

Repeat Rifaximin for Irritable Bowel Syndrome: No Clinically Significant Changes in Stool Microbial Antibiotic Sensitivity

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Abstract

Background Rifaximin has demonstrated efficacy and safety for diarrhea-predominant irritable bowel syndrome (IBS-D).

Aim To determine the rifaximin repeat treatment effect on fecal bacterial antibiotic susceptibility.

Methods Patients with IBS in Trial 3 (TARGET 3) study who responded to open-label rifaximin 550 mg three times daily for 2 weeks, with symptom recurrence within 18 weeks, were randomized to double-blind treatment: two 2-week repeat courses of rifaximin or placebo, separated by

10 weeks. Prospective stool sample collection occurred before and after open-label rifaximin, before and after the first repeat course, and at the end of the study. Susceptibility testing was performed with 11 antibiotics, including rifaximin and rifampin, using broth microdilution or agar dilution methods.

Results Of 103 patients receiving open-label rifaximin, 73 received double-blind rifaximin ($n = 37$) or placebo ($n = 36$). A total of 1429 bacterial and yeast isolates were identified, of which Bacteroidaceae (36.7%) and Enterobacteriaceae (33.9%) were the most common. In the double-blind phase, *Clostridium difficile* was highly susceptible to rifaximin [minimum inhibitory concentration (MIC) range 0.008–1 $\mu\text{g}/\text{mL}$] and rifampin (MIC range 0.004–0.25 $\mu\text{g}/\text{mL}$). Following double-blind rifaximin treatment, *Staphylococcus* isolates remained susceptible to rifaximin at all visits (MIC₅₀ range ≤ 0.06 –32 $\mu\text{g}/\text{mL}$). Rifaximin exposure was not associated with long-term cross-resistance of Bacteroidaceae, Enterobacteriaceae, and Enterococcaceae to rifampin or nonrifamycin antibiotics tested.

Conclusions In this study, short-term repeat treatment with rifaximin has no apparent long-term effect on stool microbial susceptibility to rifaximin, rifampin, and nonrifamycin antibiotics.

ClinicalTrials.gov Identifier NCT01543178.

Keywords Irritable bowel syndrome · Diarrhea · Microbiology · Rifaximin

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Introduction

Irritable bowel syndrome (IBS) is a chronic, recurrent functional bowel disorder characterized by abdominal pain with alterations in bowel habit [1, 2]. Subtypes of IBS are

characterized by stool pattern and include diarrhea-predominant IBS (IBS-D), constipation-predominant IBS, and mixed IBS [1]. The etiology of IBS remains to be fully elucidated; however, IBS is considered a multifactorial condition that is affected by both host (e.g., alterations in gut motility and/or gut microbiota) and environmental factors (e.g., diet) [2]. Indeed, the gut microbiota of patients with IBS-D differ both quantitatively and qualitatively from those of healthy individuals [3–6]. Antibiotics (e.g., rifaximin, neomycin) have been shown to improve symptoms of IBS [7–9]. However, administration of systemic antibiotics may be limited in IBS because of the potential for the development of bacterial antibiotic resistance and adverse events and because of the increased risk of *Clostridium difficile* infection [10–12].

