoscopic lesions of ulcers and/or malignancies.

The protocol of this study was approved by the Ethics and Research Committees of the hospital, and all patients gave informed consent to the study according to the Declaration of Helsinki (WHO, 1993).

2.2. Sample and Biopsy Collection

To collect samples from the targeted population and avoid any misinterpretation

of the results, the following conditions were applied: patients had to be adults suffering from various dyspeptic symptoms and must not have received nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, H_2 receptor antagonists, or any proton pump inhibitors for at least one month prior to the study. Patients were followed up by their physicians, and all upper endoscopy abnormalities were recorded. Clinical specimens were collected, stored, and transported according to established protocols.

Four antral biopsies were collected during upper endoscopy: one biopsy for the rapid urease test (RUT), taken from a position adjacent to normal gastric epithelium within 5 cm of the pylorus to avoid gastric atrophy or gastrointestinal metaplasia, with a thickness of about 1.8 to 3.3 mm; the other three biopsies, each about 5 mm thick, were used for molecular investigations targeting 16S rRNA, GLM, HPU, *vacA*, *cagA*, and *iceA* genes. The three biopsy specimens for molecular analysis were preserved immediately at −80˚C.

2.3. Extraction of DNA from Biopsy Specimens

According to the manufacturer, the QIAamp Tissue DNA Extraction Kit was used to extract genomic DNA from frozen biopsy specimens. Briefly, 25 mg of the crushed biopsy specimen was transferred into a microcentrifuge tube, followed by the addition of 180 µL of lysis buffer ATL. Then, 20 µL of proteinase K solution was added, the mixture was vortexed, and then incubated at 56˚C until the tissue was completely lysed. Lysates were spun at 8000 rpm for 15 seconds, and then 200 µL of AL buffer was added, vortexed, and incubated at 70˚C for 10 minutes. Ethanol (96% - 100%) 200 µL was added to the sample, vortexed for 15 seconds, and spun at 8000 rpm for 15 seconds. The lysates were then applied to the QIAamp Mini spin column and spun at 8000 rpm for 1 minute. Five hundred microliters of wash buffer AW1 were added to the QIAamp Mini spin column and spun at 8000 rpm for 1 minute. The column was transferred to a clean collection tube, and then 500 µL of wash buffer AW2 was added to the column and spun at 14,000 rpm for 3 minutes. The column was transferred to a new Eppendorf tube, and the DNA sample was eluted by adding 200 µL of buffer AE directly onto the membrane of the column. The column was left for 5 minutes at room temperature and then spun at 8000 rpm for one minute. Genomic DNA samples were stored at −20˚C for further investigations.

2.4. Molecular Confirmation of *H. pylori*

Polymerase chain reaction (PCR) was performed on extracted DNA from biopsies using primers specific for *H. pylori* 16S rRNA under the following conditions: initial denaturation at 95˚C for 5 minutes, followed by 37 cycles of denaturation at 95˚C for 60 seconds, annealing at 60˚C for 60 seconds, and extension at 72˚C for 60 seconds, with a final extension time of 72˚C for 5 minutes. The PCR amplification was performed using a thermocycler system (T3 thermocycler, Whatman Biometra, USA). Each 25 µL PCR reaction mixture contained 12.5 µL of PCR

master mix (Promega, GoTaq® Green Master Mix, USA), 1.5 μL of each primer (Metabion, Planegg, Germany), 3 μL of template DNA, and 6.5 μL of PCR-grade water. For each PCR experiment, appropriate positive and negative controls were included. The *H. pylori* strain J99 and nuclease-free water were used as positive and negative controls, respectively.

2.5. Detection of PCR Amplicons

To detect the amplified product, 5 μL of amplicons were visualized by electrophoresis through a 2% agarose gel (Promega, USA) at 100 V for 60 minutes in 1 × TBE buffer and stained with ethidium bromide (500 ng/mL) (Sigma-Aldrich, USA) using the gel photo documentation system (Bio-Rad Laboratories, Inc., American developer). The bands were identified by comparing the band sizes with molecular weight markers (Promega, USA). Samples were considered positive when the visible band was the same size as the positive control DNA. The primer for the 110 bp product of the 16S rRNA sequence was represented by the forward primer sequence: 5'-CTG GAG AGA CTA AGC CCT CC-3' and the reverse one: 5'-ATT ACT GAC GCT GAT TGT GC-3' (Metabion International AG, Germany).

2.6. Detection of *H. pylori* Virulence Genes

Virulence genes, including GLM, HPU, *vacA* (subtypes: s1, s2, m1, and m2), *cagA*, and *iceA*, were detected using PCR-specific primers (Table 1) with the same amplification conditions and assay protocol as described earlier.

Table 1. Primer sequences for PCR detection of *H. pylori* virulence genes.

Target Genes	Primer Sequences (5'-3')	Amplicon Size (bp)
16S rRNA-F	5'-CTG GAG AGA CTA AGC CCT CC-3'	110
16S rRNA-R	5'-ATT ACT GAC GCT GAT TGT GC-3'	
GlmM-F	5'-AAG CTT TTA GGG GTG TTA GGG GTT T-3'	294
GlmM-R	5'-AAG CTT ACT TTC TAA CAC TAA CGC-3'	
HPU1	5'-GCC AAT GGT AAA TTA GTT-3'	411
HPU2	5'-CTC CTT AAT TGT TTT TAC-3'	
cagA-F	5'-AAT ACA CCA ACG CCT CCA-3'	400
cagA-R	5'-TTG TTG CCG CTT TTG CTC TC-3'	
vacA (s1/s2)-F	5'-ATG GAA ATA CAA CAA ACA CAC-3'	259/286
vacA (s1/s2)-R	5'-CTG CTT GAA TGC GCC AAA C-3'	
vacA (m1/m2)-F	5'-CAA TCT GTC CAA TCA AGC GAG-3'	570/642
vacA (m1/m2)-R	5'-GCG TCT AAA TAA TTC CAA GG-3'	
*iceA*1-F	5'-GTG TTT TTA ACC AAA GTA TC-3'	558
*iceA*1-R	5'-CTA TAG CCA ATT TCT TTG CA-3'	

2.7. Statistical Analysis

Data were analyzed using chi-square tests to determine the association between *H. pylori* positivity and epidemiological risk factors, virulence factors, and gastroduodenal disease diagnosis. Briefly, a chi-squared test was used to compare frequencies between various groups, and Fisher's exact test was used to analyze two-by-two tables of categorical data. The relationship between virulence genes, clinical outcomes, age, and gender was evaluated using two-way ANOVA. *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1. Patient Demographics and Clinical Characteristics

A total of 144 patients were enrolled in this study, comprising 73 females and 71 males, with a mean age of 51.09 ± 15.39 years (range: 18 - 80 years). The distribution of gastroduodenal diseases diagnosed via esophagogastroduodenoscopy (EGD) is detailed in Table 2. Gastritis was the most prevalent condition, observed in 66 patients (45.83%), followed by peptic ulcer disease (PUD) in 26 patients (18.06%), mucosa-associated lymphoid tissue lymphoma (MALT lymphoma) in 7 patients (4.86%), and gastric adenocarcinoma in 3 patients (2.08%). Other conditions—including gastroenteropathy, gastric angiodysplasia, grade 2 esophagitis, and gastroesophageal reflux disease (GERD)—were identified in 20 patients (13.89%). Normal EGD findings were reported in 22 patients (15.28%).

Table 2. Distribution of gastroduodenal diseases among the study population.

