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The clinical relevance of *Helicobacter pylori* virulence genes (*VacA*, *OipA*, *IceA1*, *IceA2*, and *BabA*) in relation to clinicopathological findings among Jordanian patients with gastric complaints

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Abstract

Helicobacter pylori (*H. pylori*) establishes chronic gastric infection through key virulence genes- *BabA, VacA, IceA,* and *OipA*- that enable adhesion, immune evasion, and mucosal damage. Despite extensive research, it remains a major health concern globally due to its complex virulence profile, varied disease outcomes, and possible oral transmission. Emerging evidence suggests that the oral cavity, particularly dental plaque, may serve as a reservoir for *H. pylori*, underscoring the need to understand oral-gastric transmission pathways for effective prevention and management. This study investigated the prevalence, distribution of virulence genes, and oral–gastric transmission patterns of *H. pylori* among 125 Jordanian patients presenting with gastric complaints. A total of 250 gastric biopsies were characterized by endoscopy and assessed using histopathology and polymerase chain reaction (PCR), while 67 subgingival samples were analyzed using PCR. *H. pylori* was detected in 71.2% of biopsies by histopathology and in 66.4% by PCR. Uniplex and multiplex PCR showed high prevalence of *BabA* (95.2%), *IceA2* (81.9%), and *VacA* (74.7%) gene, while *OipA* (43.3%) and *IceA1* (21.7%) were less common. The notably high prevalence of *BabA* and *IceA2* suggests improved strain persistence, enhanced mucosal adhesion, and a greater tendency toward chronic infection. Significant associations were identified between virulence genotypes and the severity of histopathological changes, providing the first detailed genotype- disease severity map for Jordan. Subgingival samples showed *H. pylori* positivity in 83.6% of patients, with strong agreement between oral and gastric PCR results. The highest oral detection occurred in patients with gastritis (69.6%), supporting the role of the oral cavity as a potential reservoir and source of reinfection. Future research in this extent can contribute to improved prevention strategies and innovative clinical approaches for managing infection. The findings of the current study may have provided additional regional data on *H. pylori* virulence patterns and their clinicopathological significance, emphasizing the importance of integrating gastrointestinal and oral health management.

Keywords: *Helicobacter pylori*; Dental Plaque; Histopathology; Endoscopy; PCR.

1. Introduction

H. pylori is a very common human pathogen that adheres to and colonizes the gastric epithelium's luminal surface in about half of the world's population. Once acquired, *H. pylori* can establish a chronic infection leading to a range of gastroduodenal disorders, including chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and MALT lymphoma (Boden *et al*., 2021; Rendón-Huerta *et al*., 2021). Globally, infection rates exceed 50% of the population (Ansari *et al*., 2017), with transmission primarily occurring through ingestion of contaminated food or water, or via direct person-to-person contact. The prevalence increases with age and is particularly high in low-resource settings, with a notable regional rise in children under five years old (Kayali *et al*., 2024; Rostam *et al*., 2024). The World Health Organization designates *H. pylori* as a Class I carcinogen (Kotilea *et al*., 2019).

H. pylori is a Gram-negative, spiral-shaped bacterium approximately 3 µm in length, equipped with multiple flagella and characterized by microaerophilic growth, as well as urease, catalase, and oxidase activities. Under adverse environmental conditions, it may convert from a culturable spiral form to a non-culturable coccoid state. This transformation can be triggered by nutrient limitation, prolonged incubation, temperature extremes, alkaline pH, shifts from nutrient-rich media to water, CO_2 deficiency, or exposure to antimicrobial agents (Gladyshev *et al*., 2020; Jung *et al*., 2024; Rendón-Huerta *et al*., 2021).

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The virulence factors of *H. pylori* include blood group antigen-binding adhesin (*BabA*), Sialic-acid biding adhesin (*SabA*), Outer inflammatory protein A (*OipA*), *H. pylori* outer membrane protein Q and Z (*HopQ* and *HopZ*), which enable colonization and pathogenesis (Rendón-Huerta *et al*., 2021; Odenbreit *et al*., 2009). Key virulence factors, including *VacA* (vacuolating cytotoxin A), *CagA* (cytotoxin-associated gene A), *IceA* (induced by contact with epithelium gene A), *HtrA* (high temperature requirement A), *Hsps* (heat shock proteins), catalase, urease, phospholipases, and mucinase, contribute to tissue damage by inducing cytotoxicity and inflammatory responses (Baj *et al*., 2021). Subtyping of strains, typically based on *VacA* and *CagA* expression, provides important information on disease outcomes and may inform both diagnostic and therapeutic approaches (Bordin *et al*., 2021; Cho *et al*., 2021). Al-Hyassat *et al.* (2025) reported that *VacA* genotyping identified s2/m2 allelic variants as predominantly associated with mild gastritis, while s1/m1 and s1/m2 were linked to more severe gastric pathology.

