

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

21-361

MICROBIOLOGY REVIEW(S)

MICROBIOLOGY REVIEW
DIVISION OF SPECIAL PATHOGEN AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)

NDA # 21,361

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DRUG CATEGORY Anti-bacterial

INDICATION Treatment of travelers' diarrhea

DOSAGE FORM Tablets for oral administration

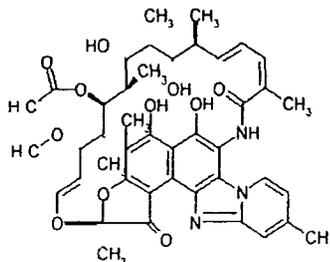
PRODUCT NAMES

a PROPRIETARY XIFAXAN™

b NONPROPRIETARY Rifaximin

c CHEMICAL [2S-[2R*, 16Z, 18E, 20R*, 21R*, 22S*, 24S*, 25R*-26S*, 27R*, 28e)-25-(Acetyloxy)-5,6,21,23-tetrahydroxy-27-methoxy-2, 4,11,16,20,22,24,26-octamethyl-2, 7-[epoxypentadecal[1,11,13]-trienimino]benzofuro[4,5-e]pyrido[1,2-a]benzimidazole-1,15(2H)-dione

STRUCTURAL FORMULA



Molecular weight 785 90
Empirical Formula C₄₃H₅₁N₃O₁₁

SUPPORTING DOCUMENTS IND —

Rifaximin

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1 EXECUTIVE SUMMARY

The subject of this NDA is Xifaxan™ (rifaximin) tablets for the treatment of patients (≥ 12 years of age) with travelers' diarrhea caused by *Escherichia coli*. Rifaximin is a derivative of rifamycin that binds to the β -subunit of bacterial DNA-dependent RNA polymerase thereby inhibiting protein synthesis and the subsequent growth of the organism.

The *in vitro* activity of rifaximin and other antimicrobial agents against enteropathogens isolated as a cause of travelers' diarrhea was evaluated using National Committee for Clinical Laboratory Standards (NCCLS) agar dilution method. With the exception of *Campylobacter jejuni* (MIC > 256 $\mu\text{g/ml}$), rifaximin demonstrated *in vitro* activity against *Shigella*, *Salmonella*, *Aeromonas* and *E. coli* with MIC ranging from 8-64 $\mu\text{g/ml}$.

The antimicrobial susceptibility to rifaximin of 408 clinical isolates of *Vibrio cholera* was determined by the NCCLS disc diffusion method. Based on studies by Jiang *et al.* 2000, the MIC of rifaximin against all *Vibrio cholera* isolates tested in this study was significantly below the fecal concentrations of the drug as measured following the administration of rifaximin (800 mg/day for 3 days): 7,961 $\mu\text{g/g}$ one day after initiation of oral therapy, 7,425 $\mu\text{g/g}$ on the second day, 4,405 $\mu\text{g/g}$ on the third, 2,891 $\mu\text{g/g}$ on the fourth, 3,266 $\mu\text{g/g}$ on the fifth, and 154 $\mu\text{g/g}$ on the sixth day.

The *in vitro* activity of rifaximin against 93 clinical isolates of *Clostridia difficile* was compared to that of vancomycin and metronidazole. Although rifaximin demonstrated activity, it had a higher MIC against *C. difficile* relative to vancomycin and metronidazole.

The effect of rifaximin on fecal micro-flora, in comparison with that of neomycin, using a rat model was investigated. Rifaximin treatment produced a significant reduction in the number of total aerobic bacteria and *Salmonella* spp, whereas, neomycin caused a reduction in *Salmonella* spp but had no significant effect in the total bacterial count.

The emergence of spontaneous resistant mutants was assessed by the NCCLS broth dilution and agar dilution methods under anaerobic and aerobic conditions. *C. difficile* demonstrated lower frequency of spontaