

Clinical Outcomes of Rifaximin Combined with Probiotics in Patients with Functional Diarrhea A Prospective Cohort Study

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Abstract

Background

Functional diarrhea is a chronic disorder of gut-brain interaction with limited treatment options. Evidence for rifaximin in this population remains scarce.

Aims

To evaluate whether rifaximin plus probiotics was associated with improved stool consistency and related symptoms in adults with functional diarrhea.

Methods

This single-center, non-randomized prospective cohort study enrolled consecutive adults with functional diarrhea from April 2022 to June 2024. After physician counseling, patients chose rifaximin plus probiotics or probiotics alone. Stool consistency assessed using the Bristol Stool Form Scale was the primary endpoint. Secondary endpoints included mean daily bowel movements, urgency of defecation, and incomplete evacuation. Outcomes were assessed at baseline, end of treatment, and 1 month after treatment.

Results

Among 121 patients completing follow-up, 62 received rifaximin plus probiotics and 59 received probiotics alone. Stool consistency improved in both groups. Although the overall group-by-time interaction was not significant, the 1-month group-by-time estimate favored rifaximin plus probiotics (OR = 0.399, 95% CI 0.165–0.964, $P = 0.041$). Daily bowel movements decreased in both groups, with fewer bowel movements in the rifaximin-plus-probiotics group at 1 month (mean difference = -0.568, 95% CI -1.036 to -0.101; adjusted $P = 0.005$). No clear between-group differences were observed for urgency or incomplete evacuation. No serious adverse events were reported.

Conclusion

Rifaximin plus probiotics was associated with better maintenance of stool consistency improvement and lower stool frequency at 1 month than probiotics alone. These exploratory findings require confirmation in randomized controlled trials.

Introduction

Functional diarrhea (FDr) is a prevalent gastrointestinal disorder characterized by chronic loose or watery stools (Sperber et al., 2021), affecting approximately 5.6% of the population in China (Xiong et al., 2004). While symptom-based treatments often utilize probiotics to regulate gut microbiota, their efficacy as monotherapy can be inconsistent (Ford et al., 2018; Niu and Xiao, 2020; Preidis et al., 2020; Su et al., 2020). Rifaximin, a non-absorbable broad-spectrum antibiotic, effectively manages similar microbiota-related gastrointestinal disorders without systemic side effects (Schoenfeld et al., 2014; Weingarden and Ko, 2024). However, conclusive evidence supporting its specific use in FDr remains lacking (Lacy et al., 2016).

Therefore, this study aimed to evaluate the efficacy and safety of combining rifaximin with probiotic therapy (live *Bacillus licheniformis* and *Lactobacillus acidophilus*) compared to probiotics alone in patients with FDr.

Methods

Study Design and Setting

This was a single-center, non-randomized prospective cohort study conducted at the Gastroenterology outpatient department of the Second Affiliated Hospital of Soochow University, Suzhou, China, from April 2022 to June 2024. Treatment allocation was based on patient preference after physician counseling rather than randomization. This observational study was not prospectively registered.

Participants

Adults aged 18–65 years with functional diarrhea (FDr) were screened for eligibility according to the Rome IV criteria. Detailed inclusion and exclusion criteria are listed below.

Inclusion Criteria

1. Age between 18 and 65 years;
2. Diagnosed with FDr according to Rome IV criteria;
3. Ability to understand the research details and voluntarily sign informed consent;
4. No FDr-related medication within two weeks prior to enrollment.

Exclusion Criteria

1. Colonoscopy indicating inflammatory bowel disease, colon cancer, ischemic bowel disease, or other organic bowel diseases;
2. Severe cardiovascular, renal, hepatic, or other critical organ failure;
3. Advanced malignancy or severe cachexia;
4. Pregnancy or breastfeeding status;
5. Allergy to rifaximin or any components of the probiotics used;
6. Severe mental illness or cognitive impairments preventing understanding of or compliance with the study protocol;
7. History of alcohol or substance abuse.

Sample size estimation

At the study design stage, the sample size was estimated using the formula for comparing two proportions (SK and S, 1991):

$$n = ((Z_{\alpha/2} + Z_{\beta})^2 \times [p_1(1 - p_1) + p_2(1 - p_2)]) / (p_2 - p_1)^2.$$

Based on prior literature and preliminary clinical observations, the anticipated response rate was 50% in the control group and 75% in the combination-therapy group. With a two-sided significance level of 0.05 and 80% power, the required sample size was calculated as 54.88 participants per group, which was rounded up to 55. Assuming a 10% dropout rate, the target enrollment was 61 participants per group, for a total planned sample size of 122 participants. Because this estimate was made during the study planning stage based on the originally defined response-rate comparison, it should be interpreted as a planning reference rather than a formal power calculation for the revised primary outcome in the present analysis.

Ethics Statement

The study was approved by the Ethics Committee of the Second Affiliated Hospital of Soochow University (Approval No. LC2024009). All participants provided written informed consent before enrollment. The study was conducted in accordance with the 2013 revision of the Declaration of Helsinki.

