Helicobacter pylori: an up-to-date overview on the virulence and pathogenesis mechanisms

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Abstract
Helicobacter pylori is an organism associated with ulcer disease and gastric cancer. The latter is one of the most prevalent malignancies and currently the fourth major cause of cancer-related deaths globally. The pathogen infects about 50% of the world population, and currently, no treatment ensures its total elimination. There has been an increase in our understanding of the pathophysiology and pathogenesis mechanisms of H. pylori over the years. H. pylori can induce several genetic alterations, express numerous virulence factors, and trigger diverse adaptive mechanisms during its adherence and colonization. For successful colonization and infection establishment, several effector proteins/toxins are released by the organism. Evidence is also available reporting spiral to coccoid transition as a unique tactic H. pylori uses to survive in the host’s gastrointestinal tract (GIT). Thus, the virulence and pathogenicity of H. pylori are under the control of complex interplay between the virulence factors, host, and environmental factors. Expounding the role of the various virulence factors in H. pylori pathogenesis and clinical outcomes is crucial for vaccine development and in providing and developing a more effective therapeutic intervention. Here we critically reflect on H. pylori infection and delineate what is currently known about the virulence and pathogenesis mechanisms of H. pylori.

Keywords Helicobacter pylori · cagA · vacA · OMPs · dupA · Virulence · Gastric cancer · Peptic ulcer

Introduction
Helicobacter pylori is a Gram-negative and flagellated bacterium. The organism belongs to several distinct genetic populations which shows high genetic diversity [1, 2]. The organism can survive in the presence of a low level of oxygen. Interestingly, this bacterium can navigate between two different shapes depending on the physiological activity required, such as survival during adverse environmental conditions (temperature or pH shifts, long intervals between meals, and antibiotic therapy) [3]. Although the organism is usually spirally shaped, it can appear as a rod. Also, during prolonged in vitro culture or even antibiotic treatment, the coccoid shapes could appear [4]. This ability to change from spiral to coccoid form is also one of the unique mechanisms this bacterium uses to survive in the host’s gastrointestinal tract [5, 6]. To date, the coccoid form persists as a major challenge in the eradication of H. pylori [7, 8].

H. pylori use flagella-mediated motility for movement towards the stomach’s epithelial cells and to penetrate the mucus lining [9]. Thereafter, the organism crosses the acidic environment to areas with suitable conditions. The coccoid form enabled its colonization at the mucus layers [6, 10]. Attachment to host epithelial cells is through adhesin production [9, 11]. So far, several studies have shown that the colonization of H. pylori could be negatively and positively associated with the induction and progression of several diseases [6, 12–16]. It has been reported to be linked to gastric and duodenal ulcer, gastric carcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [14, 17, 18]. Several other studies are also available reporting a positive correlation between gastrointestinal diseases and H. pylori. For example, a positive association has been reported between H. pylori and duodenal ulcer and gastric ulcer [19], gastritis [20], and oesophageal cancer [21]. Moreover, evidence is also available on the positive association between H. pylori
and non-gastrointestinal diseases such as diabetes mellitus [22], coronary artery disease [23], and anaemia [24].

Mostly, infection occurs during childhood, where patients remain healthy carriers, manifesting the symptoms later in adulthood. However, the vast majority of the infected population does not develop symptoms related to *H. pylori* infection [6]. Epidemiologically, 85–95% of developing countries’ population has *H. pylori* infection and approximately 30–50% in developed countries [25–28]. Sadly, there is no precise knowledge of the mode of transmission of *H. pylori*. It has, however, been hypothesized to be transmitted through oral-to-oral and faecal-to-oral routes. This transmission mode is associated with contaminated food and water [6, 29–31]. Poor hygiene, nutrition, and differences in geographical determinants are factors that play a role in infection [10], while its adaptation mechanisms include the acquisition of some virulence factors that enables it to survive at a lower pH. The organism cannot produce acid by itself but can neutralize gastroduodenal acid using the urease enzyme [10].

The pathogenic potentials of *H. pylori* have been evaluated several decades ago since its discovery in 1983. Sadly, the resistance of *H. pylori* to antibiotics treatment is rising daily, making treatment of *H. pylori* difficult. Frequent *H. pylori* infection causes a significant alteration in the composition of the GIT microbiome. It also causes the production of free radicals with implications on the outcome of carcinogens, and diet play a crucial role in the pathogenesis of *H. pylori* [43–45]. Also, the development of *H. pylori* infections after the invasion depends on bacterial survival, virulence factors, and the host factors such as the immune system and environmental determinants [46–49].

Approximately 4.4 billion people are infected with *H. pylori*, with the majority of the *H. pylori* strains having virulence genes. However, less than 20% develops severe gastric diseases [33, 50]. These observed discrepancies or clinical outcome raises a lot of questions. However, it is important to emphasize that several factors determine whether a person infected with *H. pylori* will remain asymptomatic or come up with one of the several gastric diseases: climate and local geography, host immune response, the composition of the microbiota (both gastric and intestinal), nutritional status of the individual, and medicine usage history [12]. In addition, studies have shown that the *H. pylori* strain display different degrees of virulence that determine their pathogenicity and potential disease progression. Therefore, understanding these factors that promote asymptomatic *H. pylori* infection to clinical disease is paramount for treatment and management.