Urease is a key virulence factor of *H. pylori*, enabling gastric colonization by locally buffering acidity through the production of ammonia and carbon dioxide. This process maintains a near-neutral cytoplasmic pH, supports motility, nutrient acquisition, and proton motive force, and prevents self-toxicity. After penetrating the mucus layer, the bacteria attach to epithelial cells via *AlpA*, *AlpB*, and *OipA*, promoting immune evasion and effector protein delivery through the type IV secretion system (Cáceres-Delpiano *et al*., 2015). The expression of SabA occurs during gastric disease progression together with outer membrane proteins (OMPs) such as OipA and BabA, mediating gastric atrophy and neutrophil infiltration (Baj *et al*., 2021). Concurrently, these proteins, along with lipopolysaccharides (LPS), trigger host immune responses by stimulating cytokines such as IL-10, IL-12, IL-18, IFN-γ, and TNF-α, which attract immune cells that generate oxidative stress. *H. pylori* also exploits trefoil factor family protein 1 (TFF1), a protective mucus-associated peptide, to strengthen its attachment to the gastric lining. The infection stimulates TFF1 expression, which enhances mucin and pepsinogen secretion and disrupts normal gastric function (Baj *et al*., 2020). Meanwhile, virulence factors such as *CagA* and *VacA* create a proinflammatory environment that activates the JAK/STAT pathway, driving epithelial cell proliferation, inflammation, and apoptosis, thereby contributing to severe gastritis and potentially gastric cancer (Järvå *et al*., 2020).

Evidence indicates that the degree of bacterial adherence is determined by *BabA* binding to the ABO/Lewis^b (Leb) and H-type-1 antigens present in stomach tissue (Doohan *et al*., 2021). Granulocyte infiltration, the release of pro-inflammatory mediators, the production of IL-8, and the expression of *VacA* are all consequences of this process. Alzahrani *et al*. (2014) claim that the simultaneous activation of many mechanisms accelerates the development of cancer and the degradation of mucosal tissue. In addition, *BabA* and *SabA* promote *H. pylori* adherence to the spasmolytic polypeptide-expressing metaplasia (SPEM), a metaplastic linked to the initiation of carcinogenesis (Sáenz *et al*. 2019). **Moreover,** the primary survival strategy utilized by *H. pylori* to persist in the stomach is its ability to produce ammonia through either encoding the urease enzyme from the *ureA* gene or the urea-independent pathway by hydrolyzing amide. Both pathways result in a neutral cytoplasmic and periplasmic pH. After penetration of the mucus layer, the bacteria attached to the gastric epithelial cells through lipoproteins *AlpA* and *AlpB*, and *OipA*, which allows it to escape immune clearance and inject bacterial proteins into host cells using type IV secretion system. In particular, *OpiA* assist in colonization, adherence, and progression of gastrointestinal diseases. Binding of *OipA* to the gastric epithelial cells activates the apoptotic pathways like Bcl-2 and increased secretion of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, IL-11 IL-17, matrix metalloproteinase 1 (MMP-1), and tumor necrosis factor α (TNF-α). It also downregulates the cluster of differentiation 40 (CD40), the cluster of differentiation (CD86), and major histocompatibility complex-II (MHC-II) expression on the cell surface, leading to the suppression of dendritic cells' maturation.

In recent years, the incidence of *H. pylori* infection in Jordan has shown a notable increase. A cross-sectional study by Obaidat and Roess (2019) reported that 88% of Jordanians were seropositive, indicating a substantially high burden of infection. Prevalence rates tend to be higher in developing countries than in developed nations, a pattern often attributed to inadequate hygiene practices within facilities and infrastructure, as well as limited public health awareness (Alaridah *et al.*, 2023). The clinical implications of helicobacteriosis are significant, with documented complications including iron deficiency, peptic ulcer disease, and gastric neoplasms (Cho *et al*., 2021). According to reports from the Jordanian Ministry of Health (2017), gastric cancer is the ninth most commonly diagnosed cancer in the country and is considered the seventh to tenth most prevalent cancer among Jordanians overall (Alaridah *et al*., 2023). Efforts aimed at improving preventive and public health policies must consider the prevalence rate and the factors influencing infection rates.

H. pylori is a globally prevalent pathogen transmitted through multiple routes including fecal–oral and oral–oral (Zhang *et al.,* 2022; Burucoa and Axon, 2017). Its distribution varies widely across geographic regions and population groups, with higher rates in developing countries. Although its transmission pathways remain unclear, evidence suggests that the oral cavity may serve as an additional reservoir linked to gastric infection. Non-invasive oral detection methods show promise for improving diagnosis and reducing re-infection. Advancing research in this area could lead to better prevention strategies and innovative clinical approaches for managing *H. pylori* infection. The close relationship between the oral and gastric microbiota suggests that the oral cavity may play a significant role in the movement and persistence of *H. pylori*. Growing interest has focused on the presence **of** *H. pylori* **i**n the oral cavity, as the mouth has been proposed as a potential extra-gastric reservoir. Although the exact role of *H. pylori* within the oral microbiome remains uncertain, its presence may disrupt microbial homeostasis, influencing the oral–intestinal axis and contributing to both oral and systemic disease. Evidence to date is mixed, with recent reviews reporting inconsistent links between oral *H. pylori* and oral health outcomes **(**Cuba *et al*., 2025**).** Nonetheless, several studies suggest that the oral cavity may support *H. pylori* colonization and transmission, complicating eradication, particularly in the

presence of poor oral hygiene and dental disease (Liang *et al*., 2021; Popovska *et al*., 2022; Author *et al*., 2022).