Endoscopic Finding	Number of Patients (%)
Gastritis	66 (45.83%)
Peptic ulcer disease	26 (18.06%)
MALT lymphoma	7 (4.86%)
Gastric adenocarcinoma	3 (2.08%)
Other conditions	20 (13.89%)
Normal findings	22 (15.28%)
Total	**144 (100%)**

3.2. Gender-Specific Distribution of Gastroduodenal Diseases

The mean age of male patients was 52.66 ± 13.41 years (range: 18 - 78 years), and that of female patients was 49.58 ± 17.14 years (range: 18 - 80 years). The gender-specific distribution of gastroduodenal diseases is presented in Figure 1. Gastric adenocarcinoma was observed exclusively in males (3/3, 100%). MALT lymphoma was more prevalent in males (6/7, 85.71%) compared to females (1/7, 14.29%). Conversely, gastritis and normal EGD findings were more common in females, accounting for 60.61% and 63.64% of cases, respectively.

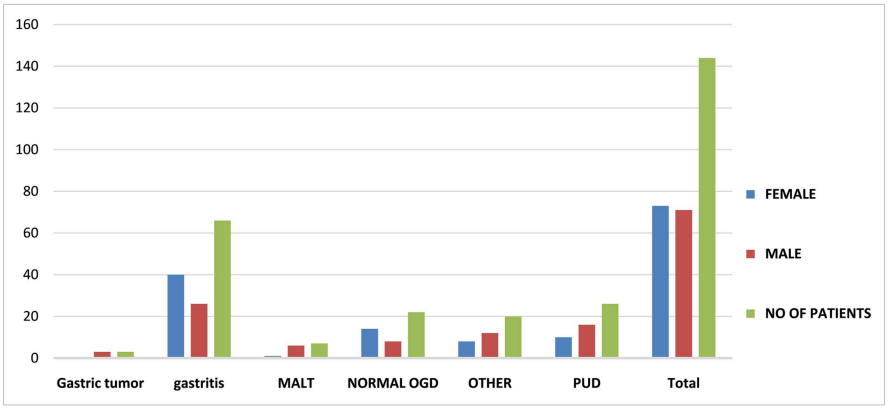


Figure 1. Gender distribution of participants across various gastroduodenal disease diagnoses. The chart illustrates the prevalence of different gastroduodenal diseases among male and female participants. Diseases include gastric tumors, gastritis, MALT (mucosa-associated lymphoid tissue) lymphoma, normal endoscopic findings (NORM EGD), and peptic ulcer disease (PUD), among others. The total number of patients is also presented, with distinct bars for each gender, highlighting the variation in disease incidence between males and females across diagnostic categories.

3.3. Molecular Detection of *H. pylori* Housekeeping Genes

The presence of *H. pylori* DNA in gastric biopsy specimens was confirmed by PCR amplification of housekeeping genes: 16S rRNA, glmM, and HPU. Among the 144 specimens, 91 (63.19%) were positive for both 16S rRNA and glmM genes, and 90 (62.50%) were positive for the HPU gene. Gel electrophoresis images confirmed the expected amplicon sizes for these genes.

3.4. Association with Gender

As shown in Table 3, the detection rates of housekeeping genes were similar between females and males, with no statistically significant differences observed ($p > 0.05$).

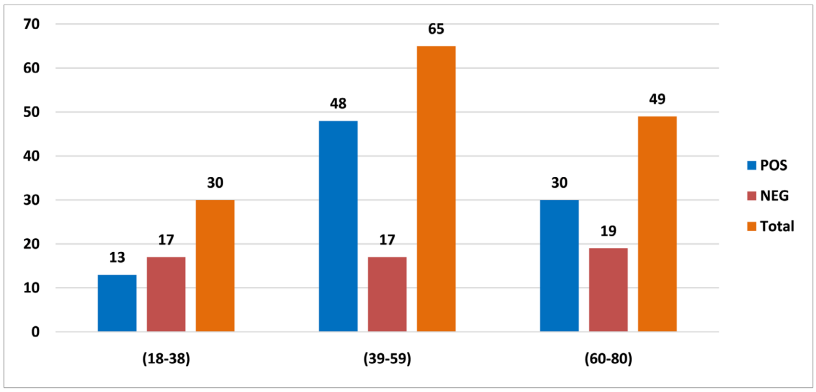
Table 3. Detection of *H. pylori* housekeeping genes by gender.

Housekeeping Gene	Gender	Positive (%)	Negative (%)	χ^2	p-Value
16S rRNA	Female	45 (61.64%)	28 (38.36%)		
	Male	46 (64.79%)	25 (35.21%)	0.153	0.696
glmM	Female	45 (61.64%)	28 (38.36%)		
	Male	46 (64.79%)	25 (35.21%)	0.153	0.696
HPU	Female	44 (60.27%)	29 (39.73%)		
	Male	46 (64.79%)	25 (35.21%)	0.313	0.576

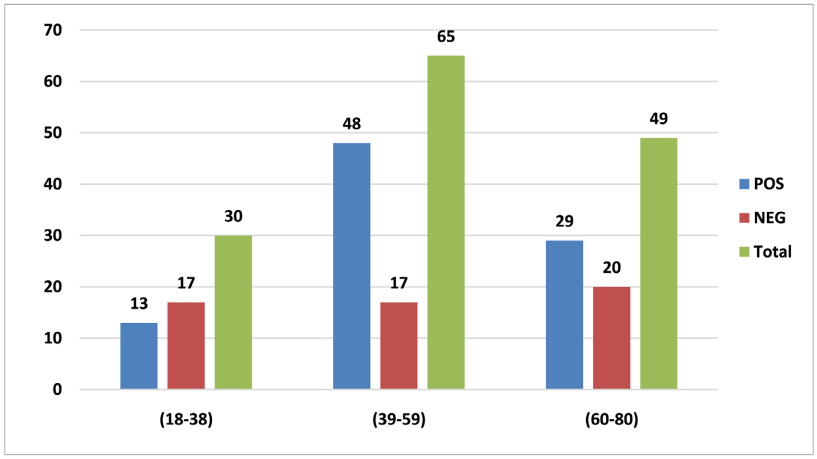
3.5. Association with Age Groups

The prevalence of *H. pylori* housekeeping genes varied significantly across

different age groups (Figure 2(A) and Figure 2(B)). The highest detection rate was in the 39 - 59 years group (48/65, 73.85%), followed by the 60 - 80 years group (30/49, 61.22%) and the 18 - 38 years group (13/30, 43.33%). Statistical analysis indicated a significant association between age and the presence of housekeeping genes ($\chi^2 = 8.34$- 8.50, $p < 0.05$). As shown in Figure 3, the agarose gel electrophoresis results demonstrate successful amplification of key *H. pylori* housekeeping genes. The genus-specific 16S rRNA gene (110 bp), as shown in Figure 3(A), the glmM gene (294 bp), as indicated in Figure 3(B), and the HPU gene (411 bp), as shown in Figure 3(C) were consistently detected in patient biopsy samples, indicating the presence of *H. pylori* across the majority of tested samples.

