

# SCIENTIFIC REPORTS



OPEN

## Probiotic supplementation does not improve eradication rate of *Helicobacter pylori* infection compared to placebo based on standard therapy: a meta-analysis

Received: 14 October 2015

Accepted: 04 March 2016

Published: 21 March 2016

Chao Lu, Jianzhong Sang, Haijian He, Xingyong Wan, Yiming Lin, Lan Li, Youming Li & Chaohui Yu

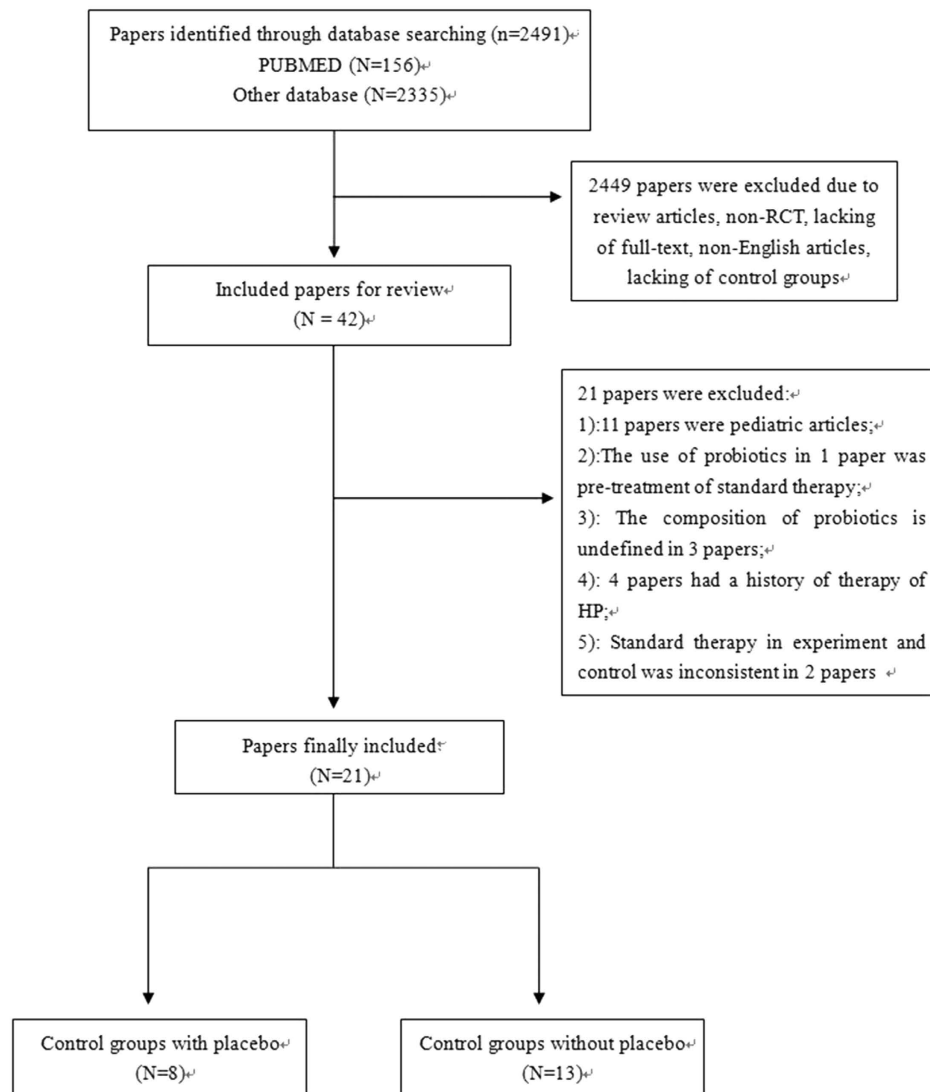
This meta-analysis included eligible randomized controlled trials (RCTs) with the aim of determining whether probiotic supplementation can improve *H. pylori* eradication rates. PUBMED, EBSCO, Web of Science, and Ovid databases were searched. We included RCTs that investigated the effect of combining probiotics, with or without a placebo, with standard therapy. A total of 21 RCTs that reported standard therapy plus probiotics were included. Compared to the placebo group, the probiotics group was 1.21 (OR 1.21, 95% CI: 0.86, 1.69) and 1.28 (OR 1.28, 95% CI: 0.88, 1.86) times more likely to achieve eradication of *H. pylori* infection in intent-to-treat (ITT) analysis and per protocol (PP) analysis, respectively. Probiotics with triple therapy plus a 14-day course of treatment did not improve the eradication of *H. pylori* infection (OR 1.44, 95% CI: 0.87, 2.39) compared to the placebo. Moreover, the placebo plus standard therapy did not improve eradication rates compared to standard therapy alone ( $P = 0.816$ ). However, probiotics did improve the adverse effects of diarrhea and nausea. These pooled data suggest that the use of probiotics plus standard therapy does not improve the eradication rate of *H. pylori* infection compared to the placebo.

*Helicobacter pylori* (*H. pylori*) is a Gram-negative microaerophilic bacterium that dwells in the human gastric mucosa. It is commonly associated with gastroduodenal diseases in humans such as gastric mucosa-associated lymphoid tissue lymphoma<sup>1</sup>, peptic ulcer disease<sup>2</sup>, and even gastric cancer<sup>3,4</sup>. Almost 50% of the worldwide human population is infected, with people living in developing countries showing higher rates of infection<sup>5</sup>. Triple therapy, which has been proposed as a first approach for *H. pylori* eradication, includes a proton pump inhibitor (PPI), clarithromycin and either amoxicillin or metronidazole. Other choices include sequential therapy and quadruple therapy<sup>6</sup>. However, the eradication rate using standard therapy was reported to be unsatisfactory using first-line or second-line treatments due to increased resistance to antibiotics and patient non-compliance<sup>7-9</sup>. Probiotics appear to be promising supplements for standard therapy of *H. pylori* infection.

Probiotics are defined as living microbial species that can induce anti-inflammatory and anti-oxidative mechanisms that may improve bowel microecology and general health<sup>10,11</sup>. Probiotics contain *Lactobacillus*, *Saccharomyces boulardii*, *Bifidobacterium*, and other bacteria and yeasts. Some meta-analyses have reported that probiotic supplementation can improve the eradication rate of *H. pylori* compared to standard therapy alone<sup>12-14</sup>. It is widely accepted that probiotics can improve *H. pylori* eradication and reduce side effects during standard therapy.

However, we found that the control groups in RCTs in previous meta-analyses were mostly without a placebo. Placebo preparations matched the probiotic preparation in color, size, shape and weight, and had no pharmacological effect. Surprisingly, we found that the eradication rate of *H. pylori* had no statistical significance between probiotic supplementation groups and placebo supplementation groups in most studies. A placebo may also

Department of Gastroenterology, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China. Correspondence and requests for materials should be addressed to C.Y. (email: ych623@sina.com)



**Figure 1. Flow diagram for searching studies.**

influence the eradication rate of *H. pylori* by a placebo effect acting through the alteration of systemic and enteric levels of hormones<sup>15</sup>. Nevertheless, there is no direct research on placebo and *H. pylori* to support this viewpoint.

This study aimed to select RCTs, and establish whether probiotic supplementation could improve tolerance to *H. pylori* standard triple eradication therapy compared to the placebo. We included RCTs without a placebo for comparison.

