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Effects of non-viable *Lactobacillus reuteri* combining with 14-day standard triple therapy on *Helicobacter pylori* eradication: A randomized double-blind placebo-controlled trial

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Abstract

Background: Viable probiotics have shown effects on the eradication of *Helicobacter pylori*, but the role of non-viable probiotics in *H. pylori* eradication is unclear. This study aimed to evaluate the effects of non-viable *Lactobacillus reuteri* DSM17648 combining with 14-day standard triple therapy on *H. pylori* eradication.

Materials and Methods: Two hundred treatment-naive *H. pylori*-positive adult patients were randomized equally to receive non-viable *L. reuteri* DSM17648 (LR group) or placebo for 4 weeks, with the latter 2 weeks treated together with triple therapy. The Gastrointestinal Symptom Rating Scale (GSRS) was completed before and after treatment. Stool samples were collected for 16S rRNA gene sequencing at week0, week2, and week8.

Results: Eradication rates in the LR group and the placebo group were 81.8% and 83.7% in ITT analysis ($p = 0.730$), 86.2% and 87.2% in PP analysis ($p = 0.830$), respectively. After treatment, the mean GSRS score decreased significantly in the LR group as compared with the placebo group (1.9 ± 0.2 vs. 2.7 ± 0.3; $p = 0.030$). Significantly less patients in the LR group as compared with the placebo group reported abdominal distention (5.1% vs. 16.3%; $p = 0.010$) and diarrhea (11.1% vs. 23.5%; $p = 0.022$). The relative abundance of *Proteobacteria* phylum and *Escherichia-Shigella* genus in the placebo group was about 4.0-fold and 8.1-fold of that in the LR group at wk2, respectively. Significant changes of diversity and enhancements of *Fusicatenibacter*, *Subdoligranulum*, and *Faecalibacterium* were observed in the LR group compared with the placebo group.

Conclusions: Supplementation of non-viable *L. reuteri* DSM17648 with triple therapy did not improve the eradication rate of *H. pylori*, but it helped to build up a beneficial microbial profile and reduced the frequencies of abdominal distention, diarrhea, and the GSRS score.

KEYWORDS
gut microbiota, *Helicobacter pylori*, *Lactobacillus reuteri*, randomized controlled trial, triple therapy

Chenghai Yang and Liping Liang contributed equally to this article.

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1 | INTRODUCTION

Helicobacter pylori (*H. pylori*) is still infecting more than half of the population in many countries, although the prevalence is decreasing.[1] As a main cause of chronic gastritis, peptic ulcer, and malignant gastric tumors, *H. pylori* places a heavy burden on developing countries and regions with high infection rate. In the last decade, the eradication rates of conventional regimens based on proton pump inhibitors (PPIs) plus antibiotics have been decreasing. Antibiotic resistance and decrease of drug compliance caused by adverse effects were the two main reasons for eradication failure.[2] Moreover, *H. pylori* treatment causes dysbiosis of gut microbiota and increases the expression of antibiotic resistance gene.[3,4] Therefore, eradication of *H. pylori* is facing a great challenge, and effective and safe methods are needed.

To reduce adverse effects, improve drug compliance and increase eradication rates, certain probiotics were added to conventional regimens in several clinical studies. Sheu et al. reported that compared with 7-day triple therapy alone, supplementation of AB-yoghurt containing *Lactobacillus* and *Bifidobacterium* for five weeks significantly increased the eradication rate (91% vs. 78%, $p < 0.05$) and reduced adverse effects.[5] However, McNicholl et al. found that probiotics containing *Lactobacillus Plantarum* and *Pediococcus acidilactici* did not improve the effects of eradication therapy.[6] But in this study, probiotics were given along with antibiotics,[6] which might affect the result. Meta-analyses of randomized controlled trials also came to inconsistent conclusions.[7,8] As for *L. reuteri*, several studies reported that it did not significantly improved the *H. pylori* eradication rates.[9-11] However, *L. reuteri* did show significant anti-*H. pylori* effects in some other studies.[12,13] In brief, probiotics were more or less shown to reduce adverse effects in the vast majority of clinical studies, but whether probiotics can improve the eradication rate of *H. pylori* remains controversial.

Meanwhile, several studies focusing on the impact of probiotics on gut microbiota during *H. pylori* eradication have been published recently.[14-17] All of these studies used viable probiotics. Viable probiotics are generally considered to be safe, but for some severely ill patients treated with viable *Lactobacillus* probiotic, bacteremia was reported.[18] Increasing evidence has shown that in addition to living probiotics, dead cells, or even cell fractions may also exert biological effects.[19-22] In view of this, non-viable probiotics could be a safe alternative and have some advantages over viable probiotics, such as product stability and standardization.[19] *L. reuteri* DSM17648 strain was found by screening hundreds of *Lactobacillus* strains of a large culture collection.[23] It was tested for antibiotic resistance, and no resistance was identified.[23] In previous studies, it was found that spray-dried non-viable *L. reuteri* DSM17648 could specifically co-aggregate *H. pylori in vitro* and reduce the value of ^{13}C-UBT *in vivo*.[23,24] However, no clinical trials have reported the effect of non-viable *L. reuteri* DSM17648 used as an adjuvant combining with traditional regimen on *H. pylori* eradication.