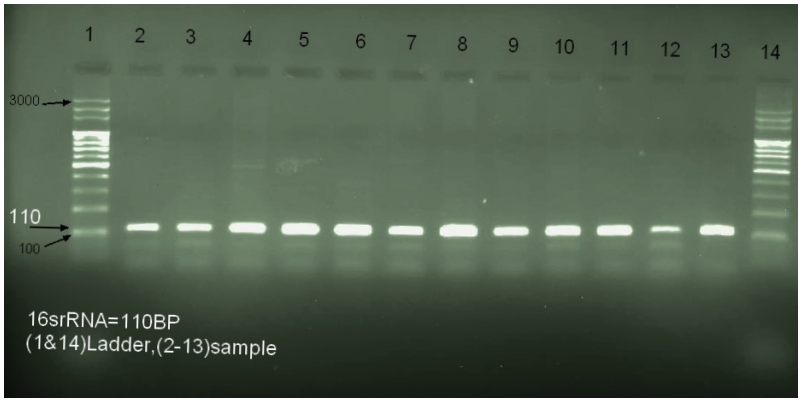


(A)

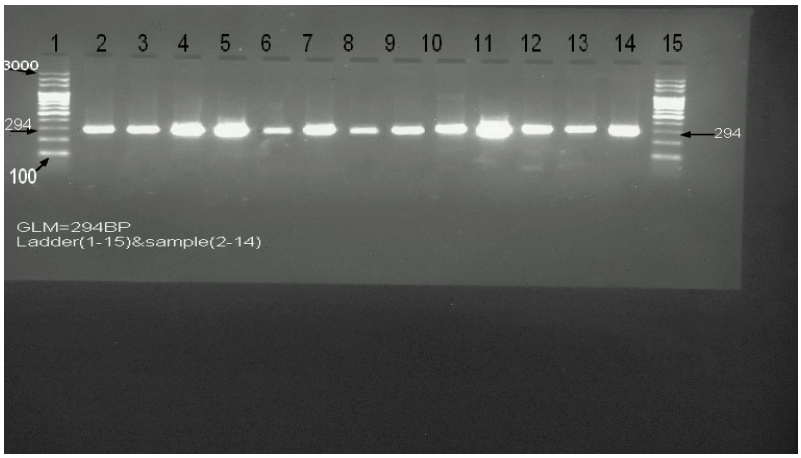


(B)

Figure 2. (A) Age group distribution of positive and negative samples for 16S rRNA and GLM gene detection. This figure presents the distribution of positive and negative samples for 16S rRNA and GLM gene across different age groups (18 - 38, 39 - 59, and 60 - 80 years). The positive and negative results are shown in distinct bars for each age category, providing insight into the prevalence of gene detection with age; (B) Age group distribution of positive and negative samples for HPU gene detection. This figure shows the distribution of positive and negative samples for the HPU gene across different age groups (18 - 38, 39 - 59, and 60 - 80 years). Each age group is represented with bars indicating the number of positive and negative cases and the total number of participants in each group, highlighting the gene's prevalence in relation to age.



(A)



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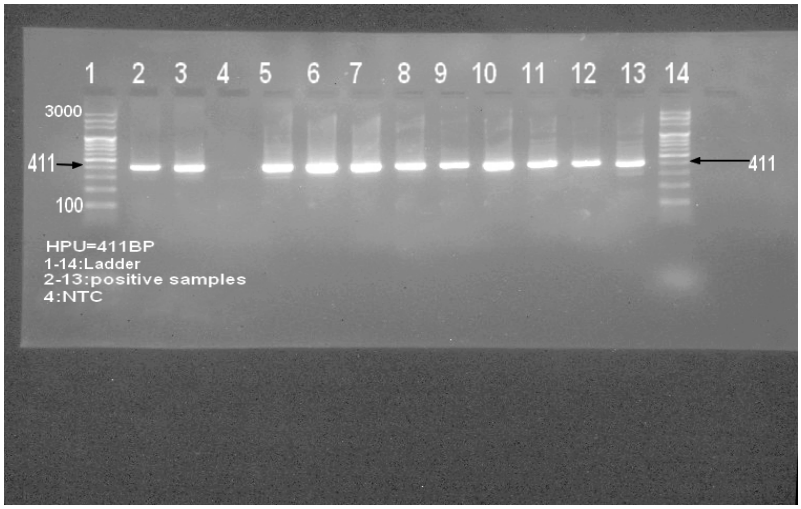


(C)

Figure 3. (A) Gel electrophoresis of genus-specific 16S rRNA PCR products from *H. pylori* isolates, with an expected product size of 110 bp. Lanes 1 and 14 contain DNA ladders, while lanes 2 - 12 correspond to patient biopsy samples; (B) PCR amplification of the *H. pylori* glmM gene, yielding a product size of 294 bp. Lanes 1 and 15 contain DNA ladders, with lanes 2 - 14 representing patient biopsy samples; (C) PCR amplification for the *H. pylori* HPU gene, producing a product size of 411 bp. Lanes 1 and 14 contain DNA ladders, while lanes 2 - 13 represent patient biopsy samples.

3.6. Association with Clinical Outcomes

A significant association was detected between housekeeping genes and clinical outcomes ($p \leq 0.0001$). The highest detection rates were observed in patients with MALT lymphoma (7/7, 100%), PUD (20/26, 76.92%), and gastritis (50/66, 75.76%), as indicated in Table 4.

Table 4. Detection of housekeeping genes by clinical outcomes.

Endoscopic Finding	16S rRNA Positive (%)	glmM Positive (%)	HPU Positive (%)	χ^2	*p*-Value
Gastritis	50 (75.76%)	50 (75.76%)	50 (75.76%)		
Peptic ulcer disease	20 (76.92%)	20 (76.92%)	20 (76.92%)		
MALT lymphoma	7 (100%)	7 (100%)	7 (100%)		
Gastric adenocarcinoma	2 (66.67%)	2 (66.67%)	2 (66.67%)		
Other conditions	5 (25.00%)	5 (25.00%)	5 (25.00%)		
Normal findings	7 (31.82%)	7 (31.82%)	6 (27.27%)	32.53	≤0.0001
Total	**91 (63.19%)**	**91 (63.19%)**	**90 (62.50%)**		

3.7. Prevalence of Virulence Genes

PCR analysis detected one or more virulence genes (*vacA*, *cagA*, and *iceA*1) in 87 out of 91 patients (95.60%) who were positive for housekeeping genes. Four patients (4.40%) were negative for all tested virulence genes, as indicated in Table 5.

Table 5. Prevalence of *H. pylori* virulence genes.

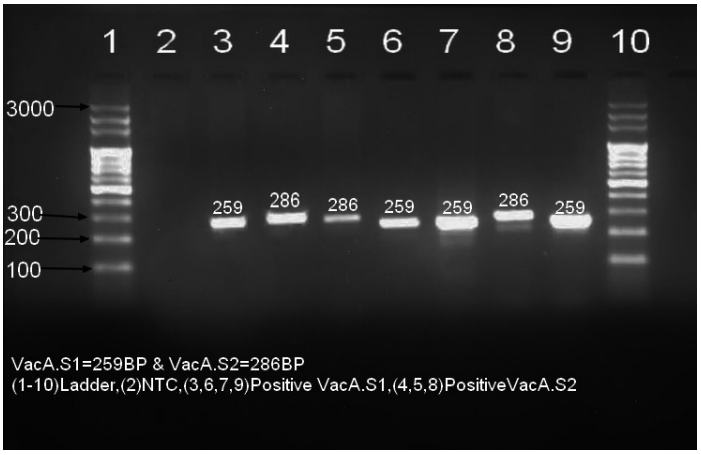
Virulence Gene	Positive (%)	Negative (%)	Total Patients
vacA	77 (84.62%)	14 (15.38%)	91
cagA	53 (58.24%)	38 (41.76%)	91
*iceA*1	27 (29.67%)	64 (70.33%)	91

3.8. Distribution of *vacA* Genotypes

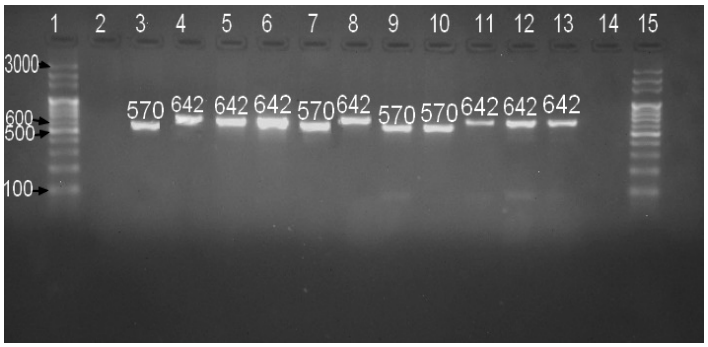
Among the 77 *vacA*-positive patients, the s1 allele was detected in 41 patients (53.25%), as shown in Figure 4(A), and the s2 allele in 10 patients (12.99%) (Figure 4(B)). The m1 allele was found in 33 patients (42.86%), and the m2 allele in 29 patients (37.66%). The most common allelic combinations were s1/m2 (16 patients, 20.78%) and s1/m1 (13 patients, 16.88%), as shown in Table 6.

