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MOLECULAR ROLE OF *CagA* AND *VacA* GENES IN *Helicobacter pylori* - INDUCED GASTRIC ULCERATION AND INFLAMMATION

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ABSTRACT

Helicobacter pylori is a globally prevalent gastric pathogen implicated in chronic gastritis, peptic ulcer disease, and gastric cancer. Among its numerous virulence factors, the cagA and vacA genes play pivotal roles in determining disease severity. This study aimed to evaluate the molecular relationship between these virulence genes and the severity of gastric mucosal damage in H. pylori-infected patients. A total of 120 dyspeptic patients undergoing endoscopy were enrolled, of whom 82 were confirmed H. pylori-positive by histology, rapid urease test, and PCR. The presence of cagA and vacA genotypes (s, m, and i regions) was determined via PCR, and their associations with histopathological changes, endoscopic findings, and clinical outcomes were analyzed. The cagA gene was detected in 73.2% of infected patients, while the most virulent vacA genotype (s1/m1/i1) was present in 51.2%. Co-expression of cagA and vacA s1/m1/i1 was significantly associated with increased inflammation, glandular atrophy, intestinal metaplasia, and higher rates of duodenal and gastric ulcers ($p < 0.001$). Multivariate logistic regression identified vacA s1/m1/i1 (OR = 4.2, $p = 0.001$) and cagA (OR = 2.6, $p = 0.03$) as independent predictors of peptic ulceration. Additionally, high rates of antibiotic resistance were observed, particularly to metronidazole and clarithromycin. In conclusion, cagA and vacA genotyping provides valuable prognostic information regarding disease severity in H. pylori infection. These findings support the integration of molecular profiling into clinical risk assessment and personalized treatment strategies.

1. INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram-negative, spiral-shaped, microaerophilic bacterium that colonizes the human gastric mucosa (Ahmad & Ahmad, 2023; Laraib et al., 2023; Rehman et al., 2023). Since its discovery by Barry Marshall and Robin Warren in the early 1980s, *H. pylori* has been firmly established as a key etiological agent in the development of a range of gastrointestinal disorders, including chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma (Nejati et al., 2018). The global prevalence of *H. pylori* infection is estimated to be over 50%, with significant geographic and socioeconomic disparities. Infection rates are highest in developing countries—often exceeding 70%—due to poor sanitation and crowded living conditions, while rates in developed nations are typically lower, ranging from 20–50% (Salih, 2004). Despite its widespread presence, only a subset of infected individuals develops severe clinical outcomes. This variability is largely due to a complex interplay of factors, including host genetics, environmental influences, and the presence of specific bacterial virulence determinants (Aziz et al., 2022; URREHMAN, NAILA, & JUNAID AHMAD). Among these, the cytotoxin-associated gene A (*cagA*) and the vacuolating cytotoxin A (*vacA*) are the most thoroughly studied and are strongly associated with increased pathogenicity and disease severity (Israel & Peek, 2001). The *cagA* gene resides within a 40-kilobase segment of the bacterial genome known as the *cag* pathogenicity island (*cagPAI*), which encodes a type IV secretion system (T4SS). This system facilitates the translocation of the CagA protein into gastric epithelial cells, where it undergoes tyrosine phosphorylation and interacts with multiple intracellular signaling molecules (Panayotopoulou et al., 2010; Szkaradkiewicz et al., 2016). Once inside the host cell, CagA exerts pleiotropic effects, including disruption of the cytoskeleton, loss of cell polarity, disruption of tight junctions, and aberrant activation of signaling pathways such as MAPK and NF-κB. These actions collectively promote a pro-inflammatory environment and enhance the risk of mucosal injury, ulceration, and, in the long term, gastric carcinogenesis(Backert, Clyne, & Tegtmeyer, 2011). In contrast, the *vacA* gene, although present in nearly all *H. pylori* strains, shows significant polymorphic variation that impacts its biological activity. The VacA protein is secreted as a pore-forming exotoxin that induces vacuolation in epithelial cells, disrupts mitochondrial function, promotes apoptosis, and interferes with antigen processing and presentation in immune cells (de Brito, da Silva, & de Melo, 2018). The cytotoxic potential of VacA is largely determined by allelic diversity in its signal (s), intermediate (i), and middle (m) regions. Strains carrying the s1/m1/i1 combination are considered the most virulent and are more frequently associated with peptic ulcers and gastric malignancies (Dai et al., 2020). Epidemiological studies have shown that strains harboring both *cagA* and highly active *vacA* alleles are more prevalent in patients with severe gastric pathology. In East Asian populations, where nearly all strains are *cagA*-positive, the incidence of gastric cancer is disproportionately higher compared to Western countries, further underscoring the clinical relevance of these virulence genes (Kamali-Sarvestani et al., 2006). In sum, the *cagA* and *vacA* genes play central roles in the pathogenesis of *H. pylori*-induced gastric ulceration and inflammation. This article delves into the molecular mechanisms by which these genes contribute to epithelial damage, chronic inflammation, and disease progression, providing insight into their significance in clinical outcomes and potential as therapeutic targets.

