**Review Article**

**Treating bacteria with bacteria: the role of probiotics in the eradication of Helicobacter pylori**

Shanyan Zhang, Jianqiang Guo, Lan Liu

*Department of Gastroenterology, The Second Hospital of Shandong University, Jinan, Peoples Republic of China*

Received October 27, 2016; Accepted November 23, 2016; Epub March 15, 2017; Published March 30, 2017

**Abstract:** Helicobacter pylori (H. pylori), with more than 50% global population infection, is an etiology of chronic gastritis and peptic ulcer and a risk factor for gastric malignancies. Due to the increase of H. pylori-resistant strains and treatment-related adverse effects, the success rate of eradication therapies has declined to 70%, far below the initial rate of 90%. To improve the eradication rate, researchers have started to look into alternative treatments for H. pylori, one of which is probiotics. In this article, we elaborated the mechanisms of action of probiotics against H. pylori infection into four aspects: immunomodulation, antimicrobial substances, interfering with adhesion, and mucosal barrier. Although probiotics are varied, we only conducted studies on Lactobacillus, Bifidobacterium, and yeasts such as Saccharomyces boulardii most commonly used in the therapy. The effects of Lactobacillus, Bifidobacterium, and Saccharomyces boulardii as monotherapy or adjuvant therapy combined with antibiotic-PPI for H. pylori eradication in children and adults showed diverse outcomes in eradication rate and adverse effects. Extensive studies indicated probiotics plays an important role in H. pylori infection. However, many uncertain problems still need to be considered in the clinical application of probiotics.

**Keywords:** Helicobacter pylori, mechanism, Lactobacillus, Bifidobacterium, Saccharomyces boulardii, eradication rate, adverse effects

**Introduction**

Helicobacter pylori (H. pylori) are a group of highly prevalent human pathogens infecting more than half of the global population [1]. The prevalence of the bacteria strongly differs between developing and developed countries, where prevalence rate in adults is approximately 60-80% and <40%, respectively. Individuals with a low socioeconomic status, poor sanitary living condition and lower educational level have been associated with a high risk of H. pylori infection [2]. Long-term H. pylori infection leads to a diverse spectrum of gastrointestinal disorders including chronic gastritis, peptic ulcer, gastric malignancies and gastric mucosa-associated lymphoid tissue lymphoma [3]. Moreover, evidence has been presented linking H. pylori to the aetiology of several extragastrointestinal diseases, such as unexplained iron-deficiency anaemia, idiopathic thrombocytopenic purpura, and vitamin B\textsubscript{12} deficiency [4]. Once infection is acquired, spontaneous clearance is relatively rare. Thus, eradication of H. pylori is an effective measure to reduce the risk of diseases and, to some extent, provides a feasible method for treatment of diseases.

Triple therapy, consisting of a proton pump inhibitor (PPI), amoxicillin, and clarithromycin or metronidazole, was proposed at the first Maastricht conference in 1996 and has been widely used for twenty years, although an increasing number of data have shown that this combination treatment has lost some efficacy and provides an unsatisfactory result, with the eradication rate only achieving 70%, far lower than the rate at the beginning [5]. The decline of the eradication rate is mainly due to the increase in antibiotic resistance especially of clarithromycin and metronidazole. High incidence of treatment-related adverse effects is another important cause, resulting in the low compliance of patients and, consequently, incomplete therapy. The latest Maastricht V/Florence consensus reportin 2016 recommended the bismuth-containing quadruple therapy as a first-line empirical treatment in areas of high clarithromycin...
Mechanism and clinical trials of probiotics against *H. pylori* infection

resistance and an alternative treatment in areas of low clarithromycin resistance. Sequential therapy, concomitant therapy (non-bismuth quadruple therapy), and levofloxacin containing triple therapy were proposed under different situations in the report [4]. Despite these efforts, however, considering increasing antibiotic resistance, frequent adverse effects, vast medical expense and complicated dosing regimens, some patients are disinclined to accept and complete treatment in the clinic.

In light of current unideal therapeutic regimens, researchers have begun to search for new alternative therapies. One potential alternative therapy is probiotics. Probiotics are defined as live microorganism that, when administered in adequate amount, confer a health benefit on the host [6]. A wide variety of probiotics have been found in the current stage and some were used in food, dietary supplements, and drugs. Effect of each probiotic strain is specific and dose-dependent. Additionally, certain strains can imitate commensals playing an important role in the digestive system [7]. Evolving evidence presented probiotics have significant effects in alleviating the symptoms of several diseases, such as acute diarrhea, antibiotic-associated diarrhea, functional gastrointestinal disorders, and inflammatory bowel disease [8]. Much research has been performed with the genera *Lactobacillus*, *Bifidobacterium*, and yeasts such as *Saccharomyces boulardii* (S. boulardii) to explore the interaction between *H. pylori* and probiotics, both in vivo and in vitro. Albeit some researchers showed no obvious effects of probiotics on *H. pylori* infections, promising results in a large portion of cell experiments, animal studies and human trials have been proven [9-11]. In this review, we introduced the microbiota that commonly interact with *H. pylori* in the stomach, summarized the possible mechanisms of action of probiotics against *H. pylori* infection, and reviewed the outcomes of studies that used probiotics as monotherapy or adjuvant therapy in eradication protocols. In the end, we discussed the challenges and opportunities for probiotics in clinical application.