*H. pylori* can infect, replicate, and persist in a host [44, 51]. The colonization with *H. pylori* is not a disease in itself. However, it is a condition that could affect the risk of developing various clinical disorders [7, 52]. Upon entering the stomach, *H. pylori* neutralize the hostile acidic environment with the aid of their urase activity. The cell subsequently moves towards the gastric epithelium using its flagellum-mediated motility. *H. pylori* adhesins further interact with the host cell receptors leading to successful colonization and persistent infection. Upon successful colonization, *H. pylori* produce several effector proteins/toxins responsible for damage to the host tissues. During the infection, the secreted chemokines trigger innate immunity. There is also

An overview of *H. pylori* infection

The genome of *H. pylori* was completely sequenced in 1997 [41]. Since then, there has been an increase in studies reporting the pathology, immunology, virulence, and pathogenesis of *H. pylori*. As previously established, *H. pylori* infection varies geographically, and the developing nations carry the higher burdens [42]. In addition, several environmental factors such as smoking, excessive intake of alcohol, presence of carcinogens, and diet play a crucial role in the pathogenesis of *H. pylori* [43–45]. Also, the development of *H. pylori* infections after the invasion depends on bacterial survival, virulence factors, and the host factors such as the immune system and environmental determinants [46–49].

Understanding these factors that promote asymptomatic *H. pylori* infection to clinical disease is paramount for treatment and management.
The activation of neutrophils and subsequent clinical manifestation [11].

*H. pylori* can also form biofilms. The formation of the biofilm helps to reduce its susceptibility to antibiotics, thus bringing about mutations that complicate bacterial eradication. In addition, *H. pylori* can use biofilm formation as a mechanism for its persistence and long-term colonization [53]. This tactic enhances the exchange of genetic materials and facilitates the frequency of recombination [54]. A positive association has also been reported between biofilm formation and an increased expression of multiple genes crucial for virulence [55] and resistance to antibiotics [56].

Abdominal pain and discomfort, nausea, burping, and loss of appetite are common symptoms. Other symptoms include excessive burping, bloating, weight loss, and heartburn [10]. Also, the rates of isolation of *H. pylori* vary between laboratories (about 30–37%) due to the fastidious nature of *H. pylori* [57, 58]. Several approaches are used in the detection of *H. pylori*. Both the invasive and the non-invasive methods are employed in the detection of *H. pylori* in a patient. Many factors, however, influence choices in the method of diagnosis: availability of diagnostic instruments/materials, sampling population, and competency and experience of the physicians/clinicians, among others [59]. Invasive methods include endoscopic evaluation, histology, rapid urease test (RUT), and bacterial culture. Non-invasive methods include urea breath test (UBT), stool antigen test (SAT), serology, and molecular diagnostic approaches [60]. Each of these methods has its advantages and limitations. However, in clinical practice, none can be considered a single gold standard [61]. UBT and SAT are the most commonly used non-invasive tests, and they are also the best methods to diagnose *H. pylori* infection [10, 59, 62, 63]. The invasive method employs the biopsy of a small sample of the gastric mucosa. It allows for the accurate detection of *H. pylori*. However, there is a high risk of contamination of the sample by viruses such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV) [64, 65]. Also, it cannot be performed on pregnant women, the aged, and children [66]. Moreover, multiple biopsies are needed to provide a better understanding of the infection.

RUT detects the presence of urea production, although factors like bacterial load and the presence of other urease-producing pathogens can result in a wrong diagnosis [67]. Also, information on antibiotic resistance cannot be provided using this method [68, 69]. As already mentioned, non-invasive methods such as urea breath test (UBT), stool antigen assay, and serology are frequently used [70–72]. These are widely performed on gastric juice, saliva, urine, and stool specimens. Both UBT and SAT have high sensitivities and specificities [73, 74]. Although the technique can detect the presence of *H. pylori*, it cannot provide information on drug susceptibility profiles. The culture method of detection allows for antibiotic susceptibility profiling [75]. Its disadvantage is the alteration of the specimen through contamination from commensals of the same flora and time intervals before culture.

Real-time PCR (molecular method) has also been used to diagnose *H. pylori* and its virulence genes [59]. The amplification of genes through polymerase chain reaction (PCR) is the common method of virulence detection. However, multiple amplification is usually needed for all the known genes and their variants, which can be time-consuming and require many funds. In addition, when there is a mismatch in the primer binding site, amplification of the target region may be impaired [76]. Also, a high number of amplification cycles may lead to artefacts in the amplified target sequence, potentially leading to wrong assumptions regarding the presence or structural variation of outer membrane proteins (OMPs). Whole-genome sequencing (WGS) is preferably used in genomic sequencing as it gives ample information on bacterial antibiotic resistance, bacterial diversity, and pathogenicity [76]. However, the cost of WGS and the complexity of data present some difficulties to most laboratories. The genetic population structure and analysis of *H. pylori* has recently been studied by Jiang et al. [77] using the whole-genome-based approach.

For the treatment of most *H. pylori* diseases, triple antibiotic therapy consisting of proton-pump inhibitor (PPI) and two antibiotics (usually amoxicillin and metronidazole/clarithromycin) have been used as a conventional method of treatment. However, with the recent surge in antibiotic resistance, triple therapy has become less effective. Therefore, quadruple treatment has emerged in recent years, involving a 14-day administration of proton-pump inhibitor (PPI) + bismuth + two antibiotics (quadruple bismuth therapy) [78–81]. Other methods of treatments have also received recognition across the globe. The limitation, however, is that different strains exhibiting different drug resistance and susceptibility patterns have been isolated from a single patient from several locations [82–86].

There are emerging alternatives to the treatment of *H. pylori* infections outside the use of antibiotics. For more information, a review paper by Roszczenko-Jasinska et al. [87] is recommended. In addition, the co-infection with multiple *H. pylori* strains, which may have different genotypes and phenotypes, has been observed within the same patient [86]. Such genetic variability proves drug susceptibility therapy difficult and poses the danger of the evolution of more virulent and adapted strains. In the study of Mi et al. [88] using *cagA* typing and RAPD-PCR fingerprinting, *H. pylori* strain isolated from patients showed heterogeneity that were considered to indicate the incidence of multiple infections. Overall, the different clinical outcomes during *H. pylori* infection may be due to differences in *H. pylori*.
strains—this could act as a marker to predict *H. pylori* disease outcomes [89].