Rifaximin is an oral nonsystemic gastrointestinal-targeted antibiotic with broad-spectrum activity in vitro against Gram-negative and Gram-positive aerobic and anaerobic bacteria, including *C. difficile* [13–15]. Rifaximin binds the β subunit of DNA-dependent RNA polymerase of target bacteria, inhibiting the synthesis of bacterial RNA [13, 16]. Rifaximin has demonstrated efficacy and safety in the treatment of IBS-D and was approved in 2015 by the US Food and Drug Administration for the treatment of IBS-D in adults [17–21]. In two phase 3 identically designed, randomized, double-blind studies [Trial 1 and 2 (TARGET 1 and 2)], a significantly greater percentage of patients with IBS-D receiving rifaximin 550 mg three times daily for 14 days reported adequate relief of global IBS symptoms for ≥ 2 of the first 4 weeks post-treatment compared with patients receiving placebo (pooled data: 40.7 vs. 31.7%, respectively; $P < 0.001$) [17]. In a double-blind study evaluating the efficacy and safety of repeat treatment with rifaximin [Trial 3 (TARGET 3)], patients who experienced symptom relapse within 18 weeks of observation after initial response ($\geq 30\%$ decrease from baseline in mean weekly pain score and $\geq 50\%$ decrease from baseline in number of days/week with Bristol Stool Scale type 6 or 7 stool for ≥ 2 of first 4 weeks post-treatment) to open-label rifaximin 550 mg three times a day for 2 weeks were randomized to receive repeat treatments of rifaximin 550 mg three times a day or placebo for 14 days. The percentage of responders to repeat treatment was significantly greater with rifaximin than with placebo (38.1 vs. 31.5%, $P = 0.03$) [21].

The safety profile of rifaximin was generally comparable to that of placebo according to the results of a pooled safety analysis of patients with nonconstipation forms of IBS participating in a phase 2 study and Trial 1 and 2 ($n = 1932$) [22]. No patients in the pooled population developed *C. difficile* colitis during dosing. The risk of bacteria developing resistance with rifaximin is thought to

be low [10], possibly because of the minimal systemic absorption of rifaximin [23, 24], the requirement of a stable mutation in host cell DNA (in contrast with plasmid-based mechanisms) [25], and the inability of resistant bacteria to effectively colonize the gastrointestinal tract [26, 27]. Mutations associated with rifaximin resistance following exposure to high concentrations of rifaximin have been shown to occur in approximately 1.0×10^{-8} to 2.6×10^{-6} bacteria, with some variation among bacterial species [14, 28]. The aim of this substudy was to examine the susceptibility of stool bacteria to select antibiotics, including rifaximin and rifampin, isolated from a subgroup of patients with IBS-D who participated in Trial 3.

Methods

Study Design and Patients

The patient population and the study design of Trial 3 (TARGET 3) have been described previously [21]. Briefly, Trial 3 was a randomized, double-blind, placebo-controlled phase 3 study (ClinicalTrials.gov identifier NCT01543178). Patients aged ≥ 18 years were eligible to participate if they had a diagnosis of IBS (based on Rome III criteria) and did not experience adequate relief of global IBS symptoms and bloating during a placebo screening phase. Patients were excluded if (after beginning diary assessments) they were taking probiotics or taking rifaximin or any other antibiotic 14 days prior to providing written informed consent [21].

Patients received open-label rifaximin 550 mg three times daily for 2 weeks, followed by a 4-week treatment-free follow-up to assess response (improvement in abdominal pain due to IBS ($\geq 30\%$ reduction from baseline in mean weekly pain score) and stool consistency ($\geq 50\%$ reduction from baseline in number of days/week with Bristol Stool Scale type 6 or 7 stool) during ≥ 2 of the first 4 weeks post-treatment) [21]. Responders continued in the study (nonresponders were withdrawn from further participation) and were monitored during a treatment-free observation phase of up to 18 weeks to determine IBS symptom recurrence (defined as loss of treatment response for either weekly abdominal pain or stool consistency for ≥ 3 weeks of a consecutive, rolling 4-week period during the 18-week observation phase). Patients with recurrence were randomly assigned (1:1), in a double-blind manner, to two repeat 2-week courses of treatment with rifaximin 550 mg or placebo three times daily, with the two repeat phases separated by 10 weeks. All patients provided written informed consent [21].

Collection of Stool Samples

All patients participating in Trial 3 also consented to provide stool samples. For inclusion in the current substudy, patients were selected using random number generator statistical software. Patients were selected in a manner to incorporate both responders and nonresponders. Prospective fecal culture and susceptibility testing were conducted with stool samples collected from patients prior to (open-label baseline) and after open-label treatment with rifaximin (open-label week 2), and prior to (double-blind baseline) and after the first double-blind course (double-blind week 2), and at the end of the study. Fresh stool samples were collected from patients at study visits when possible; patients who could not provide a fresh stool sample received a stool collection kit for home use with instruction to refrigerate the sample immediately and to deliver it to the clinic as soon as possible. At the clinic, stool samples were divided into 2-mL aliquots in polypropylene cryovials and stored at ≤ -20 °C; samples were then shipped on dry ice for long-term storage at ≤ -70 °C.

