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Impact of supplementing *Limosilactobacillus fermentum* MN–LF23 on the eradication of *Helicobacter pylori* with 14–day standard quadruple therapy: a randomized, double–blind, placebo–controlled trial

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Abstract

Background The effect of probiotics on *Helicobacter pylori* (Hp) infection demonstrates considerable heterogeneity. This study aims to elucidate the role of *Limosilactobacillus fermentum* MN–LF23 (MN–LF23) in Hp–infected populations.

Methods A total of 94 adult patients with confirmed Hp infection were enrolled in this study and randomly allocated to the placebo or MN–LF23 group. Patients initially received either placebo or probiotics along with standard quadruple therapy for 2 weeks, followed by continued administration of either placebo or probiotics for an additional 4 weeks. The eradication of Hp, serum levels of inflammatory factors, and alterations in gastrointestinal symptoms were assessed at weeks 0, 2, and 6, while fecal samples were collected for metagenomic sequencing.

Results The results showed no significant difference ($P = 1$) in the eradication rate between the placebo group (85.11%) and the probiotic group (82.98%). Following treatment, the incidence of constipation, dyspepsia, and Gastrointestinal Symptom Rating Scale (GSRS) scores in the probiotic group were markedly lower ($P < 0.05$) compared to those observed in the placebo group. Throughout the treatment process, there were no significant differences in TNF– α and IL–1 β levels between the two groups. Compared to the placebo group, the probiotic group exhibited a significant increase in beneficial bacteria such as *Limosilactobacillus fermentum*, *Lactiplantibacillus plantarum*, *Bifidobacterium longum*, *Coprococcus caltus*, and *Clostridium butyricum*.

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Conclusion MN-LF23 supplementation did not improve the eradication rate of standard quadruple therapy. However, it significantly reduced the overall GSRS score, improved digestive and constipation symptoms, and promoted the proliferation of beneficial bacteria in the intestine.

Keywords *Helicobacter pylori*, *Limosilactobacillus fermentum*, Standard quadruple therapy, Gut microbiota, Metagenomic sequencing

Introduction

Hp is a microaerophilic Gram-negative bacterium that widely colonizes the gastric antral mucosa. *Hp* is estimated to have infected more than half of the world's population [1], with an infection rate in the Chinese mainland of approximately 44.2% [2]. In 1994, the International Agency for Research on Cancer (IARC) under the World Health Organization (WHO) classified *Hp* as a Class I carcinogen [3]. The presence of *Hp* infection is closely associated with an increased risk of gastric diseases, including chronic gastritis, peptic ulcers, and gastric cancer [4]. Eradicating *Hp* can effectively reduce the risk of developing these diseases [5].

Triple and quadruple therapy with antibiotics are widely recognized as first-line treatment options internationally [6]. However, due to the widespread use of antibiotics, there has been a steady increase in *Hp* resistance over the years, leading to a rapid decline in the eradication rate of antibiotic therapy [7, 8]. Additionally, antibiotic therapy can result in side effects such as gastrointestinal dysfunction and an imbalance in gut microbiota in patients [9, 10]. Therefore, it is imperative to explore and develop new treatment plans.

In recent years, probiotics have demonstrated significant potential in combating *Hp* infection. Studies have indicated that probiotics can enhance the eradication rate of antibiotic therapy and effectively mitigate the side effects associated with such treatment. Ismail et al. found that supplementing *Limosilactobacillus reuteri* for 4 weeks following a standard 2-week triple therapy regimen resulted in a substantial increase in the *Hp* eradication rate (91.1 vs. 68.9%; $P=0.007$) and an improvement in drug treatment side effects compared to the placebo group [11]. Additionally, Viazis et al. discovered that the addition of four mixed probiotics to a 10-day non-bismuth quadruple *Hp* eradication regimen enhanced eradication rates and reduced side effects [12]. However, some studies have indicated that supplementation with *Lactiplantibacillus plantarum* and *Pediococcus acidilactici acidophilus* in antibiotic treatment regimens does not improve eradication rates or reduce side effects [13]. Therefore, the impact of probiotics on the eradication rate and side effects of antibiotic treatment can be considered heterogeneous.

We previously demonstrated that MN-LF23, isolated from dairy products, effectively reduced the abundance of *Hp* in mice infected with the bacteria. MN-LF23

has also been shown to regulate the immune response, decrease gastric inflammation, and improve the composition of the gastric antral mucosal microbiota [14]. However, its impact on human populations infected with *Hp* remains unknown.

Therefore, we conducted a randomized, double-blind, placebo-controlled experiment to assess the impact of MN-LF23 on antibiotic therapy for eradicating *Hp*. We used metagenomic sequencing technology to analyze changes in the gastrointestinal microbiota before and after treatment. This study provides new insights for developing innovative strategies against *Hp*.

Materials and methods

Participants

From July to October 2023, 94 subjects with *Hp* infection were consecutively recruited at the First Medical Center of Chinese PLA General Hospital in Beijing. The inclusion criteria were as follows: individuals aged 18–65 years with positive results from the [13]C-Urea Breath Test ([13]C-UBT) within 2 weeks before enrollment. The exclusion criteria included a history of gastrointestinal surgery, previous treatment for *Hp* infection, use of antibiotics, probiotic products, proton pump inhibitors, H_2 receptor antagonists, bismuth agents, laxatives, anti-diarrheals, or certain antibacterial traditional Chinese medicines within the past 4 weeks; current pregnancy, lactation, or preparation for pregnancy; presence of mental disorders, immune system disorders, cardiovascular diseases, lung diseases, liver diseases, kidney diseases, malignant tumors, or coagulation disorders; contraindications or allergies to amoxicillin, clarithromycin, bismuth agents or proton pump inhibitors; and participation in other clinical trials simultaneously.