Thus, we conducted a randomized, double-blind, placebo-controlled trial aiming to evaluate the effects of non-viable *Lactobacillus reuteri* DSM17648 combining with 14-day standard triple therapy on *H. pylori* eradication.

Significance Statement

This novel study is a prospective randomized control trial which uses non-viable probiotics (as opposed to viable) in human subjects. Until now, most studies with non-viable probiotics have been mainly in animal subjects. Viable probiotics cannot completely avoid biological risks. Non-viable probiotics could be a safer alternative and have some advantages over viable ones.

2 | MATERIALS AND METHODS

2.1 | Participants

Two hundred consecutive outpatients with *H. pylori* infection were recruited at Nanfang Hospital of Southern Medical University in Guangzhou between May 2019 and July 2020 under the following inclusion criteria: aged 18–70 years; diagnosed by ^{13}C-UBT within 2 weeks before entry. Key exclusion criteria included: previous treatment for *H. pylori* infection; administration of antibiotics, probiotics/prebiotics, PPIs, H_2 receptor antagonists, bismuth preparations, laxatives, anti-diarrhoeals, and certain antimicrobial traditional Chinese medicines 4 weeks before; previous history of gastrointestinal surgery; contraindications or allergies to PPIs, amoxicillin and clarithromycin; severe or unstable diseases; pregnancy or lactation; alcoholics and drug abusers; staff of this trial. The drugs mentioned above and any other things supposed to affect the efficacy and safety evaluation were also not allowed during the trial.

The study was approved by the Medical Ethics Committee of Nanfang Hospital of Southern Medical University (No. NFEC-2019–036) and adhered to the principles of Good Clinical Practice and the Declaration of Helsinki. All the patients were fully informed of the possible benefits and potential risks of participating in the trial, and voluntarily signed the written informed consent.

2.2 | Study design and medications

This study was a prospective, randomized, double-blind, placebo-controlled trial. Eligible patients were randomly allocated into the LR group or the placebo group in 1:1 according to a computer-generated randomization sequence by using randomly permuted blocks (4 patients per block). Patients, study staff, clinical research associates, and statisticians were blinded to the randomization and study products.

After 2 weeks of pretreatment of non-viable *L. reuteri* DSM17648 or placebo (twice a day with two packets each time before breakfast and supper), patients in both groups received standard triple therapy (esomeprazole 20 mg twice daily before meals, amoxicillin 1 g, and clarithromycin 500 mg twice daily after meals) for another 2 weeks. We chose clarithromycin-based triple therapy although the eradication rate <80%, because it would be higher with probiotics. And meta-analyses showed that when probiotics were effective, the total eradication rate of the control group was always less than 80%.[25,26] If the treatment failed, 14-day bismuth quadruple therapy was given as the remedy.

The active study product contained 1×10^{10} dead cells of *L. reuteri* DSM17648 in each packet, prepared as solid powder (Vita Medical Science & Technology CO., LTD, Shanghai, China) for oral application. Dead cells were made by spray-drying, and the non-viability was confirmed by culture. The placebo was made from starch and identical to non-viable *L. reuteri* DSM17648 in weight, appearance and taste, only lacking active ingredients.

Patients had to abstain from fermented milk and alcohol use during treatment. After treatment, the residual products and medicines, and empty packing boxes were recovered. Adherence to the study protocol was evaluated. The medication compliance of more than 80% was considered as good.

2.3 | Study evaluations

Patients were evaluated at 4 visits: screening at baseline (day −14~day −1, wk0), end of the 2-week pretreatment (wk2), end of the 14-day triple therapy (wk4), and follow-up (wk8).

Patients were diagnosed by ^{13}C-UBT within 2 weeks before entry. Demographic data and medical history were collected. Detailed physical and laboratory examinations were carried out at wk0 and wk4. Trial data were collected throughout the treatment period. *H. pylori* status was reassessed by ^{13}C-UBT at wk8. A flow diagram of this trial was showed in Figure 1.

