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Virulence Factors of *Helicobacter pylori*: A Review

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ABSTRACT: *Helicobacter pylori* is a spiral-shaped Gram-negative bacterium that colonizes the human stomach and can establish a long-term infection of the gastric mucosa, a condition that affects the relative risk of developing various clinical disorders of the upper gastrointestinal tract, such as chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma. *H. pylori* presents a high-level of genetic diversity, which can be an important factor in its adaptation to the host stomach and also for the clinical outcome of infection. There are important *H. pylori* virulence factors that, along with host characteristics and the external environment, have been associated with the different occurrences of diseases. This review is aimed to analyzing and summarizing the main of them and possible associations with the clinical outcome.

KEYWORDS: *Helicobacter pylori*, virulence factors, chronic gastritis, peptic ulcer disease, gastric adenocarcinoma

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Introduction

Helicobacter pylori is a flagellate Gram-negative spiral-shaped bacterium found on the luminal surface of the gastric epithelium. *H. pylori* organisms are 2.5–5.0 µm long and 0.5–1.0 µm wide, with four to six polar-sheated flagella, which are essential for bacterial motility.[1]

Infection is generally acquired during childhood and persists life-long in the absence of antibiotic treatment. Although the first isolation of the microorganism was in 1983 by Marshall and Warren,[2] it has been demonstrated that *H. pylori* has a long period of co-evolution with humans, going back at least since human migration out of Africa about 60,000 years ago.[3,4] This co-evolution is reflected on DNA sequence signatures observed in *H. pylori* strains of different geographic origins and has enabled the mapping of human migration out of Africa. This prolonged and intimate relationship is likely to have shaped the large and diverse repertoire of strategies that *H. pylori* employs to establish robust colonization and persist in the gastric niche.[5,6]

The routes of transmission of *H. pylori* still remain unclear. Person-to-person transmission and intrafamilial spread seem to be the main route, based on the intrafamilial clustering observed in some studies.[7,8] Children are often infected by a strain, which is a genetic fingerprint identical to that of their parents, and they maintain this genotype even after moving to a different environment.[9]

The finding of strain-specific genes from the comparison of sequenced *H. pylori* strains demonstrates the high diversity of *H. pylori* genome,[10] and this high level of genetic diversity can be an important factor in its adaptation to the host stomach and also for the clinical outcome of infection, an aspect that remains unclear. However, it is thought to involve an interplay among the virulence of infecting strains, host genetics, and environmental factors,[11] and experience with other bacterial pathogens suggests that *H. pylori*-specific factors may influence the microorganism's pathogenicity.

Since pathogen isolation, *H. pylori* infection has been associated with the development of various clinical disorders of the upper gastrointestinal tract, such as chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma.[12] In 1994, *H. pylori* was classified as a group I carcinogen by The International

Agency for Research on Cancer and was regarded as a primary factor for gastric cancer (GC) development.[13] In addition, during the last years, *H. pylori* infection has also been associated with some extra-digestive diseases, such as iron-deficiency anemia,[14] idiopathic thrombocytopenic purpura (ITP),[15,16] cardiovascular diseases,[17,18] hepatobiliary diseases,[19,20] and diabetes mellitus,[21,22] among others.

As regard to the host, the genetic factors have a significant impact on the clinical outcome and anatomical distribution of *H. pylori* infection, and polymorphisms in several genes are considered to increase the risk for the development of GC. For instance, individuals carrying the proinflammatory polymorphism of the interleukin-1-beta (IL-1β) and IL-1 receptor antagonist genes have a twofold to threefold increased risk of developing GC compared with subjects who have genotypes with less proinflammatory activity.[23] Similarly, polymorphisms in the genes that regulate the tumor necrosis factor (TNF)-α and the IL-16 are also associated with an increased risk of GC.[24,25] In addition, functional polymorphisms of receptors of the innate immune response have been reported to increase risk of GC.[26]

