



Review

Helicobacter pylori: Perturbation and restoration of gut microbiome

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Alternate remedies with natural products provides unlimited opportunities for new drug development. These can be either as pure compounds or as standardized set of compounds. The phytochemicals and secondary metabolites are in great demand for screening bioactive compounds and plays an important role towards drug development. Natural products have many advantages over to synthetic chemical drugs. *Helicobacter pylori* (*H. pylori*) a Gram-negative bacteria has been classified as Class I carcinogen by World Health Organization in 1994. Current treatment regimens for *H. pylori* is ‘triple therapy’ administered for two weeks which includes a combination of two antibiotics like Amoxicillin and Clarithromycin and a proton pump inhibitor (PPI) like Lansoprazole, and for ‘quadruple therapy’ in addition to antibiotics and a PPI, Bismuth is used. Antibiotic resistance can be named as the main factor for failure of treatment of *H. pylori* infection. The need of the hour is to develop a herbal remedy that could combat the growth of *H. pylori*. Probiotics can also be used as ‘feasible’ tool for *H. pylori* infection management. Present review is an attempt to briefly discuss about the pathogenicity, genetic predisposition, perturbation of gut microbiota due to antibiotic treatment and restoration of healthy gut microbiota with phytochemicals and probiotics.

Keywords. Antibiotic resistance; *Helicobacter pylori*; medicinal plants; microbiome; probiotics; triple therapy

1. Introduction

H. pylori is a Gram-negative bacterium and causes infection in the stomach. It has the unique characteristic to thrive and survive in highly acidic environments of the stomach (Warren and Marshall 1984). The acidity of the stomach is neutralized by the enzymatic activity of the urease enzyme present in *H. pylori*. This, therefore, promotes infection of *H. pylori*. Urease protein has also been found to interact with host cells *in vitro* (Rokita *et al.* 2000), although the functional activity of this property has not been studied *in vivo*. *H. pylori* uses its flagella to reach epithelial cells of the stomach and penetrate the mucous lining, and easily moves from an acidic environment to a less acidic environment. The bacterium lies safely in the stomach’s mucous lining. There has been a study on the role

of urease surface in *H. pylori* host interaction. It was found that the surface region of this enzyme in which changes that did not affect enzymatic activity impaired bacterial persistence in a murine experimental infection model (Schoep *et al.* 2010). *H. pylori*, like any other microbe, fights against the host immunity. For example, it is recognized by toll like receptors (TLRs). TLRs are the most studied pathogen recognition receptor. TLRs are secretly involved in the regulation of inflammation during the innate immune response to *H. pylori*. They represent key activators of adaptive immunity; a serious effort has been made to enlighten their role in the recognition of *H. pylori* and its components in multiple cell types (Smith 2014).

The exact cause of *H. pylori* spread among the population is not known. However, it is believed that fecal–oral or oral–oral route through the consumption

of spoiled water or food is a very common cause (Brown 2000; Stefano et al. 2018). According to the epidemiological studies, water may be a major cause of *H. pylori* infection. Other factors responsible for infection may include poor hygiene, poor nutrition, and geographical location. *H. pylori* is ranked as a Class I carcinogen for gastric cancer by the World Health Organization (WHO). A large population worldwide is infected with *H. pylori* causing serious illness like a peptic ulcer or duodenum ulcer. This can further lead to gastric cancer (Taylor and Sherburne 1995). According to a study there is an estimation that *H. pylori*-positive patients have 10% to 20% lifetime risk of developing ulcer-related diseases and 1% to 2% risk for developing distal gastric cancer (Ernst and Gold 2000; Kuipers et al. 1995). The draft genome sequences of 42 *H. pylori* clinical strains isolated from South Indian rural populations were studied which provided further insights into the evolution and genetic makeup of Indian *H. pylori* strains (Shetty et al. 2018).

In 2016, it was reported that *H. pylori* infection is more persistent in developing countries like Bangladesh, India, and Pakistan as compared to developed Asian countries like China, Japan, and South Korea. But the rate of occurrence of gastric cancer is comparatively lower in Bangladesh, India, and Pakistan as compared to China, Japan, and South Korea (Zaidi 2016). Due to this reason *H. pylori* in no time became the subject of many studies. The subjects ranged from microbiological to histological, immunological to epidemiological, and ecological areas related to health (Lee 2009; Rotimi et al. 2000; Mitchell 2001; Velin and Michetti 2006). Burning pain or ache in the abdomen, nausea, abdominal discomfort, frequent burping, and loss of appetite are the most common symptoms. Other symptoms reported are heartburn, excessive burping, bloating, and weight loss (Talley et al. 2001). For the detection of *H. pylori*, pathogen blood tests are most common. The other tests may include breath, stool, and tissue tests (endoscopy).

The most widely practised and effective treatment of *H. pylori* infection is a two-week treatment, the 'triple therapy'. This treatment involves a combination of antibiotics (Clarithromycin, Amoxicillin) and PPI (Omeprazole or Lansoprazole). This regimen achieves an eradication rate in only 70% cases (Malfertheiner et al. 2012). It is widely believed that antibiotic resistance is the major cause of treatment failure.

There has been a postulation in 2016 that *H. pylori* may be a part of the human microbiome. It has also been reported that due to the change of human gut microbiota, *H. pylori* is gradually disappearing (Yap

et al. 2016) and affects human health indirectly. Therefore, in this study, the authors have hypothesized that the elimination of *H. pylori* may cause anxiety in the gastrointestinal microbiome. This can affect the health of the human host indirectly.

