Brazilian Journal of Microbiology (2022) 53:33–50
https://doi.org/10.1007/s42770-021-00675-0

BACTERIAL AND FUNGAL PATHOGENESIS - REVIEW

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

Hyelnaya Cletus Sharndama[1] · **Ifeanyi Elibe Mba**[1]

Received: 10 June 2021 / Accepted: 24 December 2021 / Published online: 6 January 2022

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*

✉ Ifeanyi Elibe Mba
mbaifeanyie@gmail.com

[1] Department of Microbiology, University of Nigeria, Nsukka, Enugu, Nigeria

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 gastrointestinal 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 several diseases. On the other hand, it remains to be completely understood why most individuals infected with *H. pylori* remain asymptomatic while some develop severe gastric diseases. Several other recent studies enhancing our understanding of the interaction of *H. pylori* and host cells are also available [32–36].

However, to date, the exact role of the pathogen in gastric diseases and other diseases remain elusive and controversial. *H. pylori* employ antibiotic resistance mechanisms such as genetic mutations and biofilm formation [37], while its pathogenicity involves host signalling pathways and indirect inflammatory responses induced within the gastric mucosa [38]. Furthermore, the pathogenicity of *H. pylori* depends on the exact strain of *H. pylori*, although several other factors play a role. The expression of a specific virulence factor facilitates the interplay between the host and the bacterium [39]. A recent study by Palamides et al. [40] shows that *H. pylori* isolates differ in virulence factor and disease outcome. So far, the eradication of *H. pylori* has become increasingly challenging. Moreover, breaking its link with other health issues is also another challenge. To find strategies to improve the efficacy of *H. pylori*, an understanding of the virulence and pathogenesis mechanisms is essential. Thus, this review is timely as it will help to bring to the limelight the latest update on the virulence and pathogenesis mechanisms of *H. pylori*. These virulence pathways could be leveraged for therapeutic targets.

Therefore, expounding the role of virulence factors in *H. pylori* pathogenesis and clinical outcomes would be crucial in drug development and vaccine formulation. In the present review, we discussed what is currently known about the virulence and pathogenesis mechanisms of *H. pylori*.

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].

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 flagella-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

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 heterogenicity 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

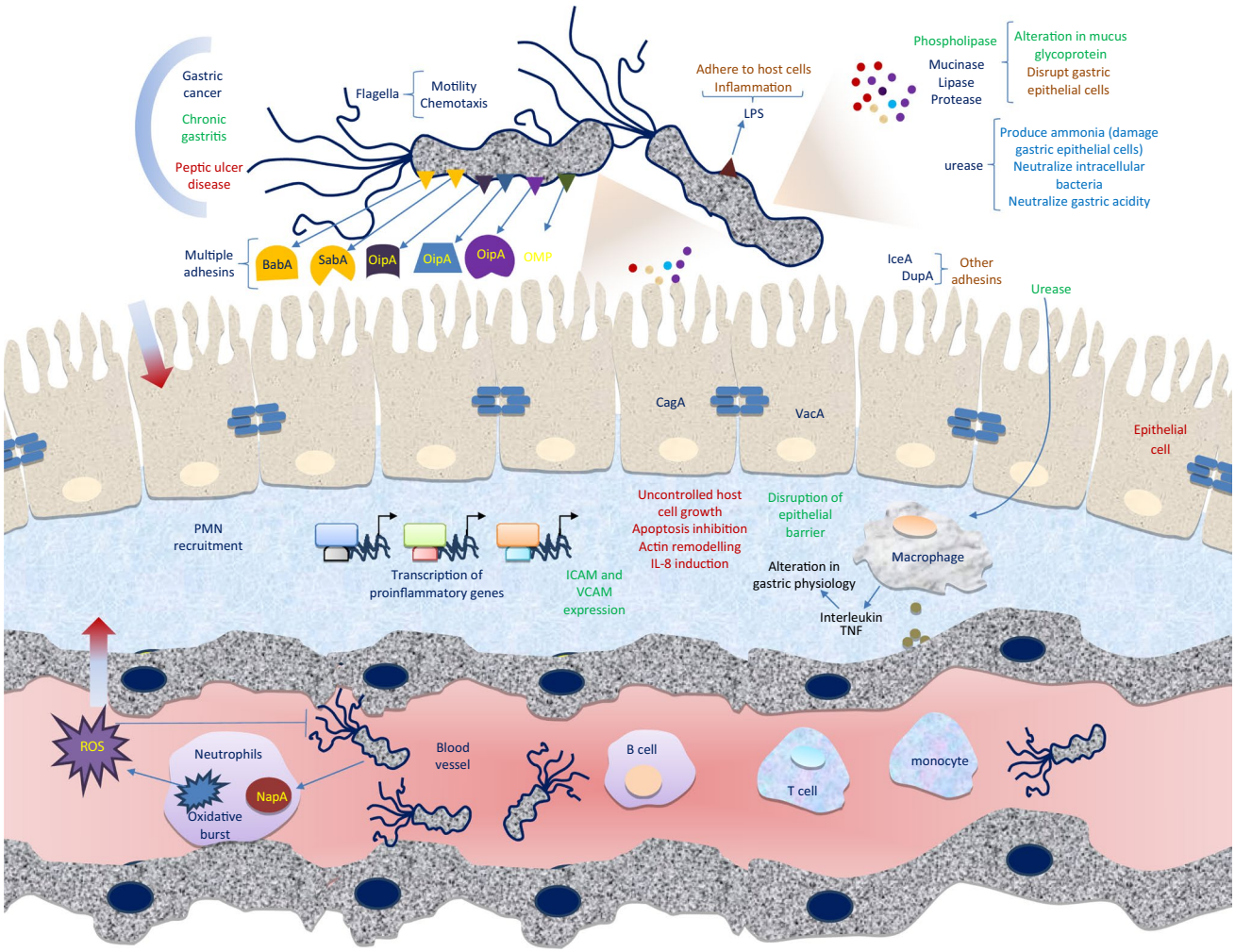

Fig. 1 Virulence and pathogenesis mechanisms of *H. pylori*. Colonization and establishment of diseases and infection by *H. pylori* depend on four major stages: (1) adaptation to the acidic environment of the gastric mucosa, (2) the movement towards the epithelial cells using the flagella, (3) penetration 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* OMPZ (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 Hpne4160, 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 adhesin (BabA), sialic acid-binding adhesin (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 adhesin 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 Hom 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, viable culturable 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 catalyses the hydrolysis of ammonia from CO_2, 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 oxygenic 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 A/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 CO_2, ammonia (NH_3), 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 (NH_3) and carbonic acid. The carbonic acid is broken down into CO_2 and H_2O. Ammonia and CO_2 participate in the lowering of pH. [139]. Ammonium (NH_4+) 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]. NH_3 disrupts the tight cell junctions, breaches cellular integrity, and damages the gastric epithelium, while CO_2 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* sla/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 Altobelli 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 (*cag*PAI) [178]. The *cag*PAI codes for the production of CagA and type (IV) secretion system (T4SS) [179]. Thus, *cag*PAI 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 (*cag*PAI). 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 cag*T4SS 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 *cag*PAI 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

Table 1 Other important virulence factors in *H. pylori*

Virulence factors	Function	Reference
Phospholipase	Activate signalling pathways (e.g. ERK1/2) Trigger chronic inflammation Enhance bacterial colonization and survival Involved in the degradation of lipids and damage to mucus layer	[221, 222]
Lipopolysaccharide	Trigger several signalling pathways Induce several inflammatory responses Induce immune responses Disrupts the mucus secretion Shield the organism against toxic materials	[223–225]
Heat shock proteins	Enhance adherence to epithelial surfaces 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	[226–229]
Arginase	Prevents bacterial killing Prevents T-cell proliferation Impair immune responses Stimulate apoptosis Help the *H. pylori* to withstand the acidic environment	[230, 231]
Superoxide dismutase (SOD)	Protect the cell from reactive oxygen species (ROS) Enhance colonization Inhibits the production of cytokines Stimulate macrophage activation	[232–235]
γ-glutamyl-transferase	Facilitates apoptosis and necrosis Induce the release of pro-inflammatory proteins Induce the release of ROS Stimulate DNA damage	[236, 237]
Cholesteryl α-glucosyltransferase (αCgT)	Shield *H. pylori* from immunological attack Stimulate the production of pro-inflammatory proteins (e.g. IL-8) Enhance bacterial growth and its resistance to antibiotics	[238, 239]

[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 *ice*A1/*ice*A2 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].