## Results

**Study characteristics.** Our search identified 2,491 references, of which eight studies with placebo groups<sup>16–23</sup> and 13 studies without placebo groups<sup>24–36</sup> met our inclusion criteria (Fig. 1). Reasons for exclusion are shown in Fig. 1. Study characteristics of therapeutic regimens are shown in Tables 1 and 2. Geographically, studies mainly originated from Europe (13/21), with other studies originating from South America (1/21) and Asia (7/21). Twenty studies used standard triple therapy and one used bismuth-quadruple therapy. <sup>13</sup>C-urea breath test (<sup>13</sup>C-UBT) was the main diagnostic method selected. A total of 3,520 participants were included in our research, in which 3,349 participants completed their respective trial. The terminal point of follow-up was reexamination of *H. pylori* infection after standard therapy, which ranged from 4 weeks to 10 weeks after the end of treatment. Characteristics of age, gender and type of patients are shown in Table 3. We found no significant difference in age (SMD = −0.05, 95% CI = −0.19, 0.09) or gender (OR = 0.92, 95% CI: 0.8, 1.06) between the two groups. Ten studies used a single probiotic and 11 used compound probiotics. Placebos were administered in the same number of sachets as the probiotics. Boxes containing active study treatments, and placebos were identical in color, size, shape, weight and taste, and contained the same number of sachets. No trademark identifications were present, either on the probiotic or the placebo sachets. The composition of a placebo in one study was capsules of acidified milk powder (skim milk biologically acidified by commercial yogurt culture)<sup>21</sup>, of which no therapeutic effect was mentioned.

| Author/year                                    | Country | Case number (probiotics/ placebo) | Diagnostic Methods               | Probiotics composition  | Eradication Therapy  | % Eradication in ITT (probiotics/ placebo) | % Eradication in PP (probiotics/ placebo) | Review of <i>H. pylori</i>                                  |
|--|---------|-----------------------------------|----------------------------------|---|--|--|---|---|
| Nista <i>et al.</i> <sup>22</sup>              | Italy   | 120 (60/60)                       | <sup>13</sup> C-UBT              | <i>Bacillus clausii</i> ( <i>B. clausii</i> )   | (rabeprazole 20 mg bid + clarithromycin 500 mg bid + amoxicillin 1 g bid) × seven days + (probiotics or placebo) × 14 days   | 72.22/71.15                                | 78/74                                     | <sup>13</sup> C-UBT six weeks after the end of treatment.   |
| Navarro- Rodriguez <i>et al.</i> <sup>21</sup> | Brazil  | 107 (55/52)                       | <sup>13</sup> C-UBT or histology | <i>Lactobacillus acidophilus</i> , <i>Lactobacillus rhamnosus</i> , <i>Bifidobacterium bifidum</i> and <i>Streptococcus faecium</i> | (lansoprazole 30 mg bid + tetracycline 500 mg bid + furazolidone 200 mg bid) × seven days + (probiotics or placebo) × 30 days  | 81.82/76.92                                | 88.24/81.63                               | <sup>13</sup> C-UBT eight weeks after the end of treatment. |
| Cremonini <i>et al.</i> <sup>18</sup>          | Italy   | 42 (21/21)                        | <sup>13</sup> C-UBT              | <i>Lactobacillus GG</i> and <i>S. boulardii</i>   | (rabeprazole 20 mg bid + clarithromycin 500 mg bid + tinidazole 500 mg bid) × seven days + (probiotics or placebo) × 14 days   | 81.82/72.73                                | 85.71/80                                  | <sup>13</sup> C-UBT 5–7 weeks after the end of treatment.   |
| Cindoruk <i>et al.</i> <sup>17</sup>           | Turkey  | 124 (62/62)                       | histology                        | <i>S. boulardii</i>   | (lansoprazole 30 mg bid + Clarithromycin 500 mg bid + amoxicillin 1 g bid) × 14 days + (probiotics or placebo) × 14 days   | 70.97/59.68                                | 70.97/59.68                               | <sup>13</sup> C-UBT six weeks after the end of treatment.   |
| Manfredi <i>et al.</i> <sup>19</sup>           | Italy   | 149 (73/76)                       | <sup>13</sup> C-UBT or SAT       | <i>Lactobacilli</i> and <i>Bifidobacteria</i>   | (esomeprazole 20 mg bid + amoxicillin 1 g bid) × first five days + (esomeprazole 20 mg bid + clarithromycin 500 mg bid + tinidazole 500 mg bid) × next five days + (probiotics or placebo) × 10 days (total) | 89.04/88.16                                | 92.86/94.37                               | SAT 8–10 weeks after the end of treatment.                  |
| Myllyluoma <i>et al.</i> <sup>20</sup>         | Finland | 47 (23/24)                        | <sup>13</sup> C-UBT              | <i>Lactobacillus rhamnosus</i> , <i>L. rhamnosus</i> , <i>Bifidobacterium breve</i> and <i>Propionibacterium freudenreichii</i> )   | (lansoprazole 30 mg bid + Clarithromycin 500 mg bid + amoxicillin 1 g bid) × seven days + (probiotics or placebo) × 28 days  | 91.30/79.17                                | 91.30/79.17                               | <sup>13</sup> C-UBT four weeks after the end of treatment.  |
| Armuzzi <i>et al.</i> <sup>24</sup>            | Italy   | 60 (30/30)                        | <sup>13</sup> C-UBT              | <i>Lactobacillus GG</i>   | (rabeprazole 20 mg bid + Clarithromycin 500 mg bid + tinidazole 500 mg bid) × seven days + (probiotics or placebo) × 14 days   | 83.33/80                                   | 83.33/80                                  | <sup>13</sup> C-UBT six weeks after the end of treatment.   |
| Shavakhi <i>et al.</i> <sup>23</sup>           | Iran    | 180 (90/90)                       | RUT or histology                 | <i>Lactobacillus</i> and <i>Bifidobacterium</i>   | (omeprazole 20 mg bid + Clarithromycin 500 mg bid + amoxicillin 1 g bid + bismuth 240 mg bid) × 14 days + (probiotics or placebo) × 14 days  | 76.67/81.11                                | 82.14/84.88                               | <sup>13</sup> C-UBT four weeks after the end of treatment.  |

**Table 1. Study characteristics with placebo.** <sup>13</sup>C-UBT: <sup>13</sup>C Urea Breath Test; SAT: Stool Antigen Test; RUT: Rapid Urease Test.