Bacteria were isolated from stool by inoculation on tryptic soy agar plates with 5% sheep's blood to identify aerobic bacteria; the anaerobes *C. difficile* and *Bacteroides* species were isolated by inoculation of stool samples onto cycloserine cefoxitin fructose agar or *Bacteroides* bile esculin agar, respectively. Identification of bacteria was achieved by morphology, Gram staining, other tests (catalase, hemolysis), and molecular identification as necessary. The presence of yeast was confirmed by wet mount, and yeast species were identified by the Vitek® 2 System (bioMérieux, Inc., Durham, NC, USA); susceptibility testing was not performed on yeast isolates.

Susceptibility Testing

Bacteria tested for antibiotic susceptibility were from the genera *Bacteroides*, *Citrobacter*, *Clostridium*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Pseudomonas*, *Serratia*, and *Staphylococcus*. Susceptibility testing was performed with 11 antibiotics using either the broth microdilution method (aerobic bacteria) or the agar dilution method (anaerobic bacteria). Aerobic bacteria were tested with rifaximin, rifampin, ceftazidime, ceftriaxone, ciprofloxacin, imipenem, meropenem, and piperacillin/tazobactam. Anaerobic bacteria were tested with rifaximin, rifampin, fidaxomicin, metronidazole, and vancomycin.

Data Analysis

Each bacterial species isolated and tested was grouped into a family [i.e., Bacteroidaceae, Clostridiaceae, Enterobacteriaceae (including *Escherichia*, *Klebsiella*, *Proteus*, *Enterobacter*, and *Citrobacter* species), Enterococcaceae, Pseudomonadaceae, or Staphylococcaceae]. The minimum

inhibitory concentration (MIC) at which $\geq 50\%$ of isolates were inhibited was the MIC₅₀ for the population, and the MIC at which $\geq 90\%$ of isolates were inhibited was the MIC₉₀ for the population. Clinical and Laboratory Standards Institute (CLSI) break points for each species were used to interpret MIC data [29]. For antibiotics with defined susceptible and intermediate categories, the susceptible classification was used for these analyses. For antibiotics without established CLSI break points, ranges for in vitro sensitivity were used if provided on the package insert. In all other cases (i.e., antibiotics without CLSI defined break points, in vitro susceptibility not provided on package insert), MIC values at or above the highest dilution tested in the MIC panel were considered resistant, and MIC values below the highest dilution were considered susceptible. The follow-up period was variable; therefore, for purposes of data analysis, the follow-up visits were combined into 4-week periods.

Results

Patient Population

A total of 103 patients were randomly selected for inclusion in the stool microbiota substudy; this was a subgroup of patients included in the Trial 3 study [21]. Patients were mostly white (82.5%), mostly female (73.8%), had a mean age of 47.9 years (range 19–85 years), and had a baseline average of 3.5 daily bowel movements. Of the 103 patients who received open-label rifaximin, 73 also participated in the double-blind phase (rifaximin, $n = 37$; placebo, $n = 36$). In the double-blind phase, a similar percentage of patients in the rifaximin and placebo groups, respectively, were female (70.3 and 72.2%) and had a similar frequency of daily bowel movements (3.1 and 2.9). Patients in the rifaximin and placebo groups had similar mean ages, respectively, of 46.9 years (range 22–85 years), and 47.7 years (range 19–74 years); however, the percentage of white patients was lower in the rifaximin group (75.7%) than in the placebo group (88.9%).

A total of 1429 bacterial and yeast isolates were identified from stool samples; the most common isolates were members of the families Bacteroidaceae (525 [36.7%]) and Enterobacteriaceae (484 [33.9%]; Table 1). A small number of Clostridiaceae (22 [1.5%]) and Pseudomonadaceae (4 [0.3%]) isolates were cultured. A total of 17 yeast isolates from 14 patients were identified (1.2%).

