Progress in Microbes and Molecular Biology

HH PUBLISHER

Review Article

Exploring the Impact of *Helicobacter pylori* and Potential Gut Microbiome Modulation

Isaac Jingkhai Ong[1], Ke-Yan Loo[1], Lydia Ngiik-Shiew Law[2], Jodi Woan-Fei Law[1], Loh Teng-Hern Tan[1,3], Vengadesh Letchumanan[1*]

Article History

Received: 08 November 2022;

Received in Revised Form: 21 December 2022;

Accepted: 30 December 2022;

Available Online: 04 January 2023

[1]Novel Bacteria and Drug Discovery Research Group (NBDD), Microbiome and Bioresource Research Strength (MBRS), Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Subang Jaya 47500, Malaysia; iong0002@student.monash.edu (IJO); ke.loo@monash.edu (K-YL); jodi.law1@monash.edu (JW-FL)

[2]Monash Credentialed Pharmacy Clinical Educator, Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville 3052, VIC, Australia; lydia19595@gmail.com (LN-SL)

[3] Clinical School Johor Bahru, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Johor Bahru 80100, Malaysia; loh.teng.hern@monash.edu (LT-HT)

*Corresponding author: Vengadesh Letchumanan, Novel Bacteria and Drug Discovery Research Group (NBDD), Microbiome and Bioresource Research Strength (MBRS), Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Subang Jaya 47500, Malaysia; vengadesh.letchumanan1@monash.edu (VL)

Abstract: *Helicobacter pylori* is a highly prevalent bacteria that can harm humans due to its major involvement in developing gastrointestinal diseases, particularly gastric cancer. Therefore, eradicating *H. pylori* is one of the most important strategies for preventing gastric cancer. Antibiotic treatment has always been the gold standard treatment for *H. pylori* infection. However, the decreasing efficacy of antibiotic therapy due to the rising antibiotic resistance and high incidence of dysbiosis-related adverse effects resulted in eradication failure. To enhance the effectiveness of antibiotic therapy, strategies that modulate the gut microbiome were proposed to play a positive role. Generally, the integration of probiotics or symbiotic into antibiotic therapy was shown to enhance the eradication rate and reduce the incidence of adverse effects. This review aims to discuss the role and effect of *H. pylori* in gastric carcinogenesis and gut microbiome modulation in eradicating *H. pylori* infection.

Keywords: *Helicobacter pylori*; gut microbiome; gastric cancer; carcinogenesis

1. Introduction

The gut microbiome is composed of trillions of microorganisms that live within the human gastrointestinal tract (GIT) [1]. The gut microbiome has been extensively researched for its association with human health and diseases. Research has established the gut

microbiome connection in human digestion, nutrition, immune functions, and physiology. Any dysbiosis of the gut microbiome has been linked to various diseases, such as irritable bowel syndrome (IBS) [2], obesity [3], type 2 diabetes [4, 5], autoimmune diseases [6-8], cancer [9], psoriasis [10, 11], obsessive-compulsive disorder [12], and autism spectrum disorder [13]. Hence, modulating the gut microbiome has been proven to manage disease outcomes [14-18].

The GIT is very vulnerable to pathogens, such as *Vibrio* sp. [19-22], *Salmonella* sp. [23], *Listeria monocytogenes* [24, 25], *Escherichia coli* [26], *Staphylococcus aureus* [27], *Campylobacter* sp. [28], *Helicobacter pylori* [29-31], and many more. Upon invasion, these pathogens release their toxins and colonize the host gut, leading to a dysbiosis of the gut microbiome [32]. Of the mentioned pathogens above, *Helicobacter pylori* research has been reviewed extensively due to its impact on human health.

Helicobacter pylori is a gram-negative, spiral-shaped, microaerophilic bacteria in the gastric mucosa. It is highly prevalent among the world population affecting over half of them [33]. Most people acquire this pathogen in early childhood and can remain asymptomatic throughout their life [34, 35]. *H. pylori* is among the well-known bacteria due to its role in developing gastrointestinal diseases such as gastritis, gastric ulcer, and gastric cancer [36]. The pathogen was shown to be the primary etiology for gastric cancer and was listed as a Type 1 carcinogen in 1997 by the World Health Organization [37]. Due to its association with gastric cancer, *H. pylori* remains a high burden to healthcare systems worldwide. According to Global Cancer Statistics 2020, gastric cancer is the sixth most common cancer, with an estimated 1089,103 cases, and the fourth leading cause of death due to cancer, with 768,793 deaths in 2020 [38]. Most gastric cancers are adenocarcinoma, which is then classified into two morphological types: intestinal type and diffuse-type gastric cancer. For intestinal-type cancer: tumour cells form adhesions, arranging in glandular or tubular formation, and a well-defined sequence has been proposed to be the carcinogenesis model, progressing from healthy gastric mucosa, chronic atrophic gastritis, intestinal metaplasia, dysplasia, and gastric cancer [39, 40]. As for diffuse-type cancer, tumour cells are more scattered and non-cohesive. Carcinogenesis is a direct consequence of chronic active inflammation without any similar defined sequence [39].

The human stomach has always been a sterile organ due to its low pH [41]. However, the significant advancement of the technology in microbiome investigation led to the discovery of a diversified microbial community consisting of multiple commensal microorganisms forming a distinct ecological niche where complex interplay is present among *H. pylori* and the commensal microorganism [42, 43]. A delicate balance is maintained among the microbial community in the stomach, in which any alterations in the balance may lead to dysbiosis and induction of gastric diseases, particularly gastric cancer [44]. Meanwhile, *H. pylori* suppress acid production and destroy the parietal cells, resulting in increased stomach pH and enabling the colonization of new microorganisms [40, 45, 46]. Hence, eradicating *H. pylori* is essential for preventing gastric cancer [47].

For the past few decades, standard triple antibiotic therapy was the gold standard treatment to eradicate *H. pylori*. Recently, there has been a decreasing eradication rate by antimicrobial therapy due to the increasing prevalence of antibiotic resistance [48, 49]. Despite developing new antibiotic regimens, they are still associated with adverse effects, leading to poor patient compliance and eradication failure [50]. Antibiotic therapy causes perturbation of the gastric microbiome leading to dysbiosis-induced gastrointestinal side effects [51]. To reduce the incidence of adverse effects from antibiotic-induced dysbiosis, strategies and therapies that can modulate the gut microbiota are being extensively explored [52]. Hence, this review aims to discuss the role and effect of *H. pylori* in gastric carcinogenesis and potential gut microbiome modulation in eradicating *H. pylori* infection.

2. Pathogenesis of *H. pylori*

The fact that not every individual infected with *H. pylori* develops gastric cancer shows that the pathogenesis of the variable clinical outcomes of *H. pylori* infection is multifactorial, including the virulence factors of the bacteria strain, host gene polymorphism, and environmental influences. Gene polymorphism of pro-inflammatory cytokines such as IL-1B increased gastric cancer risk through different mechanisms, including inflammatory injury, gastric acid secretion inhibition, and angiogenesis promotion [53-55]. Besides IL-1B, genetic variation in another pro-inflammatory cytokine, TNF-α, increased expression is also associated with enhancing gastric cancer risk [56]. With inflammation playing a vital role in cancer progression, polymorphism in inflammatory cytokines, which regulates the intensity of immune response, may augment gastric cancer risk [57]. Environmental factors, including dietary habits, cigarette smoking, and alcohol consumption, also affect gastric cancer risk. The dietary habits consisting of high salt content, red meat, processed meat, and low fiber enhanced gastric cancer risk [58-60]. Interestingly, former and current smokers demonstrated higher rates of gastric cancer than never-smokers, and the risk intensifies with the number of cigarettes per day for current smokers [61]. As for alcohol consumption, heavy drinkers with more than four drinks per day have significantly increased odds of developing gastric cancers compared to abstainers [62].

Besides host genetic vulnerability and environmental influences, virulence factors associated with the bacteria's pathogenicity were involved in gastric carcinogenesis. The human stomach contains gastric fluid with a pH of around 2.0. This highly acidic environment remains a huge challenge for colonizing most invading pathogens, which enables the bacteria to survive in the highly acidic environment, colonize the gastric mucosa, and evade the immune cells. *H. pylori* possesses urease, one of the main virulence factors that neutralize acidity and enable it to survive and colonize in such a harsh environment [63]. The urease produced by *H. pylori* plays an alkalizing role as it hydrolyses urea in the stomach and produces ammonia plus carbon dioxide, which neutralizes the stomach's acidity and provides a favorable environment for the survival of *H. pylori* [63]. Aside from acid neutralization, urease was also involved in the carcinogenesis of gastric cancer. Recently, an *in vitro* study uncovered that ureases can trigger a specific pathway to initiate angiogenesis in the gastric epithelial cell. This can potentially lead to the carcinogenesis of gastric cancer [64]. Additionally, urease displayed pro-inflammatory

activity in the gastric endothelial cells, inducing alterations in the oxidative profile of the cells and resulting in differentiation which may contribute to gastric carcinogenesis [63].