**Eradication Rates.** PP results were used to represent the final eradication rates. Total eradication rates were  $84.32 \pm 10.66\%$  and  $77.87 \pm 9.39\%$  in the probiotics and control groups, respectively. In the studies with a placebo, the eradication rate was  $84.07 \pm 14.09\%$  in the probiotics group and  $79.22 \pm 9.84\%$  in the placebo group. In studies without a placebo, the eradication rate was  $84.48 \pm 12.61\%$  in the probiotics group and  $77.04 \pm 9.4\%$  in the non-placebo group. In addition, our study revealed that in ITT analysis the probiotics group was 1.21 times more likely than the placebo group to achieve eradication of *H. pylori* infection (OR 1.21, 95% CI: 0.86, 1.69; Fig. 2) and 1.84 times more likely than the standard-therapy-alone group (OR 1.84, 95% CI: 1.51, 2.25; Fig. 2). In PP analysis, the probiotics group was 1.28 times more likely than the placebo group to achieve eradication of *H. pylori* infection (OR 1.28, 95% CI: 0.88, 1.86; Fig. 3) and 1.85 times more likely than the standard-therapy-alone group (OR 1.85, 95% CI: 1.47, 2.31; Fig. 3). Both ITT and PP analyses showed no statistically significant effect on eradication rates when the probiotics group was compared to the placebo group, but the probiotics group had a significantly higher eradication rate when compared to standard therapy alone. To avoid bias caused by the anti-*H. pylori* therapy scheme or the duration of probiotic use, we also conducted a sub-group analysis on treatment using probiotics with triple therapy plus a 14-day course of treatment. This showed that the probiotics group was not more likely to achieve the eradication of *H. pylori* infection (OR 1.44, 95% CI: 0.87, 2.39; Fig. 4) without statistical significance. In standard-therapy-alone groups, sub-group analysis on triple therapy plus a 14-day course of treatment also showed no statistical significance (OR 1.74, 95% CI: 0.96, 3.16; Fig. 4). Moreover,

| Author/year                             | Country  | Case number (probiotics/control) | Diagnostic Methods                  | Probiotics composition  | Eradication Therapy   | % Eradication in ITT (probiotics/control) | % Eradication in PP (probiotics/control) | Review of <i>H. pylori</i>                                  |
|---|----------|----------------------------------|-------------------------------------|---|---|---|--|---|
| Ziemniak <i>et al.</i> <sup>36</sup>    | Poland   | 245 (53/192)                     | UBT                                 | <i>Lactobacillus acidophilus</i> ; <i>Lactobacillus rhamnosus</i>                           | (pantoprazole 40 mg bid + clarithromycin 500 mg bid + amoxicillin 1 g bid) × 10 days + (probiotics or not) × 10 days          | 96.23/85.94                               | 96.23/85.94                              | UBT six weeks after the end of treatment.                   |
| de Bortoli <i>et al.</i> <sup>26</sup>  | Italy    | 206 (105/101)                    | <sup>13</sup> C-UBT, SAT, RUT       | <i>Lactobacillus plantarum</i> ; <i>L. reuteri</i> ; <i>Bifidobacterium infantis</i> , etc. | (esomeprazole 20 mg bid + clarithromycin 500 mg bid + amoxicillin 1 g bid) × seven days + (probiotics or not) × seven days    | 88.57/72.27                               | 92.08/76.04                              | <sup>13</sup> C-UBT eight weeks after the end of treatment. |
| Sheu <i>et al.</i> <sup>33</sup>        | China    | 160 (80/80)                      | Histology, RUT                      | <i>Lactobacillus</i> ;<br><i>Bifidobacterium</i>  | (lansoprazole 30 mg bid + clarithromycin 500 mg bid + amoxicillin 1 g bid) × seven days + (probiotics or not) × 28 days       | 91.25/78.75                               | 94.81/87.5                               | <sup>13</sup> C-UBT eight weeks after the end of treatment. |
| Song <i>et al.</i> <sup>34</sup>        | Korea    | 661 (330/331)                    | Histology, RUT                      | <i>S. boulardii</i>   | (omeprazole 20 mg bid + clarithromycin 500 mg bid + amoxicillin 1 g bid) × seven days + (probiotics or not) × 28 days         | 80/71.6                                   | 85.44/80.07                              | <sup>13</sup> C-UBT four weeks after the end of treatment.  |
| Park <i>et al.</i> <sup>31</sup>        | Korea    | 352 (176/176)                    | Histology                           | <i>Bacillus subtilis</i> ;<br><i>Streptococcus faecium</i>                                  | (omeprazole 20 mg bid + clarithromycin 500 mg bid + amoxicillin 1 g bid) × seven days + (probiotics or not) × 56 days         | 83.52/73.3                                | 85.47/78.66                              | <sup>13</sup> C-UBT four weeks after the end of treatment.  |
| Kim <i>et al.</i> <sup>29</sup>         | Korea    | 347 (168/179)                    | <sup>13</sup> C-UBT, histology, RUT | <i>L. acidophilus</i> ; <i>L. casei</i> ; <i>L. casei</i> ; <i>S. thermophilus</i>          | (PPI bid + clarithromycin 500 mg bid + amoxicillin 1 g bid) × seven days + (probiotics or not) × 21 days                      | 79.17/72.07                               | 87.5/78.66                               | <sup>13</sup> C-UBT four weeks after the end of treatment.  |
| Yasar <i>et al.</i> <sup>35</sup>       | Turkey   | 76 (38/38)                       | Histology                           | <i>Bifidobacterium</i>  | (pantoprazole 40 mg bid + clarithromycin 500 mg bid + amoxicillin 1 g bid) × 14 days + (probiotics or not) × 14 days          | 65.79/52.63                               | 65.79/52.63                              | <sup>13</sup> C-UBT four weeks after the end of treatment.  |
| Canducci <i>et al.</i> <sup>25</sup>    | Italy    | 120 (60/60)                      | <sup>13</sup> C-UBT, histology      | <i>Lactobacillus acidophilus</i>  | (Rabeprazole 20 mg bid + clarithromycin 250 mg tid + amoxicillin 500 mg tid) × seven days + (probiotics or not) × 10 days     | 86.67/70                                  | 88.14/72.41                              | <sup>13</sup> C-UBT four weeks after the end of treatment.  |
| Armuzzi <i>et al.</i> <sup>16</sup>     | Italy    | 120 (60/60)                      | <sup>13</sup> C-UBT                 | <i>Lactobacillus</i>  | (pantoprazole 40 mg bid + clarithromycin 500 mg bid + tinidazole 500 mg bid) × seven days + (probiotics or not) × 14 days     | 80/76.6                                   | 80/80.7                                  | <sup>13</sup> C-UBT six weeks after the end of treatment.   |
| Medeiros <i>et al.</i> <sup>30</sup>    | Portugal | 62 (31/31)                       | Culture                             | <i>Lactobacillus acidophilus</i>  | (esomeprazole 20 mg bid + clarithromycin 500 mg bid + amoxicillin 1 g bid) × eight days + (probiotics or not) × eight days    | 83.87/80.65                               | 83.87/80.65                              | <sup>13</sup> C-UBT 6–7 weeks after the end of treatment.   |
| Scaccianoce <i>et al.</i> <sup>32</sup> | Italy    | 31 (15/16)                       | Histology                           | <i>Lactobacillus plantarum</i> ; <i>L. reuteri</i> ; <i>Bifidobacterium Longum</i> , etc.   | (lansoprazole 30 mg bid + clarithromycin 500 mg bid + amoxicillin 1 g bid) × seven days + (probiotics or not) × 14 days       | 53.33/62.5                                | 53.33/66.67                              | <sup>13</sup> C-UBT 4–6 weeks after the end of treatment.   |
| Deguchi <i>et al.</i> <sup>27</sup>     | Japan    | 229 (115/114)                    | Culture, histology, RUT             | <i>L. gasseri</i>   | (rabeprazole 10 mg bid + clarithromycin 200 mg bid + amoxicillin 750 mg bid) × seven days + (probiotics or not) × 28 days     | 82.61/69.3                                | 85.59/74.53                              | <sup>13</sup> C-UBT 8 weeks after the end of treatment.     |
| Imase <i>et al.</i> <sup>28</sup>       | Japan    | 14 (7/7)                         | Not mentioned                       | CBM588  | (lansoprazole 30 mg bid + clarithromycin 400 mg bid + amoxicillin 750 mg bid) × seven days + (probiotics or not) × seven days | 100/87                                    | 100/87                                   | Not mentioned   |

**Table 2. Study characteristics without placebo.** <sup>13</sup>C-UBT: <sup>13</sup>C Urea Breath Test; SAT: Stool Antigen Test; RUT: Rapid Urease Test.

both ITT ( $P > Z = 0.108$ ;  $P > Z = 0.436$ ) and PP ( $P > Z = 0.108$ ;  $P > Z = 0.640$ ) meta-analyses had no publication bias under Begg's funnel plot test.