3.9. Association of *vacA* Genotypes with Clinical Outcomes

The distribution of *vacA* genotypes among different clinical outcomes is presented in Table 7. Although variations were observed in genotype frequencies among disease categories, no statistically significant association was found between *vacA* genotypes and clinical outcomes ($p > 0.05$).



(A)



(B)

Figure 4. (A) Agarose gel electrophoresis of PCR amplified products for *H. pylori vacA* S-region. Lanes 1 - 10 contain DNA ladders for reference. Lane 2 represents the no-template control (NTC). Lanes 3, 6, 7, and 9 show PCR products for the *vacA* S1 variant, while lanes 4, 5, and 8 show products for the *vacA* S2 variant; (B) Agarose gel electrophoresis of PCR amplified products for *H. pylori vacA* M-region. Lanes 1 - 15 contain DNA ladders as molecular weight references. Lane 2 represents the no-template control (NTC). Lanes 3, 7, 9, and 10 display PCR products for the *vacA* M1 variant, whereas lanes 4, 5, 6, 8, 11, 12, and 13 show products for the *vacA* M2 variant.

Table 6. Distribution of *vacA* genotypes and allele combinations.

vacA Genotype	Number of Patients (%)
s1	12 (15.58%)
s2	3 (3.90%)
m1	14 (18.18%)
m2	12 (15.58%)
s1/m1	13 (16.88%)
s1/m2	16 (20.78%)
s2/m1	6 (7.79%)
s2/m2	1 (1.30%)
Total	**77 (100%)**

Table 7. Prevalence of *vacA* genotypes in clinical outcomes.

vacA Genotype	Gastritis (%)	PUD (%)	MALT (%)	Gastric Tumor (%)	Other (%)	Normal OGD (%)	Total (%)
s1	6 (50.00%)	2 (16.67%)	0 (0%)	0 (0%)	2 (16.67%)	2 (16.67%)	12 (15.58%)
s2	1 (33.33%)	1 (33.33%)	0 (0%)	1 (33.33%)	0 (0%)	0 (0%)	3 (3.90%)
m1	10 (71.43%)	2 (14.29%)	0 (0%)	0 (0%)	1 (7.14%)	1 (7.14%)	14 (18.18%)
m2	5 (41.67%)	4 (33.33%)	1 (8.33%)	0 (0%)	1 (8.33%)	1 (8.33%)	12 (15.58%)
s1/m1	6 (46.15%)	3 (23.08%)	3 (23.08%)	0 (0%)	0 (0%)	1 (7.69%)	13 (16.88%)
s1/m2	10 (62.50%)	1 (6.25%)	3 (18.75%)	1 (6.25%)	1 (6.25%)	0 (0%)	16 (20.78%)
s2/m1	3 (50.00%)	2 (33.33%)	0 (0%)	0 (0%)	0 (0%)	1 (16.67%)	6 (7.79%)
s2/m2	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.30%)
Total	**41 (53.25%)**	**16 (20.78%)**	**7 (9.09%)**	**2 (2.60%)**	**5 (6.49%)**	**6 (7.79%)**	**77 (100%)**

3.10. Association of *vacA* Genotypes with Other Virulence Genes

A significant association was observed between *vacA* genotypes and the detection of other virulence genes (*cagA* and *iceA*1) ($\chi^2 = 62.1$, $p \leq 0.0001$) (Table 8). For instance, the s1/m1 genotype was predominantly associated with *cagA* positivity (12/13 patients, 92.31%).

Table 8. Association of *vacA* genotypes with other virulence genes.

vacA Genotype	*vacA* Alone (%)	*cagA* Positive (%)	*iceA*1 Positive (%)	*cagA* + *iceA*1 (%)	Total Patients
s1	4 (33.33%)	3 (25.00%)	1 (8.33%)	4 (33.33%)	12
s2	1 (33.33%)	2 (66.67%)	0 (0%)	0 (0%)	3
m1	4 (28.57%)	5 (35.71%)	5 (35.71%)	0 (0%)	14
m2	2 (16.67%)	4 (33.33%)	6 (50.00%)	0 (0%)	12
s1/m1	0 (0%)	12 (92.31%)	0 (0%)	1 (7.69%)	13
s1/m2	0 (0%)	11 (68.75%)	1 (6.25%)	4 (25.00%)	16
s2/m1	5 (83.33%)	0 (0%)	1 (16.67%)	0 (0%)	6
s2/m2	0 (0%)	0 (0%)	0 (0%)	1 (100%)	1
Total	**16 (20.78%)**	**37 (48.05%)**	**14 (18.18%)**	**10 (12.99%)**	**77**

3.11. *cagA* Genotype Status

The *cagA* gene was detected in 53 of 91 *H. pylori* strains (58.24%). The distribution of *cagA* positivity among different clinical outcomes is presented in Table 9. The highest prevalence of *cagA*-positive strains was observed in patients with MALT lymphoma (6/7, 85.71%) and gastric adenocarcinoma (2/2, 100%). However, no significant differences were found in the frequency of *cagA*-positive

strains between clinical groups ($p = 0.31$). The agarose gel electrophoresis of PCR amplified products for *H. pylori cagA* gene is shown in Figure 5.

Table 9. *cagA* genotype prevalence by clinical outcomes.

Clinical Outcome	*cagA* Positive (%)	*cagA* Negative (%)	Total Patients
Gastritis	25 (50.00%)	25 (50.00%)	50
Peptic ulcer disease	12 (60.00%)	8 (40.00%)	20
MALT lymphoma	6 (85.71%)	1 (14.29%)	7
Gastric adenocarcinoma	2 (100%)	0 (0%)	2
Other conditions	4 (80.00%)	1 (20.00%)	5
Normal findings	4 (57.14%)	3 (42.86%)	7
Total	**53 (58.24%)**	**38 (41.76%)**	**91**

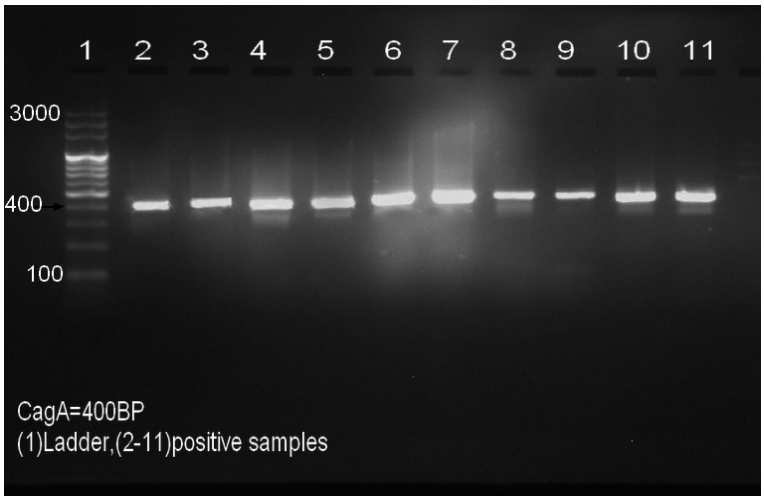


Figure 5. Agarose gel electrophoresis of PCR amplified products for *H. pylori cagA* Gene. Lane 1 contains the DNA ladder as a molecular weight reference. Lanes 2 - 11 show positive samples for the *cagA* gene in *H. pylori*.