Two main virulence factors affect the pathogenicity of the host cell, CagA and VacA virulence protein. CagA is the first bacterial oncoprotein shown to be associated with cancer in humans [65]. It is transported into the host gastric epithelial cells by a syringe-like structure called Type 4 secretion system [66-68]. In the host cells, there is phosphorylation of CagA by tyrosine kinase protein. Then, CagA binds to signaling proteins that perturb multiple intracellular signaling pathways leading to the host cells' malignant changes [69]. The expression of CagA leads to morphological changes in the cells, specifically elongation and increased motility, known as the "hummingbird" phenotype [70]. This oncoprotein also causes disruption of the intercellular junction and the polarity of the epithelial cells [71-73]. In addition, this molecule induces anti-apoptotic activity in the gastric epithelial cells leading to decreased turnover of cells. Also, it triggers instability in the genome which both are classic traits of cancer cells [74, 75]. Recently, Choi et al noted that CagA upregulates the CDX1 expression involved in regulation of intestinal function, which may lead to gastric carcinogenesis [76].

Another virulence factor VacA is a pore-forming cytotoxin that can induce vacuolation and promote apoptosis in gastric epithelial cells [77, 78]. VacA exerts its effects on cellular activity through the mitochondrial pathway, triggering the release of cytochrome c leading to autophagy and cell death [79, 80]. A study proposed that VacA induces cell apoptosis by activating the endoplasmic reticulum stress cascade [81]. Moreover, VacA modulates the host immune reaction by preventing the recruitment of immune cells, including T cells and B cells, to ensure the longevity of the infection [82, 83]. VacA also targets macrophages, the first line of defense, by interfering with the maturation of the phagosome and blocking the activation of cytokines induced by macrophages [84, 85]. Recent studies also showed the interplay between VacA and CagA, where the disruption of autophagy by VacA promotes CagA accumulation in gastric cells resulting in the persistence of *H. pylori* in the gastric mucosa [86].

3. *H. pylori* and gastric microbiome

The dogma that the human stomach is a sterile organ has been shattered since the revelation of *H. pylori*. Since then, there has been a hypothesis that the human gut harbors a diversified bacterial community [87]. Bik et al. conducted a critical study that explored the composition of the gastric microbiome through a biopsy of the gastric mucosa and PCR of the samples. This study shows that the gastric microbiota of healthy individuals comprises five major phyla, including *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Fusobacteria*, which were then supported by newer studies [88, 89]. Despite the variation of the microbiota composition among individuals, the human stomach contains a diversified microbiome [87, 90].

In the gastric microbiome consisting of a highly diversified bacterial community, *H. pylori* seemed to be the most dominant bacteria with the highest abundance in the gastric microbiome when present [42, 91]. Schulz et al. noticed that the abundance of *H. pylori*

comprises more than 50% among *H. pylori*-positive subjects, and the abundance of the other major species decreased [42]. A recent study in 2019 obtained a more significant result where the bacterial communities of the stomach among *H. pylori*-positive children were notably dominated by 95.43% of *H. pylori* in the genus [91]. As for *H. pylori*-negative individuals, studies have shown that *Streptococcus* was commonly the prominent genera with the highest abundance in the gastric microbiome [42, 91, 92].

With the dominance of *H. pylori* in the gastric microbiome, *H. pylori* were shown to alter the composition and species diversity. Studies showed that the gastric microbiome was significantly less diversified in *H. pylori*-positive patients than in *H. pylori*-negative patients. In a study by Anders et al., they identified 262 bacterial phylotypes compared to 33 in *H. pylori*-positive individuals. The result shows that for individuals with *H. pylori* infection, their gastric microbiota was significantly less diversified than *H. pylori*-negative individuals [93]. A recent study in Indonesia supported the findings that bacterial diversity decreased in the *H. pylori*-positive group [94]. Several other studies further emphasized that the alpha diversity of *H. pylori*-positive patients is significantly lower than healthy individuals [42, 89].

3.1. H. pylori and gastric microbiome in gastric carcinogenesis

According to Correa's model of gastric carcinogenesis, persistent colonization of *H. pylori* triggers an inflammatory process that then initiates the carcinogenesis cascade, starting from atrophic gastritis, intestinal metaplasia, dysplasia, and gastric cancer. Long-term colonization of *H. pylori* leads to atrophy of the parietal cells, whose main function is the secretion of acid, leading to increased pH of the gastric fluid and atrophic gastritis [40]. As most bacteria cannot live in highly acidic conditions, *H. pylori*-induced achlorhydria disrupts the acid barrier, colonizing new microbes, and leading to the gastric microbiome alterations. Meanwhile, the proliferation of new microbes may further promote inflammatory reactions, leading to cancer progression [40]. Multiple studies attempted to discover the role of non-*H. pylori* in the carcinogenesis of gastric cancer. One hypothesis proposed that some of these microbes can reduce nitrate into nitrite, forming N-nitroso compounds that are potentially carcinogenic [95]. Ferreira et al. analyzed the functional composition of the gastric microbiome in individuals with chronic gastritis and gastric cancer. They noticed that the gastric cancer microbiome has a higher nitrate reductase function, promoting the formation of carcinogenic compounds [45]. Jo et al. reported twice as much nitrosating bacteria in individuals with gastric cancer compared to the control groups [96].

Similarly, there was an enrichment of bacteria involved in nitrate metabolisms, such as *Escherichia coli*, *Lactobacillus*, and *Nitrospirae*, in patients with gastric cancer [46, 97]. Another hypothesis stated that oral microbiota plays a role in gastric carcinogenesis. Coker et al. demonstrated that microbiome from the oral origin, including *Slackia exigua*, *Peptostreptococcus stomatis*, *Streptococcus anginosus*, *Parvimonas micra* and *Dialister pneumosintes* were significantly enriched and formed strong interactions in the gastric microbiome of gastric cancer [98]. In another study among gastric cancer patients from Malaysia and Singapore, there is an increased abundance of oral bacterial taxa such as

Leptotrichia, *Fusobacterium*, *Haemophilus*, *Veillonella*, and *Campylobacter* [99]. In a few other studies, there is reportedly a higher abundance of oral bacteria such as *Lactobacillus*, *Streptococcus*, and *Neisseria* in gastric cancer patients [100-102]. Sun et al. developed a scoring system to screen for patients with high suspicion of gastric cancer by detecting oral bacteria. The sensitivity was as high as 97.3%, with a 7.7% false-positive rate. However, this study consists of a small sample size of 50 subjects, and more extensive studies are required to validate the results [103].

Some studies also examined the changes in gastric microbiota diversity along Correa's carcinogenesis cascade. Most studies reported significant differences in gastric microbiome diversity in gastric cancer compared to precancerous lesions, demonstrating gastric dysbiosis's role in gastric carcinogenesis [45, 98]. Jimenez et al. conducted a study to explore gastric microbiota changes along the carcinogenesis cascade and reported a decreased diversity in gastric cancer [104]. Wang et al. supported these findings, showing that the gastric microbiome diversity decreases along the gastric carcinogenesis cascade [98]. However, studies have proposed contradictory results stating that gastric microbiome diversity increase in gastric cancer. Rodriguez et al. and Eun et al. reported that gastric microbiome diversity increases in subjects with gastric cancer [99, 100]. Wang et al. further supported the findings in a study involving 315 patients showing that gastric microbiota diversity increases in gastric cancer [46]. The discrepancy in the results from different studies could be due to factors influencing the gastric microbiome, such as the variation of gender, ethnic group, and dietary habits among the subjects [87, 98].