In addition, we compared the eradication rates in placebo administration plus standard therapy with the standard therapy group in order to determine whether placebo treatment can improve eradication rates. Results revealed no statistical significance ( $79.22 \pm 9.84\%$  vs.  $77.04 \pm 9.4\%$ ;  $P = 0.816$ ). However, the trend still showed a potentially higher eradication rate in the placebo plus standard therapy group. Thus, RCTs on placebo plus standard therapy *versus* standard therapy alone are needed to verify our hypothesis.

| Author of study                  | Age (probiotics group) | Age (control group)* | M/F (probiotics group) | M/F (control group)* | Type of patients included                                       |
|----------------------------------|------------------------|----------------------|------------------------|----------------------|---|
| Nista <i>et al.</i>              | 46 ± 13                | 43 ± 13              | 33/27                  | 22/38                | Free of gastrointestinal symptoms                               |
| Navarro- Rodriguez <i>et al.</i> | 50.4                   | 48.4                 | 21/34                  | 19/33                | 51 PU patients and 56 dyspepsia patients                        |
| Cremonini <i>et al.</i>          | –                      | –                    | –                      | –                    | Free of gastrointestinal symptoms                               |
| Cindoruk <i>et al.</i>           | 45.82 ± 13.35          | 47.56 ± 13.53        | 26/36                  | 18/44                | Dyspepsia patients  |
| Manfredi <i>et al.</i>           | 46.4                   | 50.6                 | 39/34                  | 37/39                | Free of gastrointestinal symptoms                               |
| Shavakhi <i>et al.</i>           | 42.3 ± 13.3            | 42.2 ± 13.2          | 49/41                  | 60/30                | Patients with history of PU                                     |
| Myllyluoma <i>et al.</i>         | 57.3                   | 53.8                 | 10/13                  | 8/16                 | Free of gastrointestinal symptoms                               |
| Armuzzi <i>et al.</i>            | –                      | –                    | –                      | –                    | Free of gastrointestinal symptoms                               |
| Ziemniak <i>et al.</i>           | 44.4 ± 13.3            | 43.7 ± 10.3          | 14/39                  | 78/114               | Patients with PU or gastritis                                   |
| de Bortoli <i>et al.</i>         | 51.5 ± 13.7            | 50.1 ± 15.2          | 56/49                  | 54/47                | Free or mild of gastrointestinal symptoms, among 25 PU patients |
| Sheu <i>et al.</i>               | 47.8                   | 45.9                 | 40/40                  | 38/42                | 84 PU patients and 76 dyspepsia patients                        |
| Song <i>et al.</i>               | 49.76 ± 11.7           | 49.84 ± 11.4         | 185/145                | 219/112              | Patients with gastrointestinal symptoms, among 492 PU patients  |
| Park <i>et al.</i>               | 45.2 ± 19.8            | 47.6 ± 18.5          | 96/80                  | 95/81                | Patients with gastrointestinal symptoms, among 142 PU patients  |
| Kim <i>et al.</i>                | 48.1 ± 12.4            | 53.7 ± 12.0          | 71/97                  | 89/90                | 113 PU patients and 234 dyspepsia patients                      |
| Yasar <i>et al.</i>              | 38.32 ± 10.66          | 36.95 ± 8.62         | 11/27                  | 14/24                | Dyspepsia patients  |
| Canducci <i>et al.</i>           | –                      | –                    | –                      | –                    | Dyspepsia patients or patients with history of PU               |
| Armuzzi <i>et al.</i>            | –                      | –                    | –                      | –                    | Free of gastrointestinal symptoms                               |
| Medeiros <i>et al.</i>           | 50.7                   | 53.8                 | 18/13                  | 14/17                | Patients with history of PU                                     |
| Scaccianoce <i>et al.</i>        | 50                     | 48                   | 7/8                    | 6/10                 | Free of gastrointestinal symptoms                               |
| Deguchi <i>et al.</i>            | 55.9                   | 57.8                 | 76/39                  | 68/46                | Patients with history of PU                                     |
| Imase <i>et al.</i>              | –                      | –                    | –                      | –                    | Patients with history of PU                                     |

**Table 3. Basic characteristics of the included studies.** \*In this table, the control group contains groups with or without a placebo. \*\*PU = peptic ulcer.

**Tolerance and adverse effects.** The tolerance to the standard triple therapy itself may be affected by the probiotic supplementation. Among the included studies, only one clearly reported that there was no difference in tolerance between the probiotic and placebo groups ( $P = 0.833$ )<sup>23</sup>. Tolerance of standard therapy is affected by adverse effects. Therefore, we compared the adverse effects of diarrhea, nausea, vomiting, bloating, epigastric pain, constipation, headache and metallic taste. Between the probiotic group and the standard-therapy-alone group, we found that nausea (OR 0.43, 95% CI: 0.27, 0.7), vomiting (OR 0.3, 95% CI: 0.11, 0.86), diarrhea (OR 0.43, 95% CI: 0.21, 0.89), and constipation (OR 0.28, 95% CI: 0.13, 0.64) were improved in the probiotic group, whereas epigastric pain (OR 0.82, 95% CI: 0.48, 1.39), headache (OR 0.42, 95% CI: 0.11, 1.65), metallic taste (OR 0.69, 95% CI: 0.30, 1.58), and bloating (OR 0.54, 95% CI: 0.04, 6.64) were not different between the two groups (S1). Between the probiotic group and the placebo group, we found that nausea (OR 0.36, 95% CI: 0.21, 0.62), diarrhea (OR 0.33, 95% CI: 0.19, 0.57), and bloating (OR 0.5, 95% CI: 0.3, 0.83) were improved in probiotic group, whereas epigastric pain (OR 0.58, 95% CI: 0.25, 1.32), vomiting (OR 0.71, 95% CI: 0.31, 1.62), and constipation (OR 0.56, 95% CI: 0.31, 1.01) were not different between the two groups (S2).