2. Pathogenesis of *Helicobacter pylori*

It has been more than a century since the presence of bacteria in the stomach has been identified. The researches of Nobel Prize winners Warren and Marshall (2005) during the '80s has resulted in isolation and culture of a bacteria spiral in shape which later on was named *H. pylori*. It was shown that this bacterium could colonize in the human stomach. This resulted in inducing gastric mucosal inflammation and gastritis (Marshall 2002; Marshall et al. 1985; Morris et al. 1991; Warren and Marshall 1983). The advancement of researches associated *H. pylori* with upper gastrointestinal disorders, such as peptic ulcer, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, chronic gastritis, and gastric cancer (Parsonnet 1993). To investigate the seriousness of the infection by *H. pylori*, which is poorly understood, a large group of researchers is engaged in understanding the transmission into humans and its persistence in an environment which is devoid of *H. pylori*.

It is reported that the infection of *H. pylori* is increasing in developing countries (figure 1). The infection in the developing countries is approximately 25% in comparison to Western countries like America, London, and Canada (Pounder and Ng 1995). However, 85% of the population infected with *H. pylori* never reported any related complications or symptoms (Bytzer et al. 2011). On the basis of genotyping of *H. pylori* strains isolated from two study groups from West Bengal containing 52 adults suffering from duodenal ulcer. It was observed that geographic variations is associated with certain putative *H. pylori* virulence genes (Chattopadhyay et al. 2002).

There was a report that *H. pylori* infection is more likely in children and the aged population (Kusters et al. 2006). It is quite interesting to note that *H. pylori* use its flagella to reach epithelial cells of the stomach; after penetrating the mucous lining, it makes an easy way to move from an acidic environment to a less acidic environment (Amieva and El-Omar 2008). It was reported by Kao et al. (2016) that the attachment of *H. pylori* to host epithelial cells is favored by the production of Adhesin. Once *H. pylori* successfully colonizes, toxins get released that in turn damage the

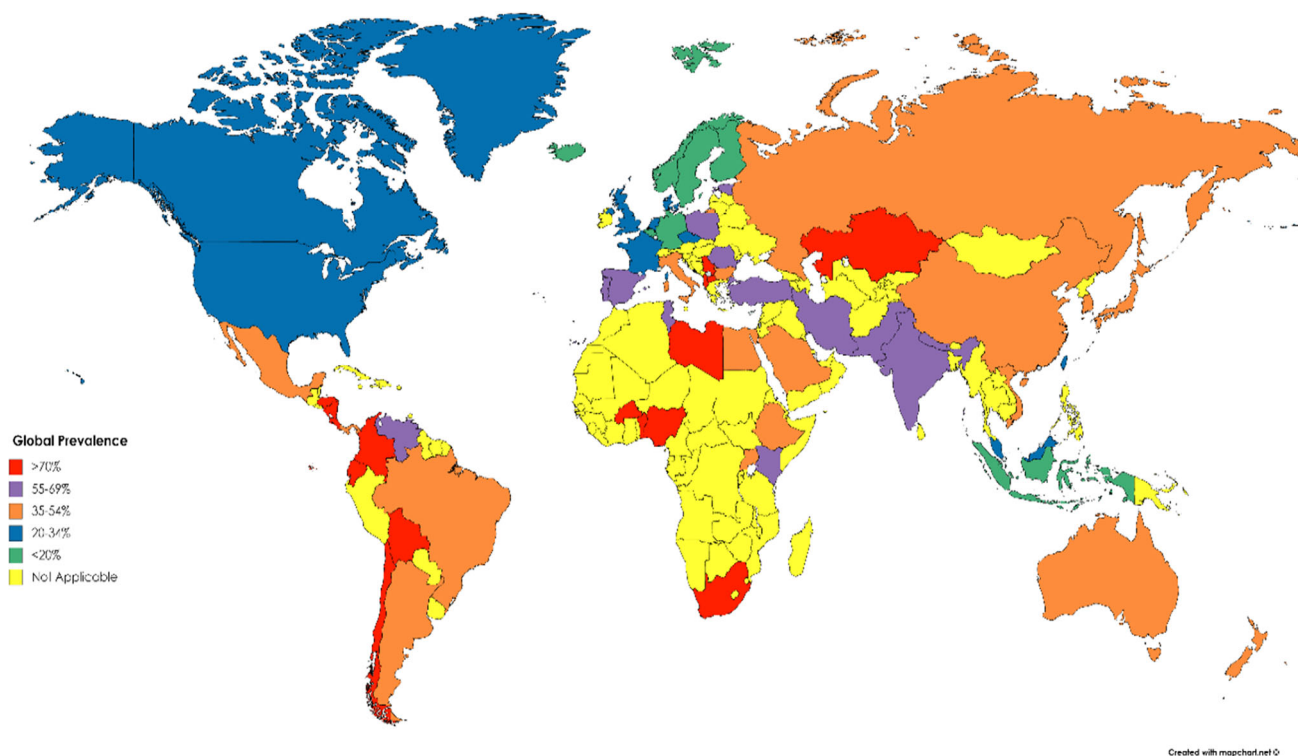


Figure 1. World map showing the spreading of *H. pylori* infection. Areas with colors have been used to show the prevalence. Red, >70%; purple, 55–69%; orange, 35–54%; blue, 21–34% and green, <20%.

host tissues. Numerous outer-membrane proteins having host-specific receptor sites act as adhesions and bind with lipids and carbohydrates available in epithelial cells. Adhesion to the epithelial cells is ensured by BabA protein that is present in the membrane of the bacteria (Ilver *et al.* 1998; Mahdavi *et al.* 2002).