2.4 | Gastrointestinal symptoms assessment and adverse effects

Patients were required to recall the history of gastrointestinal symptoms at baseline, keep the symptom diaries during the treatment and

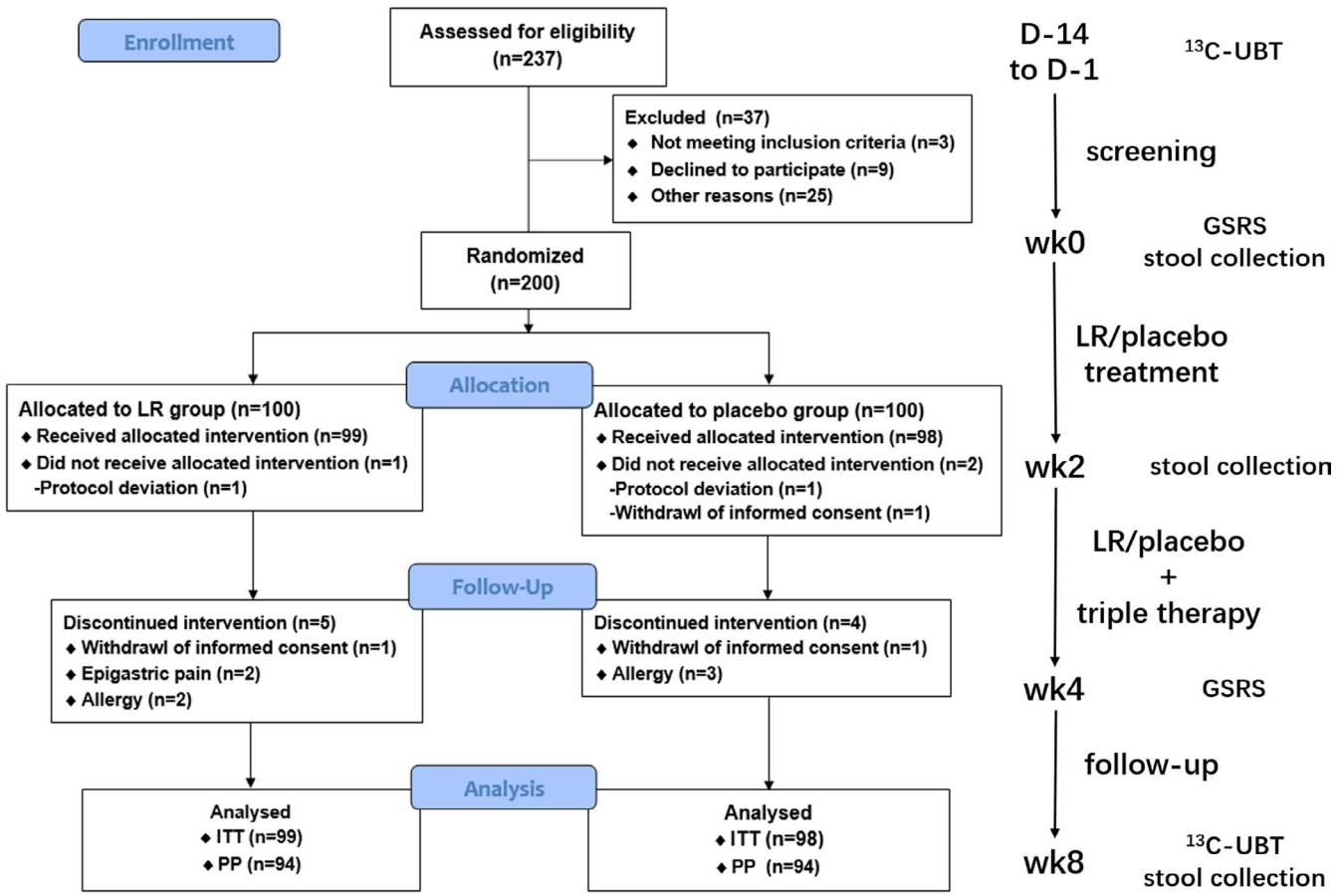

FIGURE 1 Flow diagram of this study. LR: Lactobacillus reuteri, ITT: Intention-to-treat, PP: Per-Protocol, ^{13}C-UBT: ^{13}C-Urea Breath Test, D: day, wk: week, GSRS: Gastrointestinal Symptom Rating Scale

return the diaries at wk4. Gastrointestinal symptoms were assessed and scored according to the 15-item GSRS[27] before (wk0) and after triple therapy (wk4). Special attention was given to the following symptoms: epigastric pain, heartburn, acid regurgitation, nausea or/and vomiting, abdominal distension, eructation, diarrhea, constipation. Adverse effects outside gastrointestine were also recorded and evaluated.

2.5 | Stool collection

Stool samples were collected at wk0, wk2, and wk8. Patients were instructed to collect stool samples at the medical visit site by using disposable sterile feces collection tubes. Samples were stored at −80°C after collection immediately.

2.6 | DNA extraction and 16S rRNA gene sequencing

A E.Z.N.A.® soil DNA Kit (Omega Bio-tek) was used to extract the Genomic DNA of gut microbiota from stool samples following the manufacturer's instructions. The hypervariable V3–V4 region of 16S rRNA gene was targeted and amplified. The raw reads were demultiplexed and filtered by fastp and merged by FLASH. Detailed procedures were showed in the supplementary appendix.