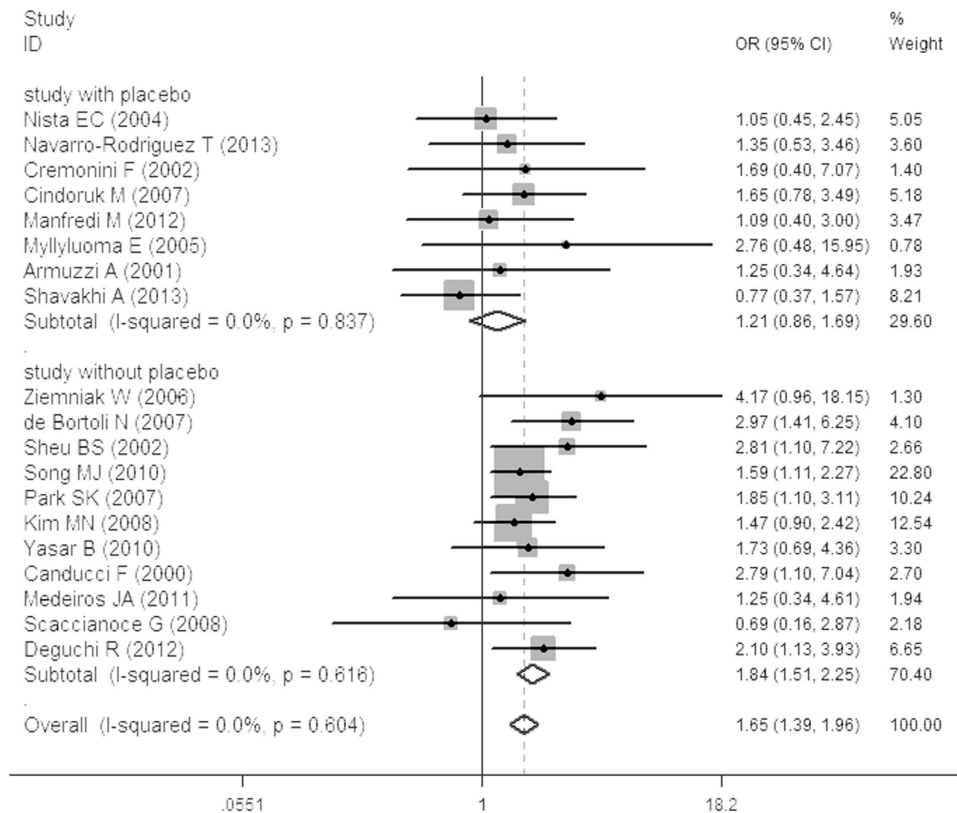
Nausea and diarrhea was clearly improved by probiotics, but it was not clear whether these two factors ultimately affected the curative effect.

## Discussion

This meta-analysis analyzed whether probiotic supplementation can improve the eradication rate of *H. pylori* infection based on standard therapy. In contrast to previously published meta-analyses<sup>13,14,37</sup>, we studied control groups given a placebo in order to determine whether placebo administration can influence eradication rates compared to probiotics. Studies without placebos were included for comparison. Our results revealed that the inclusion of probiotics to standard therapy does not increase eradication rates of *H. pylori* compared to a placebo.

Triple therapy for eradication of *H. pylori* infection is unsatisfactory throughout the world. *H. pylori* is the best known microbe that colonizes the gastric mucosa, causing gastric related diseases, as shown by Marshall<sup>38</sup>. However, Walker *et al.* revealed that the imbalance of other gastric microbiota can play an important role in affecting human health<sup>39</sup>. This may be an important factor in the lack of efficacy of standard therapy. In addition, the increasing resistance to antibiotics such as clarithromycin<sup>6</sup>, the frequency and duration of drug administration, and the occurrence of side effects can influence a patient's compliance<sup>40</sup>.

Many studies, including the meta-analysis mentioned above, have reported that probiotic supplementation can safely improve eradication rates of *H. pylori* infection and decrease side effects, although some probiotic products have been shown to increase the risk of complications in a minority of specific patient groups<sup>41</sup>. Probiotics have been shown to be useful in several illnesses such as reducing the duration and severity of rotavirus gastroenteritis<sup>42</sup>, reducing the incidence of traveler's diarrhea<sup>43</sup>, preventing and reducing relapses of *Clostridium*



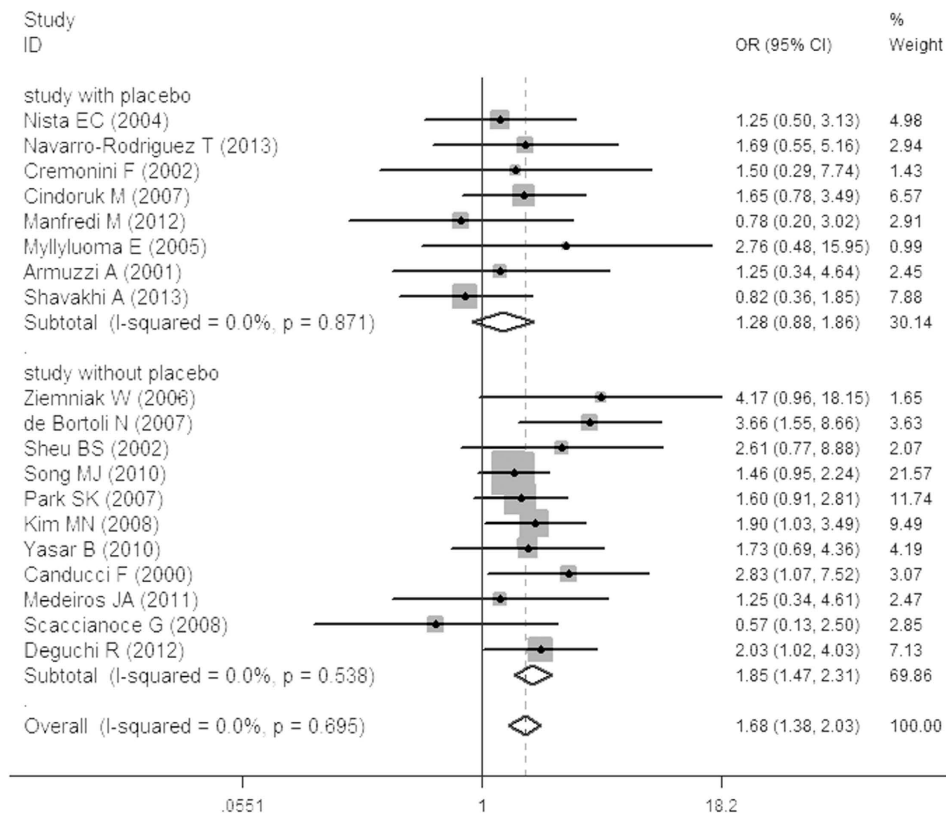
**Figure 2.** Meta-analysis of studies reporting on the eradication rate of *H. pylori* infections in the probiotics group vs. the placebo and non-placebo groups in ITT analysis and estimated the OR with a 95% confidence interval and weight percentage.

*difficile* colitis<sup>44</sup>, and anti-inflammation benefits for inflammatory bowel disease<sup>45</sup>. The mechanisms by which probiotics play their role have not been clearly defined. Many possible mechanisms have been put forward, such as inhibiting the adhesion of pathogenic bacteria to the intestinal wall and competing with microbial pathogens for a limited number of receptors present on the surface epithelium<sup>46</sup>, altering cytokine expression and the activity of intestinal-associated lymphoid tissue and epithelial cells<sup>47,48</sup>, and enhancing intestinal barrier function<sup>49</sup>. Therefore, it seems that probiotics can provide powerful supplements for the eradication of *H. pylori* infection. However, our findings were not sufficient to justify such expectations.

A placebo is a simulated or otherwise medically ineffectual treatment for a disease or other medical condition that intends to deceive the recipient. It is well-known that psychological phenomena are closely associated with gastric diseases<sup>50</sup>. In addition, the placebo effect generates alterations in the levels of systemic and enteric hormones<sup>15</sup>, and subject-expectancy effects<sup>51</sup>. The use of a placebo seems to play a potential role in treating *H. pylori* infection. Nevertheless, Asbjørn Hróbjartsson and Peter C. Gøtzsche indicated that there is little evidence for placebos having a strong clinical impact and that the formation of the placebo effect is a subjective factor<sup>52,53</sup>. In our study, merged data revealed non-significant results on eradication rates, which may be due to population selection bias of the groups included for Student's *t*-test. Thus, more evidence is needed. RCTs including standard therapy plus placebo compared to standard therapy alone are needed in order to analyze whether placebo supplementation can improve the eradication rate of *H. pylori* infection.