3.12. *iceA*1 Genotype Status

The *iceA*1 gene was identified in 27 out of 91 *H. pylori* strains (29.67%). Its distribution among clinical outcomes is shown in Table 10. No significant differences were observed in the frequency of *iceA*1-positive strains between clinical groups ($p = 0.92$). PCR amplification of the *H. pylori iceA* gene yielded a product size of 558 bp in multiple patient samples, as confirmed by agarose gel electrophoresis. Positive amplification was observed in lanes 2, 3, 4, 5, 6, 8, 9, 10, 12, 13, and 14, while the negative control (lane 7, no-template control) and a negative sample (lane 11) showed no amplification. These results indicate the presence of the *iceA* gene in most tested samples, supporting its prevalence among the isolates, as

indicated in Figure 6.

Table 10. *iceA*1 genotype prevalence by clinical outcomes.

Clinical Outcome	*iceA*1 Positive (%)	*iceA*1 Negative (%)	Total Patients
Gastritis	15 (30.00%)	35 (70.00%)	50
Peptic ulcer disease	5 (25.00%)	15 (75.00%)	20
MALT lymphoma	2 (28.57%)	5 (71.43%)	7
Gastric adenocarcinoma	1 (50.00%)	1 (50.00%)	2
Other conditions	1 (20.00%)	4 (80.00%)	5
Normal findings	3 (42.86%)	4 (57.14%)	7
Total	**27 (29.67%)**	**64 (70.33%)**	**91**

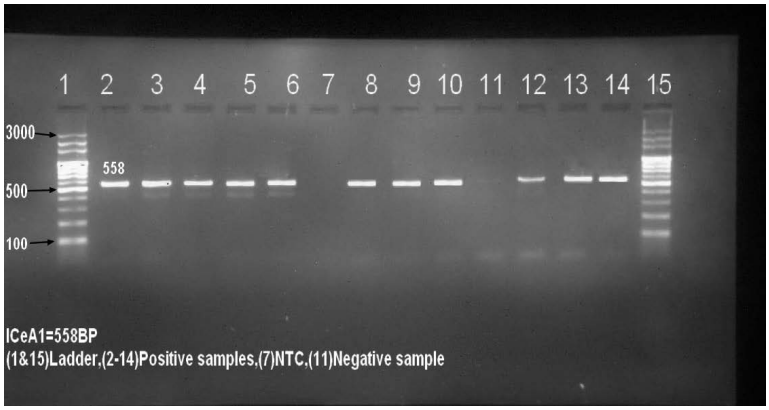


Figure 6. Agarose gel electrophoresis of PCR amplified products for *H. pylori iceA* gene. Lanes 1 and 15 contain DNA ladders as molecular weight markers. Lanes 2, 3, 4, 5, 6, 8, 9, 10, 12, 13, and 14 show positive samples for the *iceA* gene. Lane 7 represents the no-template control (NTC), and lane 11 contains a negative sample for *iceA*.

3.13. Correlation between *cagA* and *iceA*1 Genes

A significant association was detected between *cagA* and *iceA*1 genes ($p = 0.01$), as shown in Table 11. Among the 53 *cagA*-positive patients, 10 (18.87%) were also positive for *iceA*1.

Table 11. Correlation between *cagA* and *iceA*1 genes.

	*iceA*1 Positive (%)	*iceA*1 Negative (%)	Total (%)
cagA Positive	10 (18.87%)	43 (81.13%)	53 (100%)
cagA Negative	17 (44.74%)	21 (55.26%)	38 (100%)
Total	**27 (29.67%)**	**64 (70.33%)**	**91**

3.14. Association of Virulence Genes with Clinical Outcomes

An analysis of the relationship between virulence genes and clinical outcomes revealed a significant association (F = 12.5, $p \leq 0.0001$), as indicated in Figure 7. Patients with more severe gastroduodenal diseases, such as PUD, MALT lymphoma, and gastric adenocarcinoma, exhibited higher frequencies of certain virulence genes, particularly *cagA* and specific *vacA* genotypes.

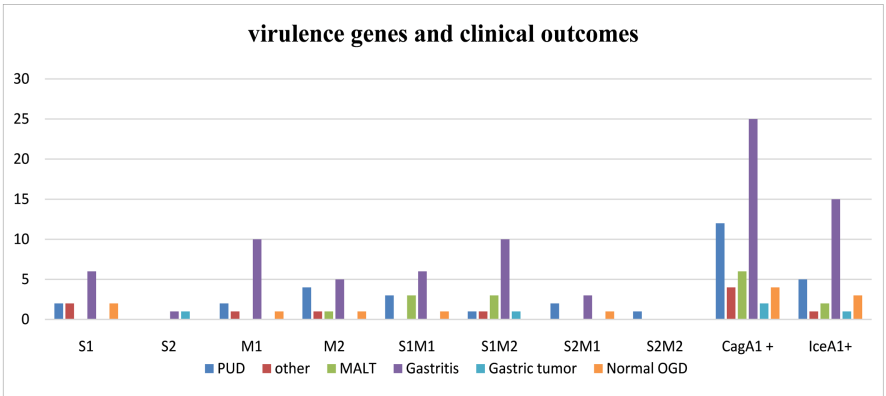
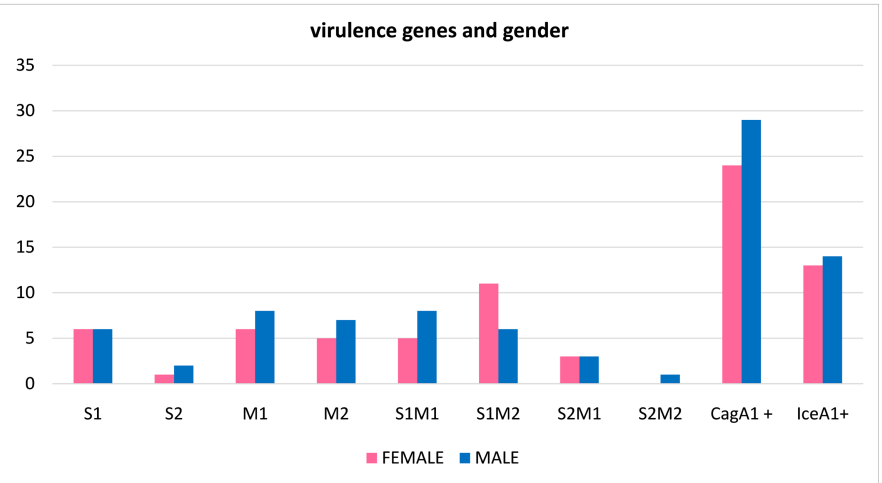


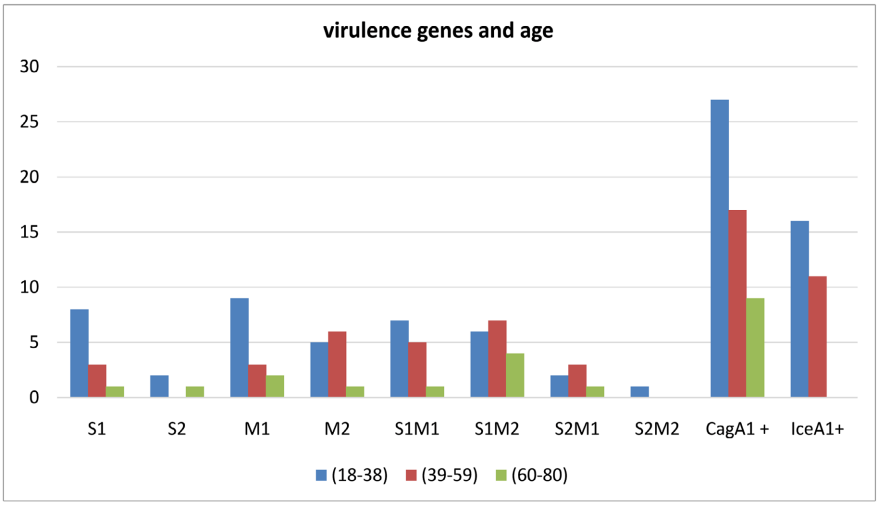
Figure 7. Relationship between *H. pylori* virulence genes and clinical outcomes. This figure illustrates the association between the presence of specific *H. pylori* virulence genes and their correlation with various clinical outcomes. The data presented highlights how different virulence factors, such as *cagA*, *vacA*, and *iceA*, influence the severity and type of clinical manifestations observed in patients.