Heat Shock Proteins (HSPs) have a role in *H. pylori*-induced gastric ulceration (Bai *et al.* 2003). HSP appears to bind gastric epithelial cells and mucin via HSP 60 and to facilitate cell damage and promote carcinogenesis (Yamaguchi *et al.* 1998; Evans *et al.* 1992). *H. pylori* have a direct damaging effect on the mucous membrane of the stomach and duodenum, contributing to the release of lysosomal enzymes, numerous cytokines leading to the inflammatory processes in the mucosa. Lipopolysaccharide (LPS) of the outer membrane of *H. pylori* triggers an immune response of the human body resulting in the development of chronic inflammation. LPS of several strains of *H. pylori* express Lewis antigens. These are quite similar to those expressed by the gastric epithelial cells (Appelmek *et al.* 1996). Pathogenic antibodies are induced by Lewis antigens. When there is an infection, *H. pylori* LPS may produce anti-Lewis antibodies that bind to bacteria. They also bind to gastric epithelium of

the host, which finally leads to tissue destruction (Appelmek *et al.* 1996). Lewis antigen mimicry provides endurance through immune invasion. Lewis antigens are also involved in colonization and adhesion (Appelmek and Vandenbroucke-Grauls 2000).

The nature and seriousness of the pathological processes that are prompted by the bacterium, *H. pylori* at various levels are determined by a set of pathogenicity factors. It is very important for a pathogen like *H. pylori* to maintain a balance between virulence and avirulence (Kalali *et al.* 2014). Removal of the bacteria from the system can happen due to a strong host immune response, which can be provoked by a virulent pathogen. Modulation of virulence for successful survival in the host is one of the mechanisms developed by *H. pylori*.

3. *H. pylori* and microbiome

The genetic material of all the microbes is the microbiome. The microbiome lives on and inside the human body. The microbiome is important for human development, nutrition, and immunity. The bacteria living in and on us are not intruders, but advantageous colonizers. Disease-causing microbes accumulate over

time. They change the gene activity and metabolic processes. This results in an abnormal immune response against substances and tissues normally present in the body (Quigley 2013). There are approximately 100 trillion microbial cells in the human microbiota. These microbial cells surmount our human cells by 10 to 1 (Savage 1977). Through the efforts of the Human Microbiome Project, the human oral and fecal microbiota have been more extensively studied than other sites in the gastrointestinal (GI) tract (Sheh and Fox 2013).

One of the major cause of public health issue is GI cancer. GI cancer is the fourth most common cancer and second leading cause of death globally (Crew and Neugut 2006). GI cancers are the end result of a complex interaction between the environment and genes. Human studies and experimental data have provided evidence that there is a role of microbiota in cancer development (Karpinski 2019; Compare and Nardone 2011; Bhatt et al. 2017). There are many factors that play a role in this, such as antibiotic treatment, diet, or lifestyle. Due to the reasonable stability of our microbiome, it is capable of halting or at least decreasing the progress of cancer. Chronic inflammation influencing the genomic stability of host cells is a crucial event in carcinogenesis. This leads to the activation of immune mechanisms (Tozun and Vardareli 2016).

The development of gastric malignancies may be influenced by the microbiome. There was an assumption that the adverse microbial community could not be supported by the gastric niche. The acidic environment of the stomach and low levels of the bacteria cultured were the reasons cited (Turnbaugh et al. 2007). A complex microbiota has been discovered, that lies in the human stomach with the power of induction of disease. The discovery of the complex microbiota was due to the advancements in DNA sequencing of conserved ribosomal genes, computational methods, and phylogenetic analysis.

The human gut microbiome is important for the maintenance of human health. It plays an intrinsic role in the absorption of nutrients, energy metabolism, and defense against pathogens attack (Gevers et al. 2012; Nicholson et al. 2012; Lozupone et al. 2012). This microbiome exists within a delicate balance. If this balance is modified, then the microbiota becomes dysbiotic. This further contributes to diverted pro-inflammatory immune responses, perceptivity to invading pathogens, and initiation of disease processes, including cancer (Schwabe and Jobin 2013). This leads to the pathogenesis of GI cancer in the esophagus

(Yamamura et al. 2016) and colon (Candela et al. 2014; Sears and Garrett 2014). Special bacterial species like *Fusobacterium nucleatum* and *Escherichia coli* are associated with the development of colorectal cancer (Castellarin et al. 2012; Bonnet et al. 2014) and gastric cancer (*H. pylori*) (Wang 2014). Figure 2 shows the primary phyla of the gastric microbiota based on the status of *H. pylori*.

The prevalence of *H. pylori* globally is high in spite of its gradually declining prevalence in many regions. The continuous evolution of clinical aspects of *H. pylori* infection and therapeutic management requires unceasing adaptation (Malfertheiner et al. 2018).

Besides bacterial resistance and the occurrence of detrimental events, there are many other factors that contribute towards the failure of *H. pylori* treatment. There are certain probiotic strains that have shown growth inhibition of *H. pylori*. The side effects that occur due to antibiotic therapy have been reduced by certain probiotic strains. This increases the *H. pylori* eradication rate (Homan and Orel 2015).

4. Genetic predisposition

According to United States National Library of Medicine, the distinguished genetic characteristic which has an effect on the probable phenotypic development of an individual organism within a population or a species under the domination of environmental conditions is genetic predisposition. The clinical outcomes in the *H. pylori* infection may be due to genetic factors and environmental conditions. The genetic factors include both host genetic predisposition to infection as well as genetic differences in *H. pylori* strains (Graham et al. 1994).

There are various treatments available in the global market of medicines to combat *H. pylori* infection. Triple therapy and sequential therapy had a success rate of 75% to 90%; however, in this approach, bacteria remain oscillating between a non-replicative state (microorganism becomes resistant to antibiotics) and a replicative state (microorganism remains susceptible to antibiotics). If the pH is between 4.0 and 6.0, the bacteria cannot enter the replicative cycle. Eradication of the microorganisms becomes difficult when they assume the resistant state (Egan et al. 2007; Wu et al. 2012; Kuol et al. 2013). The individuals may become vulnerable to the development of gastric cancer since *H. pylori* are the etiologic factor involved in the majority of the patients with peptic ulcer disease (Nagahata et al. 1996). In the human stomach, the *H.*

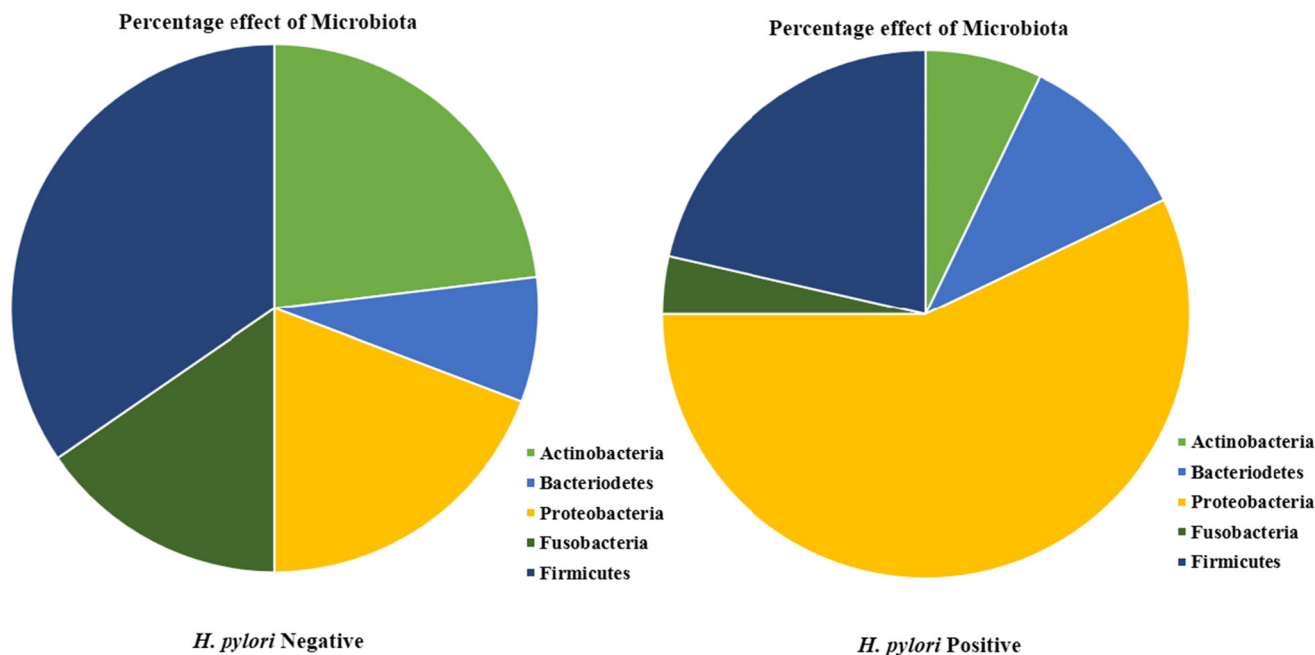


Figure 2. Effect of *H. pylori* on microbiota. *H. pylori*-negative people have a more difficult and divergent microbiota as compared with *H. pylori*-positive patients (as adapted from Noto and Peek 2017).

pylori colonize. The mode of *H. pylori* transmission is uncertain, but it can be assumed to be spread from person to person. This can be via the fecal–oral route. The socio-economic status seems to be inversely related to the widespread presence of *H. pylori* in adults (Graham 1991). Water is also thought to be a reservoir for transmission of *H. pylori* (Bellack *et al.* 2006).

The major risk factor accepted for gastric cancer development is infection with *H. pylori*. The risk of a carcinogenic effect is increased because of the damage to the mucosal barrier. This is due to the *H. pylori*-induced inflammation. Due to the genetic disposition of the bacterial strain and the host, there is an increase in the capability of gastric cancer formation. Genetic variance of VacA (Vacuolating Cytotoxin A) and CagA (Cytotoxin Associated Gene A) bacterial proteins is associated with higher gastric cancer risk. Even polymorphism and genetic changes in the host gene coding for interleukins (IL 1 β , IL 8) have also been perceived. It is astounding that *H. pylori* are able to survive in most of the hosts without activating a strong host immune system (Bornschein and Malfertheiner 2014). Immunogenic virulent factors CagA and VacA cannot deter *H. pylori* from doing so. An additional aspect of this interaction is due to the fact that *H. pylori* strains are genetically diverse. One host can have more than one strain. It was seen that the effects of methylation differ from strain to strain. This in turn creates more variability in the habitat. *H. pylori* are said to have a

limited host range and small genome coding for very few numbers of regulatory proteins. This controls the gene expression by differential methylation. Approximately 50% of all *H. pylori* strains secrete VacA. It is not essential for the growth of *H. pylori*, *in vitro* (Cover and Blaser 1992). VacA was reported to significantly contribute to gastric colonization by *H. pylori* in rodents. VacA has been reported to play an important role in the pathogenesis of peptic ulceration and gastric cancer (Salama *et al.* 2001).

The widespread presence of *H. pylori* infection is much higher in India, as compared to most Western countries. Almost all infected patients were carrying multiple strains of *H. pylori* (Suerbaum and Josenhans 2007). This diversity of the *H. pylori* population is clinically very important. The appropriate target of virulence gene or its allele may be overseen if the practice of characterization single isolate from infected individuals is done. The uneven distribution of *H. pylori* throughout the gastric mucosa should be given importance. This is because different genotypes may be prevalent at different sites. A study indicates including multiple colonies from a single host. This can be either from single or different sites in the case of multi ulceration (Patra *et al.* 2012).