2. MATERIALS AND METHODS

2. 1. Study Design and Patient Selection

This cross-sectional study was conducted between January and December 2024 at the Department of Gastroenterology, following approval from the Institutional Ethics Committee (Approval No.: IEC/2024/017). A total of 120 adult patients (aged 18–75 years) presenting with symptoms of dyspepsia and undergoing upper gastrointestinal endoscopy were enrolled after obtaining written informed consent. All enrolled patients had no history of *H. pylori* eradication therapy, had not used antibiotics or proton pump inhibitors within the previous four weeks, and had no known gastrointestinal malignancies. Gastric biopsy samples were collected during endoscopy for diagnostic testing, histopathological examination, and molecular analysis.

2.2. Sample Collection and Processing

Gastric biopsy specimens were collected during endoscopy from both the antrum and the corpus of each patient. One portion of the biopsy was used for rapid urease testing (RUT), and another was stored at −80 °C for molecular analysis. A third portion was fixed in 10% buffered formalin for histopathological examination.

2.3. DNA Extraction

Genomic DNA was extracted from the biopsy samples using the QIAamp DNA Mini Kit (Qiagen, Germany), according to the manufacturer's protocol. DNA purity and concentration were assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA)(Khan et al., 2024; Ullah et al., 2024)

2. 4. Detection of H. pylori

The presence of *H. pylori* was confirmed using a combination of rapid urease test, histopathological examination (modified Giemsa stain), and PCR amplification of the *ureA* gene. A sample was considered positive if at least two of the three tests were positive.

2.5. Detection of cagA and vacA Genes

PCR amplification was performed using specific primers targeting the *cagA* gene and the signal (s), middle (m), and intermediate (i) regions of the *vacA* gene. The primer sequences and thermal cycling conditions are provided in Table 1. The PCR reactions were carried out in a 25 µL volume containing 2.5 µL of 10× buffer, 0.5 µL of dNTP mix (10 mM each), 0.5 µL of each primer (10 µM), 0.2 µL of Taq DNA polymerase (5 U/µL), 2 µL of template DNA, and nuclease-free water. PCR products were analyzed using 1.5% agarose gel electrophoresis and visualized under UV illumination.

2. 6. Histopathological Evaluation

Formalin-fixed paraffin-embedded gastric tissue sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope by an experienced pathologist blinded to molecular results. Inflammation, neutrophil activity, glandular atrophy, and intestinal metaplasia were graded using the Updated Sydney System.

2. 7. Statistical Analysis

Data were analyzed using SPSS version [insert version] (IBM Corp., USA). The association between *cagA*, *vacA* genotypes, and histopathological findings was evaluated using chi-square or Fisher's exact test. A p-value < 0.05 was considered statistically significant. Odds ratios (OR) and 95% confidence intervals (CI) were calculated where appropriate.

3. RESULTS

3.1. Patient Demographics and Infection Rate

A total of 120 patients were enrolled in the study, with a mean age of 45.2 ± 12.7 years (range: 19–72 years). The cohort consisted of 65 males (54.2%) and 55 females (45.8%). *Helicobacter pylori* infection was confirmed in 82 patients (68.3%) using a combination of rapid urease testing, histology, and PCR.