Rifaximin and Rifampin Susceptibility

Open-Label Phase

In the open-label phase, 366 *Bacteroides* isolates were identified (Table S1). The range of MIC values for

Table 1 Most abundant isolates from stool cultures ($N = 1429$)

Bacteria family	Counts	Percentage of total
Bacteroidaceae	525	36.7
<i>Bacteroides vulgatus</i>	178	12.5
<i>Bacteroides ovatus</i>	114	8.0
<i>Bacteroides fragilis</i>	102	7.1
Enterobacteriaceae	484	33.9
<i>Escherichia coli</i>	337	23.6
<i>Klebsiella pneumoniae</i>	72	5.0
Enterococcaceae	286	20.0
<i>Enterococcus faecalis</i>	117	8.2
<i>Enterococcus faecium</i>	90	6.3
Staphylococcaceae	91	6.4
<i>Staphylococcus aureus</i>	25	1.7

Species presented in table do not represent all isolates that were cultured from stool. The isolates included in the table represent bacterial families that are $\geq 5\%$ of the total number of isolates and bacteria of clinical importance

rifaximin and rifampin was wide at each visit and across visits. The MIC₅₀ and MIC₉₀ values for rifaximin increased from baseline, although susceptible isolates were still observed at week 2 and weeks 7–32. The MIC₅₀ values for rifampin increased from baseline to week 2 and remained high through week 23, when the MIC₅₀ value returned to the baseline level.

A total of 14 *C. difficile* isolates were identified; all isolates were highly susceptible to rifaximin (MIC range 0.008–0.12 $\mu\text{g}/\text{mL}$) across visits. Similarly, most *C. difficile* isolates ($n = 14$) were highly susceptible to rifampin (MIC range 0.002–0.015 $\mu\text{g}/\text{mL}$) across all visits, with one exception. The *C. difficile* isolate from one patient had a MIC of 16 $\mu\text{g}/\text{mL}$ at week ≥ 23 ; however, this isolate was sensitive to all other antibiotics tested, including rifaximin.

A total of 328 Enterobacteriaceae isolates were identified (Table S2). MIC₅₀ and MIC₉₀ values for rifaximin increased from baseline to week 2 and remained higher until weeks 19–22, when MIC₅₀ and MIC₉₀ values decreased for the rest of the study. The baseline rifampin MIC range increased to a maximum MIC at week 2.

In addition, susceptibility of Enterococcaceae to rifaximin was consistent throughout the open-label phase (Table S3). Only three *Pseudomonas* isolates were identified in the open-label phase of the study, all in the follow-up period. Staphylococcaceae isolates were highly susceptible to rifaximin and rifampin at baseline; at week 2, the rifaximin and rifampin MIC₅₀ and MIC₉₀ values increased (Table S4). Rifaximin MIC₅₀ levels recovered to baseline levels at week 7; MIC₉₀ levels recovered to baseline levels at week 23.

Double-Blind Phase

A total of 107 and 129 *Bacteroides* isolates were identified for rifaximin susceptibility testing in samples from patients receiving double-blind rifaximin or placebo, respectively; 121 and 129 *Bacteroides* isolates were identified for rifampin susceptibility testing in samples from patients receiving double-blind rifaximin or placebo, respectively. *Bacteroides* isolates demonstrated a wide range of susceptibility to rifaximin and rifampin for both the double-blind rifaximin and placebo groups, respectively (Table 2). There were no apparent differences in susceptibility of *Bacteroides* to rifaximin or rifampin in the double-blind rifaximin or placebo groups. Rifaximin MIC₅₀ values decreased compared with baseline at week ≥ 23 following double-blind rifaximin or placebo treatment.