3.15. Association of Virulence Genes with Gender and Age

We could not detect a significant association between the presence of virulence genes and patient gender (F = 1.3, $p = 0.3$), as shown in Figure 8(A). However, a significant association was observed with age groups (F = 13.5, $p = 0.005$) (Figure 8(B)). Younger patients (18 - 38 years) had different distributions of virulence genes compared to older patients.



(A)



(B)

Figure 8. (A) Association between *H. pylori* virulence genes and gender. This bar chart displays the distribution of specific *H. pylori* virulence genes (S1, S2, M1, M2, S1M1, S1M2, S2M1, S2M2, *cagA*+, *iceA*1+) across male and female patients. The graph illustrates potential gender-based differences in the prevalence of these virulence genes, with each bar indicating the frequency of gene detection in males (blue) and females (pink); (B) Association between *H. pylori* virulence genes and age. The figure presents the distribution of *H. pylori* virulence genes across different age groups. It illustrates how the presence of specific virulence factors, such as *cagA*, *vacA*, and *iceA*, varies with patient age, potentially indicating age-related patterns in the prevalence or impact of these virulence genes.

4. Discussion

Persistent infection of the gastric mucosa with *H. pylori* has been linked to a range of gastroduodenal diseases, including gastritis, peptic ulcer disease (PUD), and gastric cancers. Epidemiological studies estimate that while most individuals colonized by *H. pylori* may develop gastritis, approximately 10% progress to PUD, and about 1% - 2% may develop gastric cancer [20] [21]. Our study, focusing on patients from Benghazi, Libya, highlights the high prevalence of *H. pylori* infection and the distribution of key virulence genes (*cagA*, *vacA*, and *iceA*1) among those with gastroduodenal diseases. This molecular characterization provides valuable insights into the pathogenic potential of *H. pylori* strains circulating in this region, emphasizing the importance of understanding local variations in virulence factors to predict clinical outcomes and inform treatment strategies.

Our study demonstrates significant associations between specific *H. pylori* virulence genes and PCR-positive results, underscoring the pathogenic role of these genetic factors in clinical outcomes ($p < 0.0001$). The *vacA* gene, presented in 84.6% of PCR-positive cases, encodes the vacuolating cytotoxin A (*vacA*), a significant contributor to cellular damage in *H. pylori* infections. *vacA* promotes the formation of vacuoles in host cells, impairs cellular function, and may trigger apoptosis, leading to tissue damage. Notably, *vacA* contains two variable regions—the signal peptide region (s1/s2) and the middle region (m1/m2)—associated with different pathogenicity levels. In line with findings from Egypt and Jordan, our

study found that *vacA* alleles s1 and m1 were more prevalent than s2 and m2, suggesting a higher virulence potential of local *H. pylori* strains [22] [23]. The predominance of *vacA* s1/m1 in our samples reflects a pattern seen in more virulent strains, often linked to severe gastroduodenal outcomes.

Regional variation in *vacA* allele prevalence is apparent, as our study's s2 allele frequency (3.9%) is consistent with findings in Türkiye (6.8%) [24] yet contrasts with the higher s2 rates reported in Saudi Arabia (41.7%) [25]. The absence of a statistically significant association between *vacA* allelic combinations and clinical outcomes in our study may be due to the relatively small sample size. However, prior research has shown that strains with *vacA* s1m1 genotypes, due to their higher vacuolating activity, are associated with increased pathogenicity and often linked to severe clinical presentations [26]. Additionally, our findings of high s1 prevalence align with reports from Kuwait [27] and highlight the need for further research to understand the clinical relevance of *vacA* variants in different populations.

The presence of specific virulence genes in *H. pylori* strains can help predict clinical outcomes. Our results showed that triple-positive strains (*vacA*/*cagA*/*iceA*1) were frequently observed in patients with PUD, gastritis, and even gastric tumors, suggesting a higher pathogenic potential. These findings align with studies reporting that *vacA* s1+/*cagA*+/*iceA*1+ strains are highly virulent and frequently associated with ulcer disease [28]. We found significant associations between *vacA* allelic combinations and the presence of *cagA* and *iceA*1 ($p \leq 0.0001$), indicating that gene combinations may enhance the pathogenicity of *H. pylori* infections. The predominance of the *vacA*/*cagA*+ genotype in gastritis patients (12 out of 37 cases) mirrors findings from Venezuela, where *cagA* is strongly associated with *vacA* s1m1+ and s2m2+ genotypes in gastritis cases [29].

The *cagA* gene, encoding the *cagA* protein, is a crucial virulence factor linked to *H. pylori* pathogenicity. *cagA* is translocated into host cells via a type IV secretion system, where it disrupts cellular processes, promotes cell proliferation, inhibits apoptosis, and induces morphological changes [30]. *cagA* is also the first identified bacterial oncoprotein associated with an increased risk of gastric adenocarcinoma, one of the deadliest cancers worldwide [31]. In our study, the *cagA* gene was detected in 58.2% of *H. pylori* strains, a prevalence consistent with studies from Türkiye [32], but lower than in East Asian countries, where nearly all strains are *cagA*-positive [33]. Although our findings did not reveal a statistically significant association between *cagA* positivity and specific clinical outcomes ($p > 0.05$), the high prevalence of *cagA* in severe gastroduodenal diseases suggests its potential role in exacerbating disease severity. These observations align with previous research linking *cagA* to severe clinical outcomes, including gastric carcinoma [34] [35].

The *iceA*1 gene's role in *H. pylori* pathogenicity appears complex and may vary by geographic location. We found that *iceA*1 was prevalent among patients with normal endoscopic findings and those with gastritis, contrasting with previous studies linking *iceA*1 to PUD (Kadi *et al*., 2014). This discrepancy might be attributed

to regional differences rather than a universal role for *iceA*1 in virulence [36] [37]. Importantly, our study found a statistically significant association between the *cagA* and *iceA*1 genes ($p = 0.01$), suggesting a possible synergy between these genes in promoting disease progression. This finding is consistent with prior studies indicating that the presence of both *cagA* and *iceA*1 may correlate with more severe clinical outcomes [38] [39].

Age and gender can influence the distribution of *H. pylori* virulence genes, potentially affecting disease risk and severity. Our study found a significant association between age and the expression of virulence genes, with older patients more frequently exhibiting virulent *H. pylori* strains. This aligns with research showing that the *cagA* gene is more common in older patients [40] and that *vacA* expression is more frequent among the elderly [41]. These findings suggest that age may be a factor in the development of more severe *H. pylori*-related diseases, potentially due to cumulative exposure and immune system changes. Although no significant association was found between gender and virulence gene prevalence in our study, other research has noted gender-related variations in disease susceptibility [42] [43]. However, our data indicate that gender may not significantly influence *H. pylori* pathogenicity, which aligns with prior findings that suggest minimal gender impact on virulence gene expression [44].

5. Conclusions

This study presents the first comprehensive molecular characterization of *Helicobacter pylori* virulence genes—*vacA*, *cagA*, and *iceA*1—and their association with gastroduodenal diseases in Benghazi, Libya. Our findings reveal a high prevalence of *H. pylori* infection (63.2%) and a substantial presence of the *vacA* gene (84.6%), particularly the s1 variant, along with significant frequencies of *cagA* (58.2%) and *iceA*1 (29.7%). These results suggest a widespread distribution of virulent *H. pylori* strains in this population.