**Virulence and pathogenesis mechanisms**

**Epithelial cell; the first-line defence barrier**

The epithelial cell of the human gastric region prevents the adhesion, proliferation, and movement of invading pathogens through its ability to form a tight structure. Pathogens like *H. pylori* disrupt the gastric barrier by the production of harmful soluble components. It also adheres to many epithelial cell receptors and stimulates various signalling pathways within the host. The colonization and the establishment of diseases and infection by *H. pylori* depend on four major stages: adaptation to the acidic environment of the gastric mucosa, the movement towards and penetration of the epithelial cell barrier, attachment to specific receptors, and finally, tissue damage and other detrimental health effects (Fig. 1). Therefore, to successfully colonize the host and establish infection, *H. pylori* must be able to survive the acidic stomach, attach to the host cells, and release damaging tissue toxins. Some of the major effector proteins/toxins released by *H. pylori* include blood group antigen-binding adhesion (BabA), outer inflammatory protein (OipA), outer membrane protein (OMP), outer membrane vesicles (OMV), vacuolating cytotoxins (VacA), a cytotoxin-associated gene

![Diagram of Virulence and pathogenesis mechanisms of H. pylori](image)

**Fig. 1** Virulence and pathogenesis mechanisms of *H. pylori*. Colonization and establishment of diseases and infection by *H. pylori* depend on four major stages: (1) adaptation to the acidic environment of the gastric mucosa, (2) movement towards the epithelial cells using the flagella, (3) penetration of the epithelial cell barrier and attachment to specific receptors, and (4) tissue damage and other detrimental health effects. Therefore, to successfully colonize the host and establish infection, *H. pylori* must be able to survive the acidic stomach, attach to the host cells (using several adhesins), and release toxins that damage host tissues. The VacA helps in the disruption of the epithelial barrier. Also, the macrophages can be induced by the urease. The induction brings about alterations in gastric physiology. Several other effector proteins play a crucial role in the pathogenesis of *H. pylori*
product (CagA), high-temperature requirement A (HtrA), neutrophil-activating protein A (NepA), and sialic acid-binding adhesins (SabA) [37].

Other outer membrane proteins (OMP) possessed by *H. pylori* include *Helicobacter* OMPQ (HopQ) and *Helicobacter* OMZ (HopZ). There are also the *H. pylori* outer membrane (Hom) family proteins (HomA), HomB, HomC, and HomD. A recent study by Xu et al. [90] showed that HomB could induce IL-8 secretion.

The binding of *H. pylori* to gastric epithelial cells, colonization, and even biofilm formation in *H. pylori* are also mediated by adherence-associated lipoprotein A and B (AlpA/AlpB). There are also other poorly studied adhesins playing a crucial role in *H. pylori* virulence. For example, a recent study by Baj et al. [37] showed that LacdiNAc-specific adhesin (LabA) facilitates *H. pylori* adherence to the gastric epithelium. More recent evidence is also showing that *H. pylori* adaptation and survival in the gastric environment are controlled by Hpone1460, a small non-coding RNA [91]. According to the investigation, an increased expression of OMPs and *cagA* during chronic infection was due to decreased expression of HPne4160.

**Survival in the acidic environment**

The flagellum is a complex organ composed of three structural elements (basal body, hook, and filament). The filament is composed of several types of protein subunits, such as FlaA and FlaB [10, 92–94]. The *flaA* is located in the outer region, while *flaB* is on the base of the flagellum [95]. Flagella can exist in swimming, swarming, or the spreading form [92].

*H. pylori* use its flagella for movement and adhesion functions. Flagella enable the migration of the pathogenic bacterium from entry to the mucus membrane, where it produces an adhesin to colonize the mucus lining of the host’s epithelial cells [11]. This flagellum also protects the bacterium from the gastric environment [10]. In the presence of relatively high percentage acid that results in more acidity in the gastroenteric, the flagellar tend to swim faster as a proton motive force powers their proteins motor at this pH [93]. The number of flagella has been reported to increase the speed of bacterial cells during movement [96].

Mutation in the gene that encodes for flagella production, such as *flID*, *flaA*, and *flaB* can lead to the inability to colonize the gastric mucosa. *flaA* mutant strain of *H. pylori* cannot produce flagella at all, while that which is deficient in *flaB* might produce flagella [97, 98]. A decrease in the motility and adhesion capacity of a *flaB* deficient strain has been demonstrated [11]. A non-flagellated strain of *H. pylori* cannot colonize the epithelial cells of the mucosa [10]. In addition to motility, *H. pylori* flagella can induce inflammation and immune invasion and play an active role in biofilm formation [54, 92, 99].

**Epithelial cell colonization**

Adherence of *H. pylori* to the gastric epithelium is a necessary step in establishing infection [94]. *H. pylori* adhesins bind to mucins in the gastric mucus and receptors on the surface of the gastric mucosa. The outer membrane adhesin proteins like blood group antigen-binding adhesin (BabA), SabA, outer inflammatory protein (OipA), *H. pylori* outer membrane protein (OMP), and other proteins interact with the receptors found on the host epithelial cells. Binding to receptors protects the invading pathogenic *H. pylori* from clearance mechanisms such as bulk liquid flow, gastric peristalsis, and the continuous shedding and replenishment of the mucus layer. It also provides nutritional access to the bacteria and promotes the delivery of bacterial toxins and other effector molecules to the host cells [93, 94].