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 secret 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 (H_2O_2) into water (H_2O). For example, in a study by Lekmeechai et al. [217], it was shown that KatA facilitated the neutralization of H_2O_2 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.

References

1. Waskito LA, Yamaoka Y (2019) The story of *Helicobacter pylori*: depicting human migrations from the phylogeography. Adv Exp Med Biol 1149:1–16
2. Hanafiah A, Lopes BS (2020) Genetic diversity and virulence characteristics of *Helicobacter pylori* isolates in different human ethnic group. Infect Genet Evol 78:104135
3. Salama NR (2020) Cell morphology as a virulence determinant: lessons from *Helicobacter pylori*. Curr Opin Microbiol 54:11–17
4. Kusters JG, Gerrits MM, Van Strijp JA, Vandenbroucke-Grauls CM (1997) Coccoid forms of *Helicobacter pylori* are the morphologic manifestation of cell death. Infect Immun 65:3672–3679
5. Reshetnyak VI, Reshetnyak TM (2017) Significance of dormant forms of *Helicobacter pylori* in ulcerogenesis. World J Gastroenterol 23:4867–4878
6. Reshetnyak VI, Burnistrov AI, Maev IV (2021) *Helicobacter pylori*: commensals, symbiont or pathogens? World J Gastroenterol 27(**7**):545

7. Krzyżek P, Grande R (2020) Transformation of *Helicobacter pylori* into Coccoid forms as a challenge for research determining activity of antimicrobial substances. Pathogens 9:184
8. Lerardi E, Losurdo G, Mileti A, Paolillo R, Giorgio F, Principi M, Di Leo A (2020) The puzzle of coccoid forms of *Helicobacter pylori*: beyond basic science. Antibiotics (Basel) 9(6):293
9. Kao C-Y, Sheu B-S, Wu J-J (2016) *Helicobacter pylori* infection: an overview of bacterial virulence factors and pathogenesis. Biomedical Journal 39(1):14–23
10. Saxena A, Mukhopadhyar AK, Nandi SP (2020) *Helicobacter pylori*: perturbation and restoration of gut microbiome. J Biosci 45(1):1–15
11. Huang Y, Wang Q-L, Cheng D-d, Xu Vot Lu, N-h, (2016) Adhesin and invasion of gastric mucosa epithelial cells by *Helicobacter pylori*. Front Cell Infect Microbiol 6:159
12. Alexander SM, Retnakumar RJ, Chouhan D, Devi TNB, Dharmaseelan S, Devadas K, Lamtha SC, Chattopadhyay S (2021) *Helicobacter pylori* in human stomach: the inconsistencies in clinical outcomes and the probable causes. Front Microbiol 12:713955
13. Hooi JK, Lai WY, Ng WK, Suen MM, Underwood FE, Tanyingoh D et al (2017) Global prevalence of *Helicobacter pylori* infection: systematic review and meta-analysis. Gastroenterology 153(2):420–429
14. Crowe SE (2019) *Helicobacter pylori* infection. N Engl J Med 380:1158–1165
15. Machlowska J, Kapusta P, Baj J, Morsink FHM, Wołkow P, Maciejewski R, Offerhaus GJA, Sitarz R (2020) High-throughput sequencing of gastric cancer patients: unravelling genetic predispositions towards an early-onset subtype. Cancers 12:1981
16. Goni E, Franceschi F (2016) *Helicobacter pylori* and extragastric diseases. Helicobacter 1:45–48
17. McColl KE (2010) *Helicobacter pylori* infection. New Eng J Med 362(17):1597–1604
18. Gravina AG, Zagari RM, de Musis C, Romano L, Loguercio C, Romano L, Loguercio C, Romano M (2018) *Helicobacter pylori* and extragastric diseases: a review. World J Gastroenterol 24(29):3204–3221
19. Narayanan M, Reddy KM, Marsicano E (2018) Peptic ulcer disease and *Helicobacter pylori* infection. Mo Med 115:219
20. Watari J, Chen N, Amenta PS, Fukui H, Oshima T, Tomita T et al (2014) *Helicobacter pylori* associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development. World J Gastroenterol: WJG 20:5461
21. Polyzos SA, Zeglinas C, Artemaki F, Doulberis M, Kazakos E, Katsinelos P et al (2018) *Helicobacter pylori* infection and esophageal adenocarcinoma: a review and a personal view. Ann Gastroenterol 31:8
22. Chen TP, Hung HF, Chen MK, Lai HH, Hsu WF, Huang KC et al (2015) *Helicobacter pylori* infection is positively associated with metabolic syndrome in Taiwanese adults: a cross-sectional study. Helicobacter 20:184–191
23. Yu XJ, Yang X, Feng L, Wang LL, Dong QJ (2017) Association between *Helicobacter pylori* infection and angiographically demonstrated coronary artery disease: a meta-analysis. Exp Ther Med 13:787–793
24. Sato Y, Yoneyama O, Azumaya M, Takeuchi M, Sasaki SY, Yokoyama J et al (2015) The relationship between iron deficiency in patients with *Helicobacter pylori*-infected nodular gastritis and the serum prohepcidin level. Helicobacter 20:11–18
25. Burucoa C, Axon A (2017) Epidemiology of *Helicobacter pylori* infection. Helicobacter 22:e12403
26. Pucułek M, Machlowska J, Wierzbicki R, Baj J, Maciejewski R, Sitarz R (2018) *Helicobacter pylori* associated factors in the development of gastric cancer with special reference to the early-onset subtype. Oncotarget 9(57):31146–31162
27. Suerbaum S, Michetti P (2002) *Helicobacter pylori* infection. N Engl J Med 347:1175–1186
28. Eusebi LH, Zagar RM, Bazzoli F (2014) Epidemiology of Helicobacter pylori infection. Helicobacter 19(S1):1–5
29. Urita Y, Watanabe T, Kawagoe N, Takemoto I, Tanaka H, Kijima S, Urita C (2013) Role of infected grandmothers in transmission of *Helicobacter pylori* to children in a Japanese rural town. J Paediat Child Health 49(5):394–398
30. Mentis A, Lehbours P, Megraud F (2015) Epidemiology and diagnosis of *Helicobacter pylori* infection. Helicobacter 20:1–7
31. Stefano K, Marco M, Federica G, Laura B, Barbara B, Gioacchino L (2018) *Helicobacter pylori*, transmission routes and recurrence of infection: state of the art. Acta Bio Medica: Atenei Parmensis 89(8):72
32. Thung I, Aramin H, Vavinskaya V, Gupta S, Park JY, Crowe SE, Valasek MA (2016) Review article: the global emergence of *Helicobacter pylori* antibiotic resistance. Aliment Pharmacol Ther 43:514–533
33. Costa L, Corre S, Michel V, et al (2019) USF1 defect drives p53 degradation during *Helicobacter pylori* infection and accelerates gastric carcinogenesis. *Gut*. 2019;gutjnl-2019–318640
34. Teng Y-S, Zhao Y-L, Li M-S et al (2020) Upexpression of BHLHE40 in gastric epithelial cells increases CXCL12 production through interaction with p-STAT3 in *Helicobacter pylori*-associated gastritis. FASEB J 34(1):1169–1181
35. Wroblewski LE, Choi E, Petersen C et al (2019) Targeted mobilization of Lrig1+ gastric epithelial stem cell populations by a carcinogenic *Helicobacter pylori* type IV secretion system. Proc Natl Acad Sci U S A 116(39):19652–19658
36. Blosse A, Levy M, Robe C, Staedel C, Copie-Bergman C, Lehours P (2019) Deregulation of miRNA in *Helicobacter pylori*-induced gastric MALT lymphoma: from mice to human. J Clin Med 8:6
37. Baj J, Forma A, Sitarz M, Portincasa P, Garruti G, Krasowska D, Maciejewski R (2021) *Helicobacter pylori* virulence factors-mechanisms of bacterial pathogenicity in the gastric microenvironment. Cells 10(1):27
38. Machlowska J, Baj J, Sitarz M, Maciejewski R, Sitarz R (2020) Gastric cancer: epidemiology, risk factors, classification, genomic characteristics and treatment strategies. Int J Mol Sci 21:4012
39. Baj J, Korona-Głowniak I, Forma A, Maani A, Sitarz E, Rahnama-Hezavah M, Radzikowska E, Portincasa P (2020) Mechanisms of the epithelial–mesenchymal transition and tumor microenvironment in *Helicobacter pylori*-induced gastric cancer. Cells 9:1055
40. Palamides P, Jolaiya T, Idowu A, Loell E, Onyekwere C, Ugaogbe R, Agbo I, Lesi D, Ndububa D (2020) *Helicobacter pylori* patient isolates from South Africa and Nigeria differ in virulence factor, pathogenicity profile and associated gastric diseases outcome. Sci Rep 10:11409
41. Tomb JF, White O, Kerlavage AR, Clayton RA, Fleischmann RD, Venter JC (1997) The complete genome sequence of the gastric pathogens *Helicobacter pylori*. Nature 388(6642):539–547
42. Hunt R, Xiao S, Megraud F, Leon-Barau R, Bazzoli F, Van der Merwe S, Le Mair A (2011) World Gastroenterology Organization Global Guideline. *Helicobacter pylori* in developing countries. August 2010: WGO global guidelines. South Afr Gastroenterol Rev 9(3):1622
43. Park YH, Kim N (2015) Review of atrophic gastric and intestinal metaplasia as a pre malignant lesion of gastric cancer. J cancer Prev 20(1):25
44. Ofori EG, Adinortey CA, Bockarie AS, Kyei F, Tagoe EA, Adinortey MB (2019) *Helicibacter pylori* infection, virulence genes' distribution and accompanying clinical outcomes: the West Africa situation. BioMed Res Int 2019