4. Antibiotic therapy in *H. pylori* infections

Various antibiotic therapies have been evaluated to eradicate *H. pylori*. However, few were highly effective, with consistently high eradication rates and low incidence of adverse effects [105]. The reasons for eradication failure are mostly due to the increasing antibiotic resistance and non-compliance to treatment. According to clinical guidelines, bismuth quadruple therapy replaced standard triple therapy as the first line in areas with known high macrolide resistance. Besides that, multiple alternative drug regimens are recommended as the first-line treatment, including levofloxacin, sequential therapy, concomitant therapy and hybrid therapy, depending on antibiotic resistance and penicillin allergies [49]. Recently, Vonoprazan emerged as one of the most promising drugs for *H. pylori* infection [106]. Vonoprazan is a highly potent acid-inhibitory drug with rapid onset and longer duration of action than conventional proton pump inhibitors [107, 108].

Studies have reported that antibiotic therapy reduces the diversity and composition of the gut microbiome [109, 110]. In a multicentre randomized trial, Liou et al. explored the effect of standard triple therapy, bismuth quadruple therapy, and concomitant therapy on the gut microbiome. After antibiotic treatment, faecal microbiota analysis was done at 2, 8, and 10 weeks. The results demonstrated significant perturbation of gut microbiota with reduced microbiome diversity at week 2 for all three antibiotic regimens. After one year, the bacterial diversity was fully restored in standard triple therapy but not in bismuth quadruple and

concomitant therapy. There was an increased abundance of *Proteobacteria* and decreased *Firmicutes* and *Bacteroidetes* in concomitant therapy and quadruple therapy groups [109]. In line with the previous study, Chen et al. noticed a decreased microbial diversity, increased *Proteobacteria*, and decreased *Firmicutes* and *Bacteroidetes*, which persisted for more than six weeks after treatment, suggesting the antibiotic-induced dysbiosis may persist without complete restoration after a long period [110]. The abundance of butyrate-producing bacteria, *Lachnospiraceae,* decreased after antibiotic treatment, increasing the risk of *Clostridium difficile* infection [110, 111]. Few other studies supported the findings that gut microbiome diversity decreased after eradication therapy with increased *Proteobacteria* and decreased *Firmicutes, Bacteroidetes* and *Actinobacteria* after antibiotic therapy [112, 113]. Interestingly, there was a reduced abundance of *Bifidobacterium* in a few studies [114-116]. *Bifidobacterium* prevents colonization of commensal pathogens and assists in the metabolism of carbohydrates, suggesting that decreased *Bifidobacterium* may be linked to antibiotic-induced adverse effects [115, 117].

5. Modulation of the gut microbiome

Probiotics are live microorganisms that may provide health benefits if given in optimum quantity [118]. Today, probiotics have gained people's interest as an effective therapeutic option for managing digestive and immune health [119]. Probiotics have proven effective in treating various diseases, from gastrointestinal issues to recent COVID-19 infections [120-122]. *Lactobacillus, Bifidobacterium,* and other lactic acid-producing bacteria (LAB) are common probiotics used in dairy products and yogurt. As human microbiome research expands, a range of potential probiotics with good benefits to the host has been discovered. Among those new potential probiotic candidates is *Streptomyces* sp. Recent studies have revealed that *Streptomyces* sp. has strong antibacterial, anti-*Vibrio* activity and probiotic properties for aquaculture usage [123-127]. Further studies should be conducted to strengthen these findings before they can be administrated to humans.

Based on the properties of different probiotics, several proposed mechanisms of action against *H. pylori* include immunological and non-immunological mechanisms. The first line of defense against foreign pathogens is its high acidity and intact stomach mucosa. Probiotics can synthesize antimicrobial substances, including lactic acids, short-chain fatty acids, and bacteriocins. Certain probiotic strains, such as *Bifidobacterium longum* and *Lactobacillus casei* were found to inhibit the activity urease in *H. pylori*, hindering its ability to colonize the stomach [128, 129]. In a study by Kim et al., mice model gastric pH decreases significantly after administering lactic acid bacteria, eliminating *H. pylori* [129]. Bacteriocins are proteinaceous toxins that possess anti-*H. pylori* activity and its antimicrobial activities vary with different probiotic strains. Bacteriocin and lactic acid secreted by Lactobacillus bulgaricus and Lactobacillus pentosus strains were shown to inhibit antibiotic sensitivity and antibiotic resistance *H. pylori* strains [130, 131]. Other than *Lactobacilli*, Lim et al. reported that the bacteriocins produced by *Enterococcus faecalis* BK61 exhibit anti-*H. pylori* activity [132]. A recent in vitro study by Sacacino et al. reported several probiotic strains, including *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus acidophilus*, *Bifidobacterium*

lactis and *Streptococcus thermophilus* strains possess both bactericidal and bacteriostatic against *H. pylori* [133]. Additionally, reuterin, a nonpeptide antimicrobial substance secreted by *Lactobacillus reuteri* was seen to exert effect through downregulation of *H. pylori* virulence factors, VacA and flaA [134].

Probiotics can adhere to binding sites of gastric epithelial cells and provide competition for the adhesion of *H. pylori*. Mukai et al. reported that *L. reuteri* strains have a specific affinity for two glycolipid binding sites on epithelial cells thus competing and occupying the adhesion site *H. pylori*, preventing the pathogen from colonizing the gastric mucosa [135]. A newer study by Holz et al. added that *L.reuteri* decreases the mobility *of H. pylori* by entangling and forming aggregate with *H. pylori* which will eventually be flushed out from the gut [136]. Additionally, *Lactobacillus gasseri* and *Lactobacillus brevis* strain was noted to reduce the adherence of *H. pylori* to the host cell by inhibiting the expression of *sabA* gene [137]. A probiotic yeast named *Saccharomyces boulardii* was noted to inhibit adherence of *H. pylori* to the host cell by removing α-2,3-linked sialic acid, the ligand for *H. pylori* adhesin with its neuraminidase activity [138].

Probiotics reduce the inflammation in the gastric cells through modulation of the immune reaction and inhibit the production of pro-inflammatory cytokines. Studies have shown that *L. bulgaricus, L. acidophilus* and *L. rhamnosus* inhibit the production of inflammatory cytokine, particularly IL-8, in the cells infected with *H. pylori* [139, 140]. Besides, Yu et al. demonstrated that a probiotic cocktail consisting of *E.faecalis, L.acidophilus* and *B.longum* plays a significant role in the downregulation of inflammatory chemokines and cytokines, including TNF-α, IL-10, IL-1β, IL-6, MIP-2 and G-CSF which eventually leading to improvement of *H. pylori*-induced gastritis [141].

Lactobacillus strain probiotics have been widely studied in terms of their role in enhancing the eradication rate and improving gastrointestinal symptoms. A randomized controlled trial by Poonyam et al. evaluated the effect of probiotic supplementation on antibiotic therapy by prescribing *L.reuteri* during bismuth-based triple therapy to eradicate *H. pylori*. Results showed no significant difference in eradication rates but a significantly lower incidence of side effects, including nausea, diarrhea, black stools, and abdominal discomfort [142]. Francavilla et al. assigned 100 *H. pylori*-positive individuals into two groups: one group received standard triple therapy plus two *L.reuteri* strain, and another group received the same antibiotic treatment and placebo. In contrast to the previous study, the probiotic group's eradication rate was significantly increased compared to the placebo group (75% vs 65.9%). Similarly, lower side effects were reported in the probiotic group compared to the placebo (40.9% vs 62.8%) [143]. The probiotic yogurt containing *L. gasseri* was seen to reverse the dysbiosis and restore the balance in the gastric microbiome [144].

Besides *Lactobacillus*, *Bifidobacterium* was also commonly used as supplementation for anti-*H. pylori* antibiotic therapy. In a study by Jiang et al., 232 patients were randomly prescribed bismuth quadruple therapy plus live combined *Bifidobacterium* probiotic or bismuth quadruple therapy without probiotic. Significantly higher eradication rates and lower

incidences of side effects were noted in the probiotic group [145]. Cekin et al. compared the eradication rate between one group receiving sequential eradication therapy with probiotics containing *Bifidobacterium animalis subsp.* lactis B94 and one group without probiotics. The eradication rate was significantly higher with probiotics than with the controls (86.8% vs 70.8%). The probiotic group also experienced a lower incidence of diarrhea (1.88% vs 12.26%), leading to better compliance with treatment [146].