This is the first meta-analysis and systemic review to compare probiotics plus standard therapy with placebo plus standard therapy for *H. pylori* infection. Although we reviewed many reports to strengthen our study, several limitations of this meta-analysis were inevitable. First, we lacked a large sample size and RCTs with sufficient case numbers in the placebo group. More large-sample RCTs would have increased the power of this analysis. Second, it is not clear whether differences in probiotics dose or composition, or the course of treatment, as well as differences in the specificity and accuracy of the diagnostic tools for *H. pylori* infection would influence the results. Third, the influence of adverse effects of probiotics should not be ignored, which may contribute to the eradication of *H. pylori* infections. In addition, due to lack of data, potentially relevant confounders such as race, smoking, lifestyle, and gene polymorphisms were not analyzed.

In conclusion, all the published research on probiotics plus standard therapy indicates that probiotics improve the eradication rate of *H. pylori* infection. However, in our study, we found that a 14-day triple therapy plus probiotics cannot improve eradication rates. In addition, the pooled data of our meta-analysis suggest that the use of probiotics plus standard therapy does not improve the eradication rate of *H. pylori* infection compared to placebo plus standard therapy, although probiotic supplementation can improve eradication rates compared to standard



**Figure 3.** Meta-analysis of studies reporting on the eradication rate of *H. pylori* infection in the probiotics group vs. the placebo and non-placebo groups in PP analysis and estimated the OR with a 95% confidence interval and weight percentage.

therapy alone. A placebo may achieve the same curative effect for the eradication of *H. pylori* infection compared to probiotics. Future research should pay more attention to the role of placebo in *H. pylori* eradication.

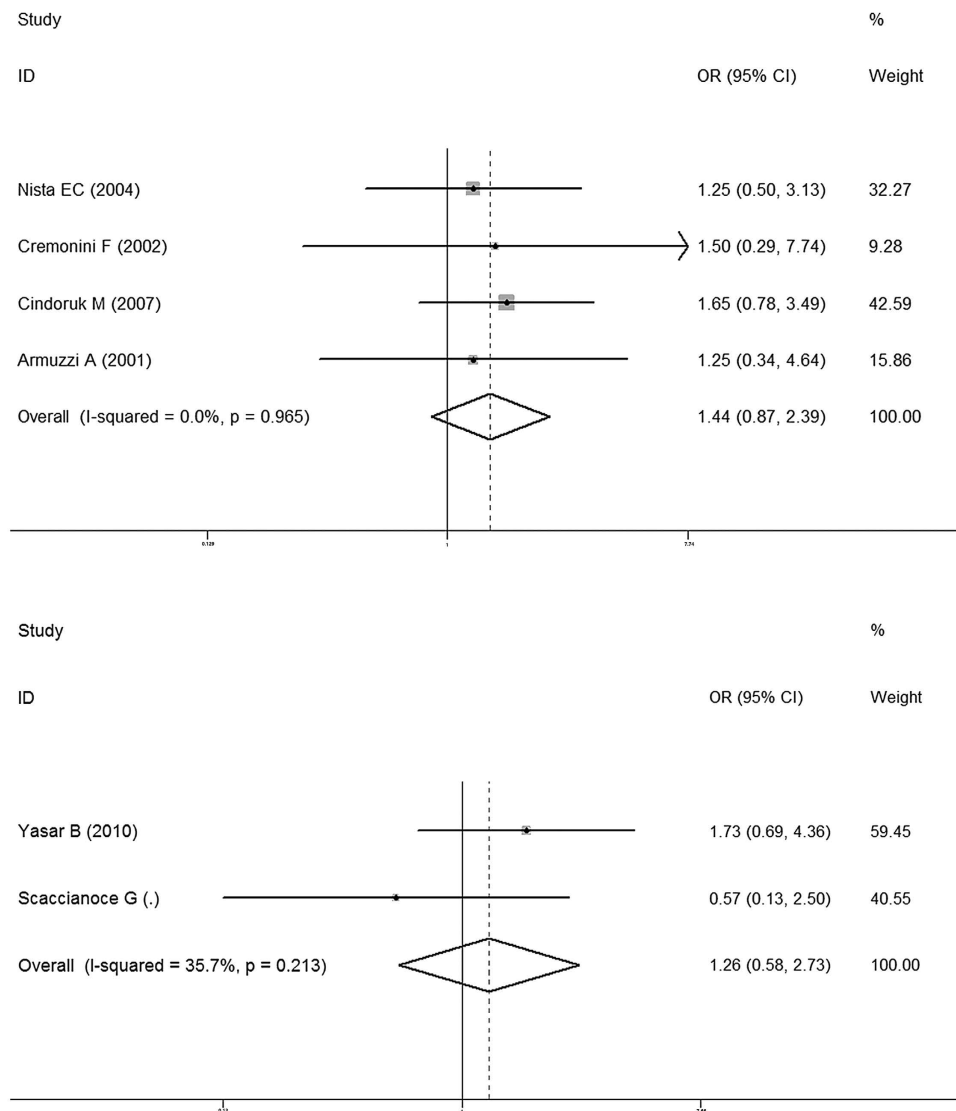
## Methods

**Search strategy and study selection.** We searched studies published up to June 1, 2015, in PubMed, EndSearch, EBSCO and Web of Science databases using the following terms: (*Helicobacter pylori* OR *H. pylori* OR *Helicobacter infection* OR *Helicobacter\** OR *HP* OR *Helicobacter pylori* (MeSH)), and (*eradication* OR *treatment* OR *therapy* OR *disease eradication* (MeSH)), and (*probiotic* OR *probiotic\** OR *prebiotic* OR *yeast* OR *yogurt* OR *symbiotic* OR *Lactobacillus* OR *Bifidobacterium* OR *Saccharomyces* OR *Lactococcus* OR *Streptococcus* OR *Enterococcus* OR *probiotic* (MeSH)). This study was limited to human and English-language randomized controlled trials (RCTs). In addition, the following criteria were used for selecting relevant studies: (1) study patients > 18 years old; (2) study populations that have not been treated for *H. pylori* infection; (3) patients in the control group received standard therapy with or without a placebo; (4) patients in the experimental group received standard therapy with probiotics; (5) availability of relative information on *H. pylori* diagnosis and successful eradication rates; and (6) same administration of standard therapy for the experimental and control groups. Standard therapy was defined as triple treatment, sequential treatment, non-bismuth quadruple therapy, or bismuth-containing quadruple therapy<sup>6</sup>.

Combining the guideline<sup>6</sup> and previous meta-analysis<sup>13</sup>, *H. pylori* infection diagnosed by at least one positive test result was considered confirmation of infection: (1) <sup>13</sup>C/<sup>14</sup>C urea breath test (UBT); (2) rapid urease test (RUT); (3) *H. pylori* culture; (4) stool antigen test; or (5) histology of biopsy staining. The primary outcome of the study was the *H. pylori* eradication rate, which had to be confirmed by a negative <sup>13</sup>C-UBT or other generally accepted method at least 4 weeks after the end of treatment. The secondary outcome measures were whether probiotics improve tolerance compared to the standard therapy. The adverse effects of interest were diarrhea, nausea, vomiting, bloating, epigastric pain, constipation, headache and metallic taste during anti-*H. pylori* therapy.