This study was registered with the Chinese Clinical Trial Registry (registration number: ChiCTR220059817) and was approved by the Medical Ethics Committee of The First Medical Center of Chinese PLA General Hospital (approval number: S2022-060-02). This study was conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki. All participants voluntarily signed a written informed consent form after fully understanding the potential benefits and risks of participating in the trial.

Study design

The doctor developed an eradication therapy based on clinical medication guidelines and the patient's past medication history. The eradication regimen utilized in this study was a 14-day standard quadruple therapy consisting of omeprazole (20 mg) and colloidal bismuth pectin (200 mg) taken before meals, as well as amoxicillin 1 g and clarithromycin (500 mg) taken after meals twice a day. All medications were to be taken during morning and evening meal times.

This is a prospective, randomized, double-blind, placebo-controlled trial. Following the prescription of the aforementioned drugs, the minimum randomization method was employed. The subjects were randomly assigned to either the placebo or probiotics group for treatment based on stratified factors, such as sex, age, and initial DOB value.

We used probiotics in the form of a single MN-LF23 freeze-dried powder, with each bag containing 1×10^{10} colony-forming units (CFU) of live cells. The placebo consisted of maltodextrin and had the same weight, appearance, and taste as the probiotic powder but lacked active ingredients. During the 14-day treatment period, both groups consumed one bag of probiotic powder or placebo 2 h after taking their medication. Within 4 weeks after the end of the medication period, both groups were instructed to take one bag of probiotic powder or placebo after breakfast and dinner.

The participants are not allowed to consume any other probiotic products during the treatment period. The adherence of subjects to antibiotics and probiotic powders was assessed via daily online questionnaires.

Study evaluations

This study was conducted in four stages: the baseline screening period (weeks -2-0, V0), a 2-week emptying period (weeks 0-2, V1), a 2-week probiotic and drug administration period (weeks 2-4, V2), and a 4-week probiotic administration period (weeks 4-8, V3).

During the V0 phase, subjects underwent the [13]C-UBT. Baseline population data on age, sex, height, weight, body mass index (BMI), smoking habits, alcohol consumption, and allergy history were collected.

Blood safety index tests were performed on the subjects at the end of phases V1 and V3. The Hp eradication rate was evaluated by the [13]C-UBT at the end of phases V2 and V3. Experimental data were collected throughout the entire intervention period.

A comprehensive timeline of the study is outlined in Fig. 1A.

Assessment of Gastrointestinal symptoms and adverse effects

The participants were requested to recall their gastrointestinal symptom history over a 2-week period at the conclusion of the V1, V2, and V3 phases. The participants were also asked to complete the GSRS in the form of a questionnaire, which consisted of 15 questions across five levels: diarrhea, indigestion, constipation, abdominal pain, and reflux [15]. A 4-point Likert scale (absent, mild, moderate, severe) was used for scoring. Any other adverse reactions occurring outside the gastrointestinal tract were documented and assessed.

Blood and stool samples

Serum and fecal samples were collected from subjects at the conclusion of phases V1, V2, and V3. The blood collector used a blood collection vessel to obtain blood from the subjects, followed by immediate centrifugation of the collected serum and storage at -80 °C. The participants were instructed to use disposable sterile fecal collection tubes for collecting fecal samples at the visit site, with immediate storage at -80 °C.

Serum indicator detection

Enzyme-linked immunosorbent assay (ELISA) was used to detect variations in serum levels of tumor necrosis factor alpha (TNF- α) and interleukin1- β (IL-1 β) during treatment, according to the instructions of the test kit (Jiangsu Meimian Industrial Co., Ltd, Yancheng, China).

DNA extraction and metagenomic sequencing

Fecal samples were subjected to whole metagenome shotgun sequencing on the Illumina NovaSeq PE150 platform at Majorbio Bio Pharm Technology Co., Ltd. (Shanghai, China). Metagenomic sequencing was conducted as previously described [16]. Briefly, total genomic DNA was extracted from human feces using an E.Z.N.A.® Soil DNA Kit (Omega Biotek, Norcross, GA, U.S.) according to the manufacturer's instructions. The concentration and purity of total genomic DNA were assessed using a TBS 380 micro fluorometer (Turner Bio Systems, USA) and a NanoDrop2000 ultra micro spectrophotometer (Thermo Fisher Scientific, USA), respectively. Raw sequencing reads underwent quality control processing using fastp (0.23.0) [17], which involved removing low-quality reads (>45 bases with a quality score <20 or >5 'N' bases), as well as low complexity and adapter-containing reads; the remaining reads were trimmed at the tails for low quality (<Q20) or 'N' bases. Human genomic reads were eliminated by mapping to the reference human genome (GRCh38) using Bowtie2 (2.4.4) [18]. The gut microbiota composition was quantified using MetaPhlAn4 (4.0.2) algorithms [19]. The alpha diversity of the gut microbiota was determined by calculating the Shannon and Simpson