Concerning to environmental factors, diet particularly plays an important role in the pathogenesis of GC. Numerous case–control epidemiological studies have shown that high intake of salted, pickled, or smoked foods, dried fish and meat, and refined carbohydrates significantly increases the risk of developing GC, whereas fiber, fresh vegetables, and fruits were found to be inversely associated with GC risk.[27–32] Nevertheless, GC comprises two main entities, the intestinal and the diffuse type, which differ considerably from an epidemiological, clinical, and molecular point of view.[33] Based on epidemiological evidence, the intestinal type, preceded by precancerous lesions, seems more closely influenced by environmental factors while the latter recognizes mainly a "genetic" substrate. It has been suggested that the dietary risk factors are common to both types of GC, but the protective factors seem to play a more important role in preventing the intestinal type. Consequently, because of the "synergistic" interplay between diet and *H. pylori* infection, *H. pylori* should always be properly considered.[34]

Some studies have reported that smoking is an important risk factor for GC development[35,36] and about 60 different components in cigarette smoke are considered to be carcinogenic. Results of a large study in Europe estimated that 17.6% of GC is related to smoking.[37] A systematic review analyzed the relationship between cigarette smoking and GC and provided evidence that smoking was significantly associated with an increased relative risk for both gastric cardia and non-cardia cancers.[38] One important study clearly demonstrated that smoking patients with CagA-positive *H. pylori* infection have a strongly increased risk of GC, demonstrating that the risk for this disease development increases dramatically in conjunction with *H. pylori* infection.[39]

Specifically regarding *H. pylori* genetic characteristics, according to Yamaoka,[40] many putative virulence genes of *H. pylori* have been reported to determine clinical outcomes, and these are generally classified into three categories. The first one contains strain-specific genes, which are present in only some *H. pylori* strains. Among them, the best studied is the cytotoxin-associated gene pathogenicity island (*cag*PAI), which encodes a bacterial type IV secretion apparatus.[41] The second group consists of phase-variable genes whose gene status can be changed during growth or under different conditions to adapt *H. pylori* physiology to the environment and ensure its survival.[42] Based on the comparison of the three first sequenced genomes of *H. pylori*, six genes encoding outer-membrane proteins (OMPs) (*oipA*, *sabA*, *sabB*, *babA*, *babC*, and *hopZ*) are thought to undergo phase variation, which is high-frequency reversible on/off switching of gene expression.[43–46] The functional status is regulated by a slipped-strand mispairing mechanism being mediated by the number of CT dinucleotide repeats in the 5' region of the genes.[40] Although variability exists in the presence of *cag*PAI among *H. pylori* strains, genes encoding OMPs are present in all *H. pylori* strains.[47–51] The last group of genes comprises variable structures and genotypes depending on the strain, such as the *vacA* gene. In addition, the structure of many genes differs between Western strains and East Asian strains, and the structural differences in some genes are reported to influence virulence.[40,52]

This review aimed to report the main genes considered as virulence factors of *H. pylori* and emphasize their functions and mechanisms, also reporting their possible relationship with the clinical outcomes of diseases associated with *H. pylori* infection.

*Cag*PAI

*Cag*PAI is a 40 kb region of chromosomal DNA encoding approximately 31 genes that forms a type IV secretion system and can be divided into two regions, cag I and cag II, according to a novel insertion sequence.[41] This secretion system forms a pilus that delivers CagA, an oncoprotein, into the cytosol of gastric epithelial cells through a rigid needle structure covered by CagY, a VirB10-homologous protein, and CagT, a VirB7-homologous protein, at the base.[53–55]

Upon delivery into host cells by the *cag* secretion system, the product of the terminal gene in the island, CagA, undergoes Src-dependent tyrosine phosphorylation and activates an eukaryotic phosphatase (SHP-2), leading to dephosphorylation of host cell proteins and cellular morphological changes.[56,57] CagA has also been shown to dysregulate β-catenin signaling[58,59] and apical–junctional complexes,[60] events that have been linked to increased cell motility and oncogenic transformation in a variety of models.[61,62] In addition, some studies have reported that *cag*PAI appears to be involved in the induction of gastric interleukin-8 (IL-8) production, a potent neutrophil-activating chemokine.[63]