Intervention

Patients in the rifaximin-plus-probiotics (RP) group received rifaximin (400 mg twice daily for 6 consecutive days), *Bacillus licheniformis* (0.25g three times daily for 12 days), and *Lactobacillus acidophilus* (0.5g three times daily for 12 days). Patients in the probiotics-only (PO) group received the same probiotic regimen without rifaximin. The rifaximin regimen reflected local clinical practice and affordability considerations and was not intended to represent a standard FDr registration regimen. The medications used in the study were:

Rifaximin: Brand name: 'Xifushen', 0.2g tablets (12 tablets per box), manufactured by Alfa Sigma Co., Ltd., approval number H20181212, valid for 36 months, stored in a light-protected, tightly sealed container.

***Bacillus licheniformis*:** Brand name: 'Zhengchangsheng', 0.25g capsules (36 capsules per bottle), manufactured by Northeast Pharmaceutical Group Shenyang No.1 Pharmaceutical Co., Ltd., approval number National Medicine Zhunzi S20073008, valid for 24 months, stored at room temperature in a light-protected, dry place.

***Lactobacillus acidophilus*:** Brand name: 'Yijinkang', 0.5g tablets (24 tablets per box), manufactured by Tonghua Jinma Pharmaceutical Group Co., Ltd., approval number National Medicine Zhunzi H10940114, valid for 24 months, stored in a light-protected, sealed container at room temperature.

Efficacy Endpoints

As no clinical trials have specifically evaluated rifaximin in functional diarrhea to date, the efficacy endpoints in this study were selected by referring to outcome measures widely adopted in rifaximin clinical trials for diarrhea-predominant irritable bowel syndrome (Pimentel et al., 2011; Garsed et al., 2014; Lembo et al., 2016).

Primary Endpoint

The primary endpoint was stool consistency assessed using the Bristol Stool Form Scale (BSFS) (Lewis and Heaton, 1997) (types 1–7) at baseline (T_1), at the end of treatment (T_2), and 1 month after treatment (T_3).

Secondary Endpoints

Secondary endpoints included mean daily bowel movements (Daily BMs) (calculated from three consecutive days), as well as the urgency of defecation and incomplete evacuation, which were both classified into five severity grades: grade 0 (not at all), grade 1 (mildly), grade 2 (moderately), grade 3 (greatly), and grade 4 (extremely).

Exploratory endpoints

Exploratory outcomes included presence or absence of frequent flatulence, borborygmi, mucus in stool, and frothy stool (recorded as yes/no).

Follow-up and Data Collection

Follow-up assessments were conducted at T_2 and T_3 through outpatient review, telephone interview, or online questionnaire. Stool consistency was assessed using patient-provided stool photographs with BSFS reference guidance whenever available. Medication adherence was assessed by pill counts, and telephone confirmation. Patients were advised not to initiate new antidiarrheal agents, antibiotics, or probiotic products during follow-up. Missing data were handled by complete-case analysis. Safety information was collected throughout treatment and follow-up, including any patient-reported adverse events, treatment interruption, treatment discontinuation, and serious adverse events.

Statistical Analysis

Baseline continuous variables were summarized as mean $\pm$ standard deviation or median (interquartile range), as appropriate, and categorical variables as frequencies and percentages.

Repeated-measures analyses were selected according to outcome type. Stool consistency was analyzed using an adjusted ordinal repeated-measures model with cumulative logit link, reporting odds ratios (ORs) and 95% confidence intervals (CIs). Daily BMs was analyzed using a repeated-measures linear model / generalized estimating equation (GEE) with identity link, reporting mean differences and 95%CIs. Urgency of defecation and incomplete evacuation were originally planned for ordinal repeated-measures modeling; however, the adjusted ordinal models did not converge because of sparse observations across some category-by-time combinations. Therefore, these two 5-level symptom scores were analyzed as approximately continuous outcomes using a repeated-measures linear model / GEE with identity link, and the results were interpreted as supportive rather than definitive ordinal-effect estimates. Binary exploratory outcomes were analyzed using GEE models for repeated binary data, with regression-based comparative estimates reported instead of within-group relative risks. An exchangeable working correlation structure was specified for GEE analyses.

All multivariable repeated-measures models were adjusted for sex and disease duration. Pairwise between-group comparisons at each time point were corrected using the Bonferroni method. Two-sided *P* values < 0.05 were considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics version 27.0.

Results

Patient Characteristics

A total of 133 patients were enrolled, including 69 in the RP group and 64 in the PO group (Fig. 1). In the RP group, 3 patients were excluded because of poor compliance, 1 discontinued participation because of temporary work-related reasons, and 3 were lost to follow-up. In the PO group, 3 patients discontinued or changed medication before completing treatment and were excluded, and 2 were lost to follow-up. Therefore, 121 patients completed treatment and follow-up and were included in the final analysis, including 62 in the RP group and 59 in the PO group. Baseline demographic and clinical characteristics were generally comparable between groups (Table 1). Baseline stool consistency, Daily BMs, urgency, incomplete evacuation, and other recorded symptoms did not differ significantly between groups (Table 2).

Table 1
Baseline characteristics of participants, by group.