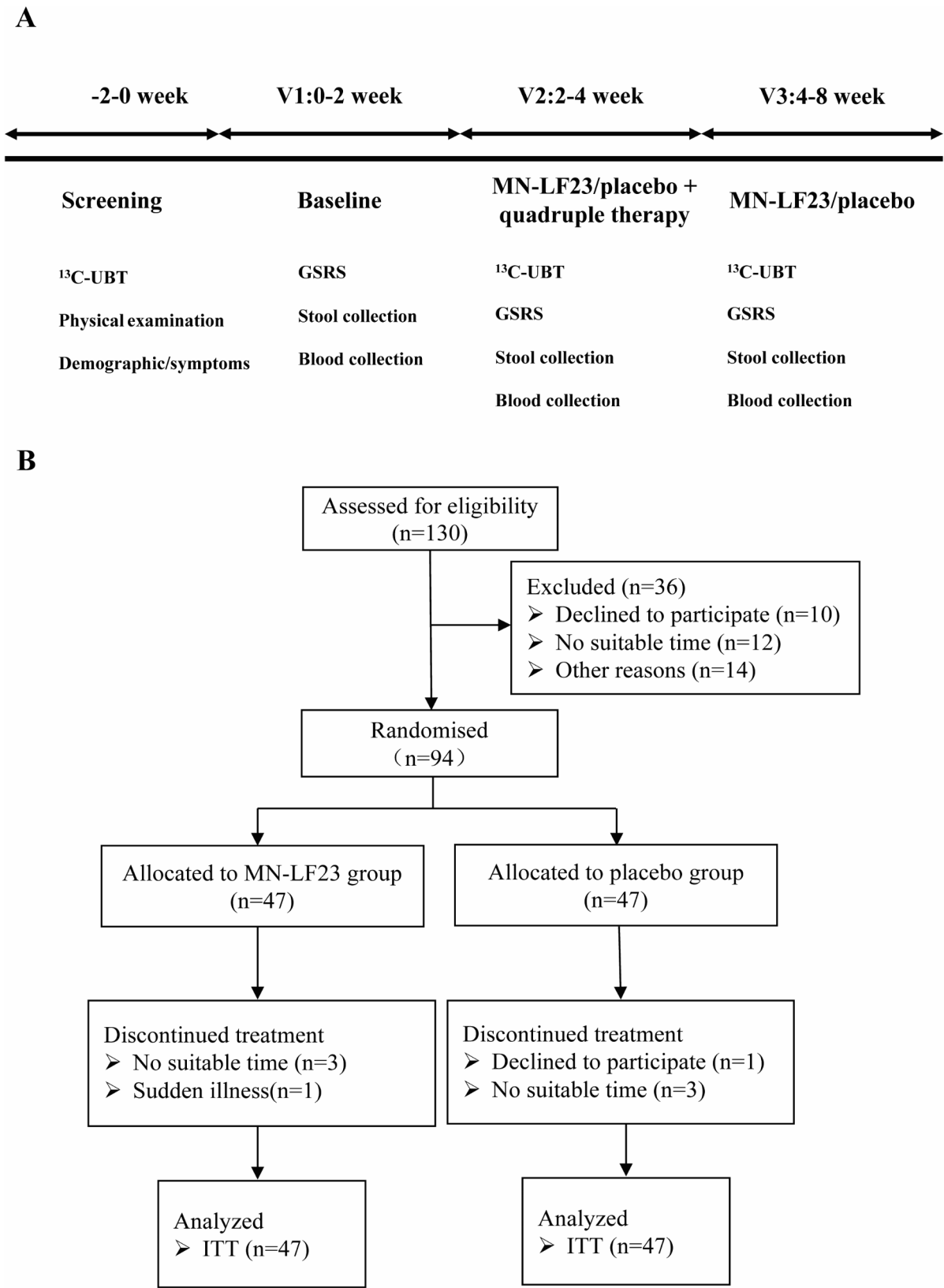


Fig. 1 Flowchart of patients throughout the study. **A:** Timeline of the study. **B:** Flow diagram of the study. ITT: Intention-to-treat

indices. The beta diversity based on principal coordinate analysis (PCoA) was estimated by calculating the Bray–Curtis distance. LEfSe was used to analyze differences in gut microbiota composition between groups.

Statistical analysis

Statistical analysis was performed using SPSS 28 software. For continuous variables, a normal or approximately normal distribution is described by mean ± standard deviation, while frequency (%) is used to describe the comparison of multiple groups of categorical variables. One-way analysis of variance was used for variables with a normal distribution or those that conformed to a normal distribution after log conversion, whereas Kruskal–Wallis rank sum test was used for non-normally distributed variables. The comparison of categorical variables between groups was conducted using the chi-square test or Fisher’s exact probability method. A *P* value < 0.05 was taken to indicate statistical significance.

Results

Baseline characteristics of patients

Figure 1B illustrates the process of subject enrollment and follow-up. Out of the 130 screened subjects, 94 eligible individuals were randomly assigned to receive placebo (*n* = 47) or probiotic (*n* = 47) intervention. Four participants from each group withdrew from the trial during the intervention process, and ITT analysis was employed to assess changes in relevant indicators throughout the intervention period. The baseline demographic data of the participants are supplemented in Table 1.

The primary outcome measure of this study was Hp eradication, as indicated by a negative.

¹³C-UBT result. The eradication rates for the placebo and probiotics groups were 85.11% (40/47) and 82.98% (39/47), respectively, with no significant differences between the groups. The changes in DOB values during the intervention period are illustrated in Fig. 2. Both

groups showed significantly lower DOB values in stages V2 and V3 compared to stage V1 (*P* < 0.001), with no significant difference observed between the groups during this period.

GSRS scores

The changes in the total GSRS score and scores for the five subscales (constipation, indigestion, reflux, abdominal pain, diarrhea) before and after treatment in both groups are depicted in Fig. 3. The probiotic group exhibited a significantly lower total GSRS score at the V3 stage than at the V1 and V2 stages (*P* < 0.01) (Fig. 3A). In terms of constipation, the V2 score of the probiotic group was significantly lower than that of the V1 group (*P* < 0.05), while the V3 score was significantly lower than that of the V1 group (*P* < 0.001), with the V3 probiotic group score also being significantly lower than that of the placebo group (*P* < 0.05) (Fig. 3B). For indigestion, the V3 score of the probiotic group was significantly lower than that of both the V1 and V2 stages (*P* < 0.01), and it was also significantly lower than that of the placebo group at this stage (*P* < 0.05) (Fig. 3C). Regarding reflux, the placebo group had a significantly lower score at stage V3 than at stage V2 (*P* < 0.05) (Fig. 3D). No significant trend was detected in the scale for abdominal pain and diarrhea (Figs. 3E, F).