Consequently, the presence of *cagA* gene has been associated with higher grades of inflammation, which may lead to

the development of the most severe gastrointestinal diseases, such as peptic ulcer disease[64] and GC.[65–69] In Western countries, it has been reported that individuals infected with *cagA*-positive strains of *H. pylori* are at a higher risk of peptic ulcer disease or GC than those infected with *cagA*-negative strains.[40,70] However, in East Asia, most strains of *H. pylori* have the *cagA* gene irrespective of the disease.[71]

Furthermore, *cagA* is a polymorphic gene that presents different numbers of repeated sequences located in its 3' region. Each repeated region of CagA protein contains Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs, including a tyrosine phosphorylation site.[72] According to the sequences flanking the EPIYA motifs, four distinct EPIYA segments, EPIYA-A, EPIYA-B, EPIYA-C, and EPIYA-D, each of which contains a single EPIYA motif, have been identified in the EPIYA-repeat region. The EPIYA-repeat region of CagA from Western *H. pylori* isolates is in arrangement of EPIYA-A, EPIYA-B, and EPIYA-C segments (A–B–C-type CagA). The EPIYA-C segment variably multiplies (mostly one to three times) in tandem among different Western CagA species. CagA from East Asian *H. pylori* isolates also possesses EPIYA-A and EPIYA-B segments, but not the repeatable EPIYA-C segment. Instead, it has a distinct EPIYA-containing segment (it is the EPIYA-D segment), which is unique to East Asian CagA. Accordingly, the EPIYA-repeat region of East Asian CagA is in an arrangement of EPIYA-A, EPIYA-B, and EPIYA-D segments (A–B–D-type CagA).[57,73]

Analysis using a series of EPIYA mutants of CagA revealed that SHP-2 specifically binds to the tyrosine-phosphorylated EPIYA-C or EPIYA-D segment. The sequence flanking the tyrosine phosphorylation site of EPIYA-D segment perfectly matches the consensus high-affinity binding sequence for the SH2 domains of SHP-2, whereas that flanking the tyrosine phosphorylation site of the EPIYA-C segment differs from the consensus sequence by a single amino acid at the pY+5 position. As a result, East Asian CagA, which contains the EPIYA-D segment, exhibits stronger SHP-2 binding than does Western CagA, which contains the EPIYA-C segment. Within Western CagA species, those having a greater number of EPIYA-C segments exhibit stronger activity to interact with SHP-2 and are more closely associated with precancerous lesions and GC.[57,73]

As regard to the function of the repeated regions, initial demonstrations suggest that *H. pylori* strains that have a larger number of EPIYA segments in their regions are less resistant to gastric acid.[71] This finding seems to indicate that *H. pylori* strains containing many EPIYA segments can survive only in the presence of advanced atrophic gastritis, in which gastric acid secretion is low.[74]

For instance, according to Yamaoka,[74] the incidence of GC is clearly higher in East Asian countries than in any other countries when age-standardized rates are considered. However, the incidence of the disease is also high in some regions where Western-type CagA strains are reported to account for the majority of *H. pylori* strains, such as Colombia and Peru. In a study comparing the number of EPIYA-C segment in Columbia and the United States, it was found that 57% of the isolates from Columbia had two EPIYA-C segments, whereas only 4% of the isolates from the USA had two EPIYA-C segments. Consequently, the number of EPIYA-C segments may explain, to some extent, the geographic difference in the incidence of GC in Western countries.[74]

In addition, an important relationship between strains *vacA* s1m1 and CagA positive has also been reported.[75,76] Although located in different genomic regions, the *cagA* gene is strongly associated with the cytotoxic activity of VacA,[77] and strains expressing the combination of these alleles and *cagA* are considered the most virulent,[78,79] causing more severe epithelial damage,[80,81] which can be associated with the development of the most severe gastric diseases.