No differences in susceptibility to rifaximin and rifampin were observed for *C. difficile* isolates identified in the double-blind rifaximin (five isolates tested) and placebo groups (nine isolates tested). *C. difficile* was highly susceptible to rifaximin (MIC range 0.008–1 $\mu\text{g}/\text{mL}$) and rifampin (MIC range 0.004–0.25 $\mu\text{g}/\text{mL}$). No *C. difficile* isolates were isolated beyond week 2 for the double-blind rifaximin group for rifaximin susceptibility testing.

Susceptibility of Enterobacteriaceae isolates to rifaximin and rifampin was consistent between the double-blind rifaximin and placebo groups (Table 3). In the double-blind rifaximin group, rifaximin MIC₅₀ values increased from baseline to week 2, then decreased to baseline levels from weeks 7 to 22 of the follow-up period. In the double-blind placebo group, rifaximin MIC₅₀ values were unchanged from baseline through the follow-up period. Rifampin MIC₅₀ values ranged between 8 and 16 $\mu\text{g}/\text{mL}$ for the double-blind rifaximin group and were 16 $\mu\text{g}/\text{mL}$ for all visits in the placebo group.

The susceptibility of Enterococcaceae to rifaximin and rifampin was consistent between the double-blind rifaximin and placebo groups (Table 4). Differences were observed in the percentages of rifampin-resistant *Enterococcus* isolates cultured from the stools of patients receiving double-blind placebo and rifaximin at baseline (53 vs. 39%, respectively), week 2 (37 vs. 50%), and during the follow-up period (64 vs. 52%).

A total of three Pseudomonadaceae isolates were identified and evaluated for susceptibility to rifaximin and rifampin. The one isolate from the double-blind rifaximin group had rifaximin and rifampin MICs of 16 and 32 $\mu\text{g}/\text{mL}$, respectively. The two isolates from the placebo group had a baseline rifaximin and rifampin MIC of 16 $\mu\text{g}/\text{mL}$ and had rifaximin and rifampin MICs of 4 and 8 $\mu\text{g}/\text{mL}$, respectively, during the follow-up period (weeks 19–22).

Table 2 Susceptibility of Bacteroidaceae to rifaximin or rifampin in the double-blind phase of the study

Timepoint ^a (patients)	Rifaximin				Rifampin			
	Isolates (n)	MIC range (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	Isolates (n)	MIC range (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)
Double-blind rifaximin								
Baseline (n = 37)	47	0.12–>1024	>1024	>1024	47	0.12–>64	>64	>64
Week 2 ^b (n = 36)	27	0.12–>1024	>1024	>1024	42	0.12–>64	>64	>64
Weeks 7–10 (n = 1)	2	512–>1024	NA	NA	2	32–>64	NA	NA
Weeks 19–22 (n = 3)	4	0.5–>1024	>1024	>1024	4	0.12–>64	>64	>64
Week ≥23 (n = 30)	27	0.12–>1024	256	>1024	26	0.03–>64	>64	>64
Double-blind placebo								
Baseline (n = 36)	45	0.25–>1024	512	>1024	45	0.06–>64	>64	>64
Week 2 ^b (n = 36)	42	0.25–>1024	512	>1024	42	0.12–>64	>64	>64
Weeks 11–14 (n = 1)	2	0.5–256	NA	NA	2	0.25–>64	NA	NA
Weeks 15–18 (n = 3)	3	128–>1024	>1024	>1024	3	32–>64	>64	>64
Weeks 19–22 (n = 6)	9	0.25–>1024	0.5	>1024	9	0.12–>64	0.12	>64
Week ≥23 (n = 26)	28	0.12–>1024	128	>1024	28	0.03–>64	16	>64

MIC minimum inhibitory concentration, MIC₅₀ MIC at which ≥50% of isolates were inhibited, MIC₉₀ MIC at which ≥90% of isolates were inhibited, NA not applicable

^a Follow-up period was variable; therefore, the follow-up visits were grouped into 4-week periods to determine whether there was an effect of time on antibiotic susceptibility of isolates

^b End of 2-week rifaximin or placebo treatment

Table 3 Susceptibility of Enterobacteriaceae to rifaximin or rifampin in the double-blind phase of the study