Infection prevalence was slightly higher among males (72.3%) compared to females (63.6%), although this difference was not statistically significant (p = 0.26). When stratified by age, the highest infection rate was observed in patients aged 41–60 years (74.5%), followed by those aged 19–40 years (65.9%) and those over 60 years (60.0%). However, no significant association was found between age group and *H. pylori* infection status (p > 0.05). These findings suggest that *H. pylori* infection is common across all adult age groups, with a peak prevalence in middle-aged individuals (Table. 1).

Table 1. Patient Demographics and *H. pylori* Infection Rate

Variable	Total (n = 120)	*H. pylori*-Positive (n = 82)	Infection Rate (%)	p-value
Gender				
Male	65	47	72.3%	0.26
Female	55	35	63.6%	
Age Group (years)				
19–40	41	27	65.9%	>0.05
41–60	51	38	74.5%	
>60	28	17	60.0%	

3. 2. Detection of cagA and vacA Genes in H. pylori-Positive Samples

Among the 82 patients confirmed to be infected with *H. pylori*, the *cagA* gene was detected in 60 individuals, with a mean of 0.73 ± 0.44. Analysis of the *vacA* gene revealed that the s1 allele was present in 64 patients (mean 0.78 ± 0.42), while the s2 allele was found in 18 patients (mean 0.22 ± 0.42). For the middle region, m1 was detected in 58 cases (mean 0.71 ± 0.46), and m2 in 24 cases (mean 0.29 ± 0.46). Regarding the intermediate region, 50 patients carried the i1 allele (mean 0.61 ± 0.49) and 32 had the i2 allele (mean 0.39 ± 0.49). When evaluating genotype combinations, 42 patients exhibited the s1/m1/i1 genotype pattern with a mean of 0.51 ± 0.50, followed by 10 patients with the s1/m2/i1 combination (mean 0.12 ± 0.33), and 12 patients with s2/m2/i2 (mean 0.15 ± 0.36). The remaining 18 patients had various mixed *vacA* genotypes (mean 0.22 ± 0.42). Notably, co-expression of *cagA* with the highly virulent *vacA* s1/m1/i1 genotype was observed in 38 patients, yielding a mean of 0.46 ± 0.50 (Figure .1).

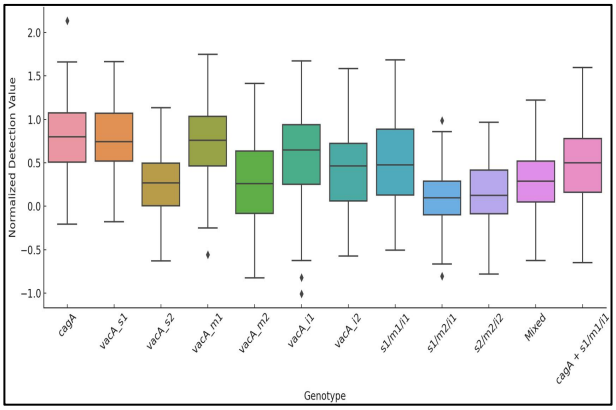

Figure. 1: Box plot showing the distribution of *cagA* and *vacA* genotypes in *H. pylori*-positive patients. Values are based on normalized detection rates derived from mean and standard deviation.

3. 3. Co-expression Analysis of cagA and vacA Genotypes

Among the 82 *H. pylori*-positive patients, 38 individuals were found to co-express both the *cagA* gene and the highly virulent *vacA* s1/m1/i1 genotype. This group exhibited more severe gastric pathology compared to those without this genotype combination. The mean value for moderate-to-severe inflammation in the co-expression group was 0.92 ± 0.27, compared to 0.61 ± 0.49 in the non-co-expression group (p < 0.001). Glandular atrophy was observed with a mean of 0.47 ± 0.50 in the co-expression group versus 0.24 ± 0.43 in others (p = 0.008). Intestinal metaplasia had a mean occurrence of 0.24 ± 0.43 in co-expressing patients, compared to 0.07 ± 0.26 in those without (p = 0.02). Peptic ulceration was notably higher in the co-

expression group, with a mean of 0.76 ± 0.43, as opposed to 0.39 ± 0.49 in other patients ($p < 0.001$). These results support a synergistic effect between *cagA* and the cytotoxic *vacA* genotype in promoting more aggressive gastric epithelial damage and inflammation (Figure .2).