45. Toh JW, Wilson RB (2020) Pathways of gastric carcinogenesis, *Helicobacter pylori* virulence and interactions with antioxidant systems, vitamin c and phytochemicals. Int J Mol Sci 21(17):6451
46. Salama NR, Hartung ML, Mueller A (2013) Life in the human stomach: persistence strategies of the bacterial pathogen *Helicobacter pylori*. Nature Rev Microbiol 11:385–399
47. Yamaoka Y, Graham DY (2014) *Helicobacter pylori* virulence and cancer pathogenesis. Future Oncol 10:1487–1500
48. Amieva M, Peek RM Jr (2016) Pathobiology of *Helicobacter pylori*-induced gastric cancer. Gastroenterology 150:64–78
49. Elhahriri M, Hanza D, Elhew R, Hamza E (2018) Occurrence of cagA+ vacA s1a m1 i1 *Helicobacter pylori* in farm animals in Egypt and ability to survive in experimentally contaminated UHT milk. Sci Rep 8(1):1–3
50. Dorer MS, Talarico S, Salama NR (2009) *Helicobacter pylori*'s unconventional role in health and disease. PLoS Pathog 5:e1000544
51. Salama NR, Hartung ML, Muller A (2013) Life in the human stomach: persistence strategies of the bacterial pathogen *Helicobacter pylori*. Natl Rev Microbiol 11:385–399
52. Kusters JG, van Vliet AHM, Kuipers E (2006) Pathogenesis of *Helicobacter pylori* infection. Clin Microbiol 19(3):449–490
53. Yanezawa H, Osaki T, Kamiya S (2015) Biofilm formation by *Helicobacter pylori* and its involvement for antibiotics resistance. Biomed Res Int 2015(914791):9
54. Hathroubi S, Hu S, Ottemann KM (2020) Genetic requirements and transcriptomics of *Helicobacter pylori* biofilm formation on abiotic and biotic surfaces. NPJ Biofilms Microbiomes 6:56
55. Hathroubi S, Servetas SL, Windham I, Merrell DS, Ottemann KM (2018) *Helicobacter pylori* biofilm formation and its potential role in pathogenesis. Microbiol Mol Biol Rev 82:e00001-e00018
56. Fauzia KA, Miftahussurur M, Syam AF, Waskito LA, Doohan D, Rezkitha YAA et al (2020) Biofilm formation and antibiotic resistance phenotype of *Helicobacter pylori* clinical isolates. Toxins 12:473
57. Patel SK, Pratap CB, Jain AK, Gulati AK, Nath G (2014) Diagnosis of *Helicobacter pylori*: what should be the gold standard? World J Gastroenterol 20:12847–12859
58. Korona-Glowniak I et al (2019) Antibiotic resistance and genotypes of *Helicobacter pylori* strains in patients with gastroduodenal disease in Southeast Poland. J Clin Med 8
59. Idowu A, Mzukwa A, Harrison U, Palamides P, Haas R, Mbao M, Njom H (2019) Detection of *Helicobacter pylori* and its virulence genes (cagA, dupA, and vacA) among patients with gastroduodenal diseases in Chris Hani Baragwanath Academic Hospital. South Africa BMC gastroenterology 19(1):1–10
60. Sabbagh P, Javanian M, Koppolu V, Vasigala VR, Ebrahimpour S (2019) *Helicobacter pylori* infection in children: an overview of diagnostic methods. Eur J Clin Microbiol Infect Dis 38(6):1035–1045
61. Wang Y-K, Kuo F-C, Liu C-J, Wu M-C, Shih H-Y, Wang SSW, Wu J-Y, Kuo C-H, Huang Y-K, Wu D-C (2015) Diagnosis of *Helicobacter pylori* infection: current options and developments. World J Gastroenterol 21(40):11221–11235
62. Yañez P, la Garza AM, Pérez-Pérez G, Cabrera L, Muñoz O, Torres J (2000) Comparison of invasive and noninvasive methods for the diagnosis and evaluation of eradication of *Helicobacter pylori* infection in children. Arch Med Res 31:415–421
63. Pellicano R, Smedile A, Ponzetto A, Berrutti M, Astegiano M, Saracco G, De Angelis C, Repici A, Morgando A, Abate ML, Fagoonee S, Rizzetto M (2005) How accurate is the culture of *Helicobacter pylori* in a clinical setting? An appraisal. Panminerva Med 47:191–194
64. Kovaleva J (2016) Infectious complications in gastrointestinal endoscopy and their prevention. Best Pract Res Clin Gastroenterol 30(5):689–704
65. Sakai E, Higurashi T, Ohkubo H, Hosono K, Ueda A, Matsuhashi N, Nakajima A (2017) Investigation of small bowel abnormalities in HIV-infected patients using capsule endoscopy. Gastroenterol Res Pract 2017
66. Megraud F, Lehours P (2007) *Helicobacter pylori* detection and antimicrobial susceptibility testing. Clin Microbiol Rev 20(2):280
67. Bilgilier C, Stadlmann A, Makristathis A, Thannesberger J, Kastner MT, Knoflach P, Steiner P, Schöniger C (2018) Prospective multicentre clinical study on inter- and intrapatient genetic resistance of *Helicobacter pylori*. Clin Microbiol Infect 24(3):267–272
68. Pantoflickova D, Scott DR, Sachs G, Dorta G, Blum AL (2003) 13C urea breath test (UBT) in the diagnosis of *Helicobacter pylori*: why does it work better with acid test meals?". Gut 52(7):933–937
69. de Francesco V, Zullo A, Perna F, Giorgio F, Hassan C, Vannella L, Lerardi E (2010) *Helicobacter pylori* antibiotic resistance and [13C] urea breath test values. J Med Microbiol 59(5):588–591
70. Ogata SK, Kawakami E, Patrício FR, Pedroso MZ, Santos AM (2001) Evaluation of invasive and non-invasive methods for the diagnosis of *Helicobacter pylori* infection in symptomatic children and adolescents. Sao Paulo Med J119:67–71
71. Korkmaz H, Kesli R, Karabagli P, Terzi Y (2013) Comparison of the diagnostic accuracy of five different stool antigen tests for the diagnosis of *Helicobacter pylori* infection. Helicobacter 18:384–391
72. Yang BL, Yeh C, Kwong WG, Lee SD (2015) A novel one-step *Helicobacter pylori* saliva antigen test. J Chin Med Assoc 78(2):96–100
73. Hooton C, Keohane J, Clair J, Azam M, O'Mahony S, Crosbie O, Lucey B (2006) Comparison of three stool antigen assays with the 13C-urea breath test for the primary diagnosis of *Helicobacter pylori* infection and monitoring treatment outcome. Eur J Gastroenterol Hepatol 18(6):595–599
74. Fischbach W, Malfertheiner P, Jansen PL, Bolten W, Bornschein J, Buderus S, Veith M (2016) S2k guideline. *Helicobacter pylori* and gastroduodenal ulcer disease. J Gastroenterol 54 (04):327–363
75. Abadi TB, Amin. (2018) Diagnosis of *Helicobacter pylori* using invasive and noninvasive approaches. J Pathog 2018:9064952
76. Imkamp F, Lauener FN, Pohl D, Lehours P, Vale FF, Jehanne Q, Wagner K (2019) Rapid characterization of virulence determinants in *Helicobacter pylori* isolated from non-atrophic gastritis patients by next-generation sequencing. J Clin Med 8(7):1030
77. Jiang X, Xu Z, Zhang T, Li Y, Li W, Tan H (2019) Whole-genome-based *Helicobacter pylori* Geographic surveillance: visualized and expanded webtool. Front Microbiol 12:687259
78. Fallone CA, Chiba N, van Zanten SV, Fischbach L, Gisbert JP, Hunt RH, Marshall JK (2016) The Toronto consensus for the treatment of *Helicobacter pylori* infection in adults. Gastroenterology 151(1):51–69
79. Chey WD, Leontiadis GI, Howden CW, Moss SF (2017) ACG clinical guideline: treatment of *Helicobacter pylori* infection. Am J Gastroenterol 112(2):212–239
80. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Helicobacter E, study group. (2012) Management of *Helicobacter pylori* infection-the Maastricht IV/Florence consensus report. Gut 61(5):646–664
81. O'Connor A, Lamarque D, Gisbert JP, O'Morain C (2017) Treatment of *Helicobacter pylori* infection 2017. Helicobacter 22:e12410
82. Ayala G, Galvan-Portillo M, Chihu L, Fierros G, Sanchez A, Carrillo B, Silva-Sanchez and study Group J (2011) Resistance