Timepoint ^a (patients)	Rifaximin				Rifampin			
	Isolates (n)	MIC range (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	Isolates (n)	MIC range (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)
Double-blind rifaximin								
Baseline (n = 37)	37	8–>128	16	>128	37	4–64	16	32
Week 2 ^b (n = 36)	30	8–>128	64	>128	30	4–>128	16	>128
Weeks 7–10 (n = 1)	4	8–64	16	64	4	8–32	8	32
Weeks 19–22 (n = 3)	3	8–16	16	16	3	4–16	8	16
Week ≥23 (n = 30)	34	8–>128	32	64	34	8–>128	16	32
Double-blind placebo								
Baseline (n = 36)	38	4–>128	32	>128	38	4–>128	16	>128
Week 2 ^b (n = 36)	41	4–>128	32	>128	41	4–>128	16	128
Weeks 11–14 (n = 1)	1	8	NA	NA	1	8	NA	NA
Weeks 15–18 (n = 3)	2	16–32	NA	NA	2	8–16	NA	NA
Weeks 19–22 (n = 6)	9	4–>128	32	>128	9	1–128	16	128
Week ≥23 (n = 26)	32	4–>128	32	>128	32	8–>128	16	32

MIC minimum inhibitory concentration, MIC₅₀ MIC at which ≥50% of isolates were inhibited, MIC₉₀ MIC at which ≥90% of isolates were inhibited, NA not applicable

^a Follow-up period was variable; therefore, the follow-up visits were grouped into 4-week periods to determine whether there was an effect of time on antibiotic susceptibility of isolates

^b End of 2-week rifaximin or placebo treatment

Staphylococcal isolates remained susceptible to rifaximin and rifampin at all visits in the double-blind placebo group. In the double-blind rifaximin group, the rifaximin

MIC₅₀ increased from baseline to week 2 (≤0.06–32 µg/mL, respectively), but the five isolates evaluated in the follow-up period had rifaximin MICs ≤ 0.06 µg/mL

Table 4 Susceptibility of Enterococcaceae to rifaximin or rifampin in the double-blind phase of the study

Timepoint ^a (patients)	Rifaximin				Rifampin			
	Isolates (n)	MIC range (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	Isolates (n)	MIC range (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)
Double-blind rifaximin								
Baseline (n = 37)	33	≤0.06–16	2	16	33	≤0.06–16	1	8
Week 2 ^b (n = 36)	20	≤0.06–32	4	16	20	≤0.06–32	2	16
Weeks 7–10 (n = 1)	1	32	NA	NA	1	8	NA	NA
Weeks 19–22 (n = 3)	0	NA	NA	NA	0	NA	NA	NA
Week ≥23 (n = 30)	21	≤0.06–32	4	32	21	≤0.06–32	4	16
Double-blind placebo								
Baseline (n = 36)	19	≤0.06–32	4	32	19	≤0.06–16	4	16
Week 2 ^b (n = 36)	30	≤0.06–32	2	16	30	≤0.06–32	2	16
Weeks 11–14 (n = 1)	0	NA	NA	NA	0	NA	NA	NA
Weeks 15–18 (n = 3)	1	2	NA	NA	1	1	NA	NA
Weeks 19–22 (n = 6)	7	0.5–16	4	16	7	0.15–16	4	16
Week ≥23 (n = 26)	17	≤0.06–32	16	32	17	≤0.06–32	8	32

MIC minimum inhibitory concentration, MIC₅₀ MIC at which ≥50% of isolates were inhibited, MIC₉₀ MIC at which ≥90% of isolates were inhibited, NA not applicable

^a Follow-up period was variable; therefore, the follow-up visits were grouped into 4-week periods to determine whether there was an effect of time on antibiotic susceptibility of isolates

^b End of 2-week rifaximin treatment

Table 5 Susceptibility of Staphylococcaceae to rifaximin or rifampin in the double-blind phase of the study