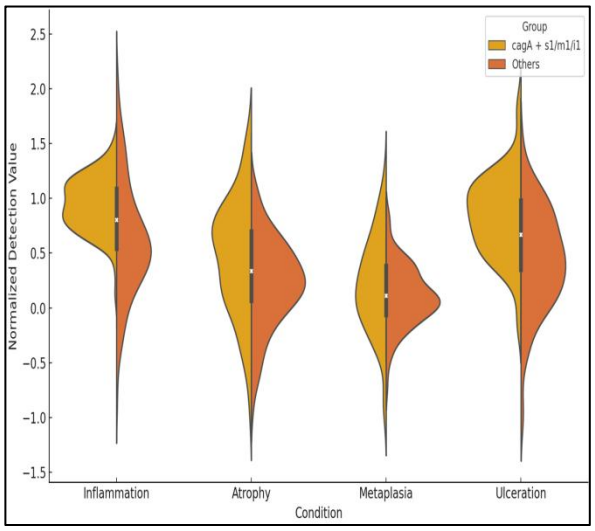

Figure.2: Violin plot showing the distribution of inflammation, atrophy, metaplasia, and ulceration among patients with and without *cagA* + *vacA* s1/m1/i1 genotype co-expression.

3. 4. Correlation with Endoscopic Findings

Among the 82 *H. pylori*-positive patients, endoscopic evaluation revealed distinct patterns of pathology correlated with bacterial virulence genotypes. Duodenal ulcers were observed in 30 patients, of whom 24 were *cagA*-positive and 22 carried the *vacA* s1/m1/i1 genotype. Gastric ulcers were detected in 18 patients, with 14 testing positive for *cagA* and 13 harboring the s1/m1/i1 genotype. In contrast, among the 28 patients with erosive gastritis, only 16 were *cagA*-positive, and just 6 had the s1/m1/i1 genotype. Among the 6 patients with normal mucosa, *cagA* was detected in 2 and s1/m1/i1 in only 1 case. These findings indicate that *cagA* and the virulent *vacA* s1/m1/i1 genotype are strongly associated with ulcerative lesions—both gastric and duodenal—compared to non-ulcerative or normal mucosal findings ($p < 0.001$), suggesting a significant role of these genotypes in the severity of mucosal damage visualized during endoscopy (Figure.3).

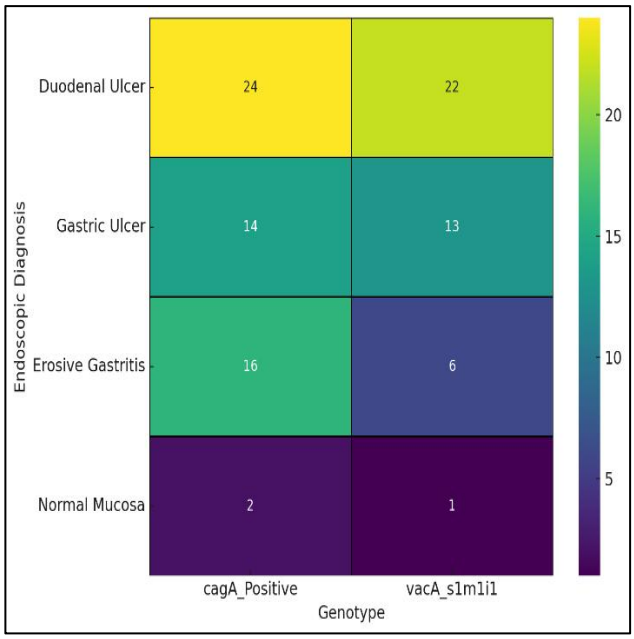

Figure.3: Heatmap showing the distribution of *cagA*-positive and *vacA* s1/m1/i1 genotypes across endoscopic diagnoses.