Additionally, the role of *H. pylori* infection and/or CagA-positive strains has been studied in several extra-gastric diseases. Researchers described an inverse relationship of CagA-positive strains with fatal cardiovascular events.[82] It was related to a positive association between *H. pylori* seroprevalence and CagA-positive strains in patients with autoimmune thyroid diseases.[83] A Japanese study demonstrated that molecular mimicry induced by CagA may be involved in the pathogenesis of *H. pylori*-associated chronic ITP.[84] Similar findings were confirmed by Kodama et al,[85] who verified that *H. pylori* eradication therapy improved the platelet count in *H. pylori*-positive patients with ITP. Another study investigated the prevalence of *cagA* and *cagE*, *vacA*, *iceA*, and *babA2*, in hepatobiliary diseases (cholangiocarcinoma/cholelithiasis) and controls. *H. pylori cagA* and *cagE* positive strains were more frequently detected in patients with cholangiocarcinoma than those with cholelithiasis or the controls.[86]

Vacuolating Cytotoxin Gene (*vacA*)

VacA is a cytotoxin secreted from bacteria as a large 140-kDa polypeptide and latter trimmed at both ends to finally deliver it in an active form to host cells, where it exerts its activity.[87]

The gene encoding VacA is present in all *H. pylori* strains and displays allelic diversity in three main regions, the s (signal), the i (intermediate), and the m (middle) regions, and consequently, the cytotoxic activity of the toxin varies between strains.[88,89] Different combinations of two major alleles of each region (s1, s2, i1, i2, m1, m2) may exist, which results in VacA toxins with distinct capability of inducing vacuolation in epithelial cells.[6,90] While *vacA* s1/m1 strains are consistently vacuolating and *vacA* s2/m2 strains are nonvacuolating, only some *vacA* s1/m2 strains are able to induce cell vacuoles.[91] Concerning the i region, s1/m2 strains that have an i1 allele are vacuolating, whereas s1/m2 strains that have an i2 allele are nonvacuolating.[92]

VacA induces multiple cellular activities and the best studied among them is the alteration in the endosomal maturation, which consequently leads to epithelial cell vacuolation.

VacA is also capable of inducing membrane-channel formation, cytochrome c release from mitochondria and binding to cell-membrane receptors activating a proinflammatory response.[88]

Strains with s1 allele secrete an active toxin and are also highly associated with ulcers and GC;[90] however, s1/s2 combination or s2 genotypes are found in patients with GC.[93] The m1 subtype demonstrates a stronger vacuolating activity than m2, and it has been associated with an increased risk of developing gastric epithelial injury and GC.[94] After the description of the *vacA* i region, it was also shown that the determinant of cytotoxicity, the i1 allele, is associated with gastric adenocarcinoma.[92,95]

In Western countries, including Latin America, the Middle East, and Africa, there have been many reports that individuals infected with s1 or m1 *H. pylori* strains have an increased risk of peptic ulcer or GC compared with individuals infected with s2 or m2 strains.[90,96] In addition, almost all *cagA*-positive strains are classified as an s1 strain, whereas almost all *cagA*-negative strains are classified as an s2/m2 strain.[90] With respect to the m region, there is a variation within East Asia; for instance, although m1 strains are common in parts of Northeast Asia, such as Japan and South Korea, m2 strains are predominant in parts of Southeast Asia, such as Taiwan and Vietnam.[97,98] Finally, concerning the i region, studies with patients from East and Southeast have reported that there is no association between this region and disease development.[99]

Duodenal Ulcer (DU) Promoting Gene (*dupA*)

H. pylori DU promoting gene (*dupA*), located in the plasticity region of *H. pylori* genome, has been initially described as a risk marker for DU development and a protective factor against GC.[100] It was the first putative specific marker whose association was described using strains obtained from both Asian (Japan and Korea) and Western (Colombia) regions, and it is thought to be a *virB4* homologue.[100,101] The *dupA* gene encompasses two continuous sequences, *jhp0917* and *jhp0918*, as described in strain J99. The *jhp0917* gene encodes a protein of 475 amino acids but lacks a region homologous to the C-terminus of *virB4*, whereas *jhp0918* gene encodes a product of 140 amino acids that is homologous to the missing *virB4* region.[9]

Originally, it was reported that the presence of *jhp0917–jhp0918* (*dupA* gene) was a marker for the development of DU disease, but some studies demonstrated that this gene can also be associated with GC development.[102,103] The function of *dupA* gene is not fully understood. It is possible that it acts in combination with other *vir* homologues in the plasticity region to form a type IV secretion system similar to the *cag*PAI.[9] In addition, it has been associated with increased IL-8 production from the antral gastric mucosa in vivo as well as from gastric epithelial cells in vitro. The gene presence is thought to be also involved in DNA uptake/DNA transfer and protein transfer, and in vitro experiments using *dupA*-deleted and complemented mutants, showing that the absence of *dupA* gene was associated with increased susceptibility to low pH.[100]

Two important studies continue to support the role of *dupA* in DU; however, one of them[104] did not investigate the association between *dupA* and GC patients, and the other one[101] did not establish an association between this disease and *dupA* positivity. Furthermore, the latter found that the occurrence of GC was significantly lower in patients with *dupA*-positive *H. pylori* strains, providing further support for *dupA* as a negative marker for GC, consistent with Lu et al.[100]

Conversely, some studies suggest that there is a possible association between *dupA* gene and GC development. Among them, Argent et al[102] studied subjects from Belgium, China, South Africa, and the United States of America, identifying *dupA* as a risk factor for GC and not as a protective factor against it, and in fact, they did not find any association between *dupA* and DU disease. Schmidt et al[103] reported a significantly higher prevalence of *dupA* gene in ethnic Chinese patients diagnosed with DU (62.5%) and GC (54.6%), as compared with those diagnosed with functional dyspepsia. Roesler et al[69] also suggest a possible association between *dupA* gene, *vacA* s1m1 and *cagA*/*cagT* positive strains, and GC development, both in early and advanced stages. In this study, it was found, in a global consideration, an expressive number of positive *dupA H. pylori* strains (31.46%) in patients with gastric adenocarcinoma. In the Swedish population, also studied by Schmidt et al,[103] there was no significant difference in the prevalence of *dupA* in isolates from patients diagnosed with DU, GC, and functional dyspepsia, which was similar to findings reported in two other Brazilian studies.[105,106] This gene was also associated with the high risk of GC development in East Asian region in a research that identified the *cagA* gene in all the studied strains and the *dupA* gene in 31.0% of these strains, suggesting that the association of these genes, in addition to virulent *vacA* genotypes, may underlie the high risk of GC in this region.[107]

Finally, a study developed by Douraghi et al[108] reported no association between *dupA* status and gastroduodenal diseases. Similarly, a systematic review and meta-analysis confirmed the importance of *dupA* gene for DU, especially in Asian countries, but there was no association between the presence of this gene, and gastric ulcer and GC.[109] Gressmann et al[110] considered that there must be a diversity in gene content that can contribute to bacterial adaptation to genetically different ethnic groups that make up the human population.

Induced by Contact with Epithelium Gene (*iceA*)

Researchers showed that *iceA* has two main allelic variants, *iceA1* and *iceA2*.[70,111] The *iceA1* is upregulated by the contact of *H. pylori* with gastric epithelial cells and exhibits sequence homology with a gene from *Neisseria lactamica*, *nlaIIIR*, which encodes a CTAG-specific restriction endonuclease.[111,112] However, *iceA2* has no homology with known genes and its function remains unclear,[113] although

some researchers have related this allele to asymptomatic gastritis and non-ulcer dyspepsia.[111]

Several reports have associated the *iceA* status with clinical outcome. According to van Doorn et al,[70] there was a significant association between the presence of *iceA1* allele and peptic ulcer disease. Conversely, the authors reinforced that the *iceA* allelic type was independent of the *cagA* and *vacA* status. Similar findings were described by Shiota et al[113] who concluded that *iceA* may be a discriminating factor for peptic ulcer disease independent of *cagA* status. In a further study, *iceA1* genotype was linked with enhanced mucosal interleukin (IL-8) expression and acute antral inflammation. Furthermore, it was demonstrated that adherence to gastric epithelial cells in vitro stimulates *iceA1* transcription *iceA1*.[111]