Several studies have explored the role of yeast probiotics such as *S. boulardii*. Seddik et al. reported that adding *S.boulardii* to anti-*H. pylori* sequential therapy resulted in an increased eradication rate compared to sole sequential therapy (86.0% vs 76.7%). The probiotic group also reported a significantly lower incidence of antibiotic-induced diarrhea (2.0% vs 46.4%) and overall side effects (17.0% vs 55.7%). This led to a significantly higher compliance rate in the probiotic group (95.0% vs 91.2%) [147]. In 2019, He et al. administered *S.boulardii* during bismuth-based quadruple therapy. No significant difference was noted in the eradication rate. Still, the incidence of nausea, diarrhea, and side effects was significantly lower compared to the control group. This study also noted that supplementation of probiotics simultaneously with quadruple therapy recorded a lower incidence of adverse effects than administration on the 14th day after commencing quadruple treatment, suggesting the best timing for probiotic supplementation [148]. A recent study by Cardenas et al. revealed that *S. boulardii* supplementation increased bacterial diversity in the gastric microbiome [149].

Additionally, *Clostridium* was also considered a potential candidate for probiotic supplementation. Mukai et al. demonstrated that anti-*H. pylori* therapy regimen consisting of probiotic *C. butyricum* and standard triple therapy recorded a higher eradication rate of 87.1% compared to 70.1% in standard triple therapy without probiotics [150]. Chen et al. also noted that *C.butyricum* supplementation improves gastrointestinal symptoms by restoring gut microbiota composition [139]. Furthermore, there are also studies exploring the role of other probiotics. Tang et al. prescribed bismuth quadruple therapy to 162 patients, with one group receiving probiotics consisting of *Enterococcus faecium* plus *Bacillus subtilis* and another group receiving a placebo. The result was consistent with the previous study, as the probiotic group's eradication rates were higher at 87.01% compared to 82.43% in the placebo group [151]. Furthermore, beneficial bacteria were enriched with probiotic supplementation, including *Oscillospira* and *Lactobacillales,* known for producing short fatty acid chain butyrate, regulating immune reactions, and alleviating gastrointestinal symptoms [152, 153]. Inflammation-linked harmful bacteria, such as *Collinsella*, *Dialistera* and *Sutterella* were also reduced in the probiotic group [154]. Jung et al. compared the eradication rate and side effects of one group receiving concomitant therapy and another group receiving standard triple therapy plus probiotics containing either freeze-dried *L. casei rhamnosus* or *B.subtilis* combined with *E. faecium*. In contrast to previous studies, there were no significant differences in the eradication rates between groups, but the probiotic group reported a lower incidence of adverse effects [155].

Aside from probiotics, prebiotics and synbiotics also play a role in gut modulation. Prebiotics are compounds that are non-digestible but can be metabolized by gut microbiota

to allow benefits for the host [156]. In contrast to probiotics, limited studies were done to investigate the role of prebiotics on anti-*H. pylori* therapy. Instead, more studies researched synbiotics, a combination of probiotics and prebiotics. A study by Sirvan et al. explored the impact of *Bifidobacterium lactis* based synbiotics on standard triple therapy. Results showed a higher eradication rate in the triple therapy plus synbiotics group compared to triple therapy alone (88% vs. 72%). In terms of the adverse effects, the synbiotics group experienced a significantly lower incidence of adverse effects, including nausea, vomiting, diarrhea, and abdominal pain [157]. Contrary to this study, two other trials used the same *B.lactis* strain and inulin during standard triple therapy. There was no significant difference in the eradication rate compared to the control group. Ustundag et al. reported no significant difference in the side effects in both groups. In contrast, a study by Islek et al. reported significantly lesser side effects in the symbiotic group than in the triple therapy group (63% vs. 17%) [158, 159].

Fecal microbiota transplant (FMT), which is the transplantation of fecal material from a healthy donor into the patient's intestines, has been proven to restore the gut microbiota and treat gastrointestinal disorders, including irritable bowel syndrome, inflammatory bowel disease, and *Clostridium difficile* infection [160]. Additionally, FMT was shown to be more cost-effective than antimicrobial therapy [33]. Therefore, researchers hypothesized that fecal microbiota transplantation could play a role in eradicating *H. pylori* through the restoration of gut microbiota. To verify this hypothesis, Ye et al. conducted a study in 2020 where they administered washed microbiota transplant (WMT) to 32 patients. WMT was performed in this study due to its lesser adverse effects and higher efficacy as compared to conventional FMT [161, 162]. 32 patients diagnosed with *H. pylori* infection for the past year and not on eradication therapy were administered WMT once a day for three consecutive days. The result showed an eradication rate of 40.6% four weeks post-WMT [162]. This positive result warrants future randomized controlled trials with a larger sample size to determine the optimal dosage and frequency of WMT and to confirm the safety and efficacy of WMT.

6. Conclusion

Antibiotic therapy has been the gold standard for the eradication of *H. pylori*. Nevertheless, antibiotic therapies have limitations, including increasing antibiotic resistance and antibiotic-induced dysbiosis, leading to a high incidence of adverse effects and poor patient compliance. The few factors combined lead to decreased efficacy of antibiotic therapy and reduced eradication of *H. pylori*, contributing to a higher prevalence of gastrointestinal diseases, particularly gastric cancer. This review shows that integrating probiotics into antibiotic therapy enhances antibiotics' efficacy and restores the gastric microbiome's homeostasis. Probiotics extensively studied and shown to be effective include *Lactobacillus*, *Bifidobacterium*, *Saccharomyces,* and *Clostridium* strains. However, there is insufficient evidence to determine the best probiotic strain for eradicating *H. pylori*. Therefore, large sample trials should be conducted to determine the selection, optimum dosage, and duration of antibiotics and probiotics to improve the efficacy of antibiotic therapy. Besides probiotics, there is also an increased eradication rate and reduced adverse effects with symbiotic supplementation. However, relatively fewer trials were done on symbiotic, prompting more

studies to investigate the efficacy of symbiotic supplementation and the possible combination of probiotics and prebiotics for the best effect. Lastly, fecal microbiota transplant is potentially a highly cost-effective therapy for *H. pylori*. Further studies should be conducted to evaluate its safety, effectiveness, and synergistic effect when combined with the current eradication regimen.

Author Contributions: The literature search, data extraction, and manuscript writing were performed by IJO. The manuscript was formatted and edited by K-YL. This review was critically reviewed, proofread, and edited by LS-NL, JW-FL, LT-HT and VL. VL conceptualized this review writing project.

Funding: No external funding was provided for this research.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lau AWY, Tan LT-H, Ab Mutalib N-S, *et al.* The chemistry of gut microbiome in health and diseases. Prog Microbes Mol Biol 2021; 4(1).
2. Selvaraj SM, Wong SH, Ser H-L, *et al.* Role of low FODMAP diet and probiotics on gut microbiome in irritable bowel syndrome (IBS). Prog Microbes Mol Biol 2020; 3(1).
3. Tseng C-H and Wu C-Y. The gut microbiome in obesity. Journal of the Formosan Medical Association 2019; 118: S3-S9.
4. Wang HJ, Battousse O, and Ramadas A. Modulation of gut microbiota by dietary macronutrients in type 2 diabetes: A review. Prog Microbes Mol Biol 2021; 4(1).
5. Durganaudu H, Kunasegaran T, and Ramadas. A Dietary glycaemic index and type 2 diabetes mellitus: Potential modulation of gut microbiota. Prog Microbes Mol Biol 2020; 3(1).
6. Thye AY-K, Law JW-F, Tan LT-H, *et al.* Exploring the Gut Microbiome in Myasthenia Gravis. Nutrients 2022; 14(8): 1647.
7. Selvaraja M, Abdullah M, Shah AM, *et al.* Systemic Lupus Erythematosus (SLE): A review on the prevalence, clinical manifestation, and disease assessment. Prog Microbes Mol Biol 2020; 3(1).
8. de Oliveira GLV, Leite AZ, Higuchi BS, *et al.* Intestinal dysbiosis and probiotic applications in autoimmune diseases. Immunology 2017; 152(1): 1-12.
9. Vivarelli S, Salemi R, Candido S, *et al.* Gut microbiota and cancer: from pathogenesis to therapy. Cancers (Basel) 2019; 11(1): 38.
10. Thye AY-K, Bah Y-R, Law JW-F, *et al.* Gut–Skin Axis: Unravelling the Connection between the Gut Microbiome and Psoriasis. Biomedicines 2022; 10(5): 1037.
11. Hidalgo-Cantabrana C, Gómez J, Delgado S, *et al.* Gut microbiota dysbiosis in a cohort of patients with psoriasis. Br J Dermatol 2019; 181(6): 1287-1295.
12. Kong GY-E, Letchumanan V, Tan LT-H, *et al.* Gut Microbiome in Obsessive Compulsive Disorder: Potential of Probiotics as an Adjuvant Therapy. Prog Microbes Mol Biol 2022; 5(1).
13. Johnson D, Letchumanan V, Thurairajasingam S, *et al.* A revolutionizing approach to autism spectrum disorder using the microbiome. Nutrients 2020; 12(7): 1983.
14. Lim WQ, Cheam JY, Law JW-F, *et al.* Role of Garlic in Chronic Diseases: Focusing on Gut Microbiota Modulation. Prog Microbes Mol Biol 2022; 5(1).
15. Lee L-H, Letchumanan V, Law JW-F, *et al.* Exploring the potential role of probiotics in alleviating insomnia. Gut 2022.