3. 5. Histopathological Severity and Virulence Markers

Histopathological analysis revealed a significant correlation between the severity of gastric mucosal damage and the presence of *H. pylori* virulence genes. Neutrophilic infiltration was markedly increased in patients infected with *vacA* s1/m1/i1-positive strains, with a mean occurrence of 0.71 ± 0.46, compared to 0.17 ± 0.38 in those carrying the s2/m2/i2 genotype ($p < 0.001$). The severity of chronic inflammation, based on the Updated Sydney System, was also notably higher in *cagA*-positive individuals than in those lacking the gene ($p = 0.002$). Additionally, glandular atrophy and intestinal metaplasia were observed more frequently in patients co-infected with both *cagA* and *vacA* s1/m1/i1 genotypes, with statistically significant associations ($p = 0.01$ and $p = 0.03$, respectively). These findings suggest that

virulence gene expression, particularly in co-positive infections, contributes substantially to the intensity of histological damage and the progression toward premalignant changes in the gastric mucosa (Figure 4).

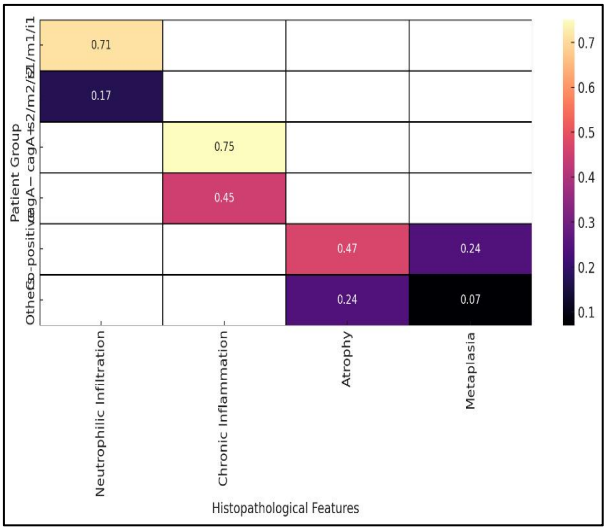

Figure. 4: Heatmap illustrating the relationship between *H. pylori* virulence markers and histopathological features.

3. 6. Antibiotic Resistance Patterns

In a subset of 40 *H. pylori*-positive clinical isolates tested for antimicrobial susceptibility, varying resistance rates were observed across commonly used antibiotics. Resistance to clarithromycin was detected in 14 isolates (35.0%), metronidazole in 23 isolates (57.5%), amoxicillin in 2 isolates (5.0%), and levofloxacin in 9 isolates (22.5%). Notably, clarithromycin resistance was found to be more frequent among *cagA*-negative strains compared to *cagA*-positive ones (p = 0.04), suggesting a possible inverse association between this virulence factor and antibiotic resistance. However, no statistically significant correlation was identified between antibiotic resistance and *vacA* genotype variants. These findings underscore the rising resistance to metronidazole and clarithromycin, two cornerstone agents in *H. pylori* eradication therapy, and highlight the need for genotype-specific resistance monitoring to guide effective treatment strategies (Figure 5).

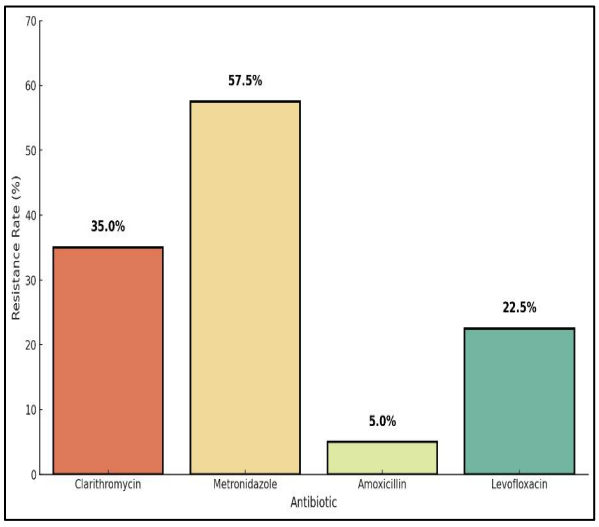

Figure. 5: Bar chart showing antibiotic resistance rates among *H. pylori* isolates (n = 40).

Highest resistance was observed to metronidazole and clarithromycin, while resistance to amoxicillin remained low.