Blood index analysis

Figure 4 illustrates the variations in the concentration of pro-inflammatory cytokines in the serum of the subjects throughout the treatment period. Both groups exhibited a significant decrease in serum TNF-α levels after treatment compared to baseline (Fig. 4A). However, no significant trend was observed in the serum IL-1β levels for either group during the treatment process (Fig. 4B). Additionally, there were no notable differences in TNF-α and IL-1β levels between the probiotic and placebo groups during the same period.

Gut microbiota analysis

The changes in gut microbiota composition and diversity throughout the treatment period are illustrated in Fig. 5. Compared to V1, both groups exhibited changes in the composition and abundance of gut microbiota at V2, which subsequently returned to baseline levels by V3. The composition of gut microbiota was similar between the two groups during the same period (Figs. 5A–C). Throughout the treatment process, the trends in alpha and beta diversity were similar in both groups. No significant differences were observed in α and β diversity between the two groups during this period (Figs. 5F–H). Relative to V1, there was a notable decrease in alpha diversity in both groups at V2, whereas beta diversity exhibited significant variations. However, both the

Table 1 Demographic and clinical characteristics of the patients included in the study

	Placebo (<i>n</i> = 47)	Probiotic (<i>n</i> = 47)	<i>P</i> value
Age (y), mean (SD)	43.85 (12.02)	43.43 (11.63)	0.862
Male, <i>n</i> (%)	15 (31.91)	15 (31.91)	1
Female, <i>n</i> (%)	32 (68.09)	32 (69.09)	1
Height (cm), mean (SD)	165.57 (6.96)	165.38 (8.47)	0.905
Weight (kg), mean (SD)	66.18 (11.53)	65.78 (10.59)	0.860
BMI (kg/m ²), mean (SD)	24.07 (3.45)	24.04 (3.34)	0.962
Smoker, <i>n</i> (%)	6 (12.77)	8 (17.02)	0.773
Drinker, <i>n</i> (%)	8 (17.02)	7 (14.89)	1
Baseline DOB, mean (SD)	35.65 (24.04)	33.56 (20.57)	0.653

¹³C-UBT results

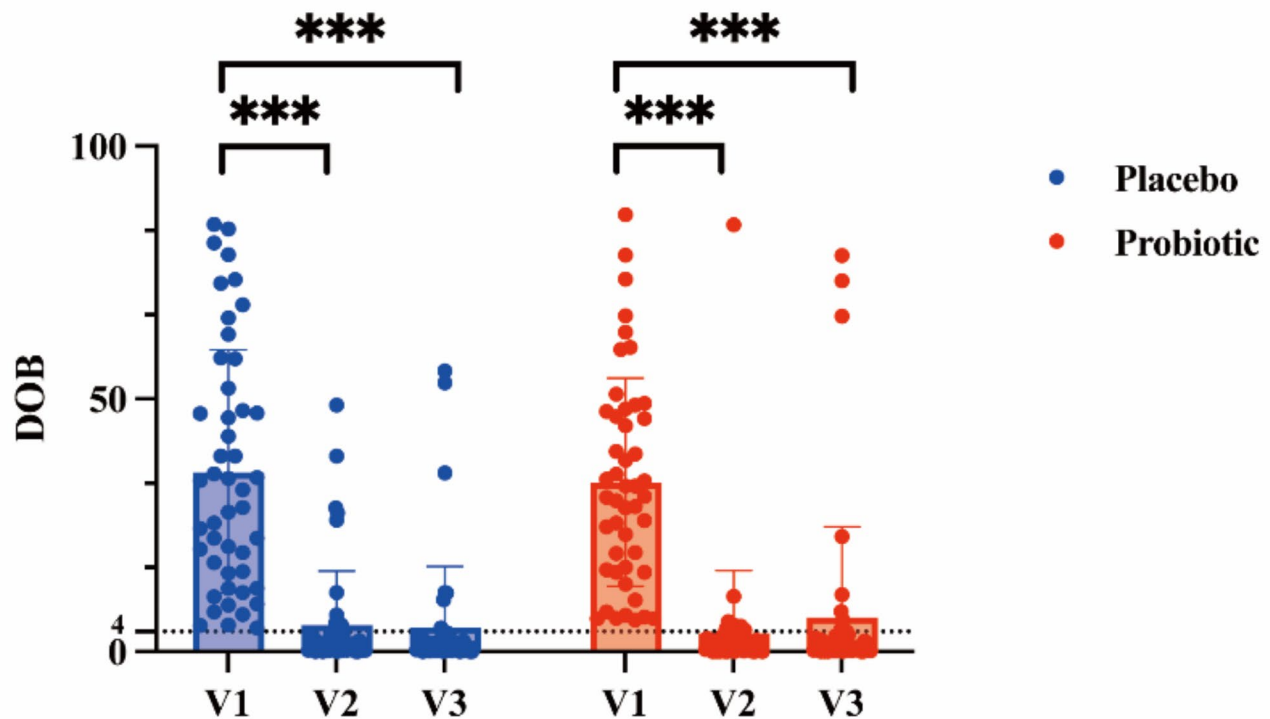


Fig. 2 Change in DOB values of the two groups during treatment. DOB: [13]C–UBT detection value, DOB > 4 indicates a positive Hp infection, whereas anything less indicates a negative result; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

alpha and beta diversity reverted to baseline levels by V3 (Figs. 5D, E, I, J).

LEfSe was used to analyze differences in gut microbiota levels between the two groups during the intervention process (Figs. 6).

During the 2-week intervention, the probiotic group exhibited a significant enrichment of beneficial bacteria, such as *Limosilactobacillus fermentum* and *Lactiplantibacillus plantarum*. In contrast, the placebo group exhibited a notable enrichment of harmful bacteria, including *Fusobacterium nucleatum*, *Prevotella bivia*, and *Fusobacterium polymorphum*.