Characteristic	Rifaximin-plus-probiotics Group(n = 62)	Probiotics-only group(n = 59)	P
Sex, n (%)	42(67.74%)	39(66.10%)	0.848
Male			
Female	20(32.26%)	20(33.90%)	
Age, y	43.81 ± 8.73	42.36 ± 11.60	0.44
Height, cm	170.05 ± 5.99	169.12 ± 9.65	0.528
Weight, kg	71.36 ± 9.66	70.58 ± 14.79	0.734
BMI, n (%)			0.338
Underweight BMI<18.5kg/m ²	0	1(1.69%)	
Normal 18.5 ≤ BMI<24kg/m ²	22(35.48%)	24(40.68%)	
Overweight 24 ≤ BMI < 28kg/m ²	31(50%)	28(47.46%)	
Obese BMI≥28kg/m ²	9(14.52%)	6(10.17%)	
Disease duration, y	5(2,10)	5(2,10)	0.68
Hypertension, n (%)	11(17.74%)	15(25.4%)	0.304
Diabetes, n (%)	2(3.23%)	3(5.08%)	0.674
Smoking, n (%)	10(16.13%)	11(18.6%)	0.715
Alcohol consumption, n (%)	10(16.13%)	13(22.0%)	0.408
Cholecystectomy, n (%)	4(6.45%)	3(5.08%)	1

Table 2

Comparison of clinical symptom outcomes between the RP group and PO group at baseline and during follow-up.

Characteristic	T ₁		P	T ₂		T ₃	
	RP Group	PO Group		RP Group	PO Group	RP Group	PO Group
	(n = 62)	(n = 59)		(n = 62)	(n = 59)	(n = 62)	(n = 59)
Main Symptoms							
Stool consistency, n (%)			0.979				
4	0	0		38(61.3%)	25(42.4%)	31(50.0%)	10(16.9%)
5	0	0		13(21.0%)	14(23.7%)	9(14.5%)	15(25.4%)
6	44(71.0%)	42(71.2%)		10(16.1%)	19(32.2%)	20(32.3%)	31(52.5%)
7	18(29.0%)	17(28.8%)		1(1.6%)	1(1.7%)	2(3.2%)	3(5.1%)
Secondary symptoms							
Daily BMs(times/day), Median (IQR)	2.25(2,3.125)	2.5(2,3.5)	0.167	1.5(1.0,2.0)	2.0(1.5,2.5)	1.5(1.0,2.125)	2.5(1.5,3.0)
Urgency of defecation, n (%)			0.502				
0	16(25.8%)	16(27.1%)		33(53.2%)	28(47.5%)	29(46.8%)	23(39.0%)
1	9(14.5%)	7(11.9%)		24(38.7%)	21(35.6%)	21(33.9%)	12(20.3%)
2	21(33.9%)	15(25.4%)		5(8.1%)	8(13.6%)	12(19.4%)	17(28.2%)
3	13(21.0%)	15(25.4%)		0	2(3.4%)	0	7(11.9%)
4	3(4.8%)	6(10.2%)		0	0	0	0
Incomplete evacuation, n (%)			0.701				
0	22(35.5%)	22(37.3%)		32(51.6%)	29(49.2%)	28(45.2%)	26(44.1%)
1	14(22.6%)	8(13.6%)		25(40.3%)	21(35.6%)	30(48.4%)	20(33.9%)
2	16(25.8%)	17(28.8%)		4(6.5%)	8(13.6%)	3(4.8%)	9(15.3%)
3	9(14.5%)	12(20.3%)		1(1.6%)	1(1.7%)	1(1.6%)	4(6.8%)
4	1(1.6%)	0		0	0	0	0
Other symptoms							
Frequent flatulence, n (%)			0.336				
RP group: Rifaximin-plus-probiotics group; PO group: Probiotics-only group. T1: Baseline; T2: At the end of treatment; T3: 1 month after treatment.							
P-values in the "T ₁ " column represent between-group comparisons at baseline, calculated using the Chi-square test, Fisher's exact test, or Mann-Whitney U test, as appropriate.							

Characteristic	T ₁			T ₂		T ₃	
	RP Group	PO Group	P	RP Group	PO Group	RP Group	PO Group
	(n = 62)	(n = 59)		(n = 62)	(n = 59)	(n = 62)	(n = 59)
Main Symptoms							
yes	43(69.35%)	36(61.02%)		10(6.13%)	20(33.90%)	15(24.19%)	27(45.76%)
no	19(30.65%)	23(38.98%)		52(83.87%)	39(66.10%)	47(75.81%)	32(54.24%)
Borborygmi, n (%)			0.682				
yes	24(38.71%)	25(42.37%)		8(12.9%)	10(16.9%)	13(20.1%)	18(30.5%)
no	38(61.29%)	34(57.6%)		54(87.1%)	49(83.1%)	49(87.1%)	41(69.5%)
Mucus in stool, n (%)			0.563				
yes	19(30.65%)	21(35.59%)		9(14.5%)	9(15.3%)	10(16.1%)	14(23.7%)
no	43(69.35%)	38(64.41%)		53(85.5%)	50(84.7%)	52(83.9%)	45(76.3%)
Frothy stool, n (%)			0.436				
yes	15(24.19%)	18(30.51%)		7(11.3%)	8(12.9%)	8(13.6%)	11(18.6%)
no	47(75.81%)	41(69.49%)		55(88.7%)	54(87.1%)	51(86.4%)	48(81.4%)
RP group: Rifaximin-plus-probiotics group; PO group: Probiotics-only group. T1: Baseline; T2: At the end of treatment; T3: 1 month after treatment.							
P-values in the "T ₁ " column represent between-group comparisons at baseline, calculated using the Chi-square test, Fisher's exact test, or Mann-Whitney U test, as appropriate.							