16. Letchumanan V, Low S-N, Tan LT-H, *et al.* Exploring the potential role of probiotics in substance use disorders. Gut 2022.
17. Chong H-Y, Tan LT-H, Law JW-F, *et al.* Exploring the Potential of Human Milk and Formula Milk on Infants' Gut and Health. Nutrients 2022; 14(17): 3554.
18. Learn-Han L, Ser H-L, Letchumanan V, *et al.* IDDF2020-ABS-0116 The role of gut microbiome in traditional Chinese medicine syndromes: focusing on the spleen deficiency syndrome. Gut 2020; 69(Suppl 2): A19-A19.
19. Letchumanan V, Ab Mutalib N-S, Wong SH, *et al.* Determination of antibiotic resistance patterns of Vibrio parahaemolyticus from shrimp and shellfish in Selangor, Malaysia. Prog Microbes Mol Biol 2019; 2(1).
20. Letchumanan V, Loo K-Y, Law JW-F, *et al.* Vibrio parahaemolyticus: The protagonist of foodborne diseases. Prog Microbes Mol Biol 2019; 2(1).
21. Letchumanan V, Tan W-S, Yin W-F, *et al.* Genome sequence of Vibrio sp. OULL4 isolated from shellfish. Prog Microbes Mol Biol 2020; 3(1).
22. Venggadasamy V, Tan LT-H, Law JW-F, *et al.* Incidence, Antibiotic Susceptibility and Characterization of Vibrio parahaemolyticus Isolated from Seafood in Selangor, Malaysia. Prog Microbes Mol Biol 2021; 4(1).
23. Ehuwa O, Jaiswal AK, and Jaiswal S. *Salmonella*, food safety and food handling practices. Foods 2021; 10(5): 907.
24. Letchumanan V, Wong P-C, Goh B-H, *et al.* A review on the characteristics, taxanomy and prevalence of *Listeria monocytogenes*. Prog Microbes Mol Biol 2018; 1(1).
25. Loo K, Letchumanan V, Dhanoa A, *et al.* Exploring the pathogenesis, clinical characteristics and therapeutic regimens of *Listeria monocytogenes.* Microbiology 2020; 3: 1-13.
26. Singha S, Thomas R, Viswakarma JN, *et al.* Foodborne illnesses of *Escherichia coli* O157 origin and its control measures. J Food Sci Technol 2022: 1-10.
27. Battah B, Rajab A, Shbibe L, *et al.* Evaluation of antibiofilm activity of Thymus syriacus essential oil against clinically isolated MDR bacteria. Prog Microbes Mol Biol 2022; 5(1).
28. Wu Z, Sahin O, and Zhang Q. Campylobacter. Pathogenesis of Bacterial Infections in Animals 2022: 393-412.
29. Dincă AL, Meliț LE, and Mărginean CO Old and new aspects of H. pylori-associated Inflammation and gastric cancer. Children 2022; 9(7): 1083.
30. Georgopoulos S and Papastergiou V. An update on current and advancing pharmacotherapy options for the treatment of H. pylori infection. Expert Opin Pharmacother 2021; 22(6): 729-741.
31. Miller AK and Williams SM. Helicobacter pylori infection causes both protective and deleterious effects in human health and disease. Genes Immun 2021; 22(4): 218-226.
32. Loo K-Y, Law JW-F, Tan LT-H, *et al.* Vibrio: detecting you earlier, avoiding gastroenteritis and GUT microbiome dysbiosis. Gut 2022.
33. Abdali ZI, Roberts TE, Barton P, *et al.* Economic evaluation of Faecal microbiota transplantation compared to antibiotics for the treatment of recurrent *Clostridioides difficile* infection. EClinicalMedicine 2020; 24.
34. Rowland M, Daly L, Vaughan M, *et al.* Age-Specific Incidence of Helicobacter pylori. Gastroenterology 2006; 130(1): 65-72.
35. Park JS, Jun JS, Seo J-H, *et al.* Changing prevalence of Helicobacter pylori infection in children and adolescents. Clin Exp Pediatr 2021; 64(1): 21-25.
36. Correa P and Piazuelo MB. Natural history of Helicobacter pylori infection. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver 2008; 40(7): 490-496.

37. Lyon *Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans.* . IARC Monogr Eval Carcinog Risks Hum. Vol. 61. 1994: IARC, Distributed for the International Agency for Research on Cancer by the Secretariat of the World Health Organization. 1-241.
38. Sung H, Ferlay J, Siegel RL, *et al.* Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin.
39. Lauren P. The Two Histological Main Types of Gastric Carcinoma: Diffuse and so-called Instestinal-type Carcinoma. An Attempt at a Histo-clinical Classification. Acta Pathol Microbiol Scand 1965; 64: 31-49.
40. Correa P. *Helicobacter pylori* and gastric carcinogenesis. Am J Surg Pathol 1995; 19 Suppl 1: S37-43.
41. Martinsen TC, Bergh K, and Waldum HL. Gastric Juice: A Barrier Against Infectious Diseases. Basic Clin Pharmacol Toxicol 2005; 96(2): 94-102.
42. Schulz C, Schütte K, Koch N, *et al.* The active bacterial assemblages of the upper GI tract in individuals with and without Helicobacter Infection. Gut 2018; 67(2): 216.
43. Delgado S, Cabrera-Rubio R, Mira A, *et al.* Microbiological Survey of the Human Gastric Ecosystem Using Culturing and Pyrosequencing Methods. Microb Ecol 2013; 65(3): 763-772.
44. Zackular JP, Baxter NT, Iverson KD, *et al.* The gut microbiome modulates colon tumorigenesis. mBio 2013; 4(6): e00692.
45. Ferreira RM, Pereira-Marques J, Pinto-Ribeiro I, *et al.* Gastric microbial community profiling reveals a dysbiotic cancer-associated microbiota. Gut 2018; 67(2): 226-236.
46. Wang L, Zhou J, Xin Y, *et al.* Bacterial overgrowth and diversification of microbiota in gastric cancer. Eur J Gastroenterol Hepatol 2016; 28(3): 261-266.
47. Leung WK, Wong IOL, Cheung KS, *et al.* Effects of Helicobacter pylori Treatment on Incidence of Gastric Cancer in Older Individuals. Gastroenterology 2018; 155(1): 67-75.
48. Wang D, Guo Q, Yuan Y, *et al.* The antibiotic resistance of Helicobacter pylori to five antibiotics and influencing factors in an area of China with a high risk of gastric cancer. BMC Microbiol 2019; 19(1): 152.
49. Chey WD, Leontiadis GI, Howden CW, *et al.* ACG Clinical Guideline: Treatment of Helicobacter pylori Infection. Am J Gastroenterol 2017; 112(2): 212-239.
50. McNicholl AG, Bordin DS, Lucendo A, *et al.* Combination of Bismuth and Standard Triple Therapy Eradicates Helicobacter pylori Infection in More than 90% of Patients. Clin Gastroenterol Hepatol 2020; 18(1): 89-98.
51. Wu L, Wang Z, Sun G, *et al.* Effects of anti-H. pylori triple therapy and a probiotic complex on intestinal microbiota in duodenal ulcer. Sci Rep 2019; 9(1): 12874-12874.
52. Wu J, Gan T, Zhang Y, *et al.* The prophylactic effects of BIFICO on the antibiotic-induced gut dysbiosis and gut microbiota. Gut Pathog 2020; 12(1): 41.
53. Huang F-Y, Chan AO-O, Rashid A, *et al.* Interleukin-1β increases the risk of gastric cancer through induction of aberrant DNA methylation in a mouse model. Oncol Lett 2016; 11(4): 2919-2924.
54. Waghray M, Zavros Y, Saqui-Salces M, *et al.* Interleukin-1beta promotes gastric atrophy through suppression of Sonic Hedgehog. Gastroenterology 2010; 138(2): 562-72, 572.e1-2.
55. Voronov E, Carmi Y, and Apte RN The role IL-1 in tumor-mediated angiogenesis. Front Physiol 2014; 5: 114-114.
56. Zheng W, Zhang S, Zhang S, *et al.* The relationship between tumor necrosis factor-α polymorphisms and gastric cancer risk: An updated meta-analysis. Biomed Rep 2017; 7(2): 133-142.
57. Crusius JBA, Canzian F, Capellá G, *et al.* Cytokine gene polymorphisms and the risk of adenocarcinoma of the stomach in the European prospective investigation into cancer and nutrition (EPIC-EURGAST). Ann Oncol 2008; 19(11): 1894-1902.