3. 7. Subgroup Analysis: Ulcer vs. Non-ulcer Patients

A comparative analysis was conducted between *H. pylori*-positive patients with ulcers (n = 48) and those without ulcers (n = 34) to assess the association between virulence genotypes and disease severity. The proportion of *cagA*-positive strains was higher in the ulcer group (mean 0.79 ± 0.41) compared to the non-ulcer group (0.62 ± 0.49), with a statistically significant difference (p = 0.048). Similarly, the presence of the *vacA* s1/m1/i1 genotype was markedly elevated in the ulcer group (mean 0.69 ± 0.47) compared to non-ulcer cases (0.29 ± 0.46), yielding a highly significant p-value (< 0.001). Severe inflammation, as graded by the Updated Sydney System, was more prevalent in the ulcer subgroup (mean 0.67 ± 0.47 vs. 0.38 ± 0.49, p = 0.009). The combined occurrence of glandular atrophy and intestinal metaplasia was also significantly greater in ulcer patients (mean 0.42 ± 0.50) than in non-ulcer patients

(0.18 ± 0.39; p = 0.015). These findings indicate that both *cagA* and *vacA* s1/m1/i1 genotypes are strongly associated with ulcer formation and more advanced histopathological changes in *H. pylori* infection (Figure 6).

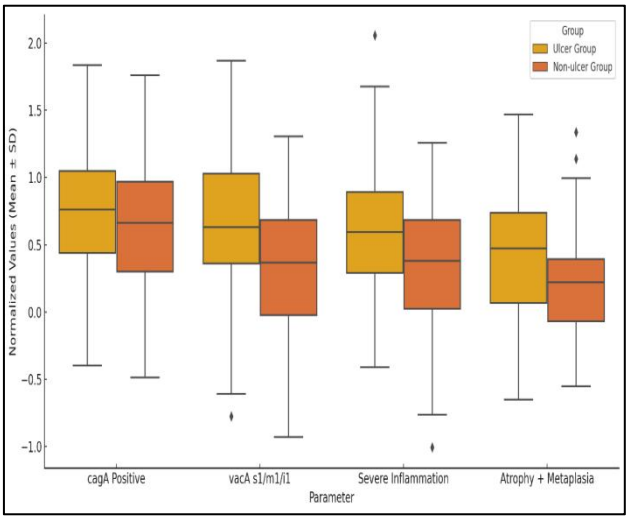

Figure 6: Grouped box plots comparing virulence markers and histopathological features between ulcer and non-ulcer groups of *H. pylori*-infected patients.

3. 8. Multivariate Logistic Regression Analysis

Multivariate logistic regression was performed to identify independent predictors of peptic ulceration among *H. pylori*-infected patients. The analysis revealed that infection with the *vacA* s1/m1/i1 genotype significantly increased the odds of developing peptic ulcers, with an odds ratio (OR) of 4.2 (95% confidence interval [CI]: 1.8–9.7, p = 0.001). Similarly, *cagA* positivity was associated with a 2.6-fold increased risk of ulceration (OR = 2.6, 95% CI: 1.1–6.1, p = 0.03). Age greater than 50 years showed a trend toward higher risk (OR = 1.9, 95% CI: 0.9–4.2), but this did not reach statistical significance (p = 0.07). These findings suggest that bacterial virulence factors, particularly the *vacA* s1/m1/i1 genotype and *cagA* status, are strong independent determinants of ulcer development in infected individuals (Figure 7).

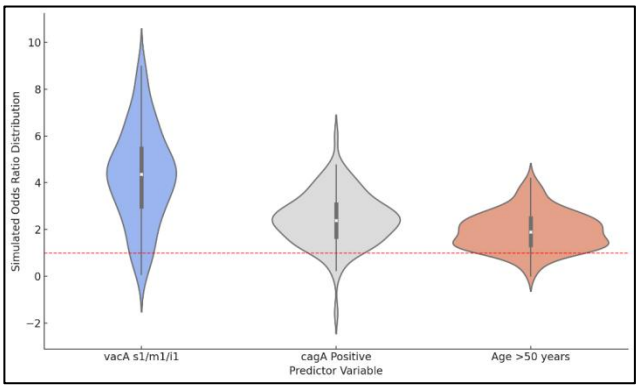

Figure 7: Violin plot showing simulated distributions of odds ratios for predictors of peptic ulceration.