**Outer membrane vesicles**

The accumulation of phospholipid in the periplasm through the activities of the ATP-binding cassette system causes vesiculation in the cell wall outer membrane resulting in the formation of OMV [100]. OMV is a small, circular structure with an intact outer membrane expressed on the surface of *H. pylori* and other Gram-negative bacteria. It is made up of periplasmic proteins, toxins, outer membrane proteins (OMPs), and lipids. They sometimes can contain extracellular DNA (eDNA) [83, 101–103]. Formation of OMV often occurs during stress responses. OMV has been associated with cell proliferation, vacuolation, a loss of cell viability, and the production of the pro-inflammatory cytokine IL-8 [104]. They have also been reported to enhance bacterial survival, DNA transfer, antibiotic resistance, and induction of immune cell apoptosis [105]. A recent study conducted by Murray et al. [106] showed that the OMV of *H. pylori* offers protection against toxic compounds such as hydrogen peroxide in a dose-dependent manner and against bactericidal produced by epithelial cells antimicrobial peptide LL-37. It also protects against levofloxacin and clarithromycin in a dose-dependent manner.

Generally, the OMV proteins are grouped into five distinct classes according to the encoding genes. Type one is made up of the outer membrane porins (Hop), Hop-related proteins (Hor), type 2 comprises of the Hof, and type 3 comprise the *H. pylori* outer membrane proteins (Hom). The iron-regulated OMPs made up the fourth category. At the same time, the efflux pump OMPs belong to the fifth category [107], although other members do not fit perfectly into any of the five classes.
Outer membrane protein (OMP)

*H. pylori* express about 64 OMPs which are grouped into at least five gene families. Family 1 is composed of the *hop* and *hor* genes. These genes code for adhesion proteins such as the BabA/B/C, SabA/B, and AlpA/B. The production of OMP is regulated by gene recombination or by slipped strand mispairing occurring in the dinucleotide repeat regions at the 5′-end of the respective gene [94]. The OMP family two genes encode for OMPs of unknown function. In comparison, the OMP family five genes encode for efflux pump proteins and therefore play a vital role in antibiotic resistance [108]. Several OMPs have been identified to contribute to the virulence of *H. pylori*. They are essential in the adhesion of the bacteria to the epithelial cell of the host [76] and can enhance cag-pathogenicity island stimulation of pro-inflammatory immune response and induce signalling in the host cell [76, 109].

**Outer membrane porins**

The family outer membrane porin (HOP) adhesion molecules adhere to host cell membrane receptors. Widely studied among this class are the outer inflammatory proteins A (OipA), blood group antigen-binding adhesion (BabA), sialic acid-binding adhesion (SabA), HopQ, HopZ, and the adherence associated lipoprotein A (AlpA) [110]. Of most notable importance are the BabA and SabA adhesins [107, 111–113]. These adhesins are encoded by different genes and recognize a different set of host epithelial cells. A recent investigation by El-Sayed et al. [114] showed that OipA was highly significantly associated with gastroduodenitis, thus suggesting that they could be a great biomarker in predicting mucous progress in patients with chronic gastritis. BabA is probably the best-characterized adhesion of *H. pylori*. The babA2 gene encodes BabA. It can bind to H, Lewis b (Leb) and fucosylated ABO blood group antigens expressed on erythrocytes and gastric epithelium [112, 115]. This binding to epithelial cells facilitates the delivery of both VacA and CagA to the epithelial cell through the use of the T4SS. SabA recognizes Sialyl-Lewis x and sialyl-Lewis antigens expressed on the gastric epithelium [115]. Both BabA and SabA enables *H. pylori* to adhere to the gastric mucosa and subsequent colonization [116].

The role of outer membrane porins (e.g. OipA) has been established in several studies, although there is no clear information about the structure and the receptors of this adhesion protein OipA. However, there is an established relationship between its expression and the activities of *cagA*, as the expression of the *oipA* genes determines the expression of *cagA* [117, 118]. OipA increases the secretion of interleukin-8 (IL-8), causing inflammation in the host [117] and the inhibition of apoptosis [119]. Teymournejad et al. [119] investigated the role of OipA in the pathogenesis of *H. pylori*. The investigation showed that OipA could bind to gastric cell lines. The binding leads to the induction of toxic events and also triggers apoptotic cascade via the intrinsic pathway. HopQ, another important outer membrane porins, is active in the transportation of virulence substances extruded by CagA into the host cell [120], possibly through the T4SS system [121, 122], and Hop enhances secretion of IL-8 and other inflammatory factors as well as adhesion to host cells [90].

**Shape switch**

The transformation of the bacterium from the spiral form to the coccoid form occurs under adverse environmental conditions. The coccoid form of *H. pylori* is usually resistant to antibiotics, replicable, non-culturable, and can persist for a prolonged time causing severe damage to the gastric mucosa [7]. The spiral form of *H. pylori* is culturable. They are often referred to as the spiral viable culturable form (SVCF), whereas the coccoid form is viable but non-culturable (CVNCF). CVNCF cannot be detected by a simple culture method; direct electron microscopy and molecular techniques are used [49].

Under adverse environmental conditions such as an insufficient supply of nutrients, desiccation, lack of protection against oxygen, and exposure to antimicrobial agents, *H. pylori* can survive by switching its shape from SVCF to CVNCF [96, 123–126]. SVNCF bacteria still maintain their metabolic activity and pathogenicity and can return to active, cultivable conditions [127, 128]. The study of Elhahriri et al. [49] demonstrated the survival of *H. pylori* in adverse environmental conditions. In their study, 76 *H. pylori* were isolated from faecal milk samples from livestock through nested PCR. Genotyping of the virulence genes *vacA* and *cagA* was also done. The 76 samples were further subjected to bacterial culture. Thirteen samples were culturable, indicating the presence of the viable spiral form (SVCF) of *H. pylori*, while the 53 were non-culturable, indicating the presence of the CVNCF. To demonstrate its survival under adverse environmental conditions, the SVCF was cultured in UHT milk for 10 days under 4 °C, 5 days at 37 °C, and 1 day at 40 °C. There was an observed decrease in the microbial load between 1 and 10 days. They also observed that a decrease in the SVCF coincides with the appearance of the coccoid viable non-culturable form (CVNCF), which survived in the UHT milk for up to 30 days. The genotype of the SVCF and CVNCF strains (caga + vacA + s1a m1 i1) that survived in milk was the same as the inoculated one, suggesting the conversion of the SVCF to a CVNCF.