Studying both gastric and oral *H. pylori* isolates is crucial to understand the dynamics of infection and transmission. The coexistence of the bacterium in both sites may contribute to reinfection, persistence, and treatment failure. Furthermore, the presence of virulence-associated genotypes in oral isolates may influence the severity of gastric disease and facilitate bacterial spread within populations, particularly in regions with high prevalence, such as Jordan (Abu-Lubad *et al*., 2018). Given the inconsistencies and methodological differences across studies, including variable detection techniques and sampling methods, integrated analysis of gastric and oral isolates is essential for defining transmission dynamics and optimizing clinical management of *H. pylori* infection. Understanding the behavior and virulence mechanisms of *H. pylori* is critical for both clinical practice and public health, especially in regions where the infection is highly prevalent. Such studies are essential because of their significant clinical and epidemiological implications. This study examines the clinical relevance of *H. pylori* virulence genes (*VacA*, *OipA*, *IceA1*, *IceA2*, and *BabA*) in gastric isolates in relation to endoscopic and histopathological findings. It also evaluates the prevalence of *H. pylori* in the oral cavity to better understand infection dynamics, guide eradication strategies, and help reduce the burden of *H. pylori*–associated diseases.

2. Materials and Methods

2.1. Gastric Mucosal Biopsy

Two hundred and fifty gastric mucosal biopsy specimens were taken from 125 patients undergoing white-light upper endoscopy. The Institutional Review Board of Al-Bashir Hospital provided ethical approval for sample collection and study procedures (MBA/IRB/3822). Before specimens were collected, each patient signed a written informed consent form. Every patient completed a prepared questionnaire. Clinical data, including age, gender, occupation, smoking status, history and length of illness, prior or current gastritis complaints, treatments or antimicrobial therapies received within the last three months, and proton pump inhibitor use within the last 15 days, were recorded. For molecular analysis, biopsy specimens were placed in 2 mL of sterile normal saline and transported on dry ice in a cooled container within four hours. Biopsy specimens intended for histopathological examination were fixed in 10% buffered formalin and transported to the histopathology laboratory.

2.2. Isolation of H. pylori from subgjinjival

To investigate the potential of the subgingival region as a reservoir for *H. pylori*, 67 dental samples were obtained from patients who underwent upper endoscopy. Only those patients who provided informed consent for dental sample collection were included in the study. Subgingival plaque samples were collected using sterile curettes. Samples were placed in Eppendorf tubes containing 2 mL of normal saline and transported on dry ice to the laboratory for analysis within four hours (Gebara *et al*., 2004).

2.3. *Molecular Identification of H. pylori using (PCR)*

2.3.1. 2.3.1. Extracting DNA from Gastric Biopsy and Subgingival Samples

Gastric biopsy samples were collected in sterile containers containing sterile normal saline. Genomic DNA was extracted using the QIAamp® DNA Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. Briefly, tissue samples were placed in 1.5 mL Eppendorf tubes containing 180 µL of cell lysis buffer, followed by the addition of 20 µL of proteinase K to inactivate nucleases and remove contaminants. The mixture was vortexed and incubated at 56 °C until complete lysis was achieved. After incubation, samples were vortexed again, and 200 µL of AL buffer (lysis buffer) was added and mixed thoroughly using a vortex mixer (Labnet International, USA). Subsequently, 200 µL of ethanol (96–100%) was added and mixed by vortexing. The mixture was then centrifuged at 8,000 rpm for 1 min using a QIAamp Mini spin column placed in a 2 mL collection tube. The flow-through and collection tube were discarded, and the spin column was transferred to a new 2 mL collection tube. DNA was washed with 500 µL of each wash buffer in two successive centrifugation steps. Finally, DNA was eluted by adding 200 µL of elution buffer.

Genomic DNA was extracted from subgingival plaque samples using the Omega DNA extraction kit (Omega, USA) following the manufacturer's instructions (Abu-Lubad *et al*., 2018). Briefly, samples were centrifuged to obtain a pellet, resuspended in TE buffer, and subjected to enzymatic lysis using lysozyme and proteinase K. RNase A was then added, and the lysate was purified using HiBind DNA Mini Columns. The extracted DNA was eluted in 50–100 µL of elution buffer. The concentration and purity of extracted DNA were determined using a NanoDrop spectrophotometer (Nabi UV/Vis Nano Spectrophotometer; Daejeon, Republic of Korea).

2.3.2. 2.3.2. DNA Amplification

Twenty-base oligonucleotide primers designated were used (Table 1) (Sedaghat *et al*., 2014). The 16S rRNA gene was amplified using; F- (5'-ATCCTGGCTCAGAGTGAACG-3') and R- (5'-GCAGGTTCACCTACGGTTACC-3') primers. A volume of 20 µL of PCR reaction mixture comprising 1× PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5% (v/v) Triton X-100), 1.5 mM $MgCl_2$, 200 µM of each dNTP, 10 pmol of each primer, 1 unit of Taq DNA polymerase, and 18 µL of template DNA was used. This reaction yielded a 395-base-pair amplified fragment. The initial denaturation was carried out at 96 °C for 5 minutes. The mixtures were then exposed to 40 cycles, each cycle consisting of three steps: denaturing at 94 °C for 1 minute, primer annealing at 62 °C for 1 minute, and extension of two minutes at 72 °C. This final extension is to make up for the complete elongation of the PCR products, together with an additional ten-minute extension time after the last cycle. A negative control prepared by adding all the master mix reagents, excluding the template, was used and executed in each batch of the PCR assay (Han and Ge, 2010).