Timepoint ^a (patients)	Rifaximin				Rifampin			
	Isolates (n)	MIC range (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	Isolates (n)	MIC range (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)
Double-blind rifaximin								
Baseline (n = 37)	9	≤0.06–64	≤0.06	64	9	≤0.06–>128	≤0.06	>128
Week 2 ^b (n = 36)	10	≤0.06–64	32	64	10	≤0.06–>128	>128	>128
Weeks 7–10 (n = 1)	0	NA	NA	NA	0	NA	NA	NA
Weeks 19–22 (n = 3)	2	≤0.06	NA	NA	2	≤0.06	NA	NA
Week ≥23 (n = 30)	3	≤0.06	≤0.06	≤0.06	3	≤0.06	≤0.06	≤0.06
Double-blind placebo								
Baseline (n = 36)	8	≤0.06–32	≤0.06	32	8	≤0.06–>128	≤0.06	>128
Week 2 ^b (n = 36)	7	≤0.06–64	≤0.06	64	7	≤0.06–>128	≤0.06	>128
Weeks 11–14 (n = 1)	0	NA	NA	NA	0	NA	NA	NA
Weeks 15–18 (n = 3)	0	NA	NA	NA	0	NA	NA	NA
Weeks 19–22 (n = 6)	1	≤0.06	NA	NA	1	≤0.06	NA	NA
Week ≥23 (n = 26)	6	≤0.06	≤0.06	≤0.06	6	≤0.06	≤0.06	≤0.06

MIC minimum inhibitory concentration, MIC₅₀ MIC at which ≥50% of isolates were inhibited, MIC₉₀ MIC at which ≥90% of isolates were inhibited, NA not applicable

^a Follow-up period was variable; therefore, the follow-up visits were grouped into 4-week periods to determine whether there was an effect of time on antibiotic susceptibility of isolates

^b End of 2-week rifaximin treatment

(Table 5). At baseline, 11 and 13% of staphylococcal isolates in the rifaximin and placebo groups, respectively, were rifampin resistant (MIC ≥ 4 µg/mL); at week 2, 70%

of staphylococcal isolates in the rifaximin group were rifampin resistant. However, repeat treatment with rifaximin did not have an apparent effect on the long-term

susceptibility of staphylococcal isolates to rifampin, as isolates recovered sensitivity to rifampin in the follow-up period (≤ 0.06 $\mu\text{g/mL}$).

Other Antibiotic Susceptibility

In the open-label phase, there was no apparent cross-resistance of Bacteroidaceae (Table S5), Enterobacteriaceae (Table S6), and Enterococcaceae (Table S7) to nonrifamycin antibiotics following exposure to rifaximin. The small number of Clostridiaceae, Pseudomonadaceae, and Staphylococcaceae isolates available limited MIC₅₀ analyses at the time points assessed with antibiotics tested.

In the double-blind phase, there was no apparent cross-resistance of Bacteroidaceae (Table S8), Enterobacteriaceae (Table S9), and Enterococcaceae (Table S10) to nonrifamycin antibiotics following rifaximin exposure. The small number of Clostridiaceae, Pseudomonadaceae, and Staphylococcaceae isolates available limited MIC₅₀ analyses at the time points assessed with the antibiotics tested.

Discussion

In the current study, short-term repeat treatment with rifaximin in patients with IBS-D was not associated with clinically meaningful resistance to rifaximin, rifampin, and nonrifamycin antibiotics tested in the long term. These findings support previous reports about rifaximin treatment of patients with hepatic encephalopathy or travelers' diarrhea, suggesting that the potential for development of cross-resistance to nonrifamycin antibiotics following treatment with rifaximin is low [30–34].