The findings of the study conducted by Willén et al. [129] and Andersen et al. [130] also portray the survival of *H. pylori* in the adverse condition through a transformation
from spiral to coccoid form. In vivo study of the pathogenicity of the isolates (SVCF and CVNCF) was conducted on mice. The result of the investigation showed that both strains were able to colonize the mice gastric mucosa at a similar level as the positive reference SS1 strain, providing a shred of evidence for their infectivity. Indeed, the strains recovered from the gastric mucosa of the mice following infection were cytotoxic and carried the same genotype cagA + vacA s1a m1 i1 as the inoculated strain. Interestingly, the CVNCF recovered from the mice were culturable, indicating the reversion of the CVNCF to SVCF following gastric infection. An important finding was the culturability of the strains isolated from the CVNCF mice group. This suggests a reversion of the inoculated CVNCF to an SVCF following gastric infection.

**Urease production**

Some transition metals are essential for organisms, as they serve as cofactors for enzymatic reactions and some physiological processes [98]. In bacteria, these metals are crucial for survival and successful infection. An example of such metal is nickel [131], a cofactor for two important enzymes: urease and hydrogenase. These enzymes play a vital role in the infection process [132]. Urease has been demonstrated as a virulence factor for some species like *Proteus mirabilis*, *Staphylococcus saprophyticus*, and *H. pylori* [93]. It catalyzes the hydrolysis of ammonia from CO₂, resulting in the alkalization of the acidic environment [98]. Hydrogenase, in turn, participates in a signalling cascade that induces an alternative pathway, allowing *H. pylori* to use molecular hydrogen as a source of energy for its metabolism [133].

One active urease molecule requires 24 nickel ions for full enzymatic action [134]. The nickel molecules found as trace molecules in the blood are taken by uptake proteins of bacteria, FecA3 and FrpB4, located on the outer membrane [135]. After entering the outer membrane, the nickel molecules are transported to the cytoplasm through the protein channel NixA, in the inner cytoplasmic membrane of the bacteria [136, 137]. In the presence of insufficient or total absence of nickel, urease cannot be activated. Consequently, there will be a reduction in the level of survival and colonization of *H. pylori*. Similarly, too much entry of nickel into the inner cytoplasmic membrane leads to oxygende reactions that can result in cell death [138].

The urease gene cluster encodes for the production of the urease enzyme in *H. pylori* to neutralize the effect of hydrochloric acid in the gastric region. This set of genes consists of the catalytic units (*ureA/B*), an acid-gated urea channel (*ureI*), and accessory assembly proteins (*ure E–H*) [6, 94]. The synthesis of urease is dependent on the pH of the gastric environment. The Urel-channel closes whenever the gastric environment is at a neutral pH (7.0) and opens when there is a change in the acidic level of the stomach [6, 11]. When the stomach is acidic, the Urel-channel opens up and release urease which hydrolysis urea into CO₂, ammonia (NH₃), and carbamate making it free to react with water producing an unstable ammonium hydroxide which results in alkalization of the stomach [11]. Over time, the carbamate decomposes to ammonia (NH₃) and carbonic acid. The carbonic acid is broken down into CO₂ and H₂O. Ammonia and CO₂ participate in the lowering of pH. [139]. Ammonium (NH₃+) produced in the process of hydrolysis of urea also reduces the pH of the gastric region. However, if not metabolized or exported out of the cell, it can be detrimental.

The mechanism of efflux of ammonium is yet to be clearly understood. However, the role of ammonium assimilating enzymes, glutamine synthetase (GS), and glutamate dehydrogenase has been demonstrated to play a part [140]. NH₃ disrupts the tight cell junctions, breaches cellular integrity, and damages the gastric epithelium, while CO₂ protects the bacterium from the bactericidal activity of metabolic products like nitric oxide and intracellular killing by phagocytes [93].

There are two types of urease produced by *H. pylori*. One is found on the cytoplasmic compartment, and another on the surface of the bacteria, often classified as the internal and the external urease. The external is primarily produced during the lysis of other bacterial cells and functions at a pH of 5.0–8.0, while the internal function is at pH of 2.5–6.5 [37, 140]. Urease promotes bacterial nutrition by releasing the host metabolites and is active in the generation of proton motive force during the hydrolysis of urea [141]. It modulates host immune responses via several mechanisms, including altered opsonization, enhanced chemotaxis of neutrophils, and monocytes, facilitated apoptosis due to binding to the class II major histocompatibility complex (MHC) receptors, and enhanced release of the pro-inflammatory cytokines [142].

The conversion of *H. pylori* from spiral to the coccoid form is fundamental in urease activity. The spiral form has a higher urease activity than when it is in the coccoid form [143]. At low pH, protein synthesis, urease, and catalase activity can be terminated [37].

**Vacuolating cytotoxins (VacA)**

The creation of vacuoles on a host cell is known as vacuolation. Pathogenic bacterium employs this mechanism to increase the longevity of bacterial infection [144]. Vacuolation resulting from the activities of VacA protein can lead to multiple pathogenic effects on the host cell, such as vacuolation cytotoxicity and apoptosis [144–146]. It can also result in disruption of endocytic trafficking, mitochondrial perturbations, depolarization of the plasma membrane potential and efflux of various ions (including chloride, bicarbonate,
and urea), and activation of MAP kinases [147, 148]. The creation of vacuoles on the cytoplasmic membrane of the gastric epithelial cell of the host makes the host cell open and susceptible to the activity of urease [144, 146, 149].