Table 1. Primers of virulence genes used to characterize *Helicobacter pylori*

Target gene	Primer sequence (5'-3')	Product Size (bp)	Annealing Temp. (ºC)
VacA gene	F- CAATCGTGTGGGTTCTGGAGC R-GCCGATATGCAAATGAGCCGC	678	63.5
BabA gene	F- AATCCAAAAAGGAGAAAAAG TATGAAA R- TGTTAGTGATTTCGGTGTAG GACA	832	56.8
OipA gene	F- GTTTTTGATGCATGGGATTT R- GTGCATCTCTTATGGCTTT	401	50.3
IceA$_1$ gene	F- GTGTTTTTAACCAAAGTATC R- CTATAGCCATTATCTTTGCA	247	52.5
IceA$_2$ gene	F- GTTGTCGTTGTTTTAATGAA R- GTCTTAAACCCCACGATTAAA	120	48.7

2.3.3. Analysis of PCR Products

PCR-amplified products were analyzed by agarose gel electrophoresis. A total of 10 µL of each PCR product was mixed with 3 µL of loading buffer, which was prepared from 20 mL of 50% glycerol, 25 mg bromophenol blue, and 30 µL of 1 N NaOH, and then loaded onto a 1% agarose gel.

Following electrophoresis, the amplified DNA fragments were verified by staining the gel with ethidium bromide and examined under a UV transilluminator and photographed with a gel documentation system (Gel Doc 2000, Bio-Rad, USA) (Han and Ge, 2010).

2.3.4. Detection of Virulence Genes Using uniplex and multiplex PCR

For PCR amplification of the *VacA, BabA, OipA, IceA1*, and *IceA2* genes, a total of 0.6 µL (0.5 µg) of template genomic DNA was added to a 25 µL reaction mixture containing 2.0 µL of 10× PCR buffer, 0.9 µL of 50 mM $MgCl_2$, 0.5 µL of 10 mM dNTPs, 1.2 µL of each primer, 0.7 µL of Taq DNA polymerase (5 U/µL), and 4.4 µL of nuclease-free water. Amplification was done in a thermal cycler at the following conditions: initial denaturation for 4 minutes at 95 °C, 31 cycles with denaturation for 44 seconds at 95 °C, annealing for 45 seconds at 51 °C, extension for 62 seconds at 72 °C , and final extension at 72 °C for 5 minutes. The products were further visualized by electrophoresis in 1.5% agarose gels stained with Gel Red as described by Oktem-Okullu *et al*, (2015).

3. Statistical Analysis

The statistical packages of social science program (IBM SPSS Modeler, New York, US) version 19.0 was used to evaluate the data. In this regard, Chi-square and Pearson's correlation analyses were performed with the view to comparing the variables.

4. Results

The study population consisted of 125 biopsies collected from patients attending gastroenterology clinics at Al-Hussein Hospital/Al-Salt and Al-Bashir Hospital/Amman with different gastric complaints including 87 females and 38 males. The mean age was 42.29 ± 17.75 years for females and 44.95 ± 19.12 years for males. Patients from Amman exhibited a mean age of 40.45 ± 18.46 years, and from Al Salt, 45.54 ± 17.63 years (Figure 1). Geographic data revealed that 60% of the examined cases were from Amman and 40% from Al- Salt city.

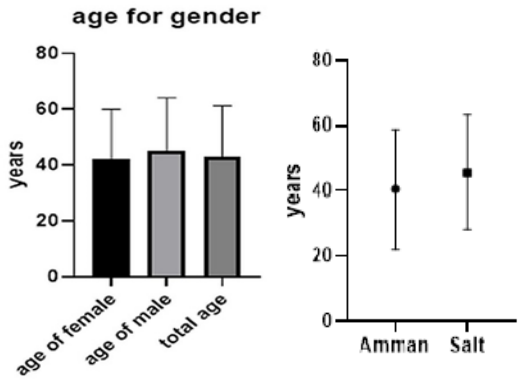

Figure 1. Mean age of the study group in relation to gender (A) and geographic distribution (B)

4.1. Characteristics of gastric biopsy by endoscopy

The endoscopic findings (in relation to the total number of cases) were categorized into normal gastric mucosa (30%), gastritis (65%), and gastric ulcer and carcinoma (5%).

4.2. Helicobacter pylori in realtion to histopathology

Corresponding to histopathology, *H. pyolri* was detected in 89 samples, and the results showed mild chronic active gastritis (10.11%), mild chronic inactive gastritis (10.11%), moderate chronic active gastritis (37.08%), moderate chronic inactive gastritis (3.37%), severe chronic active gastritis (26.97%), intestinal metaplasia (4.49%), gastric atrophy (3.37%), and reactive epithelial changes (4.49%) (Figure 2).