to antibiotics and characterization of *Helicobacter pylori* strains isolated from antrum and body from adults in Mexico. Microb Drug Resist 17(2):149–155
83. Grande R, Di Marcantonio MC, Robuffo I, Pompillio A, Celia C, Di Mario L, Mincione G (2015) *Helicobacter pylori* ATCC43629/NCTC 11639 outer membrane vesicles (OMVs) from biofilm and planktonic phase associated with extracellular DNA (eDNA). Front Microbiol 6:1369
84. Selgrad M, Tammer I, Langner C, Bornschein J, Meiβle J, Kandulski A, Malfertheiner P (2014) Different antibiotic susceptibility between antrum and corpus of the stomach, a possible reason for treatment failure of *Helicobacter pylori* infection. World J Gastroenterol 20(43):16245–16251
85. Farzi N, Malekian T, Alebouyeh M, Vaziri F, Zali MR (2015) Genotype diversity and quasi species development of *Helicobacter pylori* in a single host. Jpn J Infect Dis 68(4):176–180
86. Yin L, Liu F, Guo C, Wang Q, Pan K, Xu L, Chen Z (2018) Analysis of virulence diversity of 73 *Helicobacter pylori* strains isolated in Guizhou province. China Mol Med Rep 18(5):4611–4620
87. Roszczenko-Jasińska P, Wojtyś MI, Jagusztyn-Krynicka EK (2020) *Helicobacter pylori* treatment in the post-antibiotics era—searching for new drug targets. Appl Microbiol Biotechnol 1–15
88. Mi M, Wu F, Zhu J, Liu F, Cui G, Wen X, Chen Z (2021) Heterogeneity of *Helicobacter pylori* strains isolated from patients with gastric disorders in Guiyang. China Infect Drug Resist 14:53
89. Tag Eldeen LAT, Mohamed MA, Awad MM, Sheir MI, Shams TM, Ragheh MM (2019) A variety of *Helicobacter pylori* strains colonize the stomach of non-bleeding Egyptian patients with upper gastrointestinal disorders. Bull Natl Res Cent 43:183
90. Xu C, Soyfoo DM, Wu Y, Xu S (2020) Virulence of *Helicobacter pylori* outer membrane proteins: an updated review. Eur J Clin Microbiol Infect Dis 39:1821–1830
91. Kinoshita-Daitoku R, Kiga K, Miyakoshi M, Otsubo R, Ogura Y, Sanada T et al (2021) A bacterial small RNA regulates the adaptation of *Helicobacter pylori* to the host environment. Nat Commun 12:2085
92. Gu H (2017) Role of flagella in the pathogenesis of *Helicobacter pylori*. Curr Microbiol 74(7):863–869
93. Ansari S, Yamaoka Y (2020) *Helicobacter pylori* virulence factor cytotoxin-associated gene A (CagA)-mediated gastric pathogenicity. Int J Mol Sci 21(19):7430
94. Marcus EA, Sachs G, Scott DR (2018) Acid-regulated gene expression of *Helicobacter pylori*: insight into acid protection and gastric colonization. Helicobacter 23(3):e12490
95. Ansari S, Yamaoka Y (2017) Survival of *Helicobacter pylori* in gastric acidic territory. Helicobacter 22:4
96. Martínez LE, Hardcastle JM, Wang J, Pincus Z, Tsang J, Hoover TR, Salama NR (2016) Helicobacter pylori strains vary cell shape and flagellum number to maintain robust motility in viscous environments. Mol Microbiol 99(1):88–110
97. Clyne M, Ocroinin T, Suerbaum S, Josenhans C, Drumm B (2000) Adherence of isogenic flagellum-negative mutants of *Helicobacter pylori* and Helicobacter mustelae to human and ferret gastric epithelial cells. Infect Immun 68(7):4335
98. De Brito BB, da Silva FAF, Soares AS, Pereira VA, Santos MLC, Sampaio MM, de Melo FF (2019) Pathogenesis and clinical management of *Helicobacter pylori* gastric infection. World J Gastroenterol 25(37):5578
99. García A, Salas-Jara MJ, Herrera C, González C (2014) Biofilm and *Helicobacter pylori*: from environment to human host. World J Gastroenterol: WJG 20(19):5632
100. Roier S, Zingl FG, Cakar F, Durakovic S, Kohl P, Eichmann TO, Schild S (2016) A novel mechanism for the biogenesis of outer membrane vesicles in Gram-negative bacteria. Nat Commun 7(1):1–13
101. Olofsson A, Vaystrom A, Petzold K, Tegtmeyer N, Schleucher J, Carlsson S, Haas R, Backert S, Wai SN et al (2010) Mol Microbiol 77:1539–1555
102. Yang Y, Hong Y, Cho E, Kim GB, Kim IS (2018) Extracellular vesicles as a platform for membrane-associated therapeutic protein delivery. J Extracell Vesicles 7(1):1440131
103. Uddin MJ, Dawan J, Jeon G, Yu T, He X, Ahn J (2020) The role of bacterial membrane vesicles in the dissemination of antibiotic resistance and as promising carriers for therapeutic agent delivery. Microorganisms 8(5):670
104. Keenan J, Day T, Neal S, Cook B, Perez-Perez G, Allardyce R, Bagshaw P (2000) A role for the bacterial outer membrane in the pathogenesis of *Helicobacter pylori* infection. FEMS Microbiol Lett 182(2):259–264
105. Chmiela M, Walczak N, Rudnicka K (2018) *Helicobacter pylori* outer membrane vesicles involvement in the infection development and *Helicobacter pylori*-related diseases. J Biomed Sci 25(1):1–11
106. Murray BO, Dawson RA, Alsharaf LM, Winter JA (2020) Protective effects of *Helicobacter pylori* membrane vesicles against stress and antimicrobial agents. Microbiology 166(8):751
107. Oleastro M, Ménard A (2013) The role of *Helicobacter pylori* outer membrane proteins in adherence and pathogenesis. Biology 2(3):1110–1134
108. Bina JE, Alm RA, Uria-Nickelsen M, Thomas SR, Trust TJ, Hancock REW (2000) *Helicobacter pylori* uptake and efflux: basis for intrinsic susceptibility to antibiotics in vitro. Antimicrob Agents Chemother 44(2):248
109. Cover TL, Blaser MJ (1992) Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. J Biol Chem 267:10570–10575
110. da Costa DM, dos Santos PE, Rabenhorst SHB (2015) What exists beyond cagA and vacA? *Helicobacter pylori* genes in gastric diseases. World J Gastroenterol 21(37):10563
111. Bauwens E, Joosten M, Taganna J, Rossi M, Debraekeleer A, Tay A, Peter F, Backert S et al (2018) In silico proteomic and phylogenetic analysis of the outer membrane protein repertoire of gastric Helicobacter species Sci Rep 8:15453
112. Ishijima N, Suzuki M, Ashida H, Ichikawa Y, Kanegae Y, Saito I, Mimuro H (2011) BabA-mediated adherence is a potentiator of the *Helicobacter pylori* type IV secretion system activity. J Biol Chem 286(28):25256–25264
113. Alzahrani S, Lina TT, Gonzalez J, Pinchuk IV, Beswick EJ, Reyes VE (2014) Effect of *Helicobacter pylori* on gastric epithelial cells. World J Gastroenterol 20(36):12767
114. El-sayed MS, Musa N, Eltabbakh M, Abdelhamid DH, Mostafa SMI, Salah MM (2020) Detection of *Helicobacter pylori* oipA and dupA genes among dyspeptic patients with chronic gastritis. Alexandria Journal of Medicine 56:1
115. Mahdavi J, Sondén B, Hurtig M, Olfat FO, Forsberg L, Roche N, Borén T (2002) *Helicobacter pylori* SabA adhesin in persistent infection and chronic inflammation. Science 297(5581):573–578
116. Doohan D, Rezkitha YAA, Waskito LA, Yamaoka Y, Miftahussurur M (2021) *Helicobacter pylori* BabA – SabA key roles in the adherence phase: the synergic mechanism for successful colonization and disease development. Toxins 13:485
117. Horridge DN, Begley AA, Kim J, Aravindan N, Fan K, Forsyth MH (2017) Outer inflammatory protein a (OipA) of *Helicobacter pylori* is regulated by host cell contact and mediates CagA translocation and interleukin-8 response only in the presence of a functional cag pathogenicity island type IV secretion system. Pathog Dis 75(8):ftx113
118. Farzi N, Yadegar A, Aghdaei HA, Yamaoka Y, Zali MR (2018) Genetic diversity and functional analysis of oipA gene in association with other virulence factors among *Helicobacter pylori*