In a Malaysian study, the prevalence of *iceA1* and *iceA2* was very low, and no significant differences were noted between these virulence factors and any pathology either individually or in combination.[114] However, in a meta-analysis including 50 studies with a total of 5357 patients to confirm the relationship between the iceA allelic type and clinical outcomes, it was shown that the overall prevalence of *iceA1* was significantly higher in Asian countries than in Western countries (64.6 vs 42.1%), while *iceA2* was more prevalent in Western countries than in Asian countries (45.1 vs 25.8%). Sensitivity analysis revealed that only the *iceA1* status was significantly associated with peptic ulcer. The authors reinforced that these findings were significant in Western countries.[113]

Urease

In order to counteract the acidic environment of the stomach, *H. pylori* produces an important enzyme, urease, which hydrolyses urea into NH_3 and CO_2. It has been demonstrated that this enzyme plays an important role in the *H. pylori* colonization, being observed that urease-defective bacteria mutants are not able to colonize the gastric environment.[115] Urease causes damage to the epithelium through the production of ammonia that, in conjunction with neutrophil metabolites, forms carcinogenic agents that might participate in the development of gastric malignances.[116,117] Ammonia is capable of causing different cell alterations, including swelling of acidic intracellular compartments, alterations of vesicular membrane transport, repression of protein synthesis and ATP production, and cell-cycle arrest.[115] Urease might also help to recruit neutrophils and monocytes in the mucosa and to produce pro-inflammatory cytokines.[118]

OMPs

Studies regarding *H. pylori* virulence factors have primarily focused on urease, vacuolating cytotoxin, and cytotoxin-associated antigen.[119] However, this bacterium has a large repertoire of OMPs encoded by a family of paralogous genes.[43] This large group is probably of remarkable importance for optimal adaptation of *H. pylori* to its host.[120]

H. pylori genome contains more than 30 *omp* genes, which have been divided into *hop* (*Helicobacter* OMPs) and *hor* (*hop*-related groups) which are joined together in OMP family 1. The Hop subgroup is encoded by 21 genes[48] and included the two best studied *H. pylori* adhesins: Lewis b (Le^b) blood group antigen-binding adhesion (BabA)[49] and sialyl Lewis X antigen-binding adhesion (SabA).[50] These adhesions recognize specific carbohydrate moieties of the gastric epithelium, which promotes infection and inflammatory processes in the gastroduodenal tract.

Additionally, there are other proteins, such as AlpA (HopC), AlpB (HopB), and HopZ, which have been implicated in cell adhesion and mediate the tropism of *H. pylori* to the gastric tissue.[50,120–122] Although some functions of these OMPs have still been indefinite, researchers have focused on the study of their diagnosis, protective immunity, and pathogenicity.[119]

Blood group antigen-binding adhesion (*BabA*). BabA is the best-characterized adhesin and binds to ABO histo-blood group antigens and corresponding Le^b antigens, which are expressed on gastric human epithelial cells.[48] Although three *bab* alleles have been discovered (*babB*, *babA1*, *babA2*), only *babA2* gene product is needed for Le^b binding activity.[122]

Some researchers have demonstrated that there is an association between *babA2*-positive genotypes and occurrence of peptic ulcer disease,[48,123] although it remains controversial.[12,124] The study performed by Zambon et al[125] showed that *babA2* and *cagA*, and *vacA* s1 and m1 coexpressed by the same *H. pylori* strain work synergistically in worsening inflammation and may be a potential risk of intestinal metaplasia. A recent study with Iranian patients reported that *babA2* prevalence was significantly higher in GC patients (95%) when compared with DU patients (18.1%) and non-ulcer dyspepsia subjects (26.1%).[126]

Interestingly, another survey related that *H. pylori* infection introduced DNA double-strand breaks (DSB) in primary and transformed murine and human epithelial and mesenchymal cells.[126] The *babA* mutant was notably less capable of inducing DSB, suggesting that bacterial adhesion via *babA* is required to induce DSB. Considering that DSB induction may contribute to the genetic instability and frequent chromosomal alterations that are found in GC, the possible role of *babA* expression in GC needs further investigation.