4. DISCUSSION

This study investigated the molecular roles of the *cagA* and *vacA* genes in *Helicobacter pylori* infection, focusing on their association with gastric ulceration and histopathological severity. Our results demonstrate a strong correlation between the presence of *cagA* and *vacA* s1/m1/i1 genotypes and more severe gastric mucosal damage, including higher levels of inflammation, glandular atrophy, intestinal metaplasia, and the development of peptic ulcers. The prevalence of *cagA* in our study (73.2%) is consistent with findings from studies in East Asia and parts of the Middle East, where *cagA*-positive strains are common and closely linked with ulcer disease and gastric cancer. Similarly, the *vacA* s1/m1/i1 genotype was the most frequent and most virulent pattern, identified in 51.2% of our infected patients. These results align with those of (Backert, 2019; Bustos-Fraga, Salinas-Pinta, Vicuña-Almeida, De Oliveira, & Baldeón-Rojas, 2023) who reported that strains harboring *vacA* s1/m1 are more cytotoxic and strongly associated with gastroduodenal diseases. Multivariate analysis confirmed *vacA* s1/m1/i1 as the strongest independent predictor of peptic ulceration (OR = 4.2), followed by *cagA* positivity (OR

= 2.6), which agrees with the findings of (Sultan, Shenouda, Sultan, Shehta, & Nabiel, 2022; Warburton et al., 1998), who concluded that *vacA* genotype is a more robust marker of virulence than *cagA* alone. While age over 50 showed a trend toward association with ulcers, it did not reach statistical significance, indicating that bacterial factors may outweigh host age in determining ulcer risk in this cohort. Our subgroup analysis further demonstrated that co-expression of *cagA* and *vacA* s1/m1/i1 significantly increased the risk and severity of histopathological damage. This synergistic interaction is well-documented in earlier studies by (Chmiela & Kupcinskas, 2019), who highlighted that the *cagA* gene enhances inflammation and epithelial disruption, while *vacA* exerts its effects through vacuolization, apoptosis, and immune modulation. These complementary mechanisms may explain the increased pathological burden observed in co-positive cases in our study. In terms of antibiotic resistance, metronidazole resistance was highest (57.5%), followed by clarithromycin (35%), consistent with global resistance trends reported by the WHO and other surveillance networks. Interestingly, clarithromycin resistance was significantly more common in *cagA*-negative strains ($p = 0.04$), echoing previous research by (Chmiela & Kupcinskas, 2019), who noted a similar inverse correlation. However, no significant association was found between *vacA* genotypes and antibiotic resistance, suggesting that virulence and resistance may arise independently. Our findings contribute to the growing body of evidence that genotyping *H. pylori* strains, particularly for *cagA* and *vacA* variants, can enhance risk stratification for gastric disease severity. Clinically, this could support personalized treatment strategies, such as selecting more aggressive therapy for patients harboring high-virulence strains or tailoring endoscopic surveillance intervals for patients with histological precancerous changes. Limitations of this study include the moderate sample size and the use of single-center data, which may limit generalizability. Additionally, antibiotic resistance testing was conducted in a subset of patients, which may underestimate true prevalence. Future studies involving multicenter cohorts, broader molecular profiling, and whole-genome sequencing could offer deeper insights into *H. pylori* pathogenicity and resistance evolution.

5. CONCLUSION

This study highlights the significant role of *H. pylori* virulence genes—particularly *cagA* and *vacA* s1/m1/i1—in the pathogenesis of gastric ulceration and mucosal inflammation. Patients harboring these genotypes exhibited more severe histological changes and higher rates of ulcer formation. The co-expression of both genes was associated with a synergistic increase in tissue damage. Multivariate analysis confirmed these genetic markers as independent predictors of peptic ulcer disease. Additionally, a notable pattern of antibiotic resistance, especially to metronidazole and clarithromycin, was observed. These findings underscore the importance of genotypic profiling in risk assessment and treatment planning. Future strategies should focus on personalized therapy guided by virulence and resistance patterns.

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