At 6 weeks of intervention, the probiotic group continued to show significant enrichment of beneficial bacteria, such as *Bifidobacterium longum*, *Coprococcus caltus*, *Limosilactobacillus fermentum*, *Lactiplantibacillus plantarum*, and *Clostridium butyricum*.

Discussion

In this study, we assessed the impact of MN-LF23 supplementation on the eradication rate and side effects of 14-day standard quadruple therapy for Hp. Overall, our findings indicate that while supplementing with

MN-LF23 did not lead to a significant improvement in the eradication rate of Hp, it did demonstrate effectiveness in reducing patients' indigestion and constipation symptoms, as well as lowering GSRS scores. It was also found to have a positive effect on gut microbiota composition.

The Hp eradication rate is influenced by various factors, including antibiotic resistance, past history of Hp treatment, smoking, and alcohol consumption. A previous meta-analysis revealed that the resistance rates of Hp to metronidazole (51.1 to 83.3%), clarithromycin (21.1 to 37.5%), and levofloxacin (17.8 to 42.7%) significantly increased from 2013 to 2023, whereas the resistance rates to tetracycline (1.4 to 0.2%) and furazolidone (4 to 0%) has decreased year by year [20]. Research has indicated that a history of Hp treatment, smoking, and alcohol consumption is correlated with the failure of Hp eradication [21–23]. In the current study, both the placebo and probiotic groups demonstrated eradication rates exceeding 80%, which may be attributed to the fact that the subjects had no history of Hp treatment, as well as low rates of smoking and alcohol consumption.

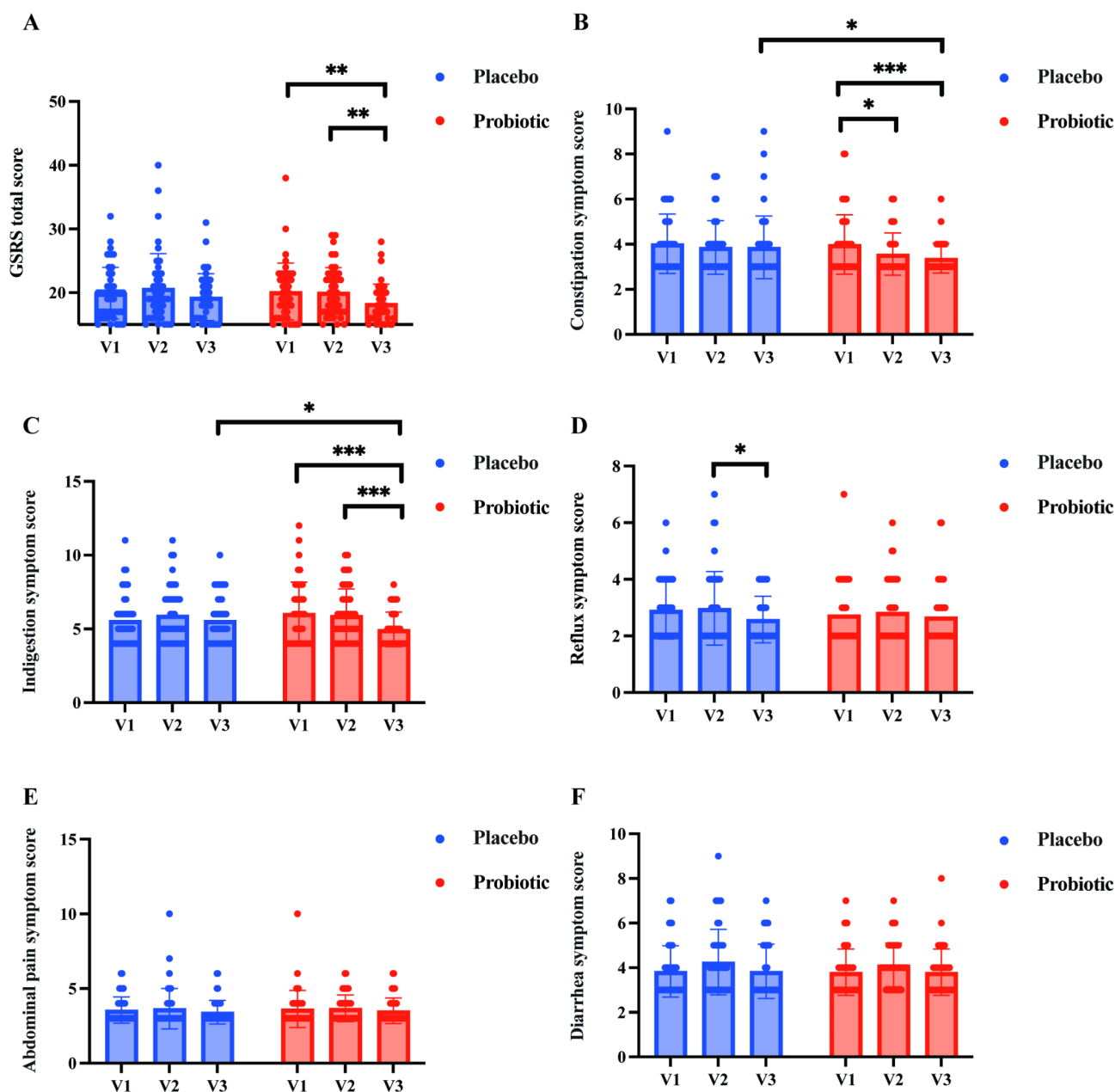


Fig. 3 Changes in the GSRS total score and the scores of its five subscales. **A:** GSRS total score. **B:** Constipation symptom score. **C:** Indigestion symptom score. **D:** Reflux symptom score. **E:** Abdominal symptom score. **F:** Diarrhea symptom score. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Our results showed that MN-LF23 did not enhance the Hp eradication rate in quadruple therapy, which is consistent with previous findings. Supplementation of *Lactiplantibacillus plantarum* and *Pediococcus acidilactici* in both triple therapy and non-bismuth quadruple therapy regimens did not enhance the Hp eradication rate [placebo 95% (95% confidence interval [CI]: 89 to 98%) vs. probiotic 97% (95% CI: 92 to 99%), $P = 0.721$]¹³. While other studies have shown that *Saccharomyces boulardii* CNCM I-745 [24] and *Lactobacillus gasseri* OLL2716 [25] can significantly improve the eradication

rate of antibiotic therapy. The comparative study design revealed significant differences in the baseline characteristics, including history of Hp treatment, average age, and location. There were also variations in probiotic use regimens, such as the duration of use, dosage, and type of intake and antibiotic used. The subjects recruited for this study had no history of Hp treatment, which may have contributed to the higher antibiotic efficacy and may have weakened the role of probiotics in the study's findings.