**The human gastric microbiota and *H. pylori***

Previously, the stomach was long-considered sterile, mainly owing to its acid milieu and past limited technologies [9]. Since the discovery of *H. pylori* in 1983, people began to break the inherent thinking and make further efforts to investigate the gastric microbiota. At first, using culture-independent methods of analysis, *Clostridium* sp, *Lactobacillus* sp and *Veillonella* sp were the most represented bacteria of the human stomach under healthy conditions. Notwithstanding, approximately 80% of microbes are not cultivable [12]. To date, in virtue of the recent development of new nucleotide sequencing techniques and advanced bioinformatic tools, the diversity and complexity of the gastric microbiota are obtained further comprehensive research. A diverse community of 128 phylotypes was identified in the stomach by using 16S rDNA sequence analysis, majority of which derive from the phyla *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Fusobacteria* [13]. Comparing to other parts of the gastrointestinal tract, the microbial load in stomach is lower, whereas an increasing number of studies showed the disruption of gastric microbiota has been associated with different diseases of the stomach, like atrophic gastritis, peptic ulcer and gastric cancer, hence, maintaining these floras in balance is essential to the health of host [14, 15].

Once infected by *H. pylori*, the stomach becomes a reservoir for *H. pylori*. Long term of *H. pylori* infection may change the composition of gastric microbiota. Andersson [16] analyzed the structure of the bacterial community in the stomach of patients of differing *H. pylori* status, which corroborated the finding that the stomach displays a diverse microbiota when *H. pylori* is absent or low in abundance. Another study further showed that positive *H. pylori* status is associated with increased relative abundance of non-*Helicobacter* bacteria from the *Proteobacteria*, *Spirochetes*, and *Acidobacteria* phyla and decreased abundance of *Actinobacteria*, *Bacteroidetes*, and *Firmicutes* [17]. With respect to the study of microbial composition of gastric mucosa from the patients with chronic gastritis, intestinal metaplasia, and gastric cancer, Eun [18] found marked differences that in gastric cancer group, the relative abundance of *Helicobacteraceae* was significantly lower than that of chronic gastritis and intestinal metaplasia, whereas the relative abundance of *Streptococcaceae* was increased.

**Mechanism of action of probiotics against *H. pylori* infection**

*H. pylori* infection, in most cases, can persist lifelong in its host in the absence of eradication therapy, because it is capable of adaptations to
Mechanism and clinical trials of probiotics against *H. pylori* infection

**Table 1. Immunomodulation mechanism**

<table>
<thead>
<tr>
<th>Author</th>
<th>Probiotic</th>
<th>Type of experiment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhou et al [23]</td>
<td><em>L. bulgaricus</em></td>
<td>Cell (SGC-7901)</td>
<td>IL-8 expression ↓</td>
</tr>
<tr>
<td>Yang et al [24]</td>
<td><em>L. acidophilus</em></td>
<td>Cell (MKN45, AGS)</td>
<td>IL-8 expression ↓</td>
</tr>
<tr>
<td>Sunanligano et al [25]</td>
<td><em>L. plantarum</em> B7</td>
<td>Animal (SD rats)</td>
<td>TNF-α, gastric MDA level, epithelial cell apoptosis and gastric inflammation ↓</td>
</tr>
<tr>
<td>Yu et al [26]</td>
<td>BIFICO†</td>
<td>Animal (C57BL/6 female mice)</td>
<td>TNF-α, IL-6, IL-1β, IL-10, G-CSF and MIP-2 expression ↓</td>
</tr>
</tbody>
</table>

†: a mixture of lactobacillus acidophilus, enterococcus faecalis and Bifidobacterium longum.

Colonize the special environment of the stomach [19]. Urease produced by *H. pylori* hydrolyzes urea to ammonia and carbon dioxide, neutralizing the pH, which allows the bacterial survival. The presence of flagella facilitates *H. pylori* penetrating into the mucus layer and reaching the gastric epithelium. As soon as *H. pylori* colonize the gastric epithelium, the host’s innate and adaptive immune systems are activated, followed by a series of inflammatory and immunological response [20]. Persistent chronic inflammation induced by *H. pylori* may progress to atrophy, metaplasia, dysplasia, and even gastric cancer, therefore alleviating gastric mucosa inflammation is of great importance for health [21].

Substantial studies have indicated probiotics play an important role in fighting against *H. pylori* infection and in this section; we will elucidate the mechanisms of action of probiotics against *H. pylori* infection into four aspects, namely, immunomodulation, antimicrobial substances, interfering with adhesion, and mucosal barrier.

**Immunomodulation mechanism**

In the process of *H. pylori* infection, the activation of the immune system can lead to the recruitment of a wide variety of inflammatory cells and mediators, and the activation of nuclear factor-κB (NF-κB) and pro- and anti-inflammatory cytokines [20]. In comparison to the uninfected ones, some cytokines such as TNF-α, IFN-γ, IL-6, IL-7, IL-8, IL-10, IL-17, and IL-18 have generally increased levels in the stomach of *H. pylori*-infected patients (Table 1). In addition, the *H. pylori*-specific CD4+ T cells only can be detectable in peripheral blood and gastric mucosa of infected humans [22].