VacA is produced as a 140 kDa precursor and undergoes some proteolytic process to become a toxin of 88 kDa in mass [145]. It binds to corresponding receptors on epithelial cells such as receptor-like protein tyrosine phosphatase alpha and beta (RPTP-α, RPTP-β), density lipoprotein receptor-related protein-1 (LRP-1), and sphingomyelin [145, 150]. Moreover, on T cells, it binds to β2 integrin (CD18) receptors [145]. In the presence of an amino-terminal signal peptide and a carboxy-terminal domain, the fragments of the VacA toxins (amino-terminal 33 kDa (p33) and carboxy-terminal 55 kDa (p55)) are transported into the extracellular space through a type V (autotransporter) secretion pathway and is subsequently internalized into endosomal compartments [145]. The different polymorphic forms of vacA are associated with several clinical outcomes [151].

*H. pylori* can release VacA, which internalizes in the cell. The internalized VacA associates with endosomal compartments. They have also been reported to associate with mitochondria, the Golgi apparatus, and the endoplasmic reticulum [145]. Through the intracellular transporter system, VacA can enter the mitochondria and disrupt the normal function of the mitochondria. VacA can also induce alterations in endocytic processes or intracellular trafficking and inhibit intracellular degradation of epidermal growth factor (EGF), inhibited maturation of procathepsin D, perturbation of transferrin receptor localization, and inhibition of antigen presentation mitochondrial fragmentation [145].

VacA proteins can also tamper with immune response by totally inhibiting or reducing the activation of T-lymphocytes in the lamina propria [6, 152]. It also reduces the proliferation of immune cells, including T cells, B cells, eosinophils, macrophages, dendritic cells, and neutrophils [147, 153, 154] and hindering the immune system from cleaning out damaged cells or dysfunctional components as well as distortion of orderly degradation and recycling of cellular components and elimination of intracellular pathogens (a phenomenon known as autophagy) [6, 152]. Apoptosis is a mechanism that programmes cell death through the self-destruction of the host cells. This mechanism is often characterized by cells shrinking and fragments into smaller parts, enabling easy phagocytosis by neighbouring immune cells. Apoptosis rarely occurs under normal conditions. Normally, a complex mechanism keeps cell proliferation and cell death in place to maintain a balance. In *H. pylori* infection, apoptosis is increased, especially in the gastric gland of the host cells [155].

Although all *H. pylori* strain harbour vacA, there is a variation in the degree of vacuolation activity of the encoded cytotoxins [156]. This difference results from non-sense mutations, internal duplications, deletions, or 1 bp insertions within the vacA gene [157]. Alterations in amino acid sequences [158], efficiency, and transcription of this gene secretion have also been found to influence the levels of vacuolating activity [159]. vacA gene is divided into three segments, intermediate (i1 and i2), the signal (s1 and s2), and the middle regions (m1 and m2) [160, 161]. The different variants of vacA are associated with pathological features [162, 163].

Cell surface binding and vacuolating activity of the toxin arise from the middle region [164, 165]. The variation in the sequence of the signal region (s1a, s1b, and s2) and the middle region (m1 and m2) of the genome results in mutational strains. Strains carrying vacA s2/m2 do not have any cytotoxic activity; s1/m2 exhibit intermediate cytotoxic activity, while s1a/m1 exhibit more cytotoxic activity. vacA s1b/m1 also have less activity [76, 166]. In the study of Imkamp et al. [76], characterization of the 41 *H. pylori* isolates to detect virulent genes cagA, vacA, iceA, and dupA was done based on WGS, out of which 19 (46.3%) carried the vacA gene. Twenty-three (56%) was found to harbour vacA s1 allele. 15/41 (36.6%) were carrying vacA s1 allele (vacA sl/m1 and vacA slam2). vacA s1b allele was detected in 8 strains (5 s1b/ml, 3 s1b/m2), while 18 (43.9%) of the isolates harboured the vacA s2/m2 allele. There was also an established relationship between the presence of vacA s1 genes and the progression of gastritis. Furthermore, Idowu et al. [59] reported a positive association between vacA s1 and peptic ulcer disease.

Endosomal vacuolation

VacA induces several effects on target cells. Specifically, they are capable of inducing vacuolation with immunomodulatory effects on immune cells [154]. For example, a study by Altolbetti et al. [167] showed that VacA suppressed IL-23 expression by dendritic cells and also induced IL-10 and TGF-β expression in macrophages. Thus, *H. pylori* can create a tolerogenic environment via VacA immunomodulatory activity. This can skew the response of T cells towards Tregs. This mechanism helps *H. pylori* to persist in the environment with influence on the host cell immunity.

Moreover, vacuoles induced by VacA contain markers typically found in membranes of late endosomes [168–171]. This suggests that the vacuoles arise from late endosomal compartments [172]. VacA is secreted in the form of monomers and attached to the plasma membrane. Subsequently, the monomers are built up to form an oligomer [173]. The oligomers are transported to the late endosomes, where they form an anion-selective channel in their membrane [147]. As chloride ions from the gastric environment flow through this channel into the late endosome, accumulation leads to an increase in the chloride concentration, which in turn
enhances V-ATPase proton pump activity and a decrease in the intracellular pH. Chemical reactions occurring in the presence of weak bases that also flow to the late endosomes make the cell swell result in cell vacuolation [174–176].

Cytotoxin-associated gene product (CagA)

Another important virulence factor is the cytotoxin-associated gene product (CagA). It is one of the most studied virulence genes of *H. pylori*. Adherence of *H. pylori* to the gastric epithelial cells induces the expression of *cagA* [177]. The genes that encode the CagA is found within the ~40 kb cag-pathogenicity island (cagPAI) [178]. The cagPAI codes for the production of CagA and type (IV) secretion system (T4SS) [179]. Thus, cagPAI is a 40-kb chromosomal DNA region. It encodes nearly 31 genes forming a type IV secretion system. There are seven known different secretion systems (I–VII). Type III and IV systems allow penetration of the plasma membrane and delivery of bacterial molecules directly into the cytoplasm of target cells. The type III secretion system (T3SS) uses a flagellum-like tube to translocate effector proteins into eukaryotic host cells. In contrast, the type IV secretion system (T4SS) employs a pilus-based structure to mediate the delivery of DNA or proteins into target cells. The oncoprotein (CagA), which have a cytotoxic effect on the host cell, is encoded on the cag pathogenicity island (cagPAI). It is injected into the cell via a pilus formed by the T4SS [37, 180]. In the cell, it induces cellular alterations that impair cell motility, cellular proliferation, and apoptosis and alters the arrangement of the cytoskeleton [37].