Multiple studies have demonstrated the efficacy of probiotics in alleviating the adverse effects of antibiotic

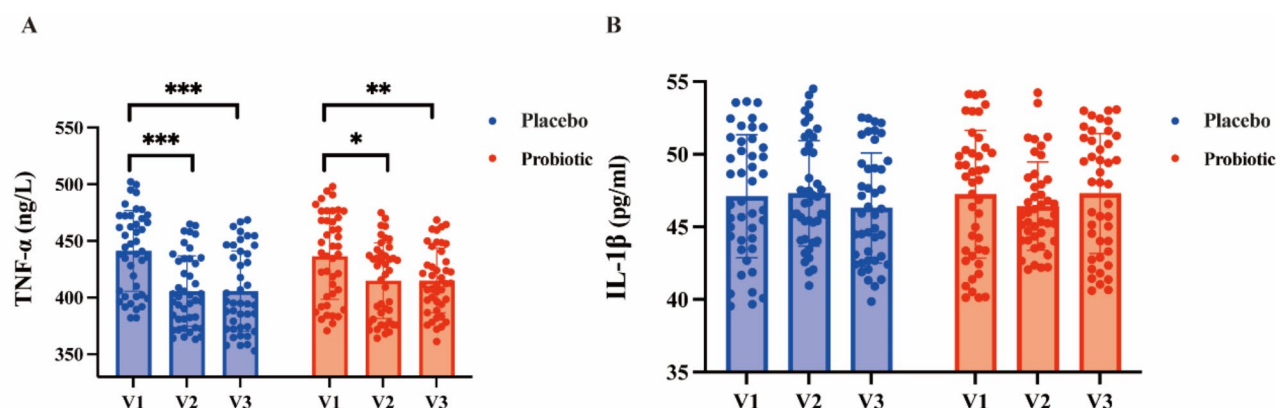


Fig. 4 Changes in the serum levels of pro-inflammatory cytokines before and after treatment. **A:** TNF- α concentration changes. **B:** IL-1 β concentration changes. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

treatment [12, 26]. Following a 2 week standard triple therapy regimen, subjects were instructed to continue taking *Lactocaseibacillus rhamnosus* DSM17648 or placebo for an additional 4 weeks. The probiotics group exhibited significant reductions in symptoms such as indigestion, constipation, abdominal pain, and overall GSRS scores post-treatment [11]. Furthermore, this study revealed that MN-LF23 effectively improved the symptoms of indigestion and constipation.

Hp infection can induce the expression of pro-inflammatory cytokines, such as TNF- α [27] and IL-1 β [28], in gastric mucosal cells, leading to gastric inflammation. However, Hp infection does not necessarily cause elevated serum proinflammatory cytokine levels. A previous meta-analysis revealed that serum TNF- α levels were significantly higher in patients with Hp infection than in uninfected individuals (15 studies, 1224 subjects; standardized mean difference [SMD] 0.88; 95% CI: 0.46, 1.29; $P \leq 0.0001$). However, there was no significant impact on serum IL-1 β levels (6 studies, 463 subjects, SMD: 0.10; 95% CI: -0.39, 0.59; $P = 0.682$) [29]. Our research findings agree with the conclusions of this meta-analysis. Following the eradication of Hp, the serum TNF- α levels of the subjects were significantly reduced, whereas there was no significant effect on IL-1 β levels. During the same period, there was no significant difference in serum inflammatory cytokine levels between the probiotic and placebo groups, which may be attributed to the initial eradication of Hp. The high efficacy of standard quadruple therapy weakened the role of probiotics.

Given the rapid development of DNA sequencing technology, the close relationship between changes in the gut microbiota and various gastrointestinal diseases has been widely confirmed [30–33]. Metagenomic sequencing technology can identify species and even strains at the species level, with a higher depth of species identification [34]. However, most studies on the impact of Hp eradication on gut microbiota are based on 16 S rRNA

sequencing analysis [9, 35, 36], which has limited effectiveness in identifying microbial species.

The administration of antibiotics can have a significant impact on the composition of the gastrointestinal microbiota in individuals [37]. The findings of this study revealed that the alpha diversity of the gut microbiota in both the placebo and probiotic groups experienced a notable decrease at 2 weeks but returned to baseline levels by 6 weeks. Additionally, both groups exhibited significant changes in beta diversity at 2 weeks, which were restored by 6 weeks. However, no substantial difference in diversity was observed between the two groups during this timeframe. These results are consistent with previous research conducted by Tang et al., which also found no significant variance in diversity between a probiotic group (supplemented with *Enterococcus faecalis* and *Bacillus subtilis*) and a placebo group [38]. However, other studies have also demonstrated that supplementation with inactivated *Limosilactobacillus reuteri* significantly enhances antibiotic-induced disruption of gut microbiota diversity [39]. In this study, although there was no discernible difference in the overall structure of the gut microbiota between the probiotic and placebo groups, the probiotic group exhibited a significant enrichment of beneficial bacteria such as *Limosilactobacillus fermentum*, *Lactiplantibacillus plantarum*, *Bifidobacterium longum*, *Coprococcus callus*, and *Clostridium butyricum*, compared to the placebo group. These bacteria contribute to gut microbiota health. Specifically, *Limosilactobacillus fermentum* [40], *Lactiplantibacillus plantarum* [41], and *Bifidobacterium longum* [42] have been shown to be beneficial in improving IBD, whereas *Coprococcus caltus* [43] and *Clostridium butyricum* [44] can produce butyric acid, which is advantageous for intestinal health. This suggests that MN-LF23 plays a positive role in mitigating antibiotic-induced disruption of the gastrointestinal microbiota and may explain why the probiotic group experienced significantly lower levels