In the pathogenesis of gastric ulcers, the role of TNF- α (tumor necrotic factor) is well established. TNF- α is a cytokine and plays an important role in gastric ulcer formation (Lyckkova *et al.* 2010). It initiates the

inflammatory process by stimulation and migration of leukocytes into the inflammatory sites. CagA is a highly immunogenic protein encoded by the *cagA* gene. This CagA was linked to the virulent property of *H. pylori*. This gene is a marker for the presence of a genomic PAI (Pathogenicity Island) (Silveira et al. 2005). Patients with peptic ulcers or gastric cancer have strains lacking the *cagPAI*, but with low frequencies. CagA affects cell shape, disturbs cell junctional activity, increases cell motility, and is responsible for gastric ulcers and gastric cancers (Buti et al. 2011). All this happens after the translocation into the host cell. *H. pylori* acid resistance is due to the urease enzyme. This enzyme converts urea into ammonia and carbamate, which spontaneously breaks down into another ammonia molecule and carbon dioxide (Burne and Chen 2000). Ammonia, which is basic in nature, then neutralizes acid in the stomach environment making it sanatorium for *H. pylori* (Pandey et al. 2010).

Even though the urease activity is present in all *H. pylori* isolates, the levels of urease activity differ remarkably between strains. The urease activity is dependent on the growth conditions (Konturek et al. 2001; Lloyd and Soll 1994). The proteins encoded by the *cagPAI* are responsible for the introduction of secretion of IL-8, forming the support and remodeling the surface of epithelial cells. *H. pylori* can be divided into two types: Type I that expresses CagA+ associated with VacA, whereas Type II does not express CagA but is associated with VacA. The ability to induce large cytoplasmic vacuoles in the eukaryotic cells is that of VacA. This can lead to cell death (Darini 2004). The transfer of CagA oncoprotein to the host cytoplasm is done by Type IV Secretion System (T4SS). Here it becomes phosphorylated and affects various cellular processes (Backert and Selbach 2008). It is considered to be the direct contact of T4SS with the epithelial cell membrane. This process is believed to be majorly responsible for the induction of IL-8 and other host immune responses. The $\alpha 5\beta 1$ epithelial integrin has been specified as one of the host receptors involved in this activity. The expression of these integrins is limited to the basolateral membrane (Kwok et al. 2007). The study of *H. pylori*-host cellular interactions could be facilitated by a monolayer *in vitro* model of epithelial cells having narrow junction integrity and measurable barrier function. A detailed examination of the apical and basolateral molecular events that occur during the infection by the pathogen can be studied (Fiorentino et al. 2013). The occurrence of monochloramine has been related to the gastric injuries detected in the presence of *H. pylori* (Weeks

and Sachs 2001). This derivative creates large amounts of ammonia, which in turn activates neutrophils that produce hypochlorous acid. This acid reacts with the amino acid taurine, in turn forming taurine-chloramine (Suzuki et al. 1998; Lapenna and Curccurolo 1996). Currently, the major virulence factors of the pathogens are urease, VacA, and CagA gene products.

It is widely believed that during infection, there is an expansion in gastrin and gastric acid secretion. This has caught the eye of many researchers from various scientific fields seeking therapeutic replacements. These alternatives may behave favorably if provided with all the factors involved (Gürbüz et al. 2002). The main habitat of *H. pylori* is considered to be gastric mucosa. This is because the bacterium survives in an acidic medium. Gastric colonization and *H. pylori* survival require the catalytic power of proteases. The serine protease HtrA (High-temperature requirement A) is considered to be important for the survival of the bacteria since it has been found in most infected individuals. Lysosomal cathepsins and metalloproteases are abundantly found during the early stages of *H. pylori*-mediated pathogenesis. Therefore, this class of protease enzymes may play a functional role in stomach cancer (Kim et al. 2013).

5. Combating *H. pylori* infection

5.1 Drugs

H. pylori infection is a severe disease and has been difficult to cure. The treatment schedule comprises of at least two high-dose antibiotics (Clarithromycin + Amoxicillin/Metronidazole) plus a proton pump inhibitor (PPI), twice daily for a 7- to 10-day period (Lind et al. 1996). In fact, it is a matter of concern that there is an emergence of resistance to antibiotics and is increasing day by day. This is thrusting a challenge to cure the diseases. Treatment failure is due to antibiotic resistance. In the near future, the development of novel drugs against infection is needed for more effective control of these diseases. Researchers are trying to develop several alternatives to cure *H. pylori* infection. The management of *H. pylori* infection is therefore very important. Challenges range from patient information to motivation of the doctors to antimicrobial treatment and patient compliance. The treatments available are drugs which have their own side-effects and the newer approach of medicinal plants along with probiotics (figure 3). An effective vaccine that could prevent and/or cure the infection, or modify