Eligibility of each study for inclusion was evaluated by two investigators. Any research-related disagreements were resolved by a third reviewer. The quality of RCTs included in this study was assessed using the Jadad scale<sup>54</sup>.

**Data abstraction.** Two authors independently extracted data from all eligible studies, and a third author checked the results. Data were extracted into Microsoft Excel (2010 edition; Microsoft, Redmond, WA, USA) to effectively organize the data. The following data were obtained from included studies: base characteristics of



**Figure 4.** Meta-analysis of studies reporting on the eradication rate of *H. pylori* infection in the probiotics group vs. the placebo and non-placebo groups (probiotics with triple therapy plus a 14-day course of treatment) and estimated the OR with a 95% confidence interval and weight percentage.

patients, authors, year of publication, country of research, details of *H. pylori* eradication therapy, details related to interventions, primary outcomes, and diagnostic methods of *H. pylori* infection.

**Statistical Analysis.** The ultimate goal of this study was to determine whether the probiotics group had a higher eradication rate than the placebo group. We also included groups without a placebo for comparison. Odds ratios (ORs) were used to measure the effect of probiotics plus standard therapy on *H. pylori* eradication rates in both intent-to-treat (ITT) and per protocol (PP). ORs were also used to measure the difference of adverse effects of interest between the probiotics group and the control group. Age and gender were analyzed by standardized mean difference (SMD) and OR, respectively. Statistical heterogeneity was analyzed with Chi-squared distribution, Cochran's Q-test and I-squared statistics. A fixed-effects model (Mantel-Haenszel) was applied for meta-analysis if the  $I^2$  statistic was under 50% and/or the Q-test was not significant at  $P < 0.05$ . We opted to stratify our analyses in this study with and without placebo. In addition, Begg's funnel plot was used to assess publication bias. Data of eradication rates of standard therapy plus placebo and standard therapy alone were merged separately, and Student's *t*-test analysis was conducted to compare these data. All analyses were carried out through the application of the commands `metan` and `metabias` in Stata 12.0 (Stata Corporation, Texas, USA), and Student's *t*-tests were performed by SPSS 16.0 (IBM, Chicago, IL, USA). Associated data were calculated and plotted using GraphPad Prism 5 (Graph Pad, San Diego, CA, USA).



## References

- Eck, M. *et al.* MALT-type lymphoma of the stomach is associated with *Helicobacter pylori* strains expressing the CagA protein. *Gastroenterology* **112**, 1482–1486 (1997).
- Peek, R. M., Jr. & Blaser, M. J. Pathophysiology of *Helicobacter pylori*-induced gastritis and peptic ulcer disease. *Am J Med.* **102**, 200–207 (1997).
- Huang, J. Q., Sridhar, S., Chen, Y. & Hunt, R. H. Meta-analysis of the relationship between *Helicobacter pylori* seropositivity and gastric cancer. *Gastroenterology* **114**, 1169–1179 (1998).
- Liu, D., He, Q. & Liu, C. Correlations among *Helicobacter pylori* infection and the expression of cyclooxygenase-2 and vascular endothelial growth factor in gastric mucosa with intestinal metaplasia or dysplasia. *J Gastroenterol Hepatol* **25**, 795–799 (2010).
- Suerbaum, S. & Michetti, P. *Helicobacter pylori* infection. *N Engl J Med.* **347**, 1175–1186 (2002).
- Malfertheiner, P. *et al.* Management of *Helicobacter pylori* infection—the Maastricht IV/ Florence Consensus Report. *Gut* **61**, 646–664 (2012).
- Graham, D. Y. & Fischbach, L. *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. *Gut* **59**, 1143–1153 (2010).
- Liou, J. M. *et al.* Levofloxacin-based and clarithromycin-based triple therapies as first-line and second-line treatments for *Helicobacter pylori* infection: a randomised comparative trial with crossover design. *Gut* **59**, 572–578 (2010).
- Megraud, F. Basis for the management of drug-resistant *Helicobacter pylori* infection. *Drugs* **64**, 1893–1904 (2004).
- Fuller, R. Probiotics in human medicine. *Gut* **32**, 439–442 (1991).
- Johnson-Henry, K. C. *et al.* Probiotics reduce bacterial colonization and gastric inflammation in *H. pylori*-infected mice. *Dig Dis Sci* **49**, 1095–1102 (2004).
- Dang, Y., Reinhardt, J. D., Zhou, X. & Zhang, G. The effect of probiotics supplementation on *Helicobacter pylori* eradication rates and side effects during eradication therapy: a meta-analysis. *PLoS One* **9**, e111030 (2014).
- Szajewska, H., Horvath, A. & Piwowarczyk, A. Meta-analysis: the effects of *Saccharomyces boulardii* supplementation on *Helicobacter pylori* eradication rates and side effects during treatment. *Aliment Pharmacol Ther* **32**, 1069–1079 (2010).
- Zhang, M. M., Qian, W., Qin, Y. Y., He, J. & Zhou, Y. H. Probiotics in *Helicobacter pylori* eradication therapy: A systematic review and meta-analysis. *World J Gastroenterol* **21**, 4345–4357 (2015).
- Kokkotou, E. *et al.* Serum correlates of the placebo effect in irritable bowel syndrome. *Neurogastroenterol Motil* **22**, 285–e281 (2010).
- Armuzzi, A. *et al.* The effect of oral administration of *Lactobacillus GG* on antibiotic-associated gastrointestinal side-effects during *Helicobacter pylori* eradication therapy. *Aliment Pharmacol Ther* **15**, 163–169 (2001).
- Cindoruk, M., Erkan, G., Karakan, T., Dursun, A. & Unal, S. Efficacy and safety of *Saccharomyces boulardii* in the 14-day triple anti-*Helicobacter pylori* therapy: a prospective randomized placebo-controlled double-blind study. *Helicobacter* **12**, 309–316 (2007).
- Cremonini, F. *et al.* Effect of different probiotic preparations on anti-*Helicobacter pylori* therapy-related side effects: a parallel group, triple blind, placebo-controlled study. *Am J Gastroenterol* **97**, 2744–2749 (2002).
- Manfredi, M. *et al.* *Helicobacter pylori* infection in clinical practice: probiotics and a combination of probiotics + lactoferrin improve compliance, but not eradication, in sequential therapy. *Helicobacter* **17**, 254–263 (2012).
- Mylylyluoma, E. *et al.* Probiotic supplementation improves tolerance to *Helicobacter pylori* eradication therapy—a placebo-controlled, double-blind randomized pilot study. *Aliment Pharmacol Ther.* **21**, 1263–1272 (2005).
- Navarro-Rodriguez, T. *et al.* Association of a probiotic to a *Helicobacter pylori* eradication regimen does not increase efficacy or decreases the adverse effects of the treatment: a prospective, randomized, double-blind, placebo-controlled study. *BMC Gastroenterol* **13**, 56 (2013).
- Nista, E. C. *et al.* *Bacillus clausii* therapy to reduce side-effects of anti-*Helicobacter pylori* treatment: randomized, double-blind, placebo controlled trial. *Aliment Pharmacol Ther.* **20**, 1181–1188 (2004).
- Shavakhi, A. *et al.* The effects of multistrain probiotic compound on bismuth-containing quadruple therapy for *Helicobacter pylori* infection: a randomized placebo-controlled triple-blind study. *Helicobacter* **18**, 280–284 (2013).
- Armuzzi, A. *et al.* Effect of *Lactobacillus GG* supplementation on antibiotic-associated gastrointestinal side effects during *Helicobacter pylori* eradication therapy: a pilot study. *Digestion* **63**, 1–7 (2001).
- Canducci, F. *et al.* A lyophilized and inactivated culture of *Lactobacillus acidophilus* increases *Helicobacter pylori* eradication rates. *Aliment Pharmacol Ther.* **14**, 1625–1629 (2000).
- de Bortoli, N. *et al.* *Helicobacter pylori* eradication: a randomized prospective study of triple therapy versus triple therapy plus lactoferrin and probiotics. *Am J Gastroenterol* **102**, 951–956 (2007).
- Deguchi, R. *et al.* Effect of pretreatment with *Lactobacillus gasseri* OLL2716 on first-line *Helicobacter pylori* eradication therapy. *J Gastroenterol Hepatol* **27**, 888–892 (2012).
- Imase, K. *et al.* Efficacy of *Clostridium butyricum* preparation concomitantly with *Helicobacter pylori* eradication therapy in relation to changes in the intestinal microbiota. *Microbiol Immunol.* **52**, 156–161 (2008).
- Kim, M. N. *et al.* The effects of probiotics on PPI-triple therapy for *Helicobacter pylori* eradication. *Helicobacter* **13**, 261–268 (2008).
- Medeiros, J. A. *et al.* Evaluation of *Helicobacter pylori* eradication by triple therapy plus *Lactobacillus acidophilus* compared to triple therapy alone. *Eur J Clin Microbiol Infect Dis* **30**, 555–559 (2011).
- Park, S. K. *et al.* The effect of probiotics on *Helicobacter pylori* eradication. *Hepatogastroenterology* **54**, 2032–2036 (2007).
- Scaccianoce, G. *et al.* Triple therapies plus different probiotics for *Helicobacter pylori* eradication. *Eur Rev Med Pharmacol Sci.* **12**, 251–256 (2008).
- Sheu, B. S. *et al.* Impact of supplement with *Lactobacillus*- and *Bifidobacterium*-containing yogurt on triple therapy for *Helicobacter pylori* eradication. *Aliment Pharmacol Ther.* **16**, 1669–1675 (2002).
- Song, M. J. *et al.* The effect of probiotics and mucoprotective agents on PPI-based triple therapy for eradication of *Helicobacter pylori*. *Helicobacter* **15**, 206–213 (2010).
- Yasar, B. *et al.* Efficacy of probiotics in *Helicobacter pylori* eradication therapy. *Turk J Gastroenterol* **21**, 212–217 (2010).
- Ziemiak, W. Efficacy of *Helicobacter pylori* eradication taking into account its resistance to antibiotics. *J Physiol Pharmacol* **57** Suppl 3, 123–141 (2006).
- Ly, Z. *et al.* Efficacy and safety of probiotics as adjuvant agents for infection: A meta-analysis. *Exp Ther Med.* **9**, 707–716 (2015).
- Marshall, B. J. & Warren, J. R. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* **1**, 1311–1315 (1984).
- Walker, M. M. & Talley, N. J. Review article: bacteria and pathogenesis of disease in the upper gastrointestinal tract—beyond the era of *Helicobacter pylori*. *Aliment Pharmacol Ther.* **39**, 767–779 (2014).
- Peura, D. *Helicobacter pylori*: rational management options. *Am J Med.* **105**, 424–430 (1998).
- Whelan, K. & Myers, C. E. Safety of probiotics in patients receiving nutritional support: a systematic review of case reports, randomized controlled trials, and nonrandomized trials. *Am J Clin Nutr.* **91**, 687–703 (2010).
- Lee, do K. *et al.* Probiotic bacteria, *B. longum* and *L. acidophilus* inhibit infection by rotavirus *in vitro* and decrease the duration of diarrhea in pediatric patients. *Clin Res Hepatol Gastroenterol* **39**, 237–244 (2015).
- Hilton, E., Kolakowski, P., Singer, C. & Smith, M. Efficacy of *Lactobacillus GG* as a Diarrheal Preventive in Travelers. *J Travel Med* **4**, 41–43 (1997).
- Friedman, G. The role of probiotics in the prevention and treatment of antibiotic-associated diarrhea and *Clostridium difficile* colitis. *Gastroenterol Clin North Am* **41**, 763–779 (2012).