Primary Endpoint: stool consistency

In the adjusted ordinal repeated-measures analysis, stool consistency (assessed using the Bristol Stool Form Scale, BSFS) improved significantly over time in both groups. Compared with baseline, the odds of being in a worse stool consistency category were markedly reduced at T₂ (OR = 0.022, 95%CI 0.010–0.047, P < 0.001) and at T₃ (OR = 0.070, 95%CI 0.032–0.154, P < 0.001). The group main effect was not statistically significant (OR = 0.713, 95%CI 0.353–1.443, P = 0.347). The overall group-by-time interaction did not reach statistical significance (P = 0.080). However, the 1-month group-by-time estimate was statistically significant (OR = 0.399, 95%CI 0.165–0.964, P = 0.041), suggesting a more favorable temporal shift in stool consistency in the RP group at T₃ (Table 3).

Table 3
Statistical analysis of stool consistency.

	Unadjusted model			Adjusted model		
	β	OR(95%CI)	P	β	OR(95%CI)	P
Stool consistency						
Group (RP vs PO)	-0.361	0.697 (0.345, 1.410)	0.315	-0.338	0.713 (0.353, 1.443)	0.347
Time						
T ₁	Reference			Reference		
T ₂	-3.894	0.020 (0.008, 0.049)	< 0.001	-3.834	0.022 (0.010, 0.047)	< 0.001
T ₃	-2.487	0.083 (0.032, 0.216)	< 0.001	-2.661	0.070 (0.032, 0.154)	< 0.001
Group * time						
Group * T ₁	Reference			Reference		
Group * T ₂	-0.425	0.654 (0.258, 1.656)	0.37	-0.498	0.608 (0.253, 1.460)	0.265
Group * T ₃	-1.082	0.339 (0.114, 1.012)	0.053	-0.919	0.399 (0.165, 0.964)	0.041
Overall Interaction P			0.152	0.08		
RP group: Rifaximin-plus-probiotics group; PO group: Probiotics-only group. T1: Baseline; T2: At the end of treatment; T3: 1 month after treatment.						
The adjusted model has been covariate-corrected for sex and disease duration.						

Descriptively, median BSFS score (corresponding to stool consistency) decreased from 6 (6, 6) to 4 (4, 5) at the end of treatment and remained 4 (4, 6) at T₃ in the RP group. In the PO group, the median BSFS score changed from 6 (6, 7) to 5 (4, 6) at the end of treatment and to 6 (5, 6) at T₃. These findings suggest improvement in both groups, with a possible advantage in maintaining stool consistency improvement in the RP group (Table 2).

Secondary Endpoint

Mean daily bowel movements (Daily BMs)

In the adjusted repeated-measures analysis, Daily BMs decreased significantly over time in both groups. Compared with baseline, Daily BMs were lower at T₂ (mean difference=-0.958, 95%CI -1.186 to -0.730, P < 0.001) and at T₃ (mean difference=-0.568, 95%CI -0.766 to -0.369, P < 0.001). The group main effect was not statistically significant (P = 0.196), but the overall group-by-time interaction was significant (P = 0.009), indicating different trajectories over time between the two treatment groups.

Bonferroni-adjusted pairwise comparisons showed no statistically significant between-group difference at the end of treatment (mean difference=-0.364, 95%CI -0.749 to 0.021; adjusted P = 0.082), whereas the RP group had lower Daily BMs at T₃ (mean difference=-0.568, 95%CI -1.036 to -0.101; adjusted P = 0.005). (Table 4)

Table 4
Statistical analysis of secondary endpoints.

	Unadjusted model		Adjusted model			
	Mean Diff (95% CI)	<i>P</i>	Bonf. <i>P</i>	Mean Diff (95% CI)	<i>P</i>	Bonf. <i>P</i>
Daily BMs						
Group (RP vs PO)	-0.253 (-0.631, 0.126)	0.191		-0.249 (-0.626, 0.128)	0.196	
Pairwise Comparisons						
RP vs PO at T ₁	-0.253 (-0.820, 0.314)		1	-0.249 (-0.814, 0.316)		1
RP vs PO at T ₂	-0.368 (-0.755, 0.020)		0.08	-0.364 (-0.749, 0.021)		0.082
RP vs PO at T ₃	-0.572 (-1.041, -0.103)		0.005	-0.568 (-1.036, -0.101)		0.005
Time						
T ₁	Reference			Reference		
T ₂	-0.958 (-1.186, -0.730)	< 0.001		-0.958 (-1.186, -0.730)	< 0.001	
T ₃	-0.568 (-0.766, -0.369)	< 0.001		-0.568 (-0.766, -0.369)	< 0.001	
Group * time						
Group * T ₁	Reference			Reference		
Group * T ₂	-0.115 (-0.391, 0.161)	0.414		-0.115 (-0.391, 0.161)	0.414	
Group * T ₃	-0.319 (-0.584, -0.055)	0.018		-0.319 (-0.584, -0.055)	0.018	
Overall Interaction <i>P</i>		0.009			0.009	
Urgency of defecation						
Group (RP vs PO)	-0.151 (-0.608, 0.306)	0.516		-0.170 (-0.620, 0.281)	0.46	
Pairwise Comparisons						
RP vs PO at T ₁	-0.151 (-0.836, 0.533)		1	-0.170 (-0.844, 0.505)		1
RP vs PO at T ₂	-0.180 (-0.574, 0.213)		1	-0.184 (-0.573, 0.205)		1
RP vs PO at T ₃	-0.410 (-0.907, 0.087)		0.233	-0.410 (-0.904, 0.083)		0.221
Time						
T ₁	Reference			Reference		
T ₂	-1.068 (-1.337, -0.798)	< 0.001		-1.068 (-1.337, -0.798)	< 0.001	
T ₃	-0.661 (-0.858, -0.464)	< 0.001		-0.661 (-0.858, -0.464)	< 0.001	
Group * time						
Group * T ₁	Reference			Reference		
Group * T ₂	-0.029 (-0.384, 0.326)	0.873		-0.014 (-0.370, 0.342)	0.938	
Group * T ₃	-0.258 (-0.557, 0.041)	0.09		-0.241 (-0.540, 0.059)	0.116	