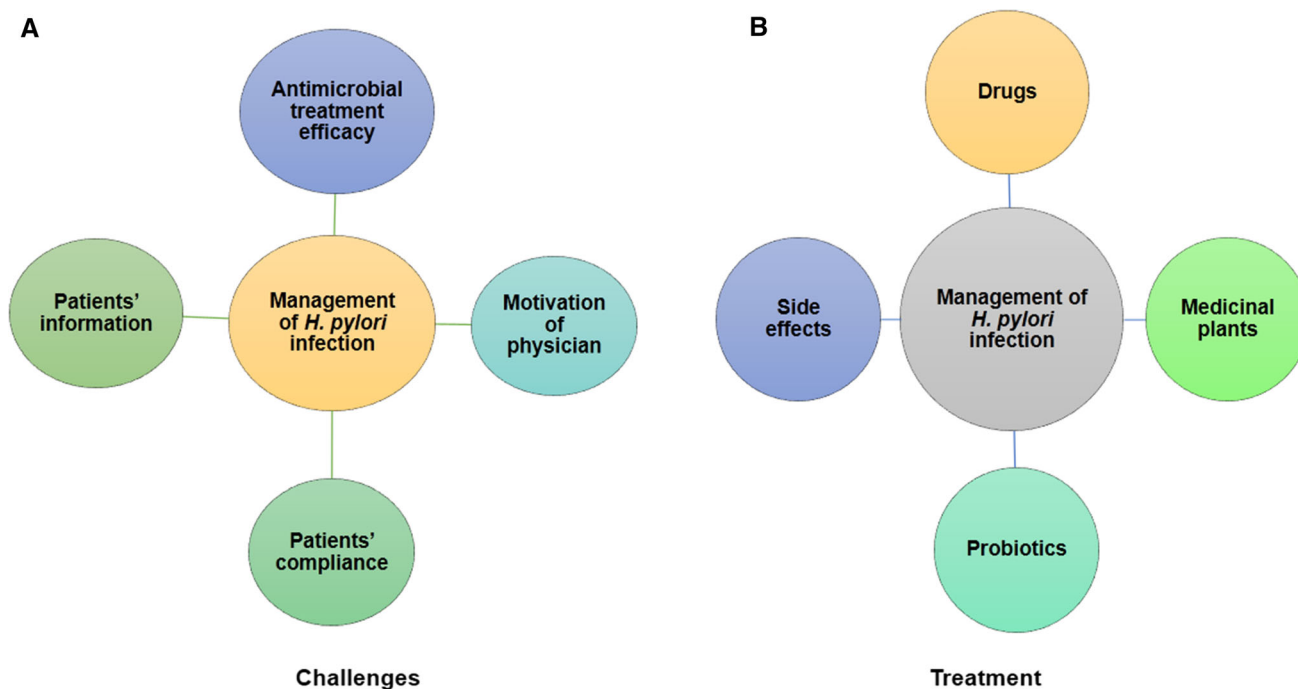


Figure 3. Management of *H. pylori* infection. (A) The challenges involved in *H. pylori* infection. (B) Treatment required in *H. pylori* infection.

the interactions of the host–pathogens in a way that prevents disease progression. There have been continuous efforts to obtain a vaccine against *H. pylori*. This vaccine development has been focused on finding the best route of delivery. In addition it would find adjuvants and antigens that favor the induction of protective immunity (Czinn and Blanchard 2011). The development of a secure and effective vaccine for human use is still not available, although some encouraging results have been obtained in animal models.

Another curative approach is to find an inhibitor of enzymes that has a crucial role in the life cycle of the pathogen and it should affect only the prokaryotes or lower eukaryotes, otherwise it might have side-effects for humans. One such example is Shikimate kinase (EC 2.7.1.71). This is the fifth enzyme of the pathway and catalyzes the specific phosphorylation of the 3-hydroxyl group of shikimic acid in the presence of adenosine triphosphate (ATP). Shikimate kinase represents a potent target for the development of new antimicrobial agents (Coggins *et al.* 2003; Herrmann and Weaver 1999; Ridley 1998; Roberts *et al.* 1998).

5.2 Medicinal plants: the upcoming weapon

In many South Asian countries like India, Nepal, Pakistan, and Bangladesh, spices are used in the

traditional system of medicine due to the presence of medicinal values. These dietary spices are also prescribed for the treatment of GI diseases (Zaidi *et al.* 2009; Zaidi and Sugiyama 2013). Studies have shown the protective effects of spices and medicinal plants against numerous diseases including *H. pylori* and GI disorders. The bactericidal and anti-adhesive activities of culinary herbs against *H. pylori* from Malaysia have also been reported (Gilani and Rahman 2005; O'Mahony *et al.* 2005). There were reports from Pakistan of the potential effect of commonly used spices and medicinal plants on *H. pylori* and its associated pathogenesis (Zaidi *et al.* 2009).

Anti-*H. pylori* compounds act as an inhibitor of enzymes (DNA gyrase, *N*-acetyltransferase, urease, dihydrofolate reductase, and myeloperoxidase) and high redox potential, adhesive activities and hydrophilic/hydrophobic natures of compounds (Wang 2014). Many natural products have anti-*H. pylori*-induced inflammation activity, such as suppression of nuclear factor- κ B, activation of mitogen-activated protein kinase pathway, and oxidative stress inhibition (Chatterjee *et al.* 2012; Takeuchi *et al.* 2014). These products include apigenin, quercetin, tea products, carotenoids-rich algae, garlic extract, finger-root extract, and polyphenol from apple peel. Numerous medicinal plant products possess anti-*H. pylori* activity, along with an anti-*H. pylori*-induced gastric







inflammatory effect. These plant products have shown promising potential as pharmaceutical candidates for *H. pylori* eradication and prevention of *H. pylori*-induced gastric disease (Wang 2014).

Table 1 illustrates anti-*H. pylori* activities of *Syzygium aromaticum* (clove), *Emblica officinalis* (amla), *Allium sativum* (garlic), *Aloe vera* (aloe), honey, and *Curcumin longa* (curcumin).