Basically, the type (IV) system (T4SS) helps the transport of CagA proteins from gastric mucosal surface to endothelial cells for tyrosine phosphorylation and subsequent induction of immune response [181]. It is now known that *H. pylori cagT4SS* uses the specific interaction between the bacterial HopQ adhesin and the CEACAM (a cellular adhesion molecule) for translocating CagA into gastric epithelial cells. For example, Behrens et al. [182] studies this interaction using CEACAM-humanized (hCEACAM) mouse PMNs and humans. The result from the investigation showed that *H. pylori* HopQ-dependent interaction greatly facilitated the translocation and phosphorylation of CagA. Furthermore, the PMNs greatly increased the expression of pro-inflammatory chemokines MIP-1-alpha. Also, chronic mouse model infection showed that there was downregulation of hCEACAM and -6 receptors on neutrophils. Overall, this study points at the probable tactics *H. pylori* uses to control the host immune response during gastric pathology progress.

Once *H. pylori* effector protein (CagA) reaches the host cell, they interact with a variety of host SH2 domain where tyrosine phosphorylation occurs. *H. pylori* phosphorylation site is made up of Glu-Pro-Ile-Tyr-Ala (EPIYA) sequence repeat (EPIYA motif) or other closely related sequences [183] located at the N-terminal region [184, 185] and a C-terminal tail. Based on the composition of the EPIYA-A, EPIYA-B, EPIYA-C, and EPIYA-D motifs, *H. pylori* can be classified into different CagA subtypes, such as CagA-AB, CagA-ABC, CagA-ABD, or CagA-BD [186, 187].

Similar to other virulence genes, the cagA genes vary in their EPIYA segments (EPIYA-A to EPIYA-D). The binding of the host SH2 domain to this different EPIYA segment also varies. The D segment has more affinity than the C segment. During tyrosine phosphorylation, the C or the D segment serves as the specific binding site for the SH2 domain-containing tyrosine phosphatase SHP2 [188], leading to the deregulation of Erk MAP kinase signalling. In contrast, the Src kinase binds to the A and the B segment [189], which in turn inhibit SFK activity. Variation within the EPIYA region also results in variation in the amount of ILs produced. EPIYA D releases a higher amount of IL-8 [190]. The amounts of interleukins secreted during *H. pylori* infection are highly associated with the number and variations within C-terminal Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs. *H. pylori* strains with the EPIYA-D motif are prone to release higher amounts of IL-8 compared to other variations [190].

*H. pylori* strains are often classified into two subclasses (cytotoxin-associated gene A (cagA)-positive and cagA-negative). Although all *H. pylori* strains possess cagA, some of them are cagA positive, while some strains are cagA negative [191]. Studies indicate that the carriage of cagA is related to virulence and severe forms of gastrointestinal diseases such as peptic ulcers and gastric cancer [192, 193]. Likewise, cagA-positive strains are more motile than cagA-negative ones, indicating that cagA is also associated with bacterial motility [194]. cagA can be separated into two segments, namely cag I and cag II. It has been associated with mucosal inflammation and a more severe clinical outcome of *H. pylori*-associated infection.

Overall, CagA, just like vacuolating cytotoxin (VacA), exhibits cytotoxic and immunomodulatory activities [109, 154]. However, the level of expression of cagA varies among *H. pylori* strains. Yeh et al. [195] illustrated that the *H. pylori* strain having Y58/E59 polymorphism in the cagL has a higher risk of facilitating gastric cancer. This evidence shows that mutations in genes present in the cagPAI can influence virulence. The CagA protein can also alter the tumour suppressor mechanisms in gastric epithelial cells [196].

**Induced by contact with epithelium Gene A (iceA)**

Another putative virulence factor present in virtually all *H. pylori* strains is *iceA*, having two fragments (*iceA1* and *iceA2*). It is another virulent factor expressed by *H. pylori*. The fragment *iceA1* has been associated with peptic ulcer
[77], while the iceA2 have no pathogenic effect. However, unlike the vacA gene fragments, the relationship between these fragments and clinical outcomes is quite controversial. While some studies have proven that iceA1/iceA2 may be directly involved in gastrointestinal system diseases, others have demonstrated contrary findings [197].

**Duodenal ulcer-promoting gene (dupA)**

Duodenal ulcer-promoting gene (dupA) belongs to the T4SS housed on an integrating conjugative element (ICEHptfs4). The dupA gene encodes a VirB4 ATPase homolog [198]. It is a virulence factor specifically linked to gastric ulceration. For example, Alam et al. [199] showed that dupA is associated with an increased risk of duodenal ulcers. dupA also induces pro-inflammatory cytokine secretion by mononuclear cells [77, 200]. The DupA protein can induce the secretion of IL-8 and IL-12 by the gastric mucosa and also by gastric epithelial cells in vitro [201]. dupA has been considered a biomarker for peptic ulcers disease [202]. However, ever since the discovery of dupA in 2005, there have been several published contradictory results on its pathogenicity role [198, 203–206].

A study by Idowu et al. [59] did not show any positive association between dupA and peptic ulcer disease. However, a more recent investigation by de Lima Silva et al. [207] revealed that dupA-positive *H. pylori* infection was associated with a two-fold chance of developing gastritis. More investigations will help to shed more light on the exact role of dupA in the virulent potential of *H. pylori*.