The significant associations observed between specific virulence gene combinations and clinical outcomes underscore the critical role of these factors in *H. pylori* pathogenicity. Strains harboring the *vacA* s1/m1 genotype and positive for *cagA* were more frequently associated with severe conditions, including peptic ulcer disease and mucosa-associated lymphoid tissue lymphoma. This highlights the potential utility of incorporating molecular profiling into clinical diagnostics to enhance risk assessment and guide targeted therapies.

Furthermore, this study provides foundational data for understanding the pathogenic potential of *H. pylori* strains in Libya, addressing an important gap in regional epidemiological knowledge. The high prevalence of virulent genotypes emphasizes the need for public health initiatives focusing on early detection, eradication strategies, and ongoing surveillance of *H. pylori* infections. Tailored diagnostic protocols based on virulence gene profiles could significantly improve patient management and reduce the burden of *H. pylori*-associated diseases in Libya and similar settings.

Future research should validate these findings in larger patient cohorts and further explore the mechanisms underlying *H. pylori*'s diverse pathogenic profiles. Investigating the interactions between virulence factors and host-specific elements and understanding regional variations in *H. pylori* genotypes will be essential in developing more effective strategies for managing *H. pylori*-associated diseases.

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We hereby affirm that all authors have equally contributed to this research endeavor.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Essawi, T., Hammoudeh, W., Sabri, I., Sweidan, W. and Farraj, M.A. (2013) Determination of *Helicobacter pylori* Virulence Genes in Gastric Biopsies by PCR. *ISRN Gastroenterology*, **2013**, Article ID: 606258. https://doi.org/10.1155/2013/606258

[2] Palframan, S.L., Kwok, T. and Gabriel, K. (2012) Vacuolating Cytotoxin a (VacA), a Key Toxin for *Helicobacter pylori* Pathogenesis. *Frontiers in Cellular and Infection Microbiology*, **2**, Article 92. https://doi.org/10.3389/fcimb.2012.00092

[3] Šebunova, N., Štšepetova, J., Sillakivi, T. and Mändar, R. (2018) The Prevalence of *Helicobacter pylori* in Estonian Bariatric Surgery Patients. *International Journal of Molecular Sciences*, **19**, Article 338. https://doi.org/10.3390/ijms19020338

[4] Quaglia, N.C. and Dambrosio, A. (2018) *Helicobacter pylori*: A Foodborne Pathogen? *World Journal of Gastroenterology*, **24**, 3472-3487. https://doi.org/10.3748/wjg.v24.i31.3472

[5] Amieva, M.R. and El-Omar, E.M. (2008) Host-Bacterial Interactions in *Helicobacter pylori* Infection. *Gastroenterology*, **134**, 306-323. https://doi.org/10.1053/j.gastro.2007.11.009

[6] Chen, Y., Mo, X., Huang, G., Huang, Y., Xiao, J., Zhao, L., *et al*. (2016) Gene Polymorphisms of Pathogenic *Helicobacter pylori* in Patients with Different Types of Gastrointestinal Diseases. *World Journal of Gastroenterology*, **22**, 9718-9726. https://doi.org/10.3748/wjg.v22.i44.9718

[7] Cavalcante, M.Q.D.F., Silva, C.I.S., Braga-Neto, M.B., Fialho, A.B.C., Nunes Fialho, A., Barbosa, A.M.C., *et al*. (2012) *Helicobacter pylori* vacA and cagA Genotypes in Patients from Northeastern Brazil with Upper Gastrointestinal Diseases. *Memórias do Instituto Oswaldo Cruz*, **107**, 561-563. https://doi.org/10.1590/s0074-02762012000400021

[8] Lina, T.T. (2014) Immune Evasion Strategies Used by *Helicobacter pylori*. *World Journal of Gastroenterology*, **20**, 12753-12766. https://doi.org/10.3748/wjg.v20.i36.12753

[9] Kabamba, E.T., Tuan, V.P. and Yamaoka, Y. (2018) Genetic Populations and Virulence Factors of *Helicobacter pylori*. *Infection, Genetics and Evolution*, **60**, 109-116. https://doi.org/10.1016/j.meegid.2018.02.022

[10] 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*. (2018) Prevalence of *Helicobacter pylori cagA* and *iceA* Genes and Their Association with Gastrointestinal Diseases. *International Journal of Microbiology*, **2018**, Article ID: 4809093. https://doi.org/10.1155/2018/4809093

[11] Talebi Bezmin Abadi, A. (2017) Strategies Used by *Helicobacter pylori* to Establish Persistent Infection. *World Journal of Gastroenterology*, **23**, 2870-2882. https://doi.org/10.3748/wjg.v23.i16.2870

[12] Logan, R. (1996) Adherence of *Helicobacter pylori*. *Alimentary Pharmacology & Therapeutics*, **10**, 3-15. https://doi.org/10.1046/j.1365-2036.1996.22164001.x

[13] Aspholm, M., Kalia, A., Ruhl, S., Schedin, S., Arnqvist, A., Lindén, S., *et al*. (2006) *Helicobacter pylori* Adhesion to Carbohydrates. *Methods in Enzymology*, **417**, 293-339. https://doi.org/10.1016/s0076-6879(06)17020-2

[14] Zheng, P. and Jones, N.L. (2003) *Helicobacter pylori* Strains Expressing the Vacuolating Cytotoxin Interrupt Phagosome Maturation in Macrophages by Recruiting and Retaining TACO (Coronin 1) Protein. *Cellular Microbiology*, **5**, 25-40. https://doi.org/10.1046/j.1462-5822.2003.00250.x

[15] Wroblewski, L.E., Peek, R.M. and Wilson, K.T. (2010) *Helicobacter pylori* and Gastric Cancer: Factors That Modulate Disease Risk. *Clinical Microbiology Reviews*, **23**, 713-739. https://doi.org/10.1128/cmr.00011-10

[16] Akeel, M., Shehata, A., Elhafey, A., Elmakki, E., Aboshouk, T., Ageely, H., *et al*. (2019) *Helicobacter pylori vacA*, *cagA* and *iceA* Genotypes in Dyspeptic Patients from Southwestern Region, Saudi Arabia: Distribution and Association with Clinical Outcomes and Histopathological Changes. *BMC Gastroenterology*, **19**, Article No. 16. https://doi.org/10.1186/s12876-019-0934-z

[17] Sarma, A., Saikia, L., Bhuyan, R.K. and Hussain, M.E. (2017) Molecular Identification and Detection of Virulent Factors in *Helicobacter pylori* from Gastric Biopsy Samples of Patients Attended at Assam Medical College and Hospital, Dibrugarh, Assam, India. *Indian Journal of Medical Microbiology*, **35**, 600-603. https://doi.org/10.4103/ijmm.ijmm_17_232

[18] Sabbagh, P., Javanian, M., Koppolu, V., Vasigala, V.R. and Ebrahimpour, S. (2019) *Helicobacter pylori* Infection in Children: An Overview of Diagnostic Methods. *European Journal of Clinical Microbiology & Infectious Diseases*, **38**, 1035-1045. https://doi.org/10.1007/s10096-019-03502-5

[19] Wang, Y. (2015) Diagnosis of *Helicobacter pylori* Infection: Current Options and Developments. *World Journal of Gastroenterology*, **21**, 11221-11235. https://doi.org/10.3748/wjg.v21.i40.11221

[20] Hatakeyama, M. (2009) *Helicobacter pylori* and Gastric Carcinogenesis. *Journal of Gastroenterology*, **44**, 239-248. https://doi.org/10.1007/s00535-009-0014-1