5.3 Probiotics

A probiotic is defined as a ‘live microbial organism which, when ingested, beneficially affects human health, including amelioration or prevention of specific disease state’ (Fuller 1992). The probiotic microorganism can be used as a ‘feasible’ tool for *H. pylori* infection management. Lactic acid bacteria, particularly *Lactobacillus* species and *Bifidobacterium* species, have been the most studied probiotics. Other organisms that are used as probiotics in humans include *Escherichia coli*, species of *Streptococcus*, *Enterococcus*, *Bacteroides*, *Bacillus*, and *Propionibacterium* (Rolfe 2000; Montrose and Floch 2005; Gill and Guarner 2004; Suvarna and Boby 2005). There is a recent study that has focused on the use of yeast as a probiotic to reduce GI tract disorders. This study shows how yeast can be an integral part of human microbiome as probiotics. *Saccharomyces boulardii* is commonly used to reduce GI tract ailments, since it is well characterized probiotic yeast (Sen and Mansell 2020). Supplementing probiotics have shown to be useful in several gastrointestinal diseases, such as irritable bowel syndrome, inflammatory bowel disease, improving the *H. pylori* eradication rates, preventing or decreasing antibiotic-associated diarrhea, and lowering the taste disturbance incidences. Probiotic supplementation may partially reinstitute physiological microecology in the intestine. This in addition provides one alternative therapy, because these bacteria have been reported to possess antimicrobial properties against pathogens. (Servin and Coconier 2003). The effect of the supernatant of *Lactobacillus acidophilus* (*L. acidophilus*) culture on the growth of *H. pylori* isolates was tested. These *H. pylori* isolates have been recovered from antral biopsies. It was found that the growth of *H. pylori* was inhibited by isolates. It was also hypothesized that all of this depends on the secretion of lactic acid from the *Lactobacillus* (Bhatia et al. 1989). It was in 1989 that the first positive *in vitro* result appeared. This was an opportunity to use probiotic species in *H. pylori* therapy. The possibility to reduce antibiotic-

Table 1. Anti- *H. pylori* properties of some selected plants and natural products

Sr. No.	Name of the plant	Activities/Uses
1.	<i>Syzygium aromaticum</i> (Clove) 	1) Inhibited the growth of <i>H. pylori</i> (Balch and Balch 2000; Kim et al. 1998) 2) Antimicrobial properties (Nazrul et al. 2010)
2.	<i>Emblica officinalis</i> (Amla) 	1) Antimicrobial activity against <i>H. pylori</i> has been reported (Rajkumar et al. 2014)
3.	<i>Allium sativum</i> (Garlic) 	1) Showed anti- <i>H. pylori</i> activity <i>in vitro</i> (Cellini et al. 1996; Sivam et al. 1997; Chung et al. 1998; Cañizares et al. 2004; Mahady et al. 2001) 2) Showed antimicrobial activity against <i>H. pylori</i> (Sivam 1997)
4.	<i>Aloe vera</i> (Aloe vera) 	1) Gel has shown antibacterial activity against <i>H. pylori</i> (Cellini et al. 2014) 2) <i>H. pylori</i> induced peptic ulcer treated in combination with Amoxicillin has been reported (Ranade et al. 2012)
5.	Honey 	1) Manuka honey has bacteriostatic properties against <i>H. pylori</i> (Somal et al. 1994) 2) Osmotic effect was inhibited thereby killing <i>H. pylori</i> (Osato et al. 1999)
6.	<i>Curcuma longa</i> (Curcumin) 	1) Anti-inflammatory (Sarkar et al. 2016) 2) Anti-oxidant (Sarkar et al. 2016) 3) Anti-cancer (Sarkar et al. 2016) 4) Anti-proliferative (Sarkar et al. 2016) 5) Anti-microbial (Sarkar et al. 2016) 6) Reduces gastric damage due to <i>H. pylori</i> infection (De et al. 2009)

associated side-effects is worthy of getting attention and needs larger trials to be confirmed. Future trials should address in particular the type of patient (asymptomatic volunteers, symptomatic patients), and choice of probiotic strain(s). This has prompted investigators to study the possibility of reducing antibiotic-associated side-effects.

To diminish the quantity of *H. pylori* by selective bacterial–bacterial cell interaction, an effective and newer method for resisting stomach microbes, was undertaken. It was thought to be an efficacious method to fight the pathogens of the stomach. The most specific binding opponent to *H. pylori* among 700 wild-type strains of *Lactobacillus* species was *Lactobacillus reuteri* (*L. reuteri*) DSM17648. The strain DSM17648 co-aggregates the pathogen both *in vivo* and *in vitro*. A specific co-aggregation occurs between different strains of *H. pylori* and *L. reuteri* DSM17648 but not with *Camylobacter jejuni* or other intestinal and oral bacteria (Holz *et al.* 2015; Buckley *et al.* 2018).

Probiotics have shown promising results in biological experiments; however, negligible studies have been done to determine their effect on humans. It is critical to identify the precise molecular mechanism that determines the action of probiotics on health and disease conditions (Qureshi *et al.* 2019). Therefore, it is important to know the mechanism probiotics use to oppose *H. pylori* infection.

The effect of probiotics on *H. pylori* infection, improvement in *H. pylori* gastritis, reduction in bacterial colonization, and achievement of complete eradication has been reviewed in *in vitro* clinical trials (Patel *et al.* 2013; Song *et al.* 2018; Ahmad *et al.* 2013). There was a study conducted on 66 children aged between 3 and 14 years infected with *H. pylori*. They were divided into two groups: Group A received antibiotic + PPI + placebo and Group B received antibiotic + PPI + probiotic. The probiotic was a combination of *Lactobacillus bulgaricus*, *L. acidophilus*, *L. casei*, *L. rhamnosus*, *Bifidobacterium infantis*, *B. breve*, and *Streptococcus thermophilus*. Both groups A and B demonstrated improvement in clinical symptoms after treatment. Group B, which was given probiotic treatment in addition to antibiotic and PPI, showed greater significant eradication rate (Khadadad *et al.* 2013). The human gastrointestinal tract is a complex and combative environment. It is unconvincing that a single probiotic bacterial strain will have the magnitude to influence the microbial ecology of the host. In another instance, a study was conducted on 14 *H. pylori*-infected patients. They were treated with *L. acidophilus*. The eradication rate of *H. pylori* increased

considerably, while the colonization rate of *H. pylori* decreased (Mrda *et al.* 1998). Even if the single probiotic strain has the enormity to influence the microbial ecology of the host, it may reduce the chances of the occurrence of diarrhea, lactose intolerance, levels of blood cholesterol, mucosal immune responses, and the induction of cancer (Dunne *et al.* 1999). Probiotics mimic the function of the human microbiota, hence act both in reducing the side-effects and also in improving the efficiency of antibiotics. Phytotherapy and bacteriophage therapy have shown great potential as an alternative to antibiotics (Vitor and Vale 2011).