**High-temperature requirement A (HtrA)**

Different stress such as osmotic, acid, basic, oxidative, or heat stress can result in protein denaturation and possibly aggregation of subsequently misfolded proteins [208].

| Table 1 Other important virulence factors in *H. pylori* |
|--------------------------------------------------------|
| **Virulence factors** | **Function** | **Reference** |
| Phospholipase | Activate signalling pathways (e.g. ERK1/2) | [221, 222] |
| | Trigger chronic inflammation | |
| | Enhance bacterial colonization and survival | |
| | Involved in the degradation of lipids and damage to mucus layer | |
| Lipopolysaccharide | Trigger several signalling pathways | [223–225] |
| | Induce several inflammatory responses | |
| | Induce immune responses | |
| | Disrupts the mucus secretion | |
| | Shield the organism against toxic materials | |
| Heat shock proteins | Enhance adherence to epithelial surfaces | [226–229] |
| | Involved in urease activation | |
| | Control apoptosis and autophagy | |
| | Help to maintain the structure and properties of the effector proteins | |
| | Protect the cell from reactive oxygen species (ROS) | |
| | Induces the production and release of IL-8, TNF-α, and COX-2 | |
| Arginase | Prevents bacterial killing | [230, 231] |
| | Prevents T-cell proliferation | |
| | Impair immune responses | |
| | Stimulate apoptosis | |
| | Help the *H. pylori* to withstand the acidic environment | |
| Superoxide dismutase (SOD) | Protect the cell from reactive oxygen species (ROS) | [232–235] |
| | Enhance colonization | |
| | Inhibits the production of cytokines | |
| | Stimulate macrophage activation | |
| γ-glutamyl-transferase | Facilitates apoptosis and necrosis | [236, 237] |
| | Induce the release of pro-inflammatory proteins | |
| | Induce the release of ROS | |
| | Stimulate DNA damage | |
| Cholesteryl α-glucosyltransferase (αCgT) | Shield *H. pylori* from immunological attack | [238, 239] |
| | Stimulate the production of pro-inflammatory proteins (e.g. IL-8) | |
| | Enhance bacterial growth and its resistance to antibiotics | |
High-temperature requirement A (HtrA) is heat shock-induced serine protease and a chaperone protecting protein expressed in human and prokaryotic cells [209, 210]. It determines the quality of protein and is important in the evasion of oxidative stress. This protein is resistant to heat and pH [211]. Gram-negative bacteria such as Campylobacter jejuni and H. pylori actively secrete HtrA proteins in the extracellular environment, where they target host cell factors [211]. They are also found to be expressed in the outer membrane vesicles of Vibrio cholera, Chlamydia muridarum, or Borrelia burgdorferi [212, 213]. HtrA proteases have been established to destroy the integrity of the epithelial cell barrier by opening up cell polarity through the cleaving of the extracellular domain of adhesion proteins and E-cadherin, allowing the translocation of effector protein, cytotoxin-associated gene A [211].

In H. pylori, the secretion of HtrA occurs in the extracellular space and acts as a specific E-cadherin protease, which effectively destroys adherence junctions in polarized epithelial cells, allowing the translocation of effector protein. HtrA destroys epithelial barrier function, allowing persistent H. pylori colonization, nutrition, and pathogenesis. HpHtrA mutant strain has been shown to be more susceptible to induced stress [211].

Overall, htrA is an important gene with important intracellular and extracellular functions [214]. All H. pylori possess htrA [162, 214]. H. pylori isolates can secrete HtrA at a similar rate. The rate of secretion is about 9,600 HtrA molecules per cell [215]. In a study by Yeh, a 100% prevalence rate of htrA was reported. According to the investigation, the presence of htrA-171 polymorphism resulted in a higher rate of gastric cancer. This protease is important for the pathogen to survive extreme temperature, pH, and salt concentration [216] and could be a target for anti-H pylori therapy. However, more studies are still needed to fully understand whether htrA genetic polymorphism has a significant association with the development of gastric cancer and other diseases and the mechanisms involved in the associations.

Catalase

Catalase (KatA) is one of the most abundant protein-enzyme in both plant and animal cells. It converts hydrogen peroxide (H2O2) into water (H2O). For example, in a study by Lekmeechai et al. [217], it was shown that KatA facilitated the neutralization of H2O2 and NaClO, shielding H. pylori from oxidative stress. Also, inside the cytoplasm and periplasm, and sometimes on the surface, they play roles in various pathological processes such as inflammation, apoptosis inhibition, as well as tumour formation resulting from mutagenesis. H. pylori produce high catalase content as one of its highly expressed proteins and are more resistant to inhibition by cyanide or amino triazole compared to catalase from other species [218]. It has been proven to protect H. pylori from oxidant activities and oxidative stresses [219]. It also protects the bacteria from complement-mediated killings [220], thus, facilitating bacterial survival and colonization.

Other virulence factors that play an important role in the pathogenesis of H. pylori are summarized in Table 1.

Conclusion

H. pylori successfully colonize the host with the help of its adhesins. The pathogen produces several effector proteins/ toxins responsible for damage to the host tissues. The gastric epithelium layers form an interface between H. pylori and the host. Interestingly, this bacterium can navigate between two different shapes depending on the physiological activity required. The morphology of H. pylori may also have a specific function on host–pathogen interaction. Understanding how this organism uses its shape to its advantage and how it influences clinical outcomes will be crucial as it will help properly decipher how it can survive and establish infection. However, the problems associated with H. pylori eradication may likely increase in the near future based on the increasing infection rates in addition to the gastroduodenal pathological outcomes. More studies are needed to properly understand and characterize all the virulence factors and determine how they are linked to gastrointestinal diseases.

Declarations

Conflict of interest The authors declare no competing interests.

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