2.7 | Study outcomes

The primary outcome was eradication rates of the two groups. Secondary outcomes comprised the changes of gut microbiota and GSRS scores[27] after treatment, symptoms improvement, adverse effects, tolerability, and compliance with therapy.

2.8 | Statistical analysis

Quantitative data were presented as mean ± standard error of mean (M ± SEM), and qualitative data were presented as *n*(%). Categorical variables, such as eradication rates and incidence rates of adverse effects between the two groups, were compared using the chi-square test or Fisher's exact test as appropriate. Numerical variables, such as age and score of GSRS, were compared using the independent sample *t* test or nonparametric Mann-Whitney *U* test as appropriate. The above-mentioned data were analyzed using the software of IBM SPSS Statistics 23.0. The α-diversity of gut microbiota was determined by calculating diversity indices. The β-diversity based on the principal coordinate analysis (PCoA) was estimated by computing Bray-Curtis distance. Wilcoxon rank-sum test was used to analyze the differences in microbial structure. If *p*-values were less than 0.05, it was considered to be statistically significant.

3 | RESULTS

3.1 | Trial profile and characteristics of patients

Two hundred eligible patients were randomly assigned to the LR group ($n = 100$) or the placebo group ($n = 100$). Patients were predominantly Han Nationality and with a predominance of women. One patient in each group underwent protocol deviation, with a negative ^{13}C-UBT. Another patient in the placebo group withdrew informed consent without taking any medication. Therefore, 99 patients in the LR group and 98 patients in the placebo group were analyzed for intention-to-treat (ITT). Nine patients discontinued treatment, with 5 patients (5.1%) from the LR group and 4 (4.1%) from the placebo group ($p = 1.000$). Thus, 94 patients in each group were analyzed for per-protocol (PP) efficacy (Figure 1). Demographic and clinical characteristics of patients in the two groups at baseline are shown in Table S1.

3.2 | Eradication rates of *H. pylori*

No significant differences were observed in the eradication rates between the LR group and the placebo group, with either ITT analysis [81.8% (95% CI: 74.1%~89.5%) vs 83.7% (95% CI: 76.2%~91.1%)] or PP analysis [86.2% (95% CI: 79.1%~93.3%) vs 87.2% (95% CI: 80.4%~94.1%)] (Table 1).

3.3 | Tolerability and compliance

During treatment, 7 patients discontinued the medication because of adverse effects: epigastric pain (2 cases, all in the LR group) or allergy (2 cases happened in the LR group and 3 in the placebo group). All the adverse effects were mild and self-limited. By counting the residual medicines and empty packing boxes, 88.9% (88/99) patients in the LR group and 80.6% (79/98) patients in the placebo group took more than 80% of their doses ($p = 0.151$). There was no significant difference in tolerability and compliance between the two groups.

3.4 | Gastrointestinal symptoms assessment and adverse effects

There was a significant difference in the mean GSRS scores comparing the LR group and the placebo group at wk4 (1.9 ± 0.2 vs. 2.7 ± 0.3, $p = 0.030$) (Figure 2). In detail, comparisons of gastrointestinal symptoms after treatment showed that patients developing

TABLE 1 Eradication rates of *H. pylori* infection in the two groups

Group	Eradication rate, %(*n*) (95% confidence interval)	
	Intention-to-treat analysis	Per protocol analysis
LR group	81.8(81/99) (74.1–89.5)	86.2(81/94) (79.1–93.3)
Placebo group	83.7(82/98) (76.2–91.1)	87.2(82/94) (80.4–94.1)
P value	0.730	0.830

Note: Values provided as *n* (%).

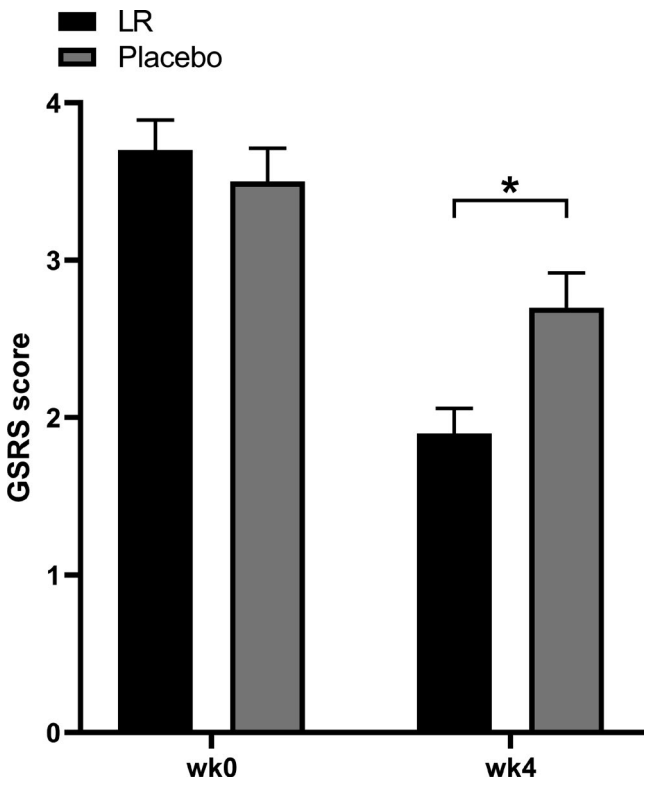

FIGURE 2 Comparisons of the mean GSRS score between the two groups before and after treatment. GSRS: Gastrointestinal Symptom Rating Scale; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