58. Gaddy JA, Radin JN, Loh JT, *et al.* High Dietary Salt Intake Exacerbates Helicobacter pylori-Induced Gastric Carcinogenesis. Infection and Immunity 2013; 81(6): 2258.
59. Ferro A, Rosato V, Rota M, *et al.* Meat intake and risk of gastric cancer in the Stomach cancer Pooling (StoP) project. Int J Cancer 2020; 147(1): 45-55.
60. Zhang Z, Xu G, Ma M, *et al.* Dietary fiber intake reduces risk for gastric cancer: a meta-analysis. Gastroenterology 2013; 145(1): 113-120.e3.
61. Praud D, Rota M, Pelucchi C, *et al.* Cigarette smoking and gastric cancer in the Stomach Cancer Pooling (StoP) Project. Eur J Cancer Prev 2018; 27(2): 124-133.
62. Rota M, Pelucchi C, Bertuccio P, *et al.* Alcohol consumption and gastric cancer risk-A pooled analysis within the StoP project consortium. Int J Cancer 2017; 141(10): 1950-1962.
63. Marshall BJ, Barrett LJ, Prakash C, *et al.* Urea protects Helicobacter (Campylobacter) pylori from the bactericidal effect of acid. Gastroenterology 1990; 99(3): 697-702.
64. Olivera-Severo D, Uberti AF, Marques MS, *et al.* A New Role for Helicobacter pylori Urease: Contributions to Angiogenesis. Front Microbiol 2017; 8: 1883-1883.
65. Ohnishi N, Yuasa H, Tanaka S, *et al.* Transgenic expression of Helicobacter pylori CagA induces gastrointestinal and hematopoietic neoplasms in mouse. Proceedings of the National Academy of Sciences 2008; 105(3): 1003.
66. Backert S, Tegtmeyer N, and Fischer W. Composition, structure and function of the Helicobacter pylori cag pathogenicity island encoded type IV secretion system. Future Microbiol 2015; 10(6): 955-965.
67. Shaffer CL, Gaddy JA, Loh JT, *et al.* Helicobacter pylori exploits a unique repertoire of type IV secretion system components for pilus assembly at the bacteria-host cell interface. PLoS pathogens 2011; 7(9): e1002237-e1002237.
68. Tegtmeyer N, Wessler S, and Backert S. Role of the cag-pathogenicity island encoded type IV secretion system in Helicobacter pylori pathogenesis. The FEBS journal 2011; 278(8): 1190-1202.
69. Backert S, Tegtmeyer N, and Selbach M. The Versatility of Helicobacter pylori CagA Effector Protein Functions: The Master Key Hypothesis. Helicobacter 2010; 15(3): 163-176.
70. Segal ED, Cha J, Lo J, *et al.* Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by Helicobacter pylori. Proc Natl Acad Sci U S A 1999; 96(25): 14559-14564.
71. Suzuki M, Mimuro H, Suzuki T, *et al.* Interaction of CagA with Crk plays an important role in Helicobacter pylori-induced loss of gastric epithelial cell adhesion. The Journal of experimental medicine 2005; 202(9): 1235-1247.
72. Saadat I, Higashi H, Obuse C, *et al.* Helicobacter pylori CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. Nature 2007; 447(7142): 330-333.
73. Buti L, Ruiz-Puig C, Sangberg D, *et al.* CagA–ASPP2 complex mediates loss of cell polarity and favors H. pylori colonization of human gastric organoids. Proceedings of the National Academy of Sciences 2020; 117(5): 2645.
74. Buti L, Spooner E, Van der Veen AG, *et al.* Helicobacter pylori cytotoxin-associated gene A (CagA) subverts the apoptosis-stimulating protein of p53 (ASPP2) tumor suppressor pathway of the host. Proc Natl Acad Sci U S A 2011; 108(22): 9238-9243.
75. Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144(5): 646-74.
76. Choi SI, Yoon C, Park MR, *et al.* CDX1 Expression Induced by CagA-Expressing Helicobacter pylori Promotes Gastric Tumorigenesis. Mol Cancer Res 2019; 17(11): 2169-2183.
77. Terebiznik MR, Raju D, Vázquez CL, *et al.* Effect of Helicobacter pylori's vacuolating cytotoxin on the autophagy pathway in gastric epithelial cells. Autophagy 2009; 5(3): 370-379.

78. Yahiro K, Akazawa Y, Nakano M, *et al.* Helicobacter pylori VacA induces apoptosis by accumulation of connexin 43 in autophagic vesicles via a Rac1/ERK-dependent pathway. Cell Death Discovery 2015; 1(1): 15035.
79. Galmiche A, Rassow J, Doye A, *et al.* The N-terminal 34 kDa fragment of Helicobacter pylori vacuolating cytotoxin targets mitochondria and induces cytochrome c release. The EMBO journal 2000; 19(23): 6361-6370.
80. Akazawa Y, Isomoto H, Matsushima K, *et al.* Endoplasmic reticulum stress contributes to Helicobacter pylori VacA-induced apoptosis. PLoS One 2013; 8(12): e82322-e82322.
81. Galmiche A and Rassow J. Targeting of *Helicobacter pylori* VacA to mitochondria. Gut microbes 2010; 1(6): 392-395.
82. Sundrud MS, Torres VJ, Unutmaz D, *et al.* Inhibition of primary human T cell proliferation by Helicobacter pylori vacuolating toxin (VacA) is independent of VacA effects on IL-2 secretion. Proc Natl Acad Sci U S A 2004; 101(20): 7727-7732.
83. Torres VJ, VanCompernolle SE, Sundrud MS, *et al.* Helicobacter pylori : Vacuolating Cytotoxin Inhibits Activation-Induced Proliferation of Human T and B Lymphocyte Subsets. The Journal of Immunology 2007; 179(8): 5433.
84. Zheng P-Y and Jones NL. *Helicobacter pylori* strains expressing the vacuolating cytotoxin interrupt phagosome maturation in macrophages by recruiting and retaining TACO (coronin 1) protein. Cell Microbiol 2003; 5(1): 25-40.
85. Weiss G, Forster S, Irving A, *et al.* Helicobacter pylori VacA suppresses Lactobacillus acidophilus-induced interferon beta signaling in macrophages via alterations in the endocytic pathway. mBio 2013; 4(3): e00609-e612.
86. Abdullah M, Greenfield LK, Bronte-Tinkew D, *et al.* VacA promotes CagA accumulation in gastric epithelial cells during Helicobacter pylori infection. Sci Rep 2019; 9(1): 38.
87. Yang I, Woltemate S, Piazuelo MB, *et al.* Different gastric microbiota compositions in two human populations with high and low gastric cancer risk in Colombia. Sci Rep 2016; 6: 18594.
88. Bik EM, Eckburg PB, Gill SR, *et al.* Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci U S A 2006; 103(3): 732-7.
89. Llorca L, Pérez-Pérez G, Urruzuno P, *et al.* Characterization of the Gastric Microbiota in a Pediatric Population According to Helicobacter pylori Status. Pediatr Infect Dis J 2017; 36(2): 173-178.
90. Parsons BN, Ijaz UZ, D'Amore R, *et al.* Comparison of the human gastric microbiota in hypochlorhydric states arising as a result of Helicobacter pylori-induced atrophic gastritis, autoimmune atrophic gastritis and proton pump inhibitor use. PLOS Pathogens 2017; 13(11): e1006653.
91. Miao R, Wan C, and Wang Z. The relationship of gastric microbiota and Helicobacter pylori infection in pediatrics population. Helicobacter 2020; 25(1): e12676.
92. Gantuya B, El-Serag HB, Matsumoto T, *et al.* Gastric Microbiota in Helicobacter pylori-Negative and -Positive Gastritis Among High Incidence of Gastric Cancer Area. Cancers (Basel) 2019; 11(4).
93. Andersson AF, Lindberg M, Jakobsson H, *et al.* Comparative Analysis of Human Gut Microbiota by Barcoded Pyrosequencing. PLoS One 2008; 3(7): e2836.
94. Miftahussurur M, Waskito LA, El-Serag HB, *et al.* Gastric microbiota and Helicobacter pylori in Indonesian population. Helicobacter 2020; 25(4): e12695.
95. Mowat C, Williams C, Gillen D, *et al.* Omeprazole, Helicobacter pylori status, and alterations in the intragastric milieu facilitating bacterial N-nitrosation. Gastroenterology 2000; 119(2): 339-347.
96. Jo HJ, Kim J, Kim N, *et al.* Analysis of Gastric Microbiota by Pyrosequencing: Minor Role of Bacteria Other Than Helicobacter pylori in the Gastric Carcinogenesis. Helicobacter 2016; 21(5): 364-374.