Probiotics have the potential to dampen cytokine reaction triggered by *H. pylori* [9]. Currently extensive studies are focused on the genera *Lactobacillus*, *L. bulgaricus*, when co-cultured with SGC-7901 cells treated with *Helicobacter pylori* Sydney strain 1 lipopolysaccharide (*H. pylori* SS1-LPS), enable the expression of TLR4 to attenuate, and subsequently inhibit the phosphorylation of TAK1 and p38MAPK, prevent the activation of NF-κB, and consequently block IL-8 production [23]. Yang [24] demonstrated that *L. acidophilus* reduced *H. pylori*-induced IL-8 expressions and inflammation through the inactivation of the Smad7 and NF-κB pathways in vitro study. To determined the anti-*H. pylori* property of *L. plantarum* B7, Sunanliganon [25] performed an experiment which researched effects of *L. plantarum* B7 on the serum TNF-α, gastric malondialdehyde (MDA) level, apoptosis, and histopathology in gastric inflammation induced by *H. pylori* in rats, and results indicated that the group receiving *L. plantarum* B7 treatment had a significant decrease in serum TNF-α level compared with *H. pylori*-infected group. Furthermore, *L. plantarum* B7 treatments resulted in a significant improvement in stomach pathology, and decreased gastric MDA level and apoptotic epithelial cells. BIFICO, which contain a mixture of the viable bacteria *Enterococcus faecalis*, *Bifidobacterium longum*, and *L. acidophilus*, were proven to ameliorate *H. pylori*-induced gastritis by inhibiting the inflammatory response in gastric epithelial cells. In this study, BIFICO significantly inhibited *H. pylori*-induced NF-κB and MAPK signaling pathway, and decreased the expression of TNF-α, IL-1β, IL-10, IL-6, G-CSF and MIP-2 [26].

**Antimicrobial substances mechanism**

Probiotics may inhibit the growth of *H. pylori* by secreting antimicrobial substances. Short chain fatty acids (SCFAs) and bacteriocins are two main types of substances associated with inhibition of *H. pylori*. SCFAs, the final catabolites of energy metabolism, include butyrate, propionate, acetate, and lactic acid [27]. The
Mechanism and clinical trials of probiotics against \textit{H. pylori} infection

production of relatively large amount of lactic acid by \textit{Lactobacillus} has been implicated as an inhibitory factor by many researchers (Table 2). Bhatia [28] first proposed that the inhibitory action of \textit{L. acidophilus} on \textit{H. pylori} is dependent on an extracellular secretory product, probably lactic acid. Furthermore, \textit{L. acidophilus} LB, \textit{L. johnsonii} La 1, \textit{L. salivarius} and \textit{L. casei} have been demonstrated to exert an inhibitory effect on \textit{H. pylori} by lactic acid production [29, 30]. Some antimicrobial activity, in addition to lactic acid production, could exert effects through inhibiting urease activity, as found in the \textit{L. casei} Shirota and \textit{L. salivarius ssp. salicinius AP-32} [31, 32].

Bacteriocins, proteinaceous molecules synthesized at the ribosomal level, are capable to interfere with the growth of most bacteria, including \textit{H. pylori} [33]. \textit{Wissella confusa}, \textit{L. lactis}, and \textit{Bcillus subtilis} were shown to secrete bacteriocins able to inhibit \textit{H. pylori} growth in vitro [29]. Certain probiotic strain could secrete some special substances with an inhibitory effects on \textit{H. pylori}. Like reuterina, a metabolite produced by \textit{L. reuteri} ATCC 55730, effectively suppressed \textit{H. pylori} infection in humans and reduced \textit{H. pylori} load [34]. \textit{L. acidophilus} CRL 639, in mixed cultures with \textit{H. pylori} after 24 h, showed an autolytic behavior, and \textit{H. pylori} simultaneously decreased, subsequently vanished after 48 h. On the basis of the research, Lorca speculated that after cell lysis, bacteria may release a proteinaceous compound named autolysins suppressing the growth of \textit{H. pylori} [35].

\textbf{Interfering with adhesion mechanism}

Adhesion of \textit{H. pylori} to the gastric epithelium, a crucial step in the \textit{H. pylori} infection, indicates the beginning of \textit{H. pylori} establishing colonization, further interacting with epithelial cells, manipulating the cellular processes and functions, and consequently, influencing outcomes of associated diseases [20]. Probiotics may inhibit adhesion of \textit{H. pylori} through directly competing for adhesion sites, or changing the structure of adhesion sites, resulting in a failure of adhesion. \textit{Like Weissella confusa} strain PL9001 is capable of interfering \textit{H. pylori} colonization by competing for adhesion sites [36]. \textit{L. reueri} strains JCM 1081 and TM 105 show the ability to bind to asialo-GM1 and sulfatide which are putative glycolipid receptor molecules of \textit{H. pylori}, and inhibit binding of \textit{H. pylori} to both glycolipids [37]. Adhesion properties of \textit{H. pylori} to various structures have been described in the past, including evidence for sialic acid-binding [38]. Recently, Sakarya [39] discovered the neuraminidase activity expressed by \textit{S. boulardii} could remove surface α-2,3-linked sialic acid, ligand for the sialic acid-binding \textit{H. pylori} adhesin, which in turn, inhibit \textit{H. pylori} adherence to duodenal epithelial cells (Table 3). Certain strainssuch as \textit{L. acidophilus} LB and \textit{L. johnsonii} La1 can exert

### Table 2. Antimicrobial substances mechanism

<table>
<thead>
<tr>
<th>Author</th>
<th>Probiotic strain</th>
<th>Mechanism of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhatia et al [28]</td>
<td>\textit{L. acidophilus}</td>
<td>Lactic acid</td>
</tr>
<tr>
<td>Lesbros-Pantoflickova et al [29]</td>
<td>\textit{L. acidophilus} \textit{L. johnsonii} La 1</td>
<td>Lactic acid</td>
</tr>
<tr>
<td>Alba et al [30]</td>
<td>\textit{L. salivarius}</td>
<td>Lactic acid</td>
</tr>
<tr>
<td>Sgouras et al [31]</td>
<td>\textit{L. casei} Shitoya</td>
<td>Lactic acid, suppress the urease activity</td>
</tr>
<tr>
<td>Hsieh et al [32]</td>
<td>\textit{L. salivarius} ssp. salicinius AP-32</td>
<td>Suppress the urease activity</td>
</tr>
<tr>
<td>Lesbros-Pantoflickova et al [29]</td>
<td>\textit{Wissella confusa}, \textit{L. lactis}, and \textit{Bcillus subtilis}</td>
<td>Bacteriocins</td>
</tr>
<tr>
<td>Francavilla et al [34]</td>
<td>\textit{L. reuteri} ATCC 55730</td>
<td>Reuterina</td>
</tr>
<tr>
<td>Lorca et al [35]</td>
<td>\textit{L. acidophilus} CRL 639</td>
<td>Autolysins</td>
</tr>
</tbody>
</table>