abdominal distention (5.1% vs. 16.3%, $p = 0.010$) and diarrhea (11.1% vs. 23.5%, $p = 0.022$) were significantly fewer in the LR group than that in the placebo group (Table 2).

3.5 | Analysis of gut microbiota

At baseline, there were no significant differences in α-, β-diversity, and composition of gut microbiota between the two groups.

Compared with baseline, α-diversity of gut microbiota (as detected by Chao and Shannon indices at species level) increased significantly in the LR group at wk2, but were restored to baseline at wk8. Compared with the placebo group, α-diversity in the LR group was significantly higher both at wk2 and wk8 (Figure 3A,B). In the placebo group, a significant decrease of α-diversity was observed at wk8. As for β-diversity at species level, a significant change was observed in the LR group at wk2, but it was restored to baseline at wk8 (Figure 3C). Between the two groups, there was a significant difference in β-diversity at wk2 (Figure 3D).

In order to find out the main differences in gut microbiota between the two groups, we analyzed the top 20 most abundant microbes. We found that the relative abundance of *Proteobacteria* phylum and *Escherichia-Shigella* genus in the placebo group was about 4.0-fold and 8.1-fold of that in the LR group at wk2, respectively (Figure 4A,B). Meanwhile, the proportions of *Bacteroidota* phylum, *Fusicatenibacter*, and *Bacteroides* genera in the LR group were significantly higher than that in the placebo group (Figure 4A,B). At wk8, the difference between the two groups disappeared at phylum level (Figure 4C). At genus level, the proportions of *Faecalibacterium* and *Suldoligranulum* in the LR group were significantly higher than that in the placebo group (Figure 4D).

When analyzing community structure at the family, genus, and species levels in detail, we observed significant changes in the LR group, but most of them were transient (Table S2). For example, some beneficial bacteria such as *Christensenellaceae*, *Akkermansiaceae*, and *Roseburia*, increased at wk2, while some detrimental bacteria such as *Actinomyces*, *unclassified_g_ Escherichia-Shigella*, and *Erysipelatoclostridium_ramosum*, decreased at the same time, but they recovered by wk8. These results suggested that non-viable *L. reuteri* DSM17648 played the most important role in gut microbiota during pretreatment phase, but the effect weakened after triple therapy.

4 | DISCUSSION

In this randomized, double-blind, placebo-controlled trial, supplementation of non-viable *L. reuteri* DSM17648 for 4 weeks did not improve the *H. pylori* eradication rate of 14-day triple therapy. However, the probiotic reduced the frequencies of abdominal distention and diarrhea, and the GSRS score.

Many factors affect the eradication rate of *H. pylori*, such as clarithromycin resistance, smoking, and diabetes. Studies showed that being of old age and having previous treatment of *H. pylori* were independent risk factors for clarithromycin resistance.[28,29] So patients with young age and no history of *H. pylori* treatment may have low clarithromycin resistance rate. Meta-analyses have shown that smoking and diabetes were associated with treatment failure.[30,31] In our study, 14-day triple therapy based on clarithromycin achieved an eradication rate of more than 80% as a whole, which could be partly attributable to the young age, no history of *H. pylori* treatment, low smoking, and diabetes rates of the patients. This result was consistent with previous studies.[32,33]

The present study showed that non-viable *L. reuteri* DSM17648 did not improve the *H. pylori* eradication rate of triple therapy. This

TABLE 2 Comparison of symptoms and adverse effects before and after treatment

	Before (wk0)			After (wk4)		
	LR	Placebo		LR	Placebo	
Symptoms	[*n* (%)]	[*n* (%)]	*p*	[*n* (%)]	[*n* (%)]	*p*
Epigastric pain	23 (23.2)	26 (26.5)	0.592	10 (10.1)	13 (13.3)	0.489
Heartburn	12 (12.1)	11 (11.2)	0.845	2 (2.0)	2 (2.0)	1.000
Acid regurgitation	19 (19.2)	16 (16.3)	0.599	3 (3.0)	5 (5.1)	0.498
Nausea or/and vomiting	14 (13.1)	18 (18.4)	0.421	3 (3.0)	9 (9.2)	0.071
Abdominal distension	35 (35.4)	32 (32.7)	0.689	5 (5.1)	16 (16.3)	0.010
Eructation	17 (17.2)	17 (17.4)	0.974	5 (5.1)	6 (6.1)	0.743
Diarrhea	20 (20.2)	14 (14.3)	0.272	11 (11.1)	23 (23.5)	0.022
Constipation	12 (12.1)	13 (13.3)	0.809	10 (10.1)	7 (7.1)	0.460
Bitter taste	0	0		34 (34.3)	43 (43.9)	0.170
Skin rash	0	0		3 (3.0)	7 (7.1)	0.213