45. Sokol, H. *et al.* Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci. USA* **105**, 16731–16736 (2008).
46. Mack, D. R., Michail, S., Wei, S., McDougall, L. & Hollingsworth, M. A. Probiotics inhibit enteropathogenic E. coli adherence *in vitro* by inducing intestinal mucin gene expression. *Am J Physiol* **276**, G941–950 (1999).
47. Haller, D. *et al.* Non-pathogenic bacteria elicit a differential cytokine response by intestinal epithelial cell/leucocyte co-cultures. *Gut* **47**, 79–87 (2000).
48. Singh, V. P., Sharma, J., Babu, S., Rizwanulla, & Singla, A. Role of probiotics in health and disease: a review. *J Pak Med Assoc* **63**, 253–257 (2013).
49. Isolauri, E. *et al.* Lactobacillus casei strain GG reverses increased intestinal permeability induced by cow milk in suckling rats. *Gastroenterology* **105**, 1643–1650 (1993).
50. Levenstein, S., Rosenstock, S., Jacobsen, R. K. & Jorgensen, T. Psychological stress increases risk for peptic ulcer, regardless of Helicobacter pylori infection or use of nonsteroidal anti-inflammatory drugs. *Clin Gastroenterol Hepatol* **13**, 498–506 e491 (2015).
51. McDonald, C. J., Mazzuca, S. A. & McCabe, G. P., Jr. How much of the placebo 'effect' is really statistical regression? *Stat Med.* **2**, 417–427 (1983).
52. Hrobjartsson, A. & Gotzsche, P. C. Is the placebo powerless? An analysis of clinical trials comparing placebo with no treatment. *N Engl J Med.* **344**, 1594–1602 (2001).
53. Hrobjartsson, A. & Gotzsche, P. C. Is the placebo powerless? Update of a systematic review with 52 new randomized trials comparing placebo with no treatment. *J Intern Med.* **256**, 91–100 (2004).
54. Jadad, A. R. *et al.* Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* **17**, 1–12 (1996).

## Acknowledgements

This study was funded by the National Natural Science Foundation of China (81400606) and the science and technology plan projects of Zhejiang Province (2015C33102).

## Author Contributions

C.L. and C.Y. designed the research; C.L. and J.S. performed the research; H.H., X.W. and Y.L. collected and analyzed the data; L.L. gave statistical support; Y.L. and C.Y. revised the manuscript; C.L. wrote the paper. All authors reviewed the manuscript.

## Additional Information

**Supplementary information** accompanies this paper at <http://www.nature.com/srep>

**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article:** Lu, C. *et al.* Probiotic supplementation does not improve eradication rate of *Helicobacter pylori* infection compared to placebo based on standard therapy: a meta-analysis. *Sci. Rep.* **6**, 23522; doi: 10.1038/srep23522 (2016).



This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>