### Table 3. Interfering with adhesion mechanism

<table>
<thead>
<tr>
<th>Author</th>
<th>Probiotic strain</th>
<th>Mechanism of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nam et al [36]</td>
<td>\textit{Weissella confusa} strain PL9001</td>
<td>Compete for adhesion sites</td>
</tr>
<tr>
<td>Mukai et al [37]</td>
<td>\textit{L. reueri} strains JCM 1081 and TM 105</td>
<td>Compete for adhesion sites</td>
</tr>
<tr>
<td>Sakarya et al [39]</td>
<td>\textit{Saccharomyces boulardii}</td>
<td>Change structure of adhesion sites</td>
</tr>
<tr>
<td>Lesbros-Pantoflickova et al [33]</td>
<td>\textit{L. acidophilus} \textit{L. johnsonii} La1</td>
<td>Secrete antimicrobial substances</td>
</tr>
</tbody>
</table>
Mechanism and clinical trials of probiotics against *H. pylori* infection

**Table 4. Mucosal barrier mechanism**

<table>
<thead>
<tr>
<th>Author</th>
<th>Probiotic strain</th>
<th>Type of experiment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pantoflickova et al [40]</td>
<td><em>L. johnsonii</em></td>
<td>Human (H. pylori positive adults)</td>
<td>Increase mucus thickness, reduce the degree of gastric inflammation</td>
</tr>
<tr>
<td>Mack et al [43]</td>
<td><em>L. plantarum</em> 299v, and <em>L. rhamnosus</em> GG</td>
<td>Cell (HT29)</td>
<td>Increase MUC2 and MUC3 genes expression and extracellular secretion of mucin by colon cell cultures</td>
</tr>
<tr>
<td>Gomi et al [44]</td>
<td><em>Bifidobacterium bifidum</em> BF-1</td>
<td>Animal (rats)</td>
<td>Enhance MUC2AC gene expression</td>
</tr>
</tbody>
</table>