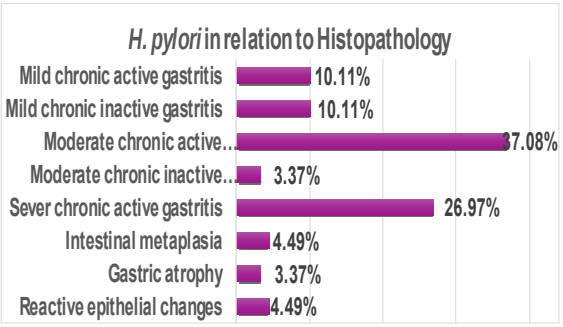

Figure 2. Percentage of *H. pylori* detected in gastric biopsy in relation to various Histopathology results

4.3. Diagnosis of Helicobacter pylori using 16S rRNA

For the detection of *H. pylori*, the *16S rRNA* gene was targeted by PCR and the amplified product was loaded on 1.5% agarose gel electrophoresis and photographed under UV light. The amplified gene gives a single band at 1471 bp. In total, 83 (66.4%) biopsy samples were *H. pylori* positive (Figure 3).

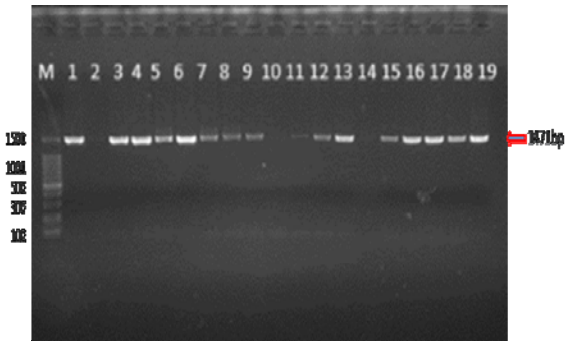

Figure 3. PCR amplification of 16SrRNA (1471 bp) gene. Lane M, 1500 bp DNA ladder, lane 1, positive control; lane 2, negative control; lane (3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19), positive *VacA* results, lane (10 and 14) negative *VacA* results

The results demonstrated that females developed infections more often than males (62.5% vs. 37.5%), and participants aged 50 years or older showed a persistently elevated risk, indicating greater susceptibility to disease (Figure 4).

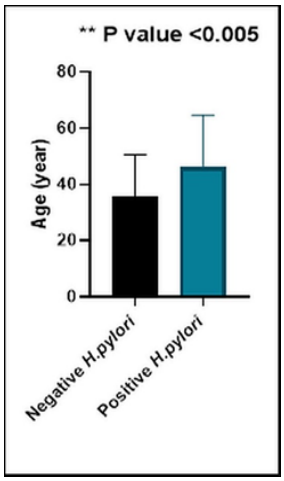

Figure 4. The prevalence of *H. pylori* in relation to age

4.4. Genotype characterization of H. pylori using uniplex and multiplex PCR

Using PCR, the presence of genes associated with pathogenicity was sought. The *VacA* gene was present in 62 (74.7%) of *H. pylori* isolates and shows a specific product with 678 bp (Figure 5).

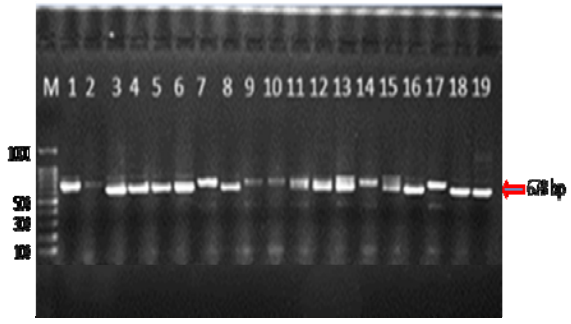

Figure 5. PCR amplification of *VacA* (678 bp) gene. Lane M, 1000 bp DNA ladder, lane 1, positivecontrol; lane 2, negative control; lanes (3-19), positive *VacA* results

OipA is a crucial gene involved in *H. pylori* colonization of the stomach mucosa. A total of 36 (43.3%) isolates had the *OipA* gene. The allelic variants of *IceA1* gene were also amplified by multiplex PCR (Figure 6). Of the isolates, 68 (81.9%) had the second allele form of *IceA2*, whereas 18 (21.7%) had a positive result for the *IceA1* (Figure 7).

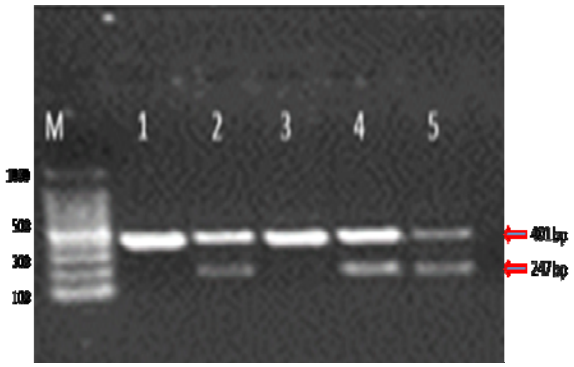

Figure 6. Amplification of *OipA* gene (401 bp) and *IceA1* gene (247 bp) applying Multiplex PCR. Lane M, 1000 bp DNA ladder; Lanes 2, 4, 5 positive results for the two genes; Lanes 1, 3 positive results for *OipA* gene only.