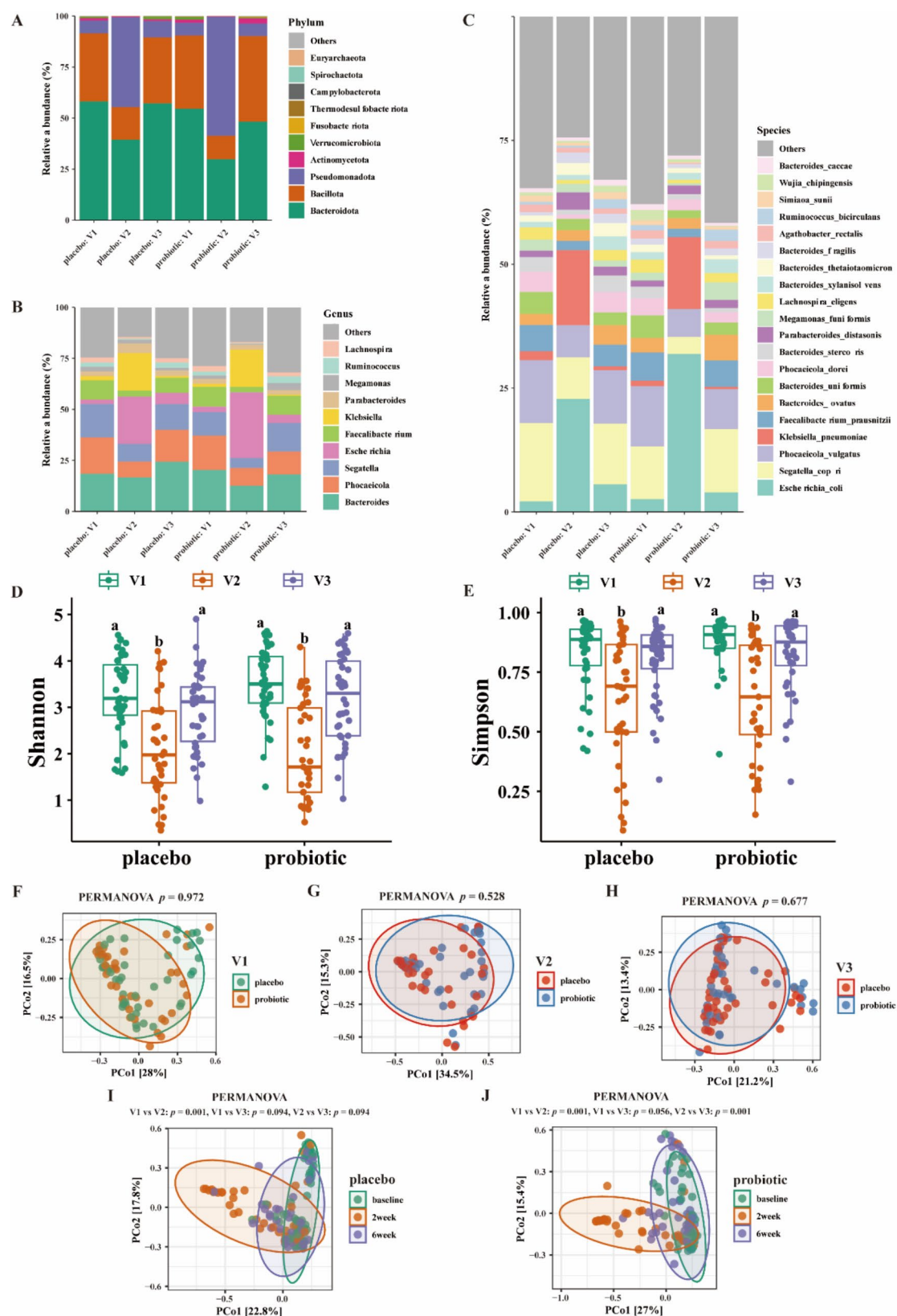


Fig. 5 Changes in gut microbiota composition and alpha and beta diversity during treatment. **A–C**: Changes in gut microbiota at the phylum, genus, and species levels. **D** and **E**: Changes in alpha diversity of gut microbiota between the two groups. **F–H**: Comparison of beta diversity between the two groups of subjects during the same period. **I** and **J**: Changes in beta diversity in the placebo group and probiotic group. Means followed by different letters indicate significant differences, $P < 0.05$