**Table 5. Probiotics as a monotherapy**

<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects</th>
<th>Diagnosis</th>
<th>Probiotic strains</th>
<th>Study design</th>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
</table>
| Cruchet et al [45]      | 252 *H. pylori*-positive, asymptomatic children | 13C-UBT                    | *L. johnsonii* La1, *L. paracasei* ST11 | R, DB, PC                             | Group 1: a product containing live La1                               | Group 1: A significant difference in Δ DOB (-7.64%; 95% CI: -14.23 to -1.03%)
|                         |                               |                           |                                 |                                       | Group 2: heat-killed La1                                              | Other groups: no differences                                           |
|                         |                               |                           |                                 |                                       | Group 3: a product containing live ST11                                |                                                                        |
|                         |                               |                           |                                 |                                       | Group 4: heat-killed ST11                                              |                                                                        |
|                         |                               |                           |                                 |                                       | Group 5: vehicle                                                      |                                                                        |
|                         |                               |                           |                                 |                                       | Group Ab: antibiotic treatment1                                       | Group Ab: A moderate but significant difference in Δ DOB (-26.6%; 95% CI: -33.9 to -19.3%)
|                         |                               |                           |                                 |                                       | Group Sbi: *S. boulardii* plus inulin                                 | Group Sbi: A moderate but significant difference in Δ DOB (-6.3%; 95% CI: -6.3 to -0.8%)
|                         |                               |                           |                                 |                                       | Group LB: LB                                                         | Group LB: no significant difference in Δ DOB (0.7%; 95% CI: -5.8 to +7.2%)
|                         |                               |                           |                                 |                                       | Eradication rate: 66% vs. 6.5% vs. 12% (P=0.001)                      |                                                                        |
| Gotteland et al [46]    | 254 *H. pylori*-positive, a-symptomatic children | 13C-UBT                    | *L. acidophilus* LB, *S. boulardii* | R, O                                  | Group 1: antibiotic treatment1                                        | In eradication study: eradication rate: 29.3% vs. 0% (P=0.038) in the prevention study: *H. pylori* infection rate: 4.1% vs. 8.1% (no statistically significant difference)
|                         |                               |                           |                                 |                                       | Group 2: placebo                                                     |                                                                        |
|                         |                               |                           |                                 |                                       | Group 2: placebo subsequent treatment was administered subsequently   |                                                                        |
| Boonyaritichai- kij et al [47] | 440 children (132 *H. pylori*-positive and 308 *H. pylori*-negative) | HpSA                       | *L. gasseri* DOL2716 (LG21)       | SB, PC, DB                             | Group 1: eradication and prevention arms                              | Group 1: no significant difference in Δ DOB (-2.0‰; 95% CI: -5.8 to +1.8‰)
|                         |                               |                           |                                 |                                       | Group 2: placebo                                                     | Group 2: A significant decrease in Δ DOB (2.0‰; 95% CI: 0.0 to 4.0‰)
|                         |                               |                           |                                 |                                       | Inactive group: placebo                                               |                                                                        |
| Francavilla et al [34]  | 40 *H. pylori*-positive, dyspeptic patients | HpSA, RUT, histopathology | *L. reuteri* ATCC 55730           | DB, PC, PC                             | Group 1: *L. reuteri* 4 w                                              | In eradication study: eradication rate: 10.5% vs. 0% (P=0.048) in the prevention study: *H. pylori* infection rate: 11.8% vs. 3.5% (no statistically significant difference)
|                         |                               |                           |                                 |                                       | Group 2: placebo                                                     |                                                                        |
|                         |                               |                           |                                 |                                       | Sequential treatment was administered subsequently                     |                                                                        |
| Gotti et al [48]       | 12 *H. pylori*-positive, asymptomatic adults | 13C-UBT                    | *L. johnsonii* La1               | O                                     | Compare DOB value after 1 w and 2 w of ingesting the product          | Twice DOB ↓
|                         |                               |                           |                                 |                                       | Group 1: a significant decrease in Δ DOB after 2 w (27.39%; 95% CI: 16.24-38.54%; P=0.043) |
| Sakamoto et al [49]    | 31 *H. pylori*-positive adults | serology 13C-UBT           | *L. gasseri* DOL2716             | O                                     | In the first part: yogurt 8 w                                          | At 0 w and 9 w: no significant decrease in DOB values (P=0.043) |
|                         |                               |                           |                                 |                                       | In the second phase: yogurt containing LG21 8 w                       |                                                                        |
|                         |                               |                           |                                 |                                       | At 18 w: a significant decrease in DOB values earlier weeks           |                                                                        |
| Miki et al [50]        | 80 healthy adults             | 13C-UBT                    | *B. bifidum* YIT4007             | R, DB, PC                             | Group 1: BF-1 beverage                                               | Group 1: a significant decrease in Δ DOB compared to placebo (P=0.027) |
|                         |                               |                           |                                 |                                       | Group 2: placebo                                                     |                                                                        |
| Myllyluoma et al [60]   | 13 adults (7 *H. pylori*-positive and 6 *H. pylori*-negative) | 13C-UBT, histopathology   | *L. rhamnosus* GG, *L. rhamnosus* LC705, *Propionibacterium freud- enreichii* JS and *B. lactis* BB12 | O                                     | Compare *H. pylori* colonization and the degree of gastric inflammation | DOB value, inflammation activity ↓ (P=0.063) |
| Wang et al [61]        | 70 *H. pylori*-positive, asymptomatic adults | 13C-UBT, serology          | Lactobacillus- and *Bifidobacterium*-containing AB-yogurt                  | PC                                    | Group 1: a bottle of AB-yogurt                                      | Group 1: significant differences in the result of 13C-UBT values between 0 and 4 w, and between 0 w and 8 w (36.2±19.4 compared with 30.1±19.6 and 36.2±29.4 compared with 28.2±15.8, P<0.05) antral biopsies showed *H. pylori* density was reduced (P=0.015) |

O: open; R: randomized; DB: double-blind; SB: single-blind; PC: placebo controlled; RUT: rapid urease test; HpSA: *H. pylori* stool antigens; w: week; d: days; antibiotic treatment: lansoprazole 1 mg/kg, bid, amoxicillin, 50 mg/kg, bid, clarithromycin 15 mg/kg, bid.
Mechanism and clinical trials of probiotics against *H. pylori* infection

their anti-adhesion activity by secreting antimicrobial substances [29].

*Mucosal barrier mechanism*

Gastric mucosal barrier, consisting of a compact epithelial cell line, a special mucus covering, and foveolar cells, represents a first line of defense against pathogenic bacteria. If the barrier is broken, acid will diffuse back into the mucosa where it can cause damage to the stomach. *H. pylori* virulence factors, such as cytoxin associated gene A (Cag A) and vacuolating cytoxin (VacA) play a major role in gastric mucosa damage [3]. Mucus, a protective gel-like coat over the surface of the gastric mucosa, protects mucosa from the invasion of bacteria. However, reduced mucus secretion in a damaged or proliferating epithelium is frequently found in *H. pylori* associated gastritis [29]. A research evaluated the effect of *L. johnsonii* intake on *H. pylori* gastritis which found regular ingestion of fermented milk containing *L. johnsonii* has an association with increase of mucus thickness [40]. Mucin, the key viscoelastic component of gastric mucus, is pH dependent, transforming from a viscous solution at neutral pH to a gel in acidic conditions [41]. In the gastric mucosa, MUC1, MUC5AC and MUC6 are three main mucin expression type [42]. *H. pylori* is known to suppress MUC5AC and MUC1 gene expression in the human gastric cell line [33]. Some probiotic strains are able to regulate mucin expression, strengthening the gastric mucosal barrier, and protect it against the adhesion of *H. pylori*. In vitro studies have shown that *L. plantarum* strain 299v and *L. rhamnosus* GG increased the expression of MUC2 and MUC3 genes and the subsequent extracellular secretion of mucin by colon cell cultures [43]. Gomi [44] indicated that *Bifidobacterium bifidum* BF-1 *B. bifidum* BF-1) has the potential to provide gastric mucosal protection by enhancing MUC5AC gene expression in an acute gastric injury rat model (Table 4).

**Clinical trials with probiotics in *H. pylori* eradication**

In this section, abundant trials and meta-analyses are introduced to provide a comprehensive description, and we will evaluate the effects of probiotics as treatment on *H. pylori*, through single use or in combination with antibiotic-PPI in children and adults in accordance with diverse genera.