isolates from Iranian patients with different gastric diseases. Infect Genet Evol 60:26–34
119. Al-Maleki AR, Loke MF, Lui SY, Ramli NSK, Khosravi Y, Ng CG, Vadivelu J (2017) *Helicobacter pylori* outer inflammatory protein A (O ip A) suppresses apoptosis of AGS gastric cells in vitro. Cellular Microbiol 19(12):e12771
120. Tegtmeyer N, Harrer A, Schmitt V, Singer BB, Backert S (2019) Expression of CEACAM1 or CEACAM5 in AZ-521 cells restores the type IV secretion deficiency for translocation of CagA by *Helicobacter pylori*. Cellular Microbiol 21(1):e12965
121. Kennemann L, Brenneke B, Andres S, Engstrand L, Meyer TF, Aebischer T, Suerbaum S (2012) In vivo sequence variation in HopZ, a phase-variable outer membrane protein of *Helicobacter pylori*. Infect Immun 80(12):4364
122. Belogolova E, Bauer B, Pompaiah M, Asakura H, Brinkman V, Ertl C, Meyer TF (2013) Helicobacter pylori outer membrane protein HopQ identified as a novel T4SS-associated virulence factor. Cellular Microbiol 15(11):1896–1912
123. Sycuro LK, Wyckoff TJ, Biboy J, Born P, Pincus Z, Vollmer W, Salama NR (2012) Multiple peptidoglycan modification networks modulate *Helicobacter pylori*'s cell shape, motility, and colonization potential. PLoS Pathog 8(3):e1002603
124. Cellini L (2014) *Helicobacter pylori*: a chameleon-like approach to life. World J Gastroenterol 20(19):5575
125. Percival SL, Suleman L (2014) Biofilms and *Helicobacter pylori*: dissemination and persistence within the environment and host. World J Gastrointest Pathophysiol 5(3):122
126. Pinto D, Santos MA, Chambel L (2015) Thirty years of viable but nonculturable state research: unsolved molecular mechanisms. Critical Rev Microbiol 41(1):61–76
127. Lleo MM, Bonato B, Tafi MC, Signoretto C, Boaretti M, Canepari P (2001) Resuscitation rate in different enterococcal species in the viable but non-culturable state. J Appl Microbiol 91(6):1095–1102
128. Senoh M, Ghosh-Banerjee J, Ramamurthy T, Colwell RR, Miyoshi SI, Nair GB, Takeda Y (2012) Conversion of viable but nonculturable enteric bacteria to culturable by co-culture with eukaryotic cells. Microbiol Immunol 56(5):342–345
129. Willén R, Carlén B, Wang X, Papadogiannakis N, Odselius R, Wadström T (2000) Morphologic conversion of *Helicobacter pylori* from spiral to coccoid form: scanning (SEM) and transmission electron microscopy (TEM) suggest viability. Upsala J Med Sci 105(1):31–40
130. Andersen AP, Elliott DA, Lawson M, Barland P, Hatcher VB, Puszkin EG (1997) Growth and morphological transformations of *Helicobacter pylori* in broth media. J Clin Microbiol 35(11):2918
131. Becker KW, Skaar EP (2014) Metal limitation and toxicity at the interface between host and pathogen. FEMS Microbiol Rev 38(6):1235–1249
132. Camilo V, Sugiyama T, Touati E (2017) Pathogenesis of *Helicobacter pylori* infection. Helicobacter 22:e12405
133. Olson JW, Maier RJ (2002) Molecular hydrogen as an energy source for *Helicobacter pylori*. Science 298(5599):1788–1790
134. Ha NC, Oh ST, Sung JY, Cha KA, Lee MH, Oh BH (2001) Supramolecular assembly and acid resistance of *Helicobacter pylori* urease. Nat Struct Biol 8(6):505–509
135. Ernst FD, Stoof J, Horrevoets WM, Kuipers EJ, Kusters JG, van Vliet AH (2006) NikR mediates nickel-responsive transcriptional repression of the *Helicobacter pylori* outer membrane proteins FecA3 (HP1400) and FrpB4 (HP1512). Infect Immun 74(12):6821
136. Fulkerson JF Jr, Mobley HL (2000) Membrane topology of the NixA nickel transporter of *Helicobacter pylori*: two nickel transport-specific motifs within transmembrane helices II and III. J Bacteriol 182(6):1722
137. Wolfram L, Bauerfeind P (2002) Conserved low-affinity nickel-binding amino acids are essential for the function of the nickel permease NixA of *Helicobacter pylori*. J bacteriol 184(5):1438
138. Mulrooney SB, Hausinger RP (2003) Nickel uptake and utilization by microorganisms. FEMS Microbiol Rev 27(2–3):239–261
139. Athmann C, Zeng N, Kang T, Marcus EA, Scott DR, Rektorschek M, Sachs G (2000) Local pH elevation mediated by the intrabacterial urease of *Helicobacter pylori* cocultured with gastric cells. J Clinical Invest 106(3):339–347
140. Miller EF, Maier RJ (2014) Ammonium metabolism enzymes aid *Helicobacter pylori* acid resistance. J Bacteriol 196(17):3074
141. Nolan KJ, McGee DJ, Mitchell HM, Kolesnikow T, Harro JM, O'Rourke J, Lee A (2002) In vivo behavior of a *Helicobacter pylori* SS1 nixA mutant with reduced urease activity. Infect Immun 70(2):685
142. Schmalstig AA, Benoit SL, Misra SK, Sharp JS, Maier RJ (2018) Noncatalytic antioxidant role for *Helicobacter pylori* urease. J Bacteriol 200(17)
143. Can F, Karahan C, Dolapci I, Demirbilek M, Tekeli A, Arslan H (2008) Urease activity and urea gene sequencing of coccoid forms of *H. pylori* induced by different factors. Curr Microbiol 56(2):150–155
144. Gebert B, Fischer W, Weiss E, Hoffmann R, Haas R (2003) *Helicobacter pylori* vacuolating cytotoxin inhibits T lymphocyte activation. Science 301(5636):1099–1102
145. McClain MS, Beckett AC, Cover TL (2017) *Helicobacter pylori* vacuolating toxin and gastric cancer. Toxins 9(10):316
146. Kakelar HM, Barzegari A, Dehghani J, Hanifian S, Saeedi N, Barar J, Omidi Y (2019) Pathogenicity of *Helicobacter pylori* in cancer development and impacts of vaccination. Gastric Cancer 22(1):23–36
147. Foegeding NJ, Caston RR, McClain MS, Ohi MD, Cover TL (2016) An overview of *Helicobacter pylori* VacA toxin biology. Toxins 8(6):173
148. Ricci V (2016) Relationship between VacA toxin and host cell autophagy in *Helicobacter pylori* infection of the human stomach: a few answers, many questions. Toxins 8(7):203
149. de Falco M, Lucariello A, Iaquinto S, Esposito V, Guerra G, De Luca A (2015) Molecular mechanisms of *Helicobacter pylori* pathogenesis. J Cell Physiol 230(8):1702–1707
150. Yahiro K, Hirayama T, Moss J, Noda M (2016) New insights into VacA intoxication mediated through its cell surface receptors. Toxins 8(5):152
151. Winter JA, Letley DP, Cook KW, Rhead KR (2014) A role for the vacuolating cytotoxin, vacA, in colonization and *Helicobacter pylori*-induced metaplasia in the stomach. J Infect Dis 210(6):954–963
152. Raju D, Hussey S, Ang M, Terebiznik MR, Sibony M, Galindo-Mata E, Jones NL (2012) Vacuolating cytotoxin and variants in Atg16L1 that disrupt autophagy promote *Helicobacter pylori* infection in humans. Gastroenterology 142(5):1160–1171
153. Djekic A, Müller A (2016) The immunomodulator VacA promotes immune tolerance and persistent *Helicobacter pylori* infection through its activities on T-cells and antigen-presenting cells. Toxins 8(6):187
154. Utsch C, Haas R (2016) VacA's induction of VacA-containing vacuoles (VCVs) and their immunomodulatory activities on human T cells. Toxins 8(6):190
155. Muzahhed M (2020) *Helicobacter pylori* oncogenicity: mechanism, prevention and risk factors. Sci World J
156. Wroblewski LE, Peek RM Jr, Wilson KT (2010) *Helicobacter pylori* and gastric cancer: factors that modulate disease risk. Clin Microbiol Rev 23(4):713