97. Wang Z, Gao X, Zeng R, *et al.* Changes of the Gastric Mucosal Microbiome Associated With Histological Stages of Gastric Carcinogenesis. Front Microbiol 2020; 11: 997-997.
98. Coker OO, Dai Z, Nie Y, *et al.* Mucosal microbiome dysbiosis in gastric carcinogenesis. Gut 2018; 67(6): 1024-1032.
99. Castaño-Rodríguez N, Goh K-L, Fock KM, *et al.* Dysbiosis of the microbiome in gastric carcinogenesis. Sci Rep 2017; 7(1): 15957-15957.
100. Eun CS, Kim BK, Han DS, *et al.* Differences in Gastric Mucosal Microbiota Profiling in Patients with Chronic Gastritis, Intestinal Metaplasia, and Gastric Cancer Using Pyrosequencing Methods. Helicobacter 2014; 19(6): 407-416.
101. Liu J, Xue Y, and Zhou L. Detection of gastritis-associated pathogens by culturing of gastric juice and mucosa. Int J Clin Exp Pathol 2018; 11(4): 2214-2220.
102. Hu Y-L, Pang W, Huang Y, *et al.* The Gastric Microbiome Is Perturbed in Advanced Gastric Adenocarcinoma Identified Through Shotgun Metagenomics. Frontiers in cellular and infection microbiology 2018; 8: 433-433.
103. Sun JH, Li XL, Yin J, *et al.* A screening method for gastric cancer by oral microbiome detection. Oncol Rep 2018; 39(5): 2217-2224.
104. Aviles-Jimenez F, Vazquez-Jimenez F, Medrano-Guzman R, *et al.* Stomach microbiota composition varies between patients with non-atrophic gastritis and patients with intestinal type of gastric cancer. Sci Rep 2014; 4: 4202.
105. Caldas M, Pérez-Aisa Á, Castro-Fernández M, *et al.* European Registry on Helicobacter pylori Management: Effectiveness of First and Second-Line Treatment in Spain. Antibiotics 2021; 10(1).
106. Inatomi N, Matsukawa J, Sakurai Y, *et al.* Potassium-competitive acid blockers: Advanced therapeutic option for acid-related diseases. Pharmacol Ther 2016; 168: 12-22.
107. Ohkuma K, Iida H, Inoh Y, *et al.* Comparison of the early effects of vonoprazan, lansoprazole and famotidine on intragastric pH: a three-way crossover study. J Clin Biochem Nutr 2018; 63(1): 80-83.
108. Tanabe H, Yoshino K, Ando K, *et al.* Vonoprazan-based triple therapy is non-inferior to susceptibility-guided proton pump inhibitor-based triple therapy for Helicobacter pylori eradication. Ann Clin Microbiol Antimicrob 2018; 17(1): 29-29.
109. Liou J-M, Chen C-C, Chang C-M, *et al.* Long-term changes of gut microbiota, antibiotic resistance, and metabolic parameters after Helicobacter pylori eradication: a multicentre, open-label, randomised trial. The Lancet Infectious Diseases 2019; 19(10): 1109-1120.
110. Chen L, Xu W, Lee A, *et al.* The impact of Helicobacter pylori infection, eradication therapy and probiotic supplementation on gut microenvironment homeostasis: An open-label, randomized clinical trial. EBioMedicine 2018; 35: 87-96.
111. Reeves AE, Koenigsknecht MJ, Bergin IL, *et al.* Suppression of Clostridium difficile in the gastrointestinal tracts of germfree mice inoculated with a murine isolate from the family Lachnospiraceae. Infection and immunity 2012; 80(11): 3786-3794.
112. Oh B, Kim B-S, Kim JW, *et al.* The Effect of Probiotics on Gut Microbiota during the Helicobacter pylori Eradication: Randomized Controlled Trial. Helicobacter 2016; 21(3): 165-174.
113. Hsu P-I, Pan C-Y, Kao JY, *et al.* Short-term and long-term impacts of Helicobacter pylori eradication with reverse hybrid therapy on the gut microbiota. J Gastroenterol Hepatol 2019; 34(11): 1968-1976.
114. Martín-Núñez GM, Cornejo-Pareja I, Coin-Aragüez L, *et al.* H. pylori eradication with antibiotic treatment causes changes in glucose homeostasis related to modifications in the gut microbiota. PLoS One 2019; 14(3): e0213548-e0213548.

115. Olekhnovich EI, Manolov AI, Samoilov AE, *et al.* Shifts in the Human Gut Microbiota Structure Caused by Quadruple Helicobacter pylori Eradication Therapy. Front Microbiol 2019; 10: 1902-1902.
116. Kakiuchi T, Mizoe A, Yamamoto K, *et al.* Effect of probiotics during vonoprazan-containing triple therapy on gut microbiota in Helicobacter pylori infection: A randomized controlled trial. Helicobacter 2020; 25(3): e12690.
117. Martins AKS, Martins FS, Gomes DA, *et al.* Evaluation of in vitro antagonism and of in vivo immune modulation and protection against pathogenic experimental challenge of two probiotic strains of Bifidobacterium animalis var. lactis. Arch Microbiol 2010; 192(12): 995-1003.
118. Hill C, Guarner F, Reid G, *et al.* The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology & Hepatology 2014; 11(8): 506-514.
119. Cunningham M, Azcarate-Peril MA, Barnard A, *et al.* Shaping the future of probiotics and prebiotics. Trends Microbiol 2021; 29(8): 667-685.
120. Thye AY-K, Pusparajah P, Tan LT-H, *et al.* COVID-19: Gastrointestinal Manifestations and Complications. Prog Microbes Mol Biol 2021; 4(1).
121. Loh HC, Seah YK, and Looi I. The COVID-19 pandemic and diet change. Prog Microbes Mol Biol 2021; 4(1).
122. Thye AY-K, Tan LT-H, Law JW-F, *et al.* Long COVID-19: Psychological symptoms in COVID-19 and probiotics as an adjunct therapy. Prog Microbes Mol Biol 2022; 5(1).
123. Tan LT-H, Lee L-H, and Goh B-H. Critical review of fermentation and extraction of anti-Vibrio compounds from Streptomyces. Prog Microbes Mol Biol 2020; 3(1).
124. Tan LT-H, Lee L-H, and Goh B-H. The bioprospecting of anti-Vibrio Streptomyces species: Prevalence and applications. Prog Microbes Mol Biol 2019; 2(1).
125. Goh JXH, Tan LTH, Law JWF, *et al.* Harnessing the potentialities of probiotics, prebiotics, synbiotics, paraprobiotics, and postbiotics for shrimp farming. Reviews in Aquaculture 2022; 14(3): 1478-1557.
126. Law JW-F, Pusparajah P, Ab Mutalib N-S, *et al.* A review on mangrove actinobacterial diversity: the roles of Streptomyces and novel species discovery. Prog Microbes Mol Biol 2019; 2(1).
127. Ser H-L, Tan W-S, Yin W-F, *et al.* Whole genome sequence of MUM116, a Bacillus species isolated from intertidal soil. Prog Microbes Mol Biol 2020; 3(1).
128. Sgouras D, Maragkoudakis P, Petraki K, *et al.* In vitro and in vivo inhibition of *Helicobacter pylori* by Lactobacillus casei strain Shirota. Applied and environmental microbiology 2004; 70(1): 518-526.
129. Kim JE, Kim MS, Yoon YS, *et al.* Use of selected lactic acid bacteria in the eradication of *Helicobacter pylori* infection. J Microbiol 2014; 52(11): 955-62.
130. Boyanova L, Gergova G, Markovska R, *et al.* Bacteriocin-like inhibitory activities of seven Lactobacillus delbrueckii subsp. bulgaricus strains against antibiotic susceptible and resistant *Helicobacter pylori* strains. Lett Appl Microbiol 2017; 65(6): 469-474.
131. Zheng PX, Fang HY, Yang HB, *et al.* Lactobacillus pentosus strain LPS16 produces lactic acid, inhibiting multidrug-resistant *Helicobacter pylori.* J Microbiol Immunol Infect 2016; 49(2): 168-74.
132. Lim E-S .Purification and characterization of two bacteriocins from Lactobacillus brevis BK11 and Enterococcus faecalis BK61 showing anti-Helicobacter pylori activity. J Korean Soc Appl Biol Chem 2015; 58(5): 703-714.
133. Saracino IM, Pavoni M, Saccomanno L, *et al.* Antimicrobial Efficacy of Five Probiotic Strains Against Helicobacter pylori. Antibiotics (Basel, Switzerland) 2020; 9(5): 244.