Figure 7 . PCR amplification of *IceA2* (120 bp) gene. Lane M, 1000 bp DNA ladder; lane 1, positive control; lane 2, negative control; lanes (3-19) positive *IceA2* results.

To emphasize the adherence ability of *H. pylori* to the gastric mucosa, *BabA* gene was amplified with a 271 bp product. The gene was detected in 79 (95.2%) of the isolates (Figure 8).

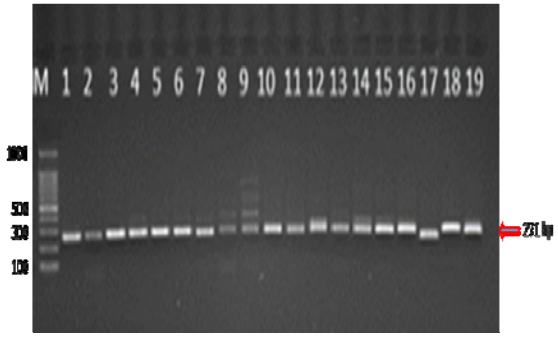

Figure 8. PCR amplification of *BabA* (271 bp) gene. Lane M, 1000 bp DNA ladder; lane 1, positive control; lanes (2-19) positive *BabA* gene results.

4.5. Association of virulence factors with histopahtology results

Variable percentages for *H. pylori* virulence genes using PCR showed that *BabA, IceA2, IceA2, VacA* and *OipA* genes are increased in cases with severe chronic active gastritis and moderate chronic active gastritis in relation to other cases (Figure 9). Statistical analysis also showed that there is a significant relation between the

VacA gene and moderate chronic inactive gastritis (p = 0.002), severe chronic active gastritis (p = 0.0009). Besides, *OipA* gene was significantly related with moderate chronic inactive gastritis (p = 0.008), *IceA2* gene with mild chronic inactive gastritis (p = 0.005), and *IceA1* gene with severe chronic active gastritis (p = 0.033). Only *BabA* gene was

found to be associated with reactive epithelial change with P-value of 0.005.

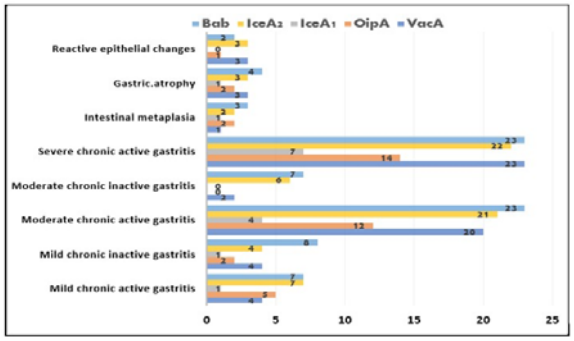

Figure 9. Histopathology results in relation to *H. pylori* genotype.

4.6. Dental samples result in relationship to gastric biopsy results

To investigate the potential role of human dental plaque as a reservoir for *H. pylori*, dental plaque samples were collected from 67 subjects who had undergone endoscopy. Only participants who provided informed consent for dental sample collection were included in the study. Fifty-six samples (83.58%) were positive for *H. pylori*. The prevalence of *H. pylori* in dental plaque was highest in patients with gastritis, n = 39 (69.6%), followed by the normal gastric mucosa, n = 16 (28.6%), and gastric ulcer & cancer, n = 1 (1.8%) (Figure 10A).

Considering the histopathology results of the 56 samples, a high percentage of *H. pylori* was detected in the dental plaque of patients diagnosed as mild chronic gastritis, n = 18 (32.1%), moderate chronic gastritis, n = 22 (39.3%). However, the lowest prevalence of dental *H. pylori* was detected in patients who had gastric atrophy, n = 4 (7.1%) and intestinal metaplasia, n = 3 (5.4%). In patients with severe chronic gastritis, *H. pylori* was identified in 9 (16.1%) samples obtained from dental plaque (Figure 10B).

The Chi-square test showed a significant relationship between dental PCR results and the findings by endoscopy (P-value < 0.05). The results revealed that 56 (83.58%) patients who were positive for *H. pylori* by PCR in gastric biopsy were also positive in dental plaque, whereas 11 (16.42%) dental plaque samples were negative for *H. pylori* and positive in gastric biopsy. Only 4 (7.14%) of the dental *H. pylori* positive patients were negative in gastric biopsy.

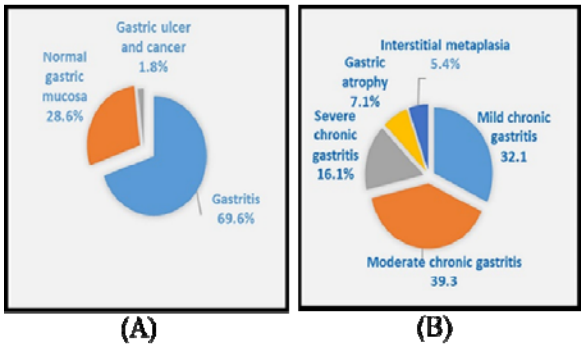

Figure 10. Prevalence of *Helicobacter pylori* in dental plaque in relation to gastric endoscopic (A) and histopathological findings (B)