157. Ito Y, Azuma T, Ito S, Suto H, Miyaji H, Yamazaki Y, Kuriyama M (1998) Full-length sequence analysis of the vacA gene from cytotoxic and noncytotoxic *Helicobacter pylori*. The J Infect Dis 178(5):1391–1398
158. Atherton JC, Cao P, Peek RM, Tummuru MK, Blaser MJ, Cover TL (1995) Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*: association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem 270(30):17771–17777
159. Forsyth MH, Atherton JC, Blaser MJ, Cover TL (1998) Heterogeneity in levels of vacuolating cytotoxin gene (vacA) transcription among *Helicobacter pylori* strains. Infect Immun 66(7):3088
160. Rhead JL, Letley DP, Mohammadi M, Hussein N, Mohagheghi MA, Hosseini ME, Atherton JC (2007) A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. Gastroenterology 133(3):926–936
161. Shiota S, Suzuki R, Yamaoka Y (2013) The significance of virulence factors in *Helicobacter pylori*. J Dig Dis 14(7):341–349
162. Chang WL, Yeh YC, Sheu BS (2018) The impacts of *H. pylori* virulence factors on the development of gastroduodenal diseases. J Biomed Sci 25:68
163. Sheikh AF et al (2018) CagA and vacA allelic combination of *Helicobacter pylori* in gastroduodenal disorders. Microb Pathog 122:144–150
164. Wang HJ, Wang WC (2000) Expression and binding analysis of GST-VacA fusions reveals that the C-terminal~ 100-residue segment of exotoxin is crucial for binding in HeLa cells. Biochem Biophys Res Commun 278(2):449–454
165. Skibinski DA, Genisset C, Barone S, Telford JL (2006) The cell-specific phenotype of the polymorphic vacA midregion is independent of the appearance of the cell surface receptor protein tyrosine phosphatase β. Infect Immun 74(1):49
166. Letley DP, Rhead JL, Twells RJ, Dove B, Atherton JC (2003) Determinants of non-toxicity in the gastric pathogen *Helicobacter pylori*. J Biol Chem 27(29):26734–26741
167. Altobelli A, Bauer M, Valez K, Cover TL, Muller A (2019) *Helicobacter pylori* VacA targets myeloid cells in the gastric lamina propria to promote peripherally induced regulatory T-cell differentiation and persistent infection. mBio 10:e00261–19
168. Papini E, De Bernard M, Milia E, Bugnoli M, Zerial M, Rappuoli R, Montecucco C (1994) Cellular vacuoles induced by *Helicobacter pylori* originate from late endosomal compartments. Proc Natl Acad Sci USA 91(21):9720–9724
169. Molinari M, Galli C, Norais N, Telford JL, Rappuoli R, Luzio JP, Montecucco C (1997) Vacuoles induced by *Helicobacter pylori* toxin contain both late endosomal and lysosomal markers. J Biol Chem 272(40):25339–25344
170. Li Y, Wandinger-Ness A, Goldenring JR, Cover TL (2004) Clustering and redistribution of late endocytic compartments in response to *Helicobacter pylori* vacuolating toxin. Mol Biol Cell 15(4):1946–1959
171. Kern B, Jain U, Utsch C, Otto A, Busch B, Jiménez-Soto L, Haas R (2015) Characterization of *Helicobacter pylori* VacA-containing vacuoles (VCVs), VacA intracellular trafficking and interference with calcium signaling in T lymphocytes. Cellular Microbiol 17(12):1811–1832
172. Bernard MD, Moschioni M, Habermann A, Griffiths G, Montecucco C (2002) Cell vacuolization induced by *Helicobacter pylori* VacA cytotoxin does not depend on late endosomal SNAREs. Cellular Microbiol 4(1):11–11
173. Cover TL, Krishna US, Israel DA, Peek RM (2003) Induction of gastric epithelial cell apoptosis by *Helicobacter pylori* vacuolating cytotoxin. Can Res 63(5):951–957
174. Ricci V, Sommi P, Fiocca R, Romano M, Solcia E, Ventura U (1997) *Helicobacter pylori* vacuolating toxin accumulates within the endosomal-vacuolar compartment of cultured gastric cells and potentiates the vacuolating activity of ammonia. J Pathog 183(4):453–459
175. Tombol F, Morbiato L, Del Giudice G, Rappuoli R, Zoratti M, Papini E (2001) The *Helicobacter pylori* VacA toxin is a urea permease that promotes urea diffusion across epithelia. J Clin Invest 108(6):929–937
176. Genisset C, Puhar A, Calore F, De Bernard M, Dell'Antone P, Montecucco C (2007) The concerted action of the *Helicobacter pylori* cytotoxin VacA and of the v-ATPase proton pump induces swelling of isolated endosomes. Cell Microbiol 9(6):1481–1490
177. Chowdhury R (2014) Host cell contact induces fur-dependent expression of virulence factors CagA and VacA in *Helicobacter pylori*. Helicobacter 19(1):17–25
178. Noto JM Jr, Peek RM (2012) The *Helicobacter pylori*:cag pathogenicity island. Methods Mol Biol 921:41–50
179. Odenbreit S, Püls J, Sedlmaier B et al (2000) Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. Science 287:1497–1500
180. Rohde M, Püls J, Buhrdorf R, Fischer W, Haas R (2003) A novel sheathed surface organelle of the *Helicobacter pylori* cag type IV secretion system. Mol Microbiol 49(1):219–234
181. Asahi M, Azuma T, Ito S et al (2000) *Helicobacter pylori* CagA protein can be tyrosine phosphorylated in gastric epithelial cells. J Exp Med 191:593–602
182. Behrens I-K, Busch B, Ishikawa-Ankerhold H et al (2020) The HopQ-CEACAM interaction controls CagA translocation, phosphorylation, and phagocytosis of *Helicobacter pylori* in neutrophils. MBio 11(1):e03256-e3319
183. Backert S, Selbach M (2005) Tyrosine-phosphorylated bacterial effector proteins: the enemies within. Trends Microbiol 13(10):476–484
184. Poppe M, Feller SM, Römer G, Wessler S (2007) Phosphorylation of *Helicobacter pylori* CagA by c-Abl leads to cell motility. Oncogene 26(24):3462–3472
185. Tammer I, Brandt S, Hartig R, König W, Backert S (2007) Activation of Abl by *Helicobacter pylori*: a novel kinase for CagA and crucial mediator of host cell scattering. Gastroenterology 132(4):1309–1319
186. Jones KR, Joo YM, Jang S, Yoo YJ, Lee HS, Chung IS, Cha JH (2009) Polymorphism in the CagA EPIYA motif impacts development of gastric cancer. J Clin Microbiol 47(4):959
187. Hatakeyama M (2011) Anthropological and clinical implications for the structural diversity of the *Helicobacter pylori* CagA oncoprotein. Cancer Sci 102(1):36–43
188. Higashi H, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, Hatakeyama M (2002) Biological activity of the *Helicobacter pylori* virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. Proc Natl Acad Sci USA 99(22):14428–14433
189. Tsutsumi R, Higashi H, Higuchi M, Okada M, Hatakeyama M (2003) Attenuation of *Helicobacter pylori* CagA· SHP-2 signaling by interaction between CagA and C-terminal Src kinase. J Biol Chem 278(6):3664–3670
190. Bridge DR, Blum FC, Jang S, Kim J, Cha JH, Merrell DS (2017) Creation and initial characterization of isogenic *Helicobacter pylori* CagA EPIYA variants reveals differential activation of host cell signaling pathways. Sci Rep 7(1):1–14
191. Suzuki H, Warren R, Marshal B (2016) *Helicobacter pylori*. (Springer Nature, 2016)
192. Roesler BM, Costa SCB, Zeitune JMR (2011) Virulence factors of *Helicobacter pylori* and their relationship with the development of early and advanced distal intestinal type gastric adenocarcinoma. *Gastritis and Gastric Cancer—New Insights in Gastroprotection, Diagnosis and Treatments* 259–280