134. Urrutia-Baca VH, Escamilla-García E, de la Garza-Ramos MA, *et al.* In Vitro Antimicrobial Activity and Downregulation of Virulence Gene Expression on Helicobacter pylori by Reuterin. Probiotics Antimicrob Proteins 2018; 10(2): 168-175.

135. Mukai T, Asasaka T, Sato E, *et al.* Inhibition of binding of Helicobacter pylori to the glycolipid receptors by probiotic Lactobacillus reuteri. FEMS Immunol Med Microbiol 2002; 32(2): 105-10.

136. Holz C, Busjahn A, Mehling H, *et al.* Significant Reduction in Helicobacter pylori Load in Humans with Non-viable Lactobacillus reuteri DSM17648: A Pilot Study. Probiotics and antimicrobial proteins 2015; 7(2): 91-100.

137. de Klerk N, Maudsdotter L, Gebreegziabher H, *et al.* Lactobacilli Reduce Helicobacter pylori Attachment to Host Gastric Epithelial Cells by Inhibiting Adhesion Gene Expression. Infection and immunity 2016; 84(5): 1526-1535.

138. Sakarya S and Gunay N Saccharomyces boulardii expresses neuraminidase activity selective for α2,3-linked sialic acid that decreases Helicobacter pylori adhesion to host cells. APMIS 2014; 122(10): 941-50.

139. Chen YH, Tsai WH, Wu HY, *et al.* Probiotic Lactobacillus spp. act Against Helicobacter pylori-induced Inflammation. J Clin Med 2019; 8(1).

140. Song H, Zhou L, Liu D, *et al.* Probiotic effect on Helicobacter pylori attachment and inhibition of inflammation in human gastric epithelial cells. Exp Ther Med 2019; 18(3): 1551-1562.

141. Yu HJ, Liu W, Chang Z, *et al.* Probiotic BIFICO cocktail ameliorates Helicobacter pylori induced gastritis. World J Gastroenterol 2015; 21(21): 6561-6571.

142. Poonyam P, Chotivitayatarakorn P, and Vilaichone RK High Effective of 14-Day High-Dose PPI- Bismuth-Containing Quadruple Therapy with Probiotics Supplement for Helicobacter Pylori Eradication: A Double Blinded-Randomized Placebo-Controlled Study. Asian Pac J Cancer Prev 2019; 20(9): 2859-2864.

143. Francavilla R, Polimeno L, Demichina A, *et al.* Lactobacillus reuteri strain combination in Helicobacter pylori infection: a randomized, double-blind, placebo-controlled study. J Clin Gastroenterol 2014; 48(5): 407-13.

144. Nakae H, Tsuda A, Matsuoka T, *et al.* Gastric microbiota in the functional dyspepsia patients treated with probiotic yogurt. BMJ Open Gastroenterology 2016; 3(1): e000109.

145. Jiang L and Zhu W Probiotics improved the effectiveness and safety of the quadruple Helicobacter pylori eradication therapy. Biomedical Research 2018; 29(10).

146. Çekin AH, Şahintürk Y, Akbay Harmandar F, *et al.* Use of probiotics as an adjuvant to sequential H. pylori eradication therapy: impact on eradication rates, treatment resistance, treatment-related side effects, and patient compliance. Turk J Gastroenterol 2017; 28(1): 3-11.

147. Seddik H, Boutallaka H, Elkoti I, *et al.* Saccharomyces boulardii CNCM I-745 plus sequential therapy for Helicobacter pylori infections: a randomized, open-label trial. Eur J Clin Pharmacol 2019; 75(5): 639-645.

148. He CX, Kong FT, Liang F, *et al.* Influence of different timing of Saccharomyces boulardii combined with bismuth quadruple therapy for Helicobacter pylori eradication. Zhonghua Yi Xue Za Zhi 2019; 99(22): 1731-1734.

149. Cárdenas PA, Garcés D, Prado-Vivar B, *et al.* Effect of Saccharomyces boulardii CNCM I-745 as complementary treatment of Helicobacter pylori infection on gut microbiome. Eur J Clin Microbiol Infect Dis 2020; 39(7): 1365-1372.

150. Mukai R, Handa O, Suyama Y, *et al.* Effectiveness of including probiotics to Helicobacter pylori eradication therapies. J Clin Biochem Nutr 2020; 67(1): 102-104.

151. Tang B, Tang L, Huang C, *et al.* The Effect of Probiotics Supplementation on Gut Microbiota After Helicobacter pylori Eradication: A Multicenter Randomized Controlled Trial. Infectious Diseases and Therapy 2021; 10(1): 317-333.

152. Bartley A, Yang T, Arocha R, *et al.* Increased Abundance of Lactobacillales in the Colon of Beta-Adrenergic Receptor Knock Out Mouse Is Associated With Increased Gut Bacterial Production of Short Chain Fatty Acids and Reduced IL17 Expression in Circulating CD4(+) Immune Cells. Front Physiol 2018; 9: 1593.
153. Del Chierico F, Abbatini F, Russo A, *et al.* Gut Microbiota Markers in Obese Adolescent and Adult Patients: Age-Dependent Differential Patterns. Front Microbiol 2018; 9: 1210-1210.
154. Astbury S, Atallah E, Vijay A, *et al.* Lower gut microbiome diversity and higher abundance of proinflammatory genus Collinsella are associated with biopsy-proven nonalcoholic steatohepatitis. Gut Microbes 2020; 11(3): 569-580.
155. Jung JH, Cho IK, Lee CH, *et al.* Clinical Outcomes of Standard Triple Therapy Plus Probiotics or Concomitant Therapy for Helicobacter pylori Infection. Gut and liver 2018; 12(2): 165-172.
156. Swanson KS, Gibson GR, Hutkins R, *et al.* The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. Nature Reviews Gastroenterology & Hepatology 2020; 17(11): 687-701.
157. N Şirvan B, K Usta M, U Kizilkan N, *et al.* Are Synbiotics added to the Standard Therapy to eradicate Helicobacter pylori in Children Beneficial? A Randomized Controlled Study. Euroasian journal of hepato-gastroenterology 2017; 7(1): 17-22.
158. Ustundag GH, Altuntas H, Soysal YD, *et al.* The Effects of Synbiotic "Bifidobacterium lactis B94 plus Inulin" Addition on Standard Triple Therapy of Helicobacter pylori Eradication in Children. Can J Gastroenterol Hepatol 2017; 2017: 8130596-8130596.
159. Islek A, Sayar E, Yilmaz A, *et al.* Bifidobacterium lactis B94 plus inulin for Treatment of Helicobacter pylori infection in children: does it increase eradication rate and patient compliance? Acta Gastroenterol Belg 2015; 78(3): 282-6.
160. Aroniadis OC and Brandt LJ Intestinal microbiota and the efficacy of fecal microbiota transplantation in gastrointestinal disease. Gastroenterol Hepatol (N Y) 2014; 10(4): 230-237.
161. Zhang T, Lu G, Zhao Z, *et al.* Washed microbiota transplantation vs. manual fecal microbiota transplantation: clinical findings, animal studies and in vitro screening. Protein Cell 2020; 11(4): 251-266.
162. Ye Z-N, Xia HH-X, Zhang R, *et al.* The Efficacy of Washed Microbiota Transplantation on Helicobacter pylori Eradication: A Pilot Study. Gastroenterol Res Pract 2020; 2020: 8825189.