Note: Values provided as *n* (%).

result was similar to Emara et al's,[34] but contrary to that of Ojetti et al.[35] Comparing our study with that of Ojetti et al, there were significant differences in mean age (30.0 years vs. 41.5 years) and type (treatment-naive vs. previous unsuccessful) of patients, eradication regimen (clarithromycin-based vs. levofloxacin-based), course of probiotic treatment (28 days vs. 14 days), dosage (2×10^{10} dead cells bid vs. 1×10^{8} CFU tid), and timing of probiotic intake (during eradication therapy and 2 weeks before vs. during eradication therapy and 1 week thereafter). These differences might contribute to the different results. Furthermore, the different living areas of patients might also be factors.[36] In another aspect, as proved in the meta-analysis, probiotics supplementation might only be efficacious in treatment regimens with eradication rates less than 80%.[37] As the eradication rates of treatment regimens increased, the pooled relative risk (RR) of probiotics was dropping (<60%: pooled RR = 1.28; 60%~69%: pooled RR = 1.18; 70%~79%: pooled RR = 1.11; >80%: pooled RR = 1.01).[37] Triple therapy achieved an eradication rate >80% in our study, which might weaken the effect of probiotic.

A role of probiotics was shown in the prevention or reduction of antibiotic-associated diarrhea.[38,39] Studies suggested that adjuvant use of probiotics significantly reduced the incidence of diarrhea during 7-day triple therapy.[40-42] In studies of 14-day triple therapy with probiotics, Emara et al. and Chotivitayatarakorn et al. also found that probiotics significantly reduced the incidence of diarrhea.[34,43] In our study, patients developing diarrhea after treatment in the LR group were significantly fewer than that of those in the placebo group (11.1% vs. 23.5%, $p = 0.022$). This result showed a positive role of non-viable *L. reuteri* DSM17648 in the prevention of diarrhea.

Probiotics can also reduce abdominal distension during *H. pylori* eradication treatment. Hauser et al. concluded that 14-day triple therapy combined with *L. rhamnosus GG* and *Bifidobacterium* significantly decreased the frequency of abdominal distension compared with placebo.[44] Our conclusion was similar, but was different from that of Emara et al, who studied the probiotic combination of *L. reuteri DSM17938* and *L. reuteri ATCC PTA 6475*.[34]

The antibiotics used to eradicate *H. pylori* can alter the indigenous gut microbiota.[45] In this study, the α-diversity of gut microbiota in the placebo group decreased significantly at wk8, suggesting microbial perturbations after triple therapy. In the LR group, α-diversity significantly increased at wk2 and then decreased after triple therapy at wk8, but it remained about the same as wk0. Furthermore, β-diversity of gut microbiota changed significantly in the LR group at wk2, but it was restored to baseline at wk8. The changes of α- and β-diversity showed the ability of non-viable *L. reuteri* DSM17648 to counteract the microbial disruption caused by triple therapy. Our results were consistent with several previous studies using triple therapy as an eradication regimen,[46,47] but were different from other studies in which decreased diversity was reported after bismuth-containing quadruple therapy, an effect that lasted for at least 6 weeks.[15,17] In the study of Tang et al,[17] no significant difference in diversity was observed between the probiotic (*Enterococcus faecium* plus *Bacillus subtilis*) group and the placebo group. But in our study, there was a significant difference in diversity between the two groups. The divergent results might be attributable to different regimens, probiotics, drug doses, and courses.

Adverse effects caused by *H. pylori* eradication, especially diarrhea, have been attributed to antibiotic-induced disturbance of gut microbiota.[15] Probiotic supplementation helped to restore the gut microbiota, thereby reducing antibiotic-associated diarrhea.[14] In our study, most of the microbes enriched in the LR group, such as *Fusicatenibacter*, *Subdoligranulum*, and *Faecalibacterium*, were considered as beneficial bacteria with the ability to produce butyrate.[48-50] However, the microbes enriched in the placebo group, especially *Escherichia-Shigella*, have been associated with diarrhea.[51] These microbial differences might be the major contributor to the significantly lower proportion of patients reporting adverse events in the LR group than that in the placebo group after treatment.