193. Bridge DR, Merrell DS (2013) Polymorphism in the *Helicobacter pylori* CagA and VacA toxins and disease. Gut microbes 4(2):101–117
194. Figura N, Trabalzini L, Mini R, Bernardini G, Scaloni A, Talamo F, Santucci A (2004) Inactivation of *Helicobacter pylori* cagA gene affects motility. Helicobacter 9(3):185–193
195. Yeh YC, Kuo HY, Chang WL. et al. (2019) *H. pylori* isolates with amino acid sequence polymorphisms as presence of both HtrA-L171 & CagL-Y58/E59 increase the risk of gastric cancer. J Biomed Sci 26:4
196. Palrasu M, Zaika E, El-Rifai W et al (2020) Bacterial CagA protein compromises tumor suppressor mechanisms in gastric epithelial cells. J Clin Invest 130(5):2422–2434
197. Chung C, Olivares A, Torres E, Yilmaz O, Cohen H, Perez-Perez G (2010) Diversity of VacA intermediate region among *Helicobacter pylori* strains from several regions of the world. J Clin Microbiol 48(3):690
198. Lu H, Hsu PI, Graham DY, Yamaoka Y (2005) Duodenal ulcer promoting gene of *Helicobacter pylori*. Gastroenterology 128:833–848
199. Alam J, Maiti S, Ghosh P, De R, Chowdhury A, Das S, Macaden R, Devarbhavi H (2012) Significant association of the dupA gene of *Helicobacter pylori* with duodenal ulcer development in a South-East Indian population. J Med Microbiol 61(9):1295–1302
200. Hussein NR, Argent RH, Marx CK, Patel SR, Robinson K, Atherton JC (2010) *Helicobacter pylori* dupA is polymorphic, and its active form induces proinflammatory cytokine secretion by mononuclear cells. J Infect Dis 202(2):261–269
201. Dadashzadeh K (2017) Relevance of *Helicobacter pylori dupA* and *oipA* genotypes and development of gastric disease. Biomed Res 28(19):8179–8183
202. Pereira WN, Ferraz MA, Zabaglia LM, de Labio RW, Orcini WA, Bianchi Ximenez JP, et al. (2014) Association among *H. pylori* virulence markers *dup*A, *cag*A and *vac*A in Brazilian patients. J Venom Anim Toxins Incl Trop Dis 20(1):1
203. Gomes LI et al (2008) Lack of association between *Helicobacter pylori* infection with dupA-positive strains and gastroduodenal diseases in Brazilian patients. Int J Med Microbiol 298:223–230
204. Zhang Z et al (2008) The *Helicobacter pylori* duodenal ulcer promoting gene, *dupA* in China. BMC Gastroenterol 8:49
205. Nguyen LT et al (2010) *Helicobacter pylori dupA* gene is not associated with clinical outcomes in the Japanese population. Clin Microbiol Infect 16:1264–1269
206. Abadi AT, Taghvaei T, Wolfram L, Kusters JG (2012) Infection with *Helicobacter pylori* strains lacking *dupA* is associated with an increased risk of gastric ulcer and gastric cancer development. J Med Microbiol 61:23–30
207. de Lima Silva LL, Oliveira AKS, Gama AR et al (2021) *Helicobacter pylori* virulence *dupA* gene: risk factor or protective factor? Braz J Microbiol 52:1921–1927
208. Zarzecka U, Modrak-Wójcik A, Figaj D, Apanowicz M, Lesner A, Bzowska A, Skorko-Glonek J (2019) Properties of the HtrA protease from bacterium *Helicobacter pylori* whose activity is indispensable for growth under stress conditions. Front Microbiology 10:961
209. Clausen T, Kaiser M, Huber R, Ehrmann M (2011) HTRA proteases: regulated proteolysis in protein quality control. Nature Rev Mol cell Biol 12(3):152–162
210. Frees D, Brøndsted L, Ingmer H (2013) Bacterial proteases and virulence. Regulated proteolysis in microorganisms 161–192
211. Hoy B, Löwer M, Weydig C, Carra G, Tegtmeyer N, Geppert T, Wessler S (2010) *Helicobacter pylori* HtrA is a new secreted virulence factor that cleaves E-cadherin to disrupt intercellular adhesion. EMBO Rep 11(10):798–804
212. Elmi A, Nasher F, Jagatia H, Gundogdu O, Bajaj-Elliott M, Wren B, Dorrell N (2016) Campylobacter jejuni outer membrane vesicle-associated proteolytic activity promotes bacterial invasion by mediating cleavage of intestinal epithelial cell E-cadherin and occludin. Cell Microbiol 18(4):561–572
213. Altindis E, Fu Y, Mekalanos JJ (2014) Proteomic analysis of Vibrio cholerae outer membrane vesicles. Proc Natl Acad Sci 111(15):E1548–E1556
214. Tegtmeyer N, Moodley Y, Yamaoka Y, Pernitzsch SR, Schmidt V, Traverso FR et al (2016) Characterisation of worldwide *Helicobacter pylori* strains reveals genetic conservation and essentiality of serine protease HtrA. Mol Microbiol 99(5):925–944
215. Neddermann M, Backert S (2019) How many protein molecules are secreted by single *Helicobacter pylori* cells: quantification of serine protease HtrA. Cell Microbiol 21(7):e13022
216. Hansen G, Hilgenfeld R (2013) Architecture and regulation of HtrA-family proteins involved in protein quality control and stress response. Cell Mol Life Sci 70(5):761–775
217. Lekmeechai S, Su Y-C, Brant M, Alvarado-Kristensson M, Vallstrom A, Obi I, Arnqvist A, Riesbeck K (2018) *Helicobacter pylori* outer membrane vesicles protect the pathogen from reactive oxygen species of the respiratory burst. Front Microbiol 9:1837
218. Switala J, Loewen PC (2002) Diversity of properties among catalases. Arch Biochem Biophys 401(2):145–154
219. Benoit SL, Maier RJ (2016) Helicobacter catalase devoid of catalytic activity protects the bacterium against oxidative stress. J Biol Chem 291(45):23366–23373
220. Richter C, Mukherjee O, Ermert D, Singh B, Su YC, Agarwal V, Riesbeck K (2016) Moonlighting of *Helicobacter pylori* catalase protects against complement-mediated killing by utilising the host molecule vitronectin. Sci Rep 6(1):1–13
221. Sitaraman R, Israel DA, Romero-Gallo J, Peek RM Jr (2012) Cell-associated hemolysis induced by *Helicobacter pylori* is mediated by phospholipases with mitogen-activated protein kinase-activating properties. J Clin Microbiol 50(3):1014
222. Lusini P, Figura N, Valassina M, Roviello F, Vindigni C, Trabalzini L, Santucci A (2005) Increased phospholipase activity in *Helicobacter pylori* strains isolated from patients with gastric carcinoma. Dig Liver Dis 37(4):232–239
223. Keenan JI, Davis KA, Beaugie CR, McGovern JJ, Moran AP (2008) Alterations in *Helicobacter pylori* outer membrane and outer membrane vesicle-associated lipopolysaccharides under iron-limiting growth conditions. Innate Immun 14(5):279–290
224. Zimmermann S, Pfannkuch L, Al-Zeer MA, Bartfeld S, Koch M, Liu J, Meyer TF (2017) ALPK1-and TIFA-dependent innate immune response triggered by the *Helicobacter pylori* type IV secretion system. Cell Rep 20(10):2384–2395
225. Yokota SI, Amano KI, Nishitani C, Ariki S, Kuroki Y, Fujii N (2012) Implication of antigenic conversion of *Helicobacter pylori* lipopolysaccharides that involve interaction with surfactant protein D. Infect Immun 80(8):2956
226. Dara M, Khashei R, Dehghani B (2017) High frequency of hopQ genotypes among Iranian *Helicobacter pylori* clinical isolates. Infez Med 25(2):123–126
227. Feige MH, Sokolova O, Pickenhahn A, Maubach G, Naumann M (2018) HopQ impacts the integrin α5β1-independent NF-κB activation by *Helicobacter pylori* in CEACAM expressing cells. Int J Med Microbiol 308(5):527–533
228. Grzeszczuk MJ, Bocian-Ostrzycka KM, Banaś AM, Roszczenko-Jasinska P, Malinowska A, Stralova H, Jagusztyn-Krynicka EK (2018) Thioloxido reductase HP0231 of *Helicobacter pylori* impacts HopQ-dependent CagA translocation. Int J Med Microbiol 308(8):977–985
229. Hsu WT, Ho SY, Jian TY, Huang HN, Lin YL, Chen CH, Liao KW (2018) *Helicobacter pylori*-derived heat shock protein 60 increases the induction of regulatory T-cells associated with persistent infection. Microb Pathog 119:152–161