**Probiotics as a monotherapy in treatment**

On account of side effects of antibiotics, single use of probiotics to eradicate *H. pylori* can provide a safer therapy for children, and numerous scholars performed studies on it (Table 5). In a double-blind, randomized, controlled clinical trial in Santiago [45], 252 asymptomatic children who were screened as *H. pylori* positive by the 13C-urea breath test (13C-UBT) were distributed into five groups. Each group, by turns, received a product containing live *L. johnsonii* La1, heat-killed *L. johnsonii* La1, live *L. Paracasei* ST11, heat-killed *L. paracasei* ST11, and a vehicle every day for 4 wk. At the end of the study period, a moderate but significant difference in Δ DOB (δ13CO2 values above baseline values before and after treatments) was detected in children receiving live *L. johnsonii* La1, whereas no differences were observed in the other groups. Gotteland [46] conducted a similar study, in which he evaluated the effect of *S. boulardii* plus inulin (Sbl) or *L. acidophilus* LB (LB) on *H. pylori* colonization in children, and used standard triple therapy as contrast, demonstrating a moderate but significant decrease in DOB (excess δ13CO2 values above baseline) values in Sbl group, however, compared with the 66% eradication rate of the standard triple therapy group, the eradication rates of the Sbl and LB groups were extremely low, at about 12% and 6.5%, respectively. Another study used *H. pylori* stool antigen test to detect *H. pylori* and investigated the effects of long-term administration of *L. gasseri* OLL2716 (LG21) strain in the *H. pylori* eradication rate in asymptomatic pre-school children [47]. After regular ingestion of cheese containing LG21 for a year, in eradication study, eradication was found in 24 of 82 subjects (eradication ratio 29.3%; 95% CI: 19.4-39.1%) in the active group, whereas no eradication was observed in the placebo group. In prevention study, rates of *H. pylori* infection were 4.1% and 8.1% in the active and placebo groups, respectively. Above studies indicate that not all probiotics have effective role on treatment of *H. pylori*, such as *L. paracasei* ST11, in addition, certain probiotics may diminish the bacterial load, but are unable to completely eradicate *H. pylori* in children.

Many probiotics, such as *L. johnsonii* La1 [48], *L. gasseri* OLL 2716 [49], *L. reuteri* ATCC 55730
## Table 6. Probiotics as adjuvant therapy combined with antibiotics-PPI in *H. pylori* eradication

<table>
<thead>
<tr>
<th>Author</th>
<th>Study design</th>
<th>Patients</th>
<th>Assessment tools</th>
<th>Probiotics strains</th>
<th>Eradication therapy</th>
<th>Results (probiotics vs. placebo)</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Du et al [52]</td>
<td>R, O</td>
<td>234 <em>H. pylori</em>-positive gastritic adults</td>
<td>RUT, histopathology, 13C-UBT/14C-UBT</td>
<td><em>L. acidophilus</em></td>
<td>Omeprazole 20 mg bid, clarithromycin 500 mg bid, amoxicillin 1000 mg bid</td>
<td>Eradication rate POCA:OCA=79.5%:60.8% P&lt;0.014</td>
<td>Symptom relieving rate POCA:OCA=85.5%:89.2%: 87.2%</td>
</tr>
<tr>
<td>Deguchi et al [53]</td>
<td>PC, R</td>
<td>229 <em>H. pylori</em>-positive adults</td>
<td>13C-UBT, HpSA</td>
<td><em>L. gasseri</em> OLL2716</td>
<td>Rabeprazole 10 mg bid, amoxicillin 750 mg bid, clarithromycin 200 mg bid, 1 w</td>
<td>95/115 (82.6%) vs. 79/114 (69.3%) P&lt;0.018</td>
<td></td>
</tr>
<tr>
<td>Ojetti et al [54]</td>
<td>PC, R</td>
<td>90 <em>H. pylori</em>-positive adults which experienced an unsuccessful anti-<em>H. pylori</em> antibiotic treatment</td>
<td>13C-UBT</td>
<td><em>L. reuteri</em> ATCC 55730</td>
<td>Esomeprazole 20 mg bid, levofloxacin 500 mg bid, amoxicillin 1 gr bid, 1w</td>
<td>36/45 (80%) vs. 28/45 (62%) P&lt;0.05</td>
<td>Nausea: 32/45 (66.7%) vs. 45/45 (100%) P&lt;0.001 diarrhea: 10/45 (22.2%) vs. 26/45 (57.7%) P&lt;0.004</td>
</tr>
<tr>
<td>Emara et al [55]</td>
<td>DB, PC, R</td>
<td>70 <em>H. pylori</em>-positive, dyspeptic adults</td>
<td>HpSA, RUT, Histopathology, Questionnaire</td>
<td><em>L. reuteri</em> DS 17938 and <em>L. reuteri</em> ATCC PTA 6475</td>
<td>Omeprazole 20 mg bid, amoxicillin 1000 mg bid, clarithromycin 500 mg bid, 2 w</td>
<td>26/35 (74.3%) vs. 23/35 (65.7%) P&lt;0.003</td>
<td><em>L. reuteri</em> group significantly decreased the GSRS scores</td>
</tr>
<tr>
<td>Francavilla et al [56]</td>
<td>DB, PC, R</td>
<td>100 <em>H. pylori</em>-positive, dyspeptic adults</td>
<td>13C-UBT, RUT, serology, histopathology, Questionnaire-naire</td>
<td><em>L. reuteri</em> DSM 17938 and <em>L. reuteri</em> ATCC PTA 6475</td>
<td>PPI-clarithromycin-amoxicillin (not clear)</td>
<td>33/44 (75%) vs. 29/44 (65.9%) absolute difference: 9.1% (OR: 1.5; 95% CI: 0.6-3.9%)</td>
<td></td>
</tr>
<tr>
<td>Dajani et al [59]</td>
<td>O, R</td>
<td>377 <em>H. pylori</em>-positive adults and children (group A: standard triple therapy, group B: probiotic concomitant, group C: pre-treatment with probiotic, group D: probiotic concomitant with sequential therapy)</td>
<td>15C-UBT</td>
<td><em>B. infantis</em> 2036</td>
<td>Standard triple therapy: PPI-amoxicillin-clarithromycin or metronidazole 10 d  The sequential therapy: PPI-amoxicillin 5 d, PPI-clarithromycin-metronidazole 5 d</td>
<td>Group A:B:C:D=68.9%:83%:86%:90.8% Improvement of clinical symptoms group A:B:C:D=28.8%:54%:52.5%:49.8%</td>
<td></td>
</tr>
<tr>
<td>Wang et al [62]</td>
<td>PC, R</td>
<td>100 <em>H. pylori</em>-positive children</td>
<td>13C-UBT</td>
<td>A mixture of <em>Lactobacillus</em>-S and *B. bifidum-12</td>
<td>PPI 0.6-0.8 mg/kg bid, clarithromycin 10-15 mg/kg bid, amoxicillin 30-50 mg/kg bid, 2 w</td>
<td>36/43 (83.7%) vs. 29/45 (64.4%) P&lt;0.05</td>
<td>5/43 (11.6%) vs. 12/45 (26.7%) P&lt;0.07</td>
</tr>
</tbody>
</table>