	Unadjusted model		Adjusted model			
	Mean Diff (95% CI)	<i>P</i>	Bonf. <i>P</i>	Mean Diff (95% CI)	<i>P</i>	Bonf. <i>P</i>
Daily BMs						
Overall Interaction <i>P</i>		0.056			0.069	
Incomplete evacuation						
Group (RP vs PO)	-0.080 (-0.491, 0.330)	0.702		-0.091 (-0.504, 0.323)	0.668	
Pairwise Comparisons						
RP vs PO at T ₁	-0.080 (-0.695, 0.535)		1	-0.091 (-0.709, 0.528)		1
RP vs PO at T ₂	-0.081 (-0.487, 0.325)		1	-0.086 (-0.491, 0.319)		1
RP vs PO at T ₃	-0.202 (-0.644, 0.239)		1	-0.206 (-0.647, 0.235)		1
Time						
T ₁	Reference			Reference		
T ₂	-0.644 (-0.842, -0.446)	< 0.001		-0.644 (-0.842, -0.446)	< 0.001	
T ₃	-0.475 (-0.659, -0.290)	< 0.001		-0.475 (-0.659, -0.290)	< 0.001	
Group * time						
Group * T ₁	Reference			Reference		
Group * T ₂	-0.001 (-0.279, 0.277)	0.994		0.005 (-0.275, 0.285)	0.974	
Group * T ₃	-0.122 (-0.392, 0.148)	0.375		-0.116 (-0.387, 0.156)	0.405	
Overall Interaction <i>P</i>		0.275			0.29	

RP group: Rifaximin-plus-probiotics group; **PO group:** Probiotics-only group. **T1:** Baseline; **T2:** At the end of treatment; **T3:** 1 month after treatment.

The adjusted model has been covariate-corrected for sex and disease duration; the table header uses Mean Difference according to the journal's guidelines, and its value is mathematically equivalent to the β value output by the linear model; Pairwise Comparisons have been corrected for multiple comparisons between groups using the Bonferroni method.

Urgency of Defecation and Incomplete Evacuation

Urgency of defecation and incomplete evacuation were recorded on a 5-level ordered severity scale. Because the adjusted ordinal model did not converge, they were analyzed as an approximately continuous supportive outcome.

Urgency improved significantly over time in both groups, with adjusted mean differences of -1.068 (95%CI -1.337 to -0.798, $P < 0.001$) at T₂ and -0.661 (95%CI -0.858 to -0.464, $P < 0.001$) at T₃. However, neither the group main effect ($P = 0.460$) nor the overall group-by-time interaction ($P = 0.069$) was statistically significant. Bonferroni-adjusted between-group comparisons were also non-significant at both post-baseline time points.

Incomplete evacuation improved significantly over time in both groups, with adjusted mean differences of -0.644 (95%CI -0.842 to -0.446, $P < 0.001$) at T₂ and -0.475 (95%CI -0.659 to -0.290, $P < 0.001$) at T₃. The group main effect ($P = 0.668$), the overall group-by-time interaction ($P = 0.290$), and Bonferroni-adjusted pairwise comparisons were all non-significant. (Table 4)

Exploratory outcomes

Among the exploratory binary outcomes, frequent flatulence showed a significant group-by-time interaction in the adjusted GEE model (overall interaction $P < 0.001$). Bonferroni-adjusted pairwise comparisons showed no significant between-group difference at T_2 , but the RP group had a lower risk of frequent flatulence at T_3 (risk difference=-0.250, 95%CI -0.505 to -0.002; adjusted $P = 0.046$).

For borborygmi, the adjusted overall interaction was nominally significant ($P = 0.042$), but Bonferroni-adjusted between-group comparisons at T_2 and at T_3 were not significant. No clear between-group differences were observed for mucus in stool or frothy stool. (Table 5)

Table 5
Statistical analysis of Exploratory outcomes.