230. Zhang J, Zhang X, Wu C, Lu D, Guo G, Mao X, Zou Q (2011) Expression, purification and characterization of arginase from *Helicobacter pylori* in its apo form. PLoS One 6(10):e26205
231. Zhang X, Zhang J, Zhang R, Guo Y, Wu C, Mao X, Zou Q (2013) Structural, enzymatic and biochemical studies on *Helicobacter pylori* arginase. Int J of Biochem cell biol 45(5):995–1002
232. Morishita K, Takeuchi H, Morimoto N, Shimamura T, Kadota Y, Tsuda M, Sugiura T (2012) Superoxide dismutase activity of *Helicobacter pylori* per se from 158 clinical isolates and the characteristics. Microbiol Immunol 56(4):262–272
233. Švagelj D, Terzić V, Dovhanj J, Švagelj M, Cvrković M, Švagelj I (2016) Superoxide dismutases in chronic gastritis. APMIS 124(4):252–256
234. Negovan A, Iancu M, Tripon F, Crauciuc A, Mocan S, Bănescu C (2018) The CAT-262 C> T, MnSOD Ala16Val, GPX1 Pro-198Leu polymorphisms related to oxidative stress and the presence of gastric lesions. J Gastrointestin Liver Dis 27(4):l371-378
235. Stent A, Every AL, Chionh YT Ng GZ. and Sutton P (2018) Superoxide dismutase from *Helicobacter pylori* suppresses the production of pro-inflammatory cytokines during in vivo infection. Helicobacter 23(1):e12459
236. Shibayama K, Wachino JI, Arakawa Y, Saidijam M, Rutherford NG, Henderson PJ (2007) Metabolism of glutamine and glutathione via γ-glutamyltranspeptidase and glutamate transport in *Helicobacter pylori*: possible significance in the pathophysiology of the organism. Mol Microbiol 64(2):396–406
237. Boonyanugomol W, Chomvarin C, Song JY, Kim KM, Kim JM, Cho MJ, Baik SC (2012) Effects of *Helicobacter pylori* γ-glutamyltranspeptidase on apoptosis and inflammation in human biliary cells. Dig Dis Sci 57(10):2615–2624
238. Morey P, Meyer TF (2019) The sweeping role of cholesterol depletion in the persistence of *Helicobacter pylori* infections. In: Backert S (eds) Molecular Mechanisms of Inflammation: Induction, Resolution and Escape by *Helicobacter pylori*. Curr Top Microbiol Immunol, Vol 421:209–227 Springer, Cham
239. Morey P, Pfannkuch L, Pang E, Boccellato F, Sigal M, Imai-Matsushima A, Meyer TF (2018) *Helicobacter pylori* depletes cholesterol in gastric glands to prevent interferon gamma signaling and escape the inflammatory response. Gastroenterology 154(5):1391–1404