O: open; R: randomized; DB: double-blind; PC: placebo controlled; RUT: rapid urease test; *H. pylori*: *Helicobacter pylori*; HpSA: *H. pylori* stool antigens; w: week; d: days; PPI: proton pump inhibitor; OCA: standard therapy group; POCA: probiotic pre-treated group; OCAP: probiotic post-treated group.
[34], and B. bifidum BF-1 [50], show the ability to modulate H. pylori colonization instead of eradicating it in adults.

**Probiotics as adjuvant therapy combined with antibiotic-PPI in H. pylori eradication**

Evaluating the effects of probiotics as adjuvant therapy combined with antibiotic-PPI in clinical trials, needs to consider two main aspects, namely, eradication rates and adverse events (Table 6). As the predominant bacteria in human stomach, Lactobacillus has been widely studied [51-56]. A study by Zheng [51] broadly examined the efficacy of eradication regimens supplemented with Lactobacillus-containing probiotic in a meta-analysis that contained nine randomized, controlled trials. Lactobacillus-containing probiotic supplementation potentially elevated H. pylori eradication rates by approximately 10%, although side effects were not reduced significantly. In the subgroup analysis, eradication rates significantly increased by 17% in the Lactobacillus alone-administered group (RR=1.25; 95% CI=1.13-1.37; NNT=6). Lactobacillus-containing probiotics also improved the eradication rates both in adults (RR=1.12; 95% CI=1.04-1.20; NNT=12) and in children (RR=1.25; 95% CI=1.01-1.53; NNT=7). Thus, Lactobacillus-containing probiotics were concluded to be effective as adjunct to eradication therapy, although side effects may not decrease. Furthermore, Lactobacillus administered alone could distinctly benefit eradication therapy.

*L. acidophilus*, a microaerophilic species, occurs naturally in the human gastrointestinal tract. Du [52] presented that administration of it could improve H. pylori eradication rate whether before or after triple therapy, however, symptom relieving rate has no significant change in probiotic group. In a randomized, controlled clinical research [53], a total of 229 patients were randomized into either 1-week triple therapy or triple therapy plus *L. gasseri*-containing yogurt. In the yogurt-plus-triple therapy groups, the yogurt containing *L. gasseri* OLL2716 was given twice daily for 4 weeks (3 weeks pretreatment and 1 week during eradication therapy). Overall, the eradication rate (ITT/PP) was 69.3%/74.5% for the triple-only group, and 82.6%/85.6% for the yogurt-plus-triple group (P=0.018/P=0.041). Eradication of primary clarithromycin-resistant strains tended to be higher for the yogurt-plus-triple therapy than the triple-only therapy (38.5 vs. 28.0%, P=0.458). Unfortunately, with respect to the report of adverse reacts were not involved in this research.

Ojetti V [54] found that in H. pylori-positive subjects, *L. reuteri* ATCC 55730 supplementation increased the eradication rate while reducing the incidence of the most common side effects associated with antibiotic therapy in a second-line treatment. Recently, the role of a new probiotic preparation (a mixture of *L. reuteri* DSM 17938 and *L. reuteri* ATCCPTA 6475) in *H. pylori* infection was studied by some scholars. Emara MH [55] demonstrated that triple therapy of *H. pylori* supplemented with *L. reuteri* combination increased the eradication rate by 8.6%, improved the GSRS score, reduced the reported side effects, and improved the histological features of *H. pylori* infection as compared with the placebo-supplemented triple therapy. The research of Francavilla R [56] is similar to that of Emara MH, although the former introduced more indexes to assess the influences of *L. reuteri* combination in *H. pylori* infection. In the study, the two groups, both with fifty patients, received *L. reuteri* combination and placebo once daily, respectively. Significantly fewer patients reported side effects in the *L. reuteri* combination as compared with the placebo (40.9% vs. 62.8%; P<0.04). Eradication rate was 75% in the *L. reuteri* combination and 65.9% in placebo, such that *L. reuteri* combination increased eradication rate by 9.1% (OR: 1.5). Thus, *L. reuteri* combination, when administered with eradication therapy, a significant reduction in antibiotic-associated side effects and (not significantly) increase of *H. pylori* eradication rate was shown.