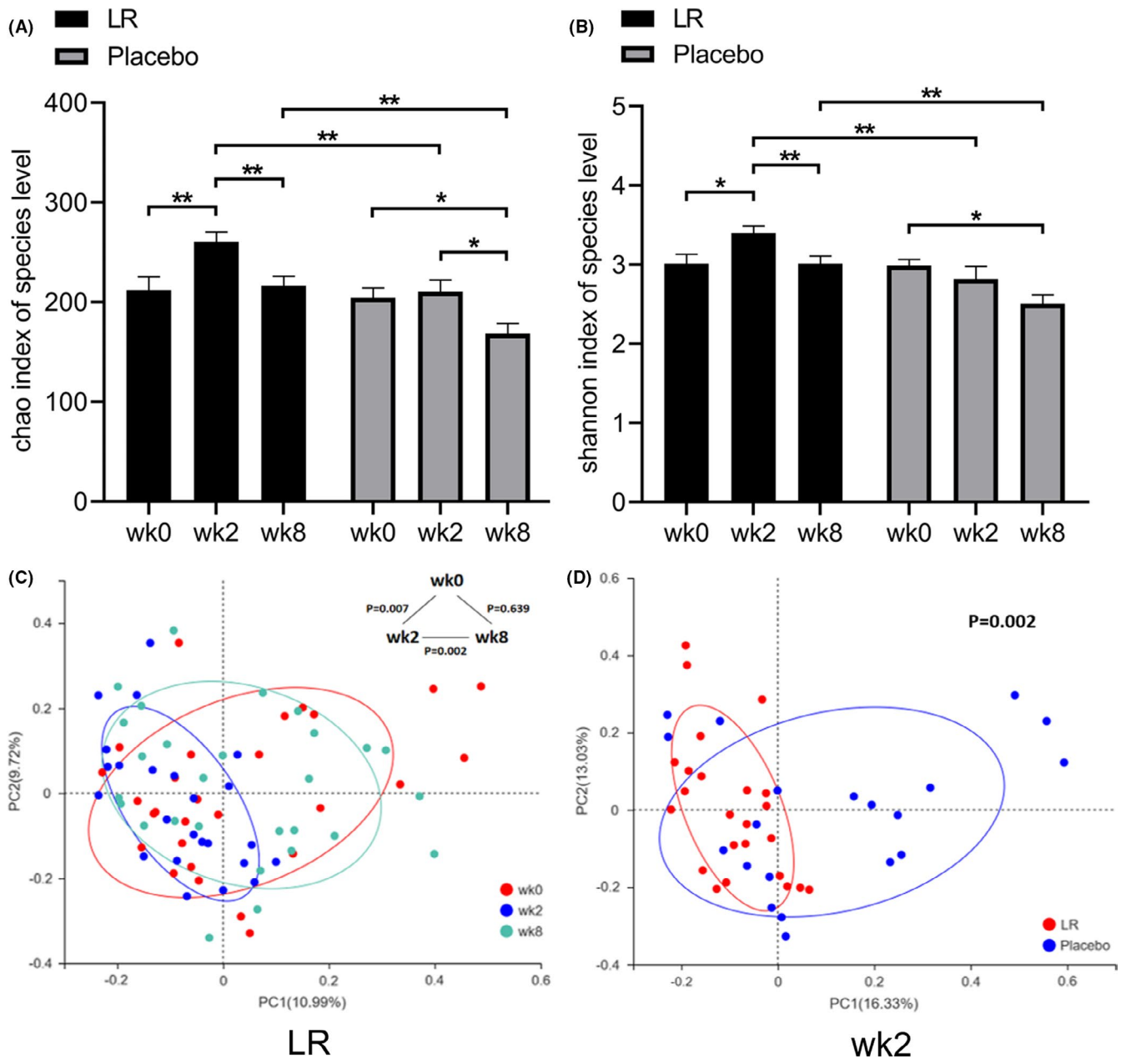

FIGURE 3 Comparisons of diversity in gut microbiota between the two groups. A,B, Comparisons of α-diversity between the two groups. C, Comparisons of β-diversity between different timepoints in the LR group. D, Comparison of β-diversity between the two groups at wk2. α-diversity: Wilcoxon rank-sum test; β-diversity: Principal coordinate analysis (PCoA) based on the Bray-Curtis dissimilarity

Some beneficial effects of *L. reuteri* DSM17648 might be mediated independent of cell viability, especially its adhesive property. Previous studies have reported the binding ability of *L. reuteri* strains to glycolipids.[52,53] This ability may be responsible for the adhesion to gastrointestinal mucosa and the competitive inhibition by sharing "receptor molecules" with targeted pathogens.[53] The adhesion to intestinal epithelial cells and the enhancement of intestinal barrier function may provide a mechanism of action.[54] And such "receptor molecules," including lipoteichoic acid and carbohydrate structures, are strain specific and can be preserved in the spray-drying process.[24]