Overall, bacterial isolates were susceptible to rifaximin and rifampin during the open-label and double-blind phases of the study. Increases in the MIC₅₀ or the MIC₉₀ of rifaximin against *Bacteroides* were within baseline MIC ranges during both the open-label and the double-blind phases of the study and within the MIC ranges previously reported in the literature [35]. The MIC₅₀ of rifaximin with Enterobacteriaceae increased at week 2 in both the open-label and the double-blind phases of this study but recovered to baseline levels in the follow-up period; this observation is consistent with the rapid elimination of rifaximin-resistant bacteria, particularly aerobes, from the stool [26]. Transient increases in rifaximin and rifampin MIC₅₀ values for staphylococcal isolates were observed after treatment with open-label rifaximin (week 2) and after repeat treatment with double-blind rifaximin (week 2); however, MIC values for rifaximin and rifampin returned to baseline levels as the duration of time post-rifaximin exposure increased (week ≥ 23). The transient increases observed in the short term do not appear to be clinically

harmful. Yeast growth can occur following treatment with some antibiotics [36, 37]; however, the small number of yeast isolates identified supports a previous finding that rifaximin does not create an environment favorable to yeast growth [27].

A previous study has shown that some patients with IBS may be predisposed to infection with *C. difficile*, although isolates from these patients were susceptible to the antibiotics tested [38]. One limitation of the current study was the lack of consistency in antimicrobial susceptibility testing for bacterial families with a small number of isolates identified (i.e., Clostridiaceae, Pseudomonadaceae). The human gut microbiome is thought to contain at least 1200 different microorganisms; thus, an additional limitation of this study was the limited number of bacterial species examined for resistance relative to the quantity and diversity of microbes in the human gut microbiome [39]. Finally, the use of fecal samples, while noninvasive, may not be entirely representative of the composition and, possibly, activity of the gut microbiota in vivo [40]. Indeed, the bacterial composition of fecal and colonic mucosa differed significantly in both healthy individuals ($P = 0.0001$) and patients with IBS-D ($P = 0.001$).

The large number of patients with prospectively collected stool samples available for microbial susceptibility testing strengthens study results. Potential limitations of the study include the small number of isolates collected from some bacterial families, thus limiting potential robustness of the susceptibility testing, uncertainty as to whether this patient sampling was representative of a general population with IBS-D, and the lack of evaluation of the potential relationship between susceptibility profiles and clinical response observed in Trial 3.

In conclusion, because of the potential for chronicity of IBS-D symptoms, patients may require repeat treatment with rifaximin for the management of symptoms [2]. Based on the results of this prospective study, there is no evidence of long-term clinically relevant antibiotic resistance with repeated courses of rifaximin for the treatment of IBS-D.

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Author's contributions Dr. Pimentel contributed to the study design, participated in the conduct of the study, analyzed data, and critically reviewed and edited the manuscript. Dr. Cash contributed to the study design, analyzed data, and critically reviewed and edited the manuscript. Dr. Lembo contributed to the study design, participated in the conduct of the study, analyzed data, and critically reviewed and edited the manuscript. Dr. Schoenfeld contributed to the study design, participated in the conduct of the study, analyzed data, and critically reviewed and edited the manuscript. Drs. Wolf and Israel analyzed data and critically reviewed and edited the manuscript. All of the

authors approved the final version of the manuscript, including the authorship list.

Compliance with ethical standards

Conflict of interest M. Pimentel reports serving as a consultant for and receiving research funding from Salix Pharmaceuticals. In addition, Cedars-Sinai Medical Center has a licensing agreement with Salix Pharmaceuticals. B.D. Cash reports serving as a speaker, consultant, and an advisory board member for Salix Pharmaceuticals. A. Lembo reports serving as a consultant and an advisory board member for Salix Pharmaceuticals, Ironwood Pharmaceuticals, Inc., Allergan plc, Ardelyx, and Valeant Pharmaceuticals. R.A. Wolf is a former employee of Salix Pharmaceuticals. R.J. Israel is an employee of Salix Pharmaceuticals or its affiliates. P. Schoenfeld reports serving as a consultant and advisory board member for Salix Pharmaceuticals, Ironwood Pharmaceuticals, Allergan, and Commonwealth Laboratories, a division of Valeant Pharmaceuticals North America LLC.

Ethical standards All procedures performed in the study were in accordance with the International Standard of Good Clinical Practice procedures and with the principles of the Declaration of Helsinki (1964) and relevant amendments. The protocol was approved by all institutional review boards and ethics committees at participating sites.

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