[21] Miernyk, K., Morris, J., Bruden, D., McMahon, B., Hurlburt, D., Sacco, F., *et al*. (2011) Characterization of *Helicobacter pylori cagA* and *vacA* Genotypes among Alaskans and Their Correlation with Clinical Disease. *Journal of Clinical Microbiology*, **49**, 3114-3121. https://doi.org/10.1128/jcm.00469-11

[22] Sayed Zaki, M.E., Elewa, A., Abdelwahab Ali, M. and Shehta, A. (2016) Study of Virulence Genes Cag A and Vac A in *Helicobacter pylori* Isolated from Mansoura University Hospital Patients by Multiplex PCR. *International Journal of Current Microbiology and Applied Sciences*, **5**, 154-160. https://doi.org/10.20546/ijcmas.2016.502.018

[23] Nimri, L.F., Matalka, I., Bani-Hani, K.E. and Ibrahim, M. (2006) *Helicobacter pylori* Genotypes Identified in Gastric Biopsy Specimens from Jordanian Patients. *BMC*

Gastroenterology, **6**, Article No. 27. https://doi.org/10.1186/1471-230x-6-27

[24] Bolek, B.K., Salih, B.A. and Sander, E. (2007) Genotyping of *Helicobacter pylori* Strains from Gastric Biopsies by Multiplex Polymerase Chain Reaction. How Advantageous Is It? *Diagnostic Microbiology and Infectious Disease*, **58**, 67-70. https://doi.org/10.1016/j.diagmicrobio.2006.12.001

[25] Momenah, A.M. and Tayeb, M.T. (2007) *Helicobacter pylori cagA* and *iceA* Genotypes Status and Risk of Peptic Ulcer in Saudi Patients. *Saudi Medical Journal*, **28**, 382-385.

[26] Marie, M.A.M. (2012) Relationship between *Helicobacter pylori* Virulence Genes and Clinical Outcomes in Saudi Patients. *Journal of Korean Medical Science*, **27**, 190-193. https://doi.org/10.3346/jkms.2012.27.2.190

[27] Qabandi, A.A., Mustafa, A.S., Siddique, I., Khajah, A.K., Madda, J.P. and Junaid, T.A. (2005) Distribution of *vacA* and *cagA* Genotypes of *Helicobacter pylori* in Kuwait. *Acta Tropica*, **93**, 283-288. https://doi.org/10.1016/j.actatropica.2005.01.004

[28] van Doorn, L., Figueiredo, C., Sanna, R., Plaisier, A., Schneeberger, P., de Boer, W., *et al*. (1998) Clinical Relevance of the *cagA*, *vacA*, and *iceA* Status of *Helicobacter pylori*. *Gastroenterology*, **115**, 58-66. https://doi.org/10.1016/s0016-5085(98)70365-8

[29] Villllalobos, L.B., Cavazza, M.E. and Ortiz-Princz, D. (2015) Detection of *cagA* Gene and Typing *vacA* Gene of *Helicobacter pylori* in Biopsies of Patients with Gastric Symptoms in Cumana, Sucre State, Venezuela. *SABER. Revista Multidisciplinaria del Consejo de Investigación de la Universidad de Oriente*, **27**, 430-440.

[30] Hatakeyama, M. (2017) Structure and Function of *Helicobacter pylori cagA*, the First-Identified Bacterial Protein Involved in Human Cancer. *Proceedings of the Japan Academy*, *Series B*, **93**, 196-219. https://doi.org/10.2183/pjab.93.013

[31] Tohidpour, A. (2016) CagA-Mediated Pathogenesis of *Helicobacter pylori*. *Microbial Pathogenesis*, **93**, 44-55. https://doi.org/10.1016/j.micpath.2016.01.005

[32] Sarıbaş, Z., Demir, H., Simşek, H., Ozen, H. and Akyön, Y. (2010) Detection of cagA Prevalence in Clinical Isolates of *Helicobacter pylori*. *Mikrobiyoloji Bulteni*, **44**, 461-465.

[33] Hooi, J.K.Y., Lai, W.Y., Ng, W.K., Suen, M.M.Y., Underwood, F.E., Tanyingoh, D., *et al*. (2017) Global Prevalence of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis. *Gastroenterology*, **153**, 420-429. https://doi.org/10.1053/j.gastro.2017.04.022

[34] Falsafi, T., Khani, A., Mahjoub, F., Asgarani, E. and Sotoudeh, N. (2015) Analysis of Vaca/cagA Genotypes/Status in *Helicobacter pylori* Isolates from Iranian Children and Their Association with Clinical Outcome. *Turkish Journal of Medical Sciences*, **45**, 170-177. https://doi.org/10.3906/sag-1311-2

[35] Kadi, R.H., Halawani, E.M. and Abdelkader, H.S. (2014) Prevalence of *H. pylori* Strains Harbouring cagA and iceA Virulence Genes in Saudi Patients with Gastritis and Peptic Ulcer Disease. *Microbiology Discovery*, **2**, Article 2. https://doi.org/10.7243/2052-6180-2-2

[36] Shiota, S., Watada, M., Matsunari, O., Iwatani, S., Suzuki, R. and Yamaoka, Y. (2012) *Helicobacter pylori* iceA, Clinical Outcomes, and Correlation with cagA: A Meta-Analysis. *PLOS ONE*, **7**, e30354. https://doi.org/10.1371/journal.pone.0030354

[37] Ciftci, I.H., Uslan, I., Dilek, F.H., Aşık, G., Ozgür, M.A. and Dilek, O.N. (2011) [Investigation of *Helicobacter pylori* iceA1 and iceA2 Genes in Patients with Chronic Gastritis and Gastric Cancer]. *Mikrobiyoloji bulteni*, **45**, 228-233.

[38] Wei, G., Chen, J., Liu, A., Zhang, M., Liu, X., Liu, D., *et al*. (2012) Prevalence of

Helicobacter pylori vacA, cagA and iceA Genotypes and Correlation with Clinical Outcome. *Experimental and Therapeutic Medicine*, **4**, 1039-1044. https://doi.org/10.3892/etm.2012.704

[39] Ben Mansour, K., Fendri, C., Zribi, M., Masmoudi, A., Labbene, M., Fillali, A., *et al*. (2010) Prevalence of *Helicobacter pylori vacA*, *cagA*, *iceA* and *oipA* Genotypes in Tunisian Patients. *Annals of Clinical Microbiology and Antimicrobials*, **9**, Article No. 10. https://doi.org/10.1186/1476-0711-9-10

[40] Nogueira, C., Figueiredo, C., Carneiro, F., Taveira Gomes, A., Barreira, R., Figueira, P., *et al*. (2001) *Helicobacter pylori* Genotypes May Determine Gastric Histopathology. *The American Journal of Pathology*, **158**, 647-654. https://doi.org/10.1016/s0002-9440(10)64006-0

[41] Park, J.Y., Forman, D., Waskito, L.A., Yamaoka, Y. and Crabtree, J.E. (2018) Epidemiology of *Helicobacter pylori* and *cagA*-Positive Infections and Global Variations in Gastric Cancer. *Toxins*, **10**, Article 163. https://doi.org/10.3390/toxins10040163

[42] Zambon, C. (2003) *Helicobacter pylori* babA2, cagA, and s1 vacA Genes Work Synergistically in Causing Intestinal Metaplasia. *Journal of Clinical Pathology*, **56**, 287-291. https://doi.org/10.1136/jcp.56.4.287

[43] Malfertheiner, P., Chan, F.K. and McColl, K.E. (2009) Peptic Ulcer Disease. *The Lancet*, **374**, 1449-1461. https://doi.org/10.1016/s0140-6736(09)60938-7

[44] Tomb, J., White, O., Kerlavage, A.R., Clayton, R.A., Sutton, G.G., Fleischmann, R.D., *et al*. (1997) Erratum: The Complete Genome Sequence of the Gastric Pathogen *Helicobacter pylori*. *Nature*, **389**, 412-412. https://doi.org/10.1038/38792