	Unadjusted model			Adjusted model		
	OR / Risk Diff (95% CI)	P	Bonf. P	OR / Risk Diff (95% CI)	P	Bonf. P
Frequent flatulence						
Group (RP vs PO)	1.446 (0.682, 3.067)	0.336		1.440 (0.668, 3.105)	0.352	
Time						
T ₁	Reference			Reference		
T ₂	0.328 (0.192, 0.558)	< 0.001		0.321 (0.187, 0.553)	< 0.001	
T ₃	0.539 (0.353, 0.824)	0.004		0.533 (0.346, 0.822)	0.004	
Group * time						
Group * T ₁	Reference			Reference		
Group * T ₂	0.259 (0.101, 0.666)	0.005		0.232 (0.087, 0.616)	0.003	
Group * T ₃	0.239 (0.111, 0.517)	< 0.001		0.220 (0.099, 0.487)	< 0.001	
Overall Interaction P		0.001		-	< 0.001	
Pairwise Comparisons						
RP vs PO at T ₁	0.083 (-0.170, 0.337)		1	0.080 (-0.172, 0.332)		1
RP vs PO at T ₂	-0.178 (-0.405, 0.049)		0.324	-0.200 (-0.434, 0.034)		0.179
RP vs PO at T ₃	-0.232 (-0.478, 0.014)		0.085	-0.250 (-0.505, -0.002)		0.046
Borborygmi						
Group (RP vs PO)	0.859 (0.415, 1.776)	0.682		0.830 (0.396, 1.739)	0.622	
Time						
T ₁	Reference			Reference		
T ₂	0.278 (0.152, 0.505)	< 0.001		0.264 (0.144, 0.485)	< 0.001	
T ₃	0.601 (0.417, 0.867)	0.006		0.588 (0.405, 0.854)	0.005	
Group * time						
Group * T ₁	Reference			Reference		
Group * T ₂	0.845 (0.345, 2.072)	0.713		0.874 (0.347, 2.203)	0.775	
Group * T ₃	0.390 (0.182, 0.835)	0.015		0.392 (0.178, 0.862)	0.02	
Overall Interaction P		0.036			0.042	
Pairwise Comparisons						
RP vs PO at T ₁	-0.037 (-0.299, 0.225)		1	-0.043 (-0.301, 0.214)		1
RP vs PO at T ₂	-0.040 (-0.231, 0.150)		1	-0.036 (-0.205, 0.134)		1

	Unadjusted model		Adjusted model			
	OR / Risk Diff (95% CI)	P	Bonf. P	OR / Risk Diff (95% CI)	P	Bonf. P
Frequent flatulence						
RP vs PO at T ₃	-0.177 (-0.394, 0.039)		0.243	-0.165 (-0.360, 0.029)		0.188
	Reference					
Mucus in stool						
Group (RP vs PO)	0.800 (0.374, 1.707)	0.563		0.769 (0.356, 1.660)	0.503	
Time						
T ₁	Reference			Reference		
T ₂	0.326 (0.179, 0.591)	< 0.001		0.329 (0.181, 0.598)	< 0.001	
T ₃	0.563 (0.353, 0.897)	0.016		0.565 (0.354, 0.903)	0.017	
Group * time						
Group * T ₁	Reference			Reference		
Group * T ₂	1.180 (0.519, 2.681)	0.693		1.081 (0.463, 2.527)	0.857	
Group * T ₃	0.773 (0.367, 1.628)	0.498		0.722 (0.332, 1.570)	0.412	
Overall Interaction <i>P</i>		0.399			0.434	
Pairwise Comparisons						
RP vs PO at T ₁	-0.049 (-0.300, 0.202)		1	-0.058 (-0.311, 0.196)		1
RP vs PO at T ₂	-0.007 (-0.197, 0.183)		1	-0.023 (-0.211, 0.166)		1
RP vs PO at T ₃	-0.076 (-0.289, 0.137)		1	-0.091 (-0.304, 0.123)		1
Frothy stool						
Group (RP vs PO)	0.727 (0.326, 1.623)	0.436		0.669 (0.290, 1.542)	0.345	
Time						
T ₁	Reference			Reference		
T ₂	0.357 (0.182, 0.703)	0.003		0.351 (0.178, 0.695)	0.003	
T ₃	0.522 (0.307, 0.886)	0.016		0.516 (0.301, 0.883)	0.016	
Group * time						
Group * T ₁	Reference			Reference		
Group * T ₂	1.116 (0.447, 2.786)	0.814		1.030 (0.392, 2.707)	0.953	
Group * T ₃	0.889 (0.415, 1.906)	0.763		0.835 (0.371, 1.876)	0.662	
Overall Interaction <i>P</i>		0.844			0.856	
Pairwise Comparisons						

	Unadjusted model		Adjusted model			
	OR / Risk Diff (95% CI)	P	Bonf. P	OR / Risk Diff (95% CI)	P	Bonf. P
Frequent flatulence						
RP vs PO at T ₁	-0.063 (-0.301, 0.174)		1	-0.073 (-0.298, 0.152)		1
RP vs PO at T ₂	-0.023 (-0.199, 0.153)		1	-0.034 (-0.186, 0.118)		1
RP vs PO at T ₃	-0.057 (-0.252, 0.137)		1	-0.066 (-0.239, 0.108)		1

RP group: Rifaximin-plus-probiotics group; **PO group:** Probiotics-only group. **T1:** Baseline; **T2:** At the end of treatment; **T3:** 1 month after treatment.

The adjusted model has been corrected for sex and disease duration; For each symptom, main effects and group-by-time interactions are reported as odds ratios (ORs), while Bonferroni-adjusted pairwise comparisons are reported as absolute risk differences (Risk Diff); the p-values for post-hoc tests between groups are corrected using the Bonferroni method.

Safety

No serious adverse events were reported during the study. Both regimens were generally well tolerated. However, because mild adverse events were not systematically graded, between-group safety comparisons should be interpreted cautiously.

Discussion

In this non-randomized prospective cohort study, both treatment strategies were associated with improvement in stool consistency and related symptoms over time. Compared with probiotics alone, rifaximin plus probiotics showed a possible advantage in maintaining improvement in stool consistency and in reducing stool frequency at 1 month after treatment. By contrast, urgency and incomplete evacuation improved in both groups without clear evidence of additional benefit from rifaximin.