Sialic acid-binding adhesion (*SabA*). *H. pylori* infection induces expression of inflammation-associated "sialylated" carbohydrate structures that are upregulated as part of complex gangliosides in inflamed gastric tissue. Therefore, adherence of bacteria to gastric mucosa is dependent on SabA and cognate sialylated/fucosylated glycans on the host cell surface. The ability to bind to the glycosylated epithelial cells is considered to be essential for *H. pylori* to cause persistent infection and disease.[120,127]

Researchers have demonstrated that *H. pylori* also binds to red blood cells in gastric mucosal blood vessels in both

infected humans and rhesus monkeys. It was verified that SabA is the bacterial surface protein that mediates *H. pylori* binding to red blood cells. Additionally, they have related that clinical *H. pylori* isolates demonstrate polymorphism in their abilities to bind various sialylated carbohydrates, and this variability may adapt the binding properties of bacteria both to individual hosts and changing epithelial glycosylation patterns during chronic inflammation.[127]

Another study has assessed the contribution of each BabA, SabA and the neutrophil-activating protein (HP-NAP) in the inflammation, using mutant strains of *H. pylori*. The authors have found that SabA was essential in phagocytosis induction, and *napA* deletion resulted in enhanced generation of reactive oxygen species and impaired adherence to host cells. They have concluded that SabA stimulates human neutrophils through selection-mimicry mechanism, and HP-NAP modulates the oxidative burst, which could adjust the impact of *H. pylori* infection for establishment of the chronic inflammation in the gastric mucosa.[128]

Outer inflammatory protein (*OipA*). OipA, a proinflammatory OMP, is called HopH. Initially, Yamaoka et al[129] discovered that it was correlated with mucosal IL-8 levels and that protein was present in 97.5% of patients with gastric or DU when compared with 70% of those with chronic gastritis. Thereafter, researchers confirmed the proinflammatory role of OipA, considering that *oipA* isogenic mutants reduced the induction of IL-8 from gastric epithelial cell lines.[130]

Another study showed that OipA status was strongly correlated with *cagA*, *vacA*, and *iceA* genotypes.[131] Kudo et al[132] related that functional oipA was significantly associated with high *H. pylori* density, severe neutrophil infiltration, and high mucosal IL-8 levels. After that, researchers have demonstrated that OipA can induce inflammation and actin dynamics through the phosphorylation of multiple signaling pathways that usually interact with *cag*PAI (CagA)-related pathways.[133–135]

Researchers have proposed that *H. pylori* virulence factors may not be independent of one another. Therefore, *cag*PAI genotype, *vacA* alleles, *oipA* status, and *dupA* presence have been linked to severity of clinical outcomes.[136] Similar findings were verified by Yamaoka et al,[137] who demonstrated that the oipA status was closely linked to specific *cag*PAI, *vacA*, and *babA2* genotypes. An independent univariate analysis showed that *oipA* "on", *cag*PAI-positive, *vacA* s1 genotype, and *babA*-positive types were all related to a risk of DU. However, a multiple logistic regression analysis showed that only the oipA "on" status was an independent predictor of DU from gastritis. Furthermore, the authors described that only a functional oipA was significantly associated with high *H. pylori* density, severe neutrophil infiltration, and high mucosal IL-8 levels.

Similar findings were confirmed by the sequencing of *oipA* in *H. pylori* strains from 58 patients with chronic gastritis. In this study, the *oipA* "on" genotype was linked to other virulence factors such as *vacA* s1, *vacA* m1, *babA2*, and most strongly, *cagA* genotypes. Additionally, *oipA* mutagenesis resulted in reduced bacterial adherence to gastric epithelia in vitro, reinforcing the role of OipA in the gastric mucosa colonization.[121]

Conclusions

In this review, we have summarized reports and studies of genes and virulence factors of *H. pylori* that are suggested to be involved in the development of several gastrointestinal diseases. Although there is much knowledge concerning the virulence factors of *H. pylori*, there are lots of questions that remain unclear, especially regarding the specificity of each virulence factor and the clinical outcomes. More studies regarding this relationship will certainly highlight the pathophysiology of *H. pylori* and gastrointestinal disease development.

Author Contributions

Contributed to the writing of the manuscript: BMR, EMARG, JMRZ. Jointly developed the structure and arguments for the paper: BMR, EMARG, JMRZ. All authors reviewed and approved of the final manuscript.

DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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