5. Discusion

Infection with *H. pylori* increases the risk of severe clinical problems which is principally caused by the presence of distinct virulence factors that collectively contribute to the disease severity. Gastric biopsies obtained by endoscopy were utilized for histological examination and molecular analysis to ensure an accurate diagnosis of *H. pylori* infection. Endoscopy reveals gastritis as the primary diagnosis which affects 65% of patients, thus requiring fast detection of gastrointestinal disorders (Ayana *et al.*, 2014; Ho and Chiou, 2023). Histopathological analysis revealed that 71.2% of patients exhibited features associated with *H. pylori* infection, while PCR targeting 16S rRNA detected the bacterium in 66.4% of biopsies, indicating a high prevalence among patients undergoing upper endoscopy. A recent study by Al-Hyassat *et al*. (2025) employed real-time PCR targeting the 16S rRNA gene to detect *H. pylori* in 106 clinical samples, revealing a positivity rate of 74.5% in gastric biopsies, which aligns with our findings. In Jordan, seroprevalence udies reported that *H. pylori* affects 88% of the population nationwide (Obaidat and Roess, 2019).

These findings are comparable to previous research indicating a high global prevalence of *H. pylori*, particularly in the Middle East and North Africa, underscoring the need for improved diagnostic approaches and highlighting the bacterium's pivotal role in gastrointestinal disease progression (Alsulaimany *et al.* 2020).

The results revealed a predominance of female patients, consistent with earlier studies reporting that women are more likely to seek medical care for stomach issues and are more susceptible to specific gastrointestinal conditions (Al-Hyassat et al., 2025; Feyisa & Woldeamanuel, 2021). Similarly, Qiao *et al*, (2024) observed a positive linear correlation between *H. pylori* infection rate and increasing age among women, with older females showing higher infection rates, lower eradication success, and greater recurrence. These findings highlight the complex interplay between age, gender, and *H. pylori* persistence. Bharucha and Lacy (2020) emphasized that digestive system disorders affect individuals across the lifespan, with negligible age disparities between sexes. Consistent with this, the current study found broadly comparable age distributions among patients, with those from Amman showing a slightly lower mean age than those from Al-

Salt; however, the difference was not statistically significant.

Transmission predominantly occurs through oral–oral or fecal–oral routes, most often in early childhood, with infections frequently remaining asymptomatic into adulthood in the absence of targeted antimicrobial intervention. The observed high prevalence may partly reflect insufficient public awareness of transmission ways and preventive health behaviors (Alaridah *et al*., 2023; Kotilea *et al*., 2019). However, identification of these fundamental elements enables healthcare professionals to create specific treatment plans (Cardos *et al*., 2021; Shah *et al*., 2024).

Histopathological examination revealed gastric impairment in all infected biopsy specimens, demonstrating the method's effectiveness in assessing tissue deterioration and detecting bacterial presence. Histological observations support the role of *H. pylori* infection in a wide range of gastrointestinal disorders, emphasizing its central involvement in disease pathophysiology. The findings further highlight the substantial contribution of *H. pylori* to the development of chronic gastritis and reinforce the diagnostic reliability of histopathological evaluation, consistent with previous reports (Dănilă *et al*., 2022). Similarly, Santacroce *et al*. (2024) corroborated these results by demonstrating the role of *H. pylori* in chronic gastritis progression, including the development of gastric atrophy and intestinal metaplasia. These findings collectively align with earlier studies confirming that histopathology remains a critical method for confirming *H. pylori* infection in gastric biopsy samples (Al-Hyassat *et al*., 2025).

The distribution of *H. pylori* virulence genes in positive isolates from the Jordanian cohort demonstrates a distinct regional pathogenic profile, with specific virulence factors significantly associated with the severity of gastric histopathological changes. Previous studies have shown that *H. pylori* infection can lead to chronic gastritis, peptic ulcers, and gastric cancer (Ansari & Yamaoka, 2019; Braga *et al*., 2019; El-Sayed *et al*., 2020). Consistent with these findings, our study highlights the role of these virulence genes as biomarkers, demonstrating a strong association with moderate to severe gastritis. Molecular analysis revealed a high prevalence of adhesin genes—*BabA* (95.2%) and *IceA2* (81.9%)—alongside the major cytotoxin *VacA* (74.7%). This pattern suggests that the dominant strains in Jordan are highly adapted for long-term colonization and persistence, facilitated by *BabA* binding to ABO/Lewis^b (Leb) antigens on gastric epithelium. Chronic colonization underlies subsequent pathology, including the 65% prevalence of chronic gastritis observed in our endoscopic findings.

The *BabA* gene encodes an adhesin that attaches to Leb antigens, promoting bacterial colonization, chronic inflammation, and epithelial changes (Gerhard *et al*., 1999; Zhao *et al*., 2020; Singh *et al*., 2024). Our findings suggest its significant association with reactive epithelial changes in gastric biopsies, highlighting its critical role in disease progression. Critically, the lower prevalence of *OipA* (43.3%) and *IceA1* (21.7%) compared to *BabA* and *VacA* indicates that, within this specific regional population, the most frequent pathogenetic route relies heavily on superior adhesion rather than solely on immediate inflammatory stimulation by *OipA*, or acute damage traditionally linked to *IceA1*. This specific genotype ratio, therefore, provides the first detailed genotype-prevalence mapping for these five key factors in Jordan, a novel contribution essential for defining local strain pathology.