The primary endpoint in the revised analysis was stool consistency(Irvine *et al.*, 2016; Singh *et al.*, 2026), because loose or watery stool is the defining symptom of FDr and therefore provides a more clinically anchored endpoint than bowel movement frequency alone(Tack, 2012; Lacy *et al.*, 2016; Lembo *et al.*, 2022). In the adjusted ordinal model, both groups showed marked improvement over time. Although the overall interaction between treatment group and time for stool consistency did not reach statistical significance, the significant 1-month group-by-time estimate suggested a possible maintenance benefit of rifaximin plus probiotics. Given the borderline nature of this signal and the observational design, this finding should be interpreted cautiously rather than as definitive evidence of superiority.

Bowel movement frequency also improved in both groups, but the between-group difference was mainly evident at 1 month after treatment rather than immediately at treatment completion. This pattern may indicate that any additional benefit from rifaximin is more relevant to short-term maintenance than to immediate relief. At the same time, the absolute between-group difference was modest, and its clinical importance should not be overstated, especially given that baseline bowel frequency was only mildly elevated in many participants(Lembo *et al.*, 2022).

Urgency of defecation and incomplete evacuation improved over time in both groups, but adjusted between-group differences were not significant. These findings suggest that these symptoms may improve as part of general symptom relief, without clear evidence that rifaximin provides additional benefit beyond probiotics. Because the adjusted ordinal models for these two endpoints did not converge, they were analyzed as approximately continuous supportive outcomes; therefore, the corresponding estimates should be interpreted with caution.

Among the exploratory outcomes, only frequent flatulence showed a more consistent signal favoring rifaximin plus probiotics at 1 month after treatment. The remaining exploratory symptom outcomes did not show stable between-group differences after adjustment and multiple-comparison correction. These analyses should therefore be regarded as hypothesis-generating rather than confirmatory.

To establish a robust baseline therapy, we selected a combination of *Bacillus licheniformis* and *Lactobacillus acidophilus* based on their complementary mechanisms (Preidis et al., 2020; Weingarden and Ko, 2024). *B. licheniformis*, a spore-forming bacterium, can survive gastric transit to effectively reverse microbial dysbiosis and mitigate intestinal inflammation (Łubkowska et al., 2023; Zeng et al., 2023, 2024). Concurrently, *L. acidophilus* exerts a direct anti-diarrheal effect by upregulating intestinal NHE3 and DRA transporters, thereby promoting critical fluid and electrolyte absorption (Singh et al., 2012; Kumar et al., 2016). Because combining probiotic strains with distinct functional properties is often more effective in regulating intestinal microecology than single-strain therapies (Wang et al., 2020), we hypothesized this specific combination would provide an optimal synergistic foundation for FDr symptom relief.

Building on this foundation, we sought to determine whether the addition of rifaximin could further complement this regimen by influencing gut luminal ecology (DuPont, 2016), host–microbe interactions (DuPont, 2015), and intestinal barrier homeostasis (Patel et al., 2022). However the present study did not include microbiome profiling or dedicated testing for small intestinal bacterial overgrowth. Accordingly, mechanistic interpretation should be framed as a hypothesis rather than a demonstrated pathway.

The distinction between FDr and IBS-D is clinically subtle. In Rome IV-based practice, abdominal pain may be present in FDr, but it is not the predominant symptom (DuPont, 2016); IBS-D and FDr are increasingly viewed as overlapping conditions on a spectrum rather than entirely discrete entities (DuPont, 2015). This diagnostic overlap should be kept in mind when interpreting the present findings. Although rifaximin has established evidence in IBS-D (Lembo et al., 2022), the regimen used in this study (rifaximin 400 mg twice daily for 6 days) differed from commonly used IBS-D protocols. This specific approach was adopted based on relevant literature, local clinical experience, and patient affordability. Additionally, as patients self-selected their therapies after being fully informed, these findings primarily reflect real-world observational practice. Therefore, the present results should not be interpreted as evidence for an optimized rifaximin dosing strategy in FDr.

No serious adverse events were observed in this cohort. This overall tolerability is consistent with the generally favorable short-course safety profile reported for rifaximin (Lembo et al., 2022) and with the broad safety experience of probiotics (DuPont, 2016), but the absence of systematic grading for mild events in the present study limits more detailed safety interpretation. FDr is distinguished from IBS-D primarily by the absence of *predominant* abdominal pain rather than absolute absence of pain, and the overlap between the two conditions is well recognized. Short-course rifaximin in IBS-D trials has shown a placebo-like safety profile, with nausea the only adverse reaction occurring at a clearly higher rate than placebo in the FDA label (DuPont, 2015), while broader reviews summarize headache, nausea, diarrhea, and abdominal pain among the more commonly reported mild events (Patel et al., 2022); probiotic reviews generally describe mild gastrointestinal complaints and occasional skin reactions without a clear excess of serious toxicity (Guo et al., 2019).