*S. boulardii*, a yeast strain, has been deemed to have a therapeutic potential for many gastrointestinal and extra-intestinal diseases in recent years [57]. Szajewska conducted two systematic reviews with *S. boulardii* as research subject, in 2010 and 2015, respectively [11, 58]. The latest study covered 11 RCTs involving a total of 2200 participants, 330 of which were children. Of the 853 patients in the *S. boulardii* group, 679 (80%, 95% CI=77-82) experienced eradication compared with 608 of the 855 patients (71%; 95% CI=68-74) in the control group (RR=1.11; 95% CI=1.06-1.17). Compared
Bifidobacterium spp.

Mechanism and clinical trials of probiotics against H. pylori infection

with the control, S. boulardii reduced the risk of overall H. pylori therapy-related adverse effects (RR=0.44; 95% CI=0.31-0.64), particularly of diarrhea (RR=0.51; 95% CI=0.42-0.62) and nausea (RR=0.6; 95% CI=0.44-0.83). The addition of S. boulardii evidently improved the eradication rate and some of the therapy-related side effects, although it did not achieve the desired level of success [4].

Compared with Lactobacillus and S. boulardii, fewer studies have been done in recent years on single Bifidobacterium spp. as adjuvant therapy in H. pylori eradication.

B. infantis, a Bifidobacterium strain, inhabits the intestines of both infants and adult which considered beneficial resulting of acid production. Dajani AI [59] evaluated the effect of adding B. infantis as adjuvant to common regimens used for H. pylori eradication. The clinical study tested three different regimens of H. pylori eradication treatment: standard triple therapy with a probiotic added concomitantly (n=100), starting the probiotic for 2 weeks before initiating standard triple therapy along with the probiotic (n=95), and the probiotic given concomitantly to sequential treatment (n=76), and traditional standard triple therapy (n=106) as a control group. The eradication rate of each group was 83%, 90.5%, 90.8%, and 68.9%, respectively. B. infantis as an adjuvant to triple and sequential therapies was thus found to be capable of significantly improving the eradication rates.

In regard to the clinical trials of multi-strain probiotics are frequently occur in the literature either as a single use or as adjuvant therapy. In the study of Myllyluoma [60], a probiotic combination including L. rhamnosus GG, L. rhamnosus LC705, P. Freudenreichii JS, and B. lactis Bb12 presents the ability to decrease DOB value and exert a beneficial effect on gastric mucosa in H. pylori infected patients. Bifidobacterium and Lactobacillus are generally used together as research subjects, and many studies and meta-analyses demonstrate this combination can decrease H. pylori density as monotherapy, in addition, effectively reduce eradication rate and incidence of total side effects as adjuvant therapy in children and adults [61-63]. However, not all mixtures were effective in eradicating H. pylori and preventing of adverse events, since the role of probiotics may depend on specific combinations [64]

Conclusion

So far, we have a comprehensive understanding of probiotics by numerous in vitro studies, animal model, and human trials. Probiotics may inhibit the infection of H. pylori through immunomodulation, producing antimicrobial substances, interfering with adhesion, and enhancing the function of the mucosal barrier. In the treatment of H. pylori, probiotics have shown diverse effects depending on certain strains. While L. paracasei ST11 has no effect on H. pylori, like L. johnsonii La1, L. acidophilus LB, L. gasseri OLL2716, L. reuteri ATCC, and B. bifidum BF-1 as a monotherapy, are capable of diminishing the bacterial load, not eradicating H. pylori in adults and children. L. acidophilus, L. gasseri OLL2716 and B. infantis, when combined with antibiotic-PPI as adjuvant therapy, can improve the eradication rate, but not obviously influence the adverse symptoms. L. reuteri and S. boulardii have the ability to increase eradication rate and reduce adverse effects, however, the eradication rate shows no significant decrease in the mixture of L. reuteri strains. Effects of multi-strain probiotics on the treatment depend on specific combination, and not all of them were valid in eradicating H. pylori.

Although extensive studies indicates probiotics plays an important role in the H. pylori infection, still many uncertain problems are needs to be considered in the clinical application of probiotics, such as the choice of probiotic, safety of certain strain, intake frequency, intake dose, and the time of adding probiotics to the eradication therapy. Moreover, if probiotics are used in combination with antibiotic-PPI, the extra cost may be a limiting factor to its use. Some scholars claim that probiotics are economical as a treatment method; however, more evidence is needed to support this statement. In the past, many researchers have deliberated whether probiotics are effective for the treatment of H. pylori infection. Based on unremitting efforts, we are now able to draw preliminary conclusions that certain probiotics are in fact capable of effectively contributing to H. pylori eradication in treatments. A discussion
Mechanism and clinical trials of probiotics against H. pylori infection

should now be opened on how to use probiotics to their best advantage in preparation for the upcoming era of probiotics.

Acknowledgements

This work was supported by the grant from the Natural Science Foundation of Shandong Province (ZR2014HL015).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Lan Liu, Department of Gastroenterology, The Second Hospital of Shandong University, 247 Beiyuan Street, Jinan 250033, Shandong Province, People’s Republic of China. Tel: +86 13791133426; Fax: +86 531 85875454; E-mail: liulandoctor@163.com

References


Mechanism and clinical trials of probiotics against *H. pylori* infection


Mechanism and clinical trials of probiotics against *H. pylori* infection