There were some advantages of this study. Firstly, this was a randomized, double-blind, placebo-controlled study focusing on non-viable probiotic supplemented to a conventional regimen for *H. pylori* eradication. Non-viable probiotic preparations do not need to be refrigerated. They are convenient for storage and transportation, which reduces the cost. In addition, non-viable probiotic preparations are safer than viable ones, without biological risks.[18] Non-viable *L. reuteri* DSM17648 is available in China and the price is reasonable. It is a good choice for patients without refrigerators or with immunodeficiency. Secondly, this study evaluated *H. pylori* eradication rates, adverse effects, and short-term change of gut microbiota at

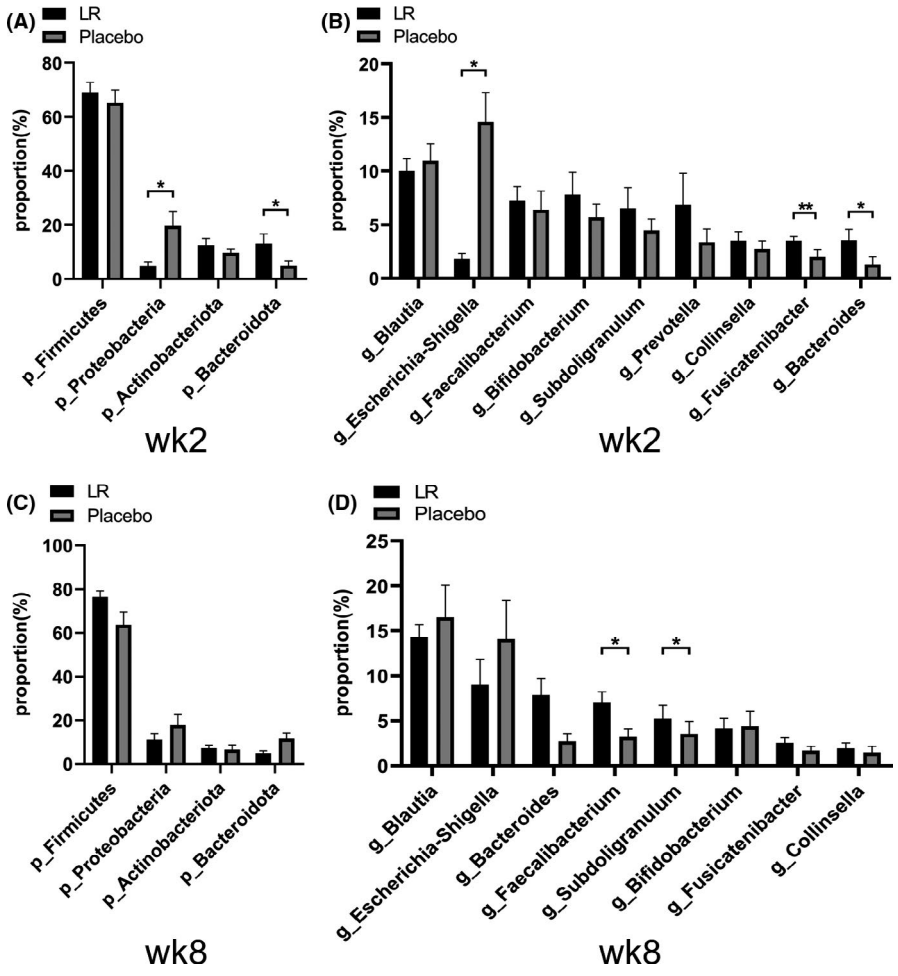

FIGURE 4 Comparisons of the main microbes between the two groups at wk2 (A,B) and wk8 (C,D). p: Phylum, g: Genus; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

the same time, providing a broad view of the impact of probiotic on *H. pylori* eradication.

However, there were some limitations of this study. First of all, this study was a single-center clinical trial and the sample size was small. Next, most of the patients included in this study were under 40 years old, making it less applicable to the older population. Moreover, this study did not investigate the mechanism by which heat-inactivated probiotics changed the microbiota and reduced gastrointestinal side effects. Lastly, stool samples of more time points, especially at wk4, were not collected to fully monitor the dynamical changes of gut microbiota.

In conclusion, the present study showed that supplementation of non-viable *L. reuteri* DSM17648 before and during 14-day standard triple therapy did not increase the eradication rate of *H. pylori*. However, it helped to build up a beneficial microbial profile in the gut and reduced the frequencies of abdominal distention and diarrhea, as well as to improve the score of GSRS after treatment.

CONFLICT OF INTEREST

No conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Chenghai Yang, Liping Liang, Le Liu, Pinjing Lv, Siqi wang, and Zhiqing Wang were involved in the acquisition and analysis of data. Ye Chen was responsible for the study design and the interpretation of data. Chenghai Yang wrote the manuscript and Liping Liang polished it. Le Liu and Ye Chen were involved in critical revision of the manuscript. All authors approved the final version of the manuscript.

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SUPPORTING INFORMATION

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