Medicinal plant products are usually selected with a protracted history of their use against diseases associated with stomach infection. Such herbs come from the folk medicine of each country. Studies through many years have revealed that many phytomedicine products have an anti-*H. pylori* activity and gastroprotective action. Although the mechanism of action is not understood completely yet, the current knowledge acts as a bridge between instrumental action of herbs with inhibition of essential enzymes of *H. pylori*, attenuation of inflammation, and modulation of the host immune system (Vale and Oleastro 2014).

Phage therapy is extensively being relooked as an alternative to antibiotics (Lin *et al.* 2017). The phage display approach is used to select peptides that specifically bound *H. pylori* urease holoenzyme and inhibited its enzymatic activity *in vitro* (Houimel *et al.* 2001). A combinatorial library and two newly constructed random peptide libraries were used to identify two peptides that specifically inhibited the activity of *H. pylori* urease. A study identified four single-chain Fv antibody fragments binding to *H. pylori* urease. The peptide sequences inhibited single-chain Fv binding to *H. pylori* urease, and the authors suggested they could be used in a vaccination strategy (Mader *et al.* 1997). There is limited knowledge of the use of phages in the treatment of *H. pylori*. Recently, there has been a study of a temperate phage of *H. pylori*, induced by ultraviolet (Vale *et al.* 2008). This along with the sequences of complete (Lehours *et al.* 2011; Uchiyama *et al.* 2012) and remnant prophages provided whole genome sequencing of *H. pylori* strains.

Probiotics were found to be useful in several gastrointestinal diseases. There are reports that have shown the defeat of the bacterium by the eradication rate of probiotics' monotherapy. Probiotics compete with *H. pylori* for host surface receptors, thereby inhibiting its adhesion to epithelial cells. It has also been demonstrated that probiotics could hamper *H. pylori* urease activity. On these basis, since probiotic

administration does not carry the risk of antibiotic resistance, it could represent an optimal strategy in selected cases (Losurdo *et al.* 2018).

Pre-treatment of *L. plantarum* ZDY 2013 played a pivotal role in preventing gastric mucosal inflammation and gastric microbiota alteration induced by *H. pylori* infection. The selective modulation in gastric microbiota posed by this interference indicated that targeting gastric microbiota through oral administration of probiotics might be an alternative strategy to prevent infection caused by *H. pylori* (Pan *et al.* 2016).

A study was performed that showed that the intestinal microbiota is composed mainly of Gram-negative anaerobic bacilli. These bacilli live in the colon microenvironment with *Bacteroides* and *Fusobacterium* as the main genera (Torres 2017). The interest for the possible interactions between the intestinal microbiota and other important pathogens, such as *H. pylori*, has grown immensely (Parker 2016).

Some serious changes in the gastric microenvironment is produced by *H. pylori* infection. This influences the composition of gastric microbiota. Large intestinal microbiota changes might have close relations with it. This action could trigger important modifications in the homeostatic functions exerted by gut commensals. Further, this would lead to a new gastrointestinal balance. Simultaneously the strategies used to eradicate *H. pylori* can modulate this physiological symbiosis, but can be also conversely affected by its properties (Lopetuso *et al.* 2018)

The critical involvement of human gut microbiota in human health and a slight alteration induces a dysbiotic situation that develops gastric cancer. Promoting health and preventing disease is explicitly functioned by the host microbiota. The dysbiosis contributes to inflammation, susceptibility to pathogens, and diseases (Noto and Peek 2017). Although there have been great advances in research to understand the complex interaction between *H. pylori* and the gastric microbiota in the development of gastric inflammation and cancer, detailed studies are still needed. Such a study should be done in a well-defined human population, in order to compare differences in the microbiota of *H. pylori*-infected patients with and without neoplastic lesions (Joo 2017).

The currently prescribed treatment for *H. pylori* infection instigates long-term disturbances in the intestinal microbiota. The combination of the probiotics appeared to result in changes in the microbiota which are minor (Myllyluoma *et al.* 2007). Therefore, following probiotic supplementation with antibiotic

therapy can be an effective way to cure infection caused by *H. pylori*.

The clinical implications are partially understood, since many studies have described *H. pylori*-dependent gut microbiota alterations. In addition to this, the mechanism of actions that sustain these processes is also not clear. This represents a undisputed challenge for future research projects which need to understand the role played by viruses, parasites, yeasts, fungi, as well as *H. pylori*.

6. Conclusion

Antibiotic therapy has a limitation mainly due to antibiotic resistance to eradicate *H. pylori*. Therefore, it becomes very pertinent to look for alternatives to fight against *H. pylori* infection. According to the research in last few decades, it clearly illustrates the tremendous progress in the pathogenicity, involvement of microbiome and genetic predisposition in understanding the severity of gastric inflammation and gastric cancer produced by *H. pylori*. The role of *H. pylori* virulence genes, such as CagA and VacA, in the development of gastric diseases is now being extensively studied. A few studies have been carried out on the role of the membrane vesicles, molecular and functional aspects, role of interaction of HopQ adhesion and HSP. The study on involvement of virulence factors in *H. pylori* pathogenesis will be highly beneficial for vaccine development and to find an alternative therapy. It appears that combination therapy consisting of phytochemicals and probiotics is a promising choice. Phytochemicals are unlikely to cause remarkable disbiosis in microbiota as these are part of a routine diet and mild perturbations, even they happen, can be restored by probiotics. Identification of the nature of active ingredient as well as mechanism of action of traditional medicines would be the requirements for research in future.

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