Limitations

This study has several limitations. First, treatment allocation was based on patient preference rather than randomization; therefore, selection bias and residual confounding cannot be fully excluded despite adjustment for available covariates. Second, this was a single-center study with a relatively small sample size and a short follow-up period, which limited the assessment of long-term efficacy, symptom recurrence, and safety. Third, symptom assessment partly relied on patient-reported information during follow-up, and abdominal pain was not systematically quantified, which may have limited the strict differentiation between functional diarrhea and overlapping IBS-D spectrum conditions. Finally, microbiome profiling and dedicated testing for small intestinal bacterial overgrowth were not performed; therefore, mechanistic interpretations should be regarded as exploratory. Further randomized controlled trials with longer follow-up and mechanistic assessments are needed to confirm these findings.

Conclusion

In this prospective non-randomized cohort study, rifaximin plus probiotics was associated with a possible advantage in maintaining improvement in stool consistency and with lower stool frequency at 1 month after treatment compared with probiotics alone. No clear additional benefit was observed for urgency or incomplete evacuation. Given the observational design, modest sample size, and short follow-up, these findings should be interpreted cautiously and require confirmation in randomized controlled trials.

Declarations

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Second Affiliated Hospital of Soochow University.

(Project No. lc2024009; Ethics approval code: jd-lc2024009-ir01)

Written informed consent was obtained from all participants prior to enrollment.

Human Ethics and Consent to Participate

The ethical approval and informed consent procedures for this study involving human participants are described above. The study was reviewed and approved by the Ethics Committee of the Second Affiliated Hospital of Soochow University, and written informed consent was obtained from all participants.

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Author Contributions

Guo-jian Yin conceptualized the study, supervised the project, conducted outpatient follow-ups, and revised the manuscript. **Jing Zhou** designed the methodology, verified the clinical data, performed the statistical analysis, and wrote the original draft. **Yu Xin Liu** and **Feng Jie Ji** were responsible for data collection and telephone follow-ups. **Guo-jian Yin** and **Jing Zhou** reviewed and edited the manuscript. All authors approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

Consent for publication: Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are not publicly available due to patient privacy and ethical restrictions, but are available from the corresponding author on reasonable request.

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Figures

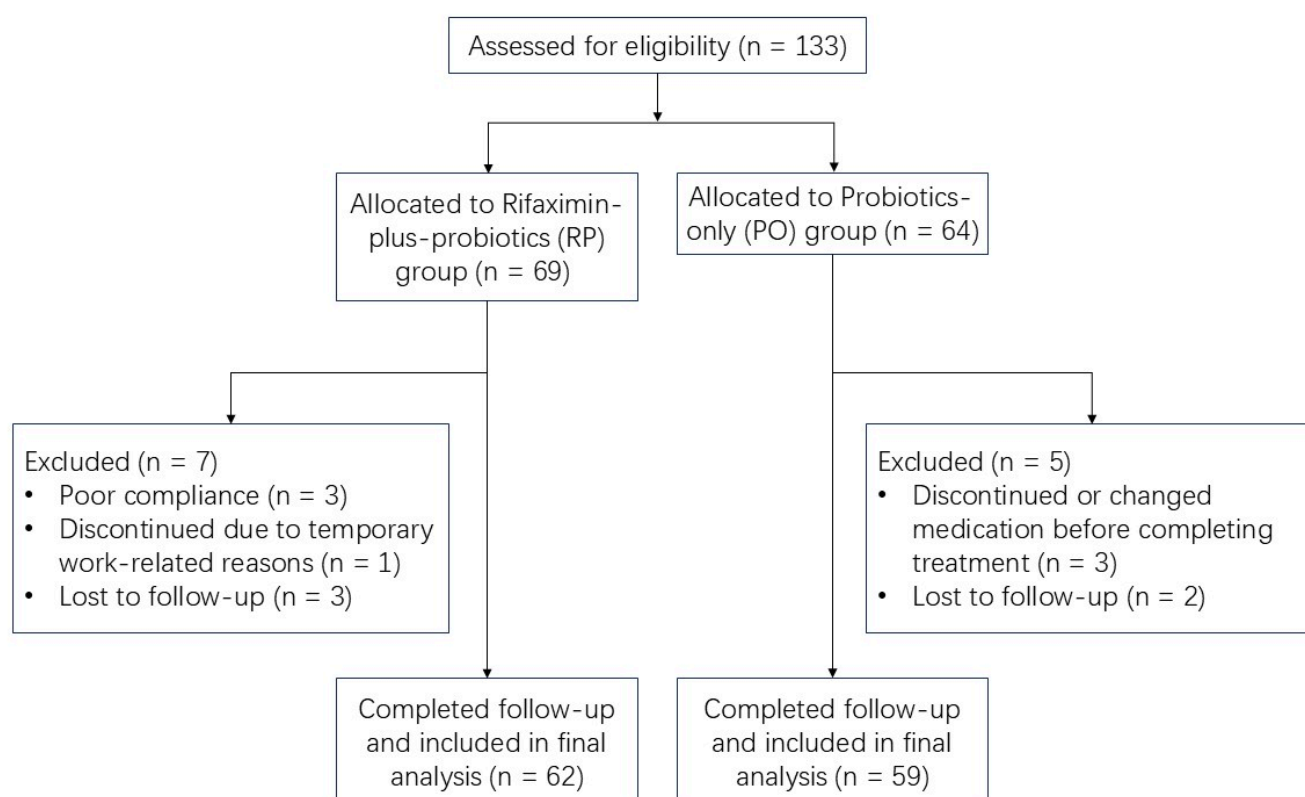


Figure 1

Participant Flow Diagram