The research investigates how *H. pylori* virulence factors affect histopathological results by studying genes which produce significant clinical effects. The higher frequency of *BabA*, *IceA2* genes in severe and moderate chronic active gastritis cases was corroborate earlier findings supporting its role in gastric diseases (Phan *et al*., 2017). The *VacA* gene plays a critical role in the progression of gastric disease. It supports regulate the host's immune response and can influence how severe an infection develops. In our study, higher *VacA* gene expression was significantly associated with both severe gastric disease (p= 0.0009) and chronic active gastritis (p= 0.002). Importantly, *VacA* was not detected in normal gastric mucosa, reinforcing its value as a marker specific to disease (Al-Ouqaili *et al*., 2023). Multiplex PCR showed a lower prevalence of *IceA1* and a higher prevalence of *IceA2* in our cohort, consistent with the findings of Yakoob *et al*. (2015) and contrasting with earlier reports for *IceA1* (Feliciano *et al*., 2015). These findings support the role of *IceA2* in severe gastrointestinal disorders, including peptic ulcers and gastric cancer through its association with IL-8 overexpression ((Yakoob *et al*., 2015; Baj *et al*., 2020). Additionally, our results show a significant association between *IceA1* and *IceA2* alleles and severe chronic active gastritis, consistent with Akeel *et al*. (2019), who reported that these genes correlate with varying degrees of gastric inflammation and pathology.

The ability of *H. pylori* to colonize the oral cavity is becoming more widely acknowledged. Once the stomach is cleared, it may act as a chronic reservoir that leads to reinfection.

This gap limits our understanding of why most patients continue to experience recurrent infection despite successful treatment. Turning to the oral environment, a successfully isolated viable *H. pylori* from infected root canals demonstrated that the bacteria can persist within protected oral tissues and supports the possibility that the oral cavity may act as a reservoir (Elger *et al*., 2024). The detection of *H. pylori* DNA, including the pathogenic *cagA* gene, was found in dental plaque and saliva (Wongsuwanlert *et al*., 2023). It has also been found more often in deeper periodontal pockets, particularly when the periodontal pathogen *Porphyromonas gingivalis* is present (Kadota *et al*., 2020). The likelihood of oral colonization appears to be influenced by lifestyle and environmental factors, such as dietary habits and oral hygiene. Poor oral hygiene has been consistently associated with higher detection rates. Although a link between oral and gastric *H. pylori* has been previously reported (Cai *et al*., 2014), the relationship between oral infection and gastrointestinal disorders remains unclear. The severity of *H. pylori* infections has not significantly declined, and even after successful eradication, recurrence rates remain high, largely due to oral colonization. In our study, a strong significant correlation was observed between *H. pylori* isolates from dental plaque and *H. pylori*-positive gastric samples, with the highest oral prevalence observed in patients with gastritis (69.6%). Miyabayashi *et al*. (2000) examined patients with *H. pylori* gastritis to evaluate the

relationship between successful gastric eradication and the persistence of oral *H. pylori* after treatment. Their conclusion indicated that oral colonization by *H. pylori* negatively impacts eradication success and may contribute to the recurrence of gastric infection.

Our study indicates that dental plaque acts as a reservoir for *H. pylori*, facilitating recurrent infections after gastric eradication therapy. The high prevalence of infection in gastritis patients (69.6%) highlights the bacterium's involvement in the early stages of gastric disease. These findings align with previous research showing higher *H. pylori* detection rates in non-atrophic gastritis (Cuba *et al*., 2023). Our results further support the inclusion of oral sites in diagnostic and therapeutic strategies to prevent reinfection (Cuba *et al*., 2025; Fan *et al*., 2025; Anand *et al*., 2025; Scholz *et al*., 2025). While oral *H. pylori* detection can serve as an early biomarker for gastritis, its clinical utility diminishes as gastric pathology progresses toward precancerous stages, underscoring the critical role of the oral cavity in the bacterium's life cycle.

6. Conclusion

This study shows that *H. pylori* virulence factors *VacA*, *BabA*, and *OipA* play a major role in severe gastric diseases. The findings highlight the importance of early detection, thorough diagnostics, and personalized treatment plans. The high prevalence of *H. pylori* in dental plaque highlights oral hygiene as a critical component of infection. The bacterium's persistence in the gastrointestinal tract can lead to chronic inflammation and increase the risk of cancer. Managing these infections effectively requires better diagnostic tools, tailored therapies, and preventive strategies, which should be priorities in both clinical care and public health. Future research should focus on large-scale, long-term studies to pinpoint the most critical virulence factors, investigate the oral cavity as a potential reservoir for recurrent infection, and refine diagnostic and treatment approaches by comparing gastric and oral bacterial strains.

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Institutional Review Board Statement

The Ministry of Health's Institutional Review Board authorized this study and was carried out in agreement with the Declaration of Helsinki, Amman, Jordan (MBA/IRB/3822).

The research required all participants to give their consent before joining the study.

The research data appears in the article as its primary original contribution. The corresponding author will receive all additional inquiries.

The authors state no conflict of interests in this work.

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