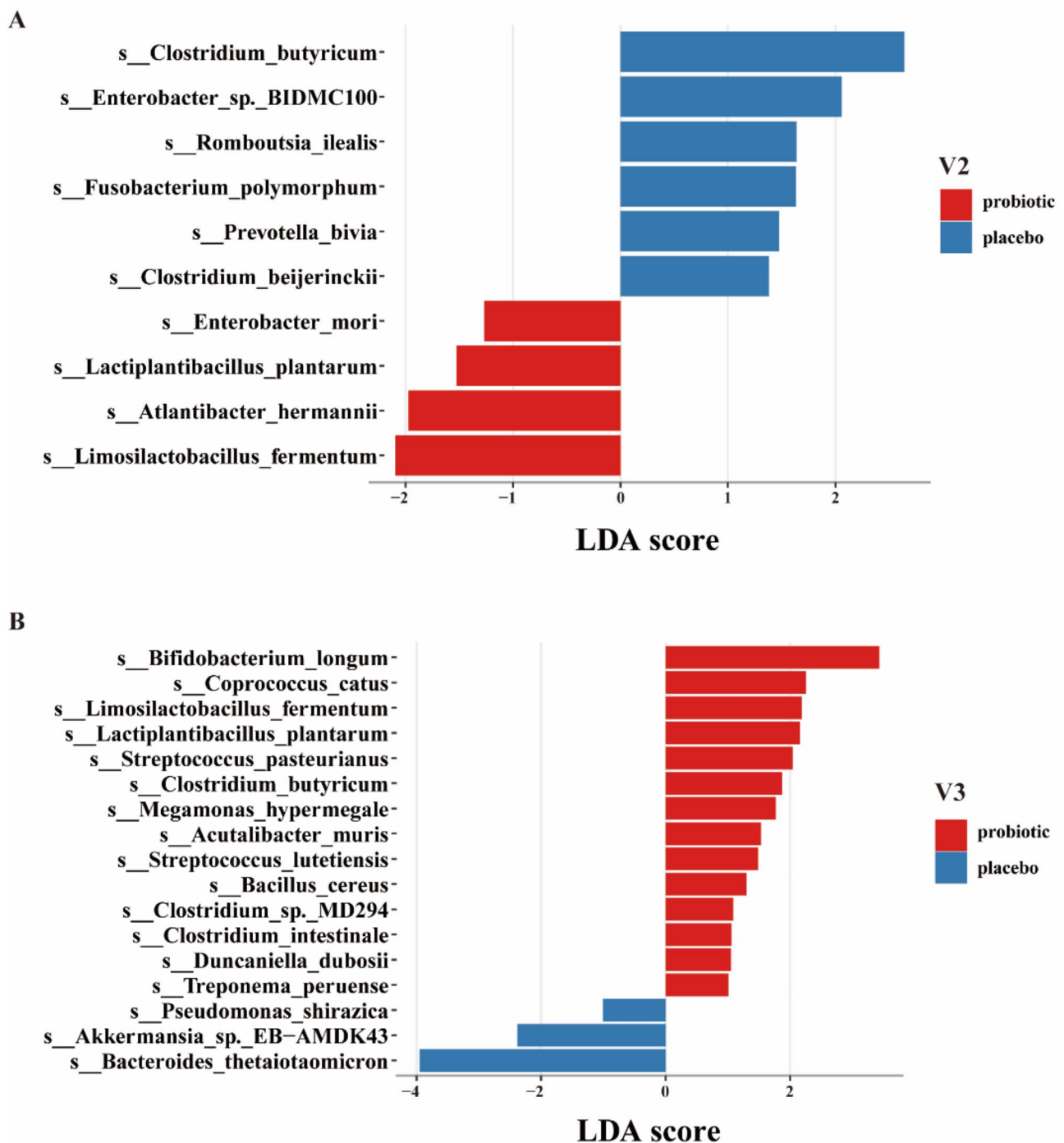


Fig. 6 The species level differences in gut microbiota between the two groups during treatment. **A:** Two-week intervention. **B:** Six-week intervention

of indigestion, constipation, and GSRS scores compared to the placebo group.

In this study, we used the independently developed MN-LF23, which has a comprehensive preliminary experimental basis from screening to animal experiments. The changes in the gastrointestinal microbiota of patients during the treatment process and the role of MN-LF23 were subjected to in-depth analysis using

metagenomic sequencing technology. We also assessed the impact of MN-LF23 on the efficacy and adverse reactions of antibiotics at the end of treatment (V2).

There are still some limitations to this study. First, the subjects included in the study were middle-aged and elderly. Second, the tracking period was relatively short and can be followed for another 4 weeks after V3 to fully detect dynamic changes in the gut microbiota.

Furthermore, the gastric mucosa of the subjects was not collected; therefore, the impact of probiotics on the patients' gastric microbiota remains unknown.

Conclusions

In conclusion, our findings suggest that supplementation with MN-LF23 does not improve the eradication rate of the 14-day standard quadruple therapy. However, MN-LF23 was effective in alleviating the side effects associated with antibiotic treatment, including reducing indigestion, constipation, and GSRS scores, as well as regulating the gut microbiota balance.

Abbreviations

Hp	<i>Helicobacter Pylori</i>
MN-LF23	<i>Limosilactobacillus Fermentum</i> MN-LF23
GSRS	Gastrointestinal Symptom Rating Scale
IARC	International Agency for Research on Cancer
WHO	World Health Organization
¹³ C-UBT	¹³ C-Urea Breath Test
CFU	Colony-forming Units
BMI	Body Mass Index
ELISA	Enzyme-linked Immunosorbent Assay
TNF-α	Tumor Necrosis Factor Alpha
IL-1β	Interleukin1-β
PCoA	Principal Coordinate Analysis
ITT	Intention-to-treat

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Author contributions

Conceptualization, LZ, YM, XZ and RW; Methodology, YZ, YZ, LZ, JH, and RW; Formal analysis and investigation, YZ, XN, LZ and QZ; Writing—original draft preparation, YZ; Writing—review and editing, FW, XZ and RW; Supervision, YZ, YM, FW and XZ; Resources, YM, FW, XZ and RW. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was registered with the Chinese Clinical Trial Registry (registration number: ChiCTR220059817) and was approved by the Medical Ethics Committee of The First Medical Center of Chinese PLA General Hospital (approval number: S2022-060-02). This study was conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki. All participants voluntarily signed a written informed consent form after fully understanding the potential benefits and risks of participating in the trial.

Consent for publication

All study subjects gave their written informed consent that their data could be used for publications in anonymized form.

Competing interests

The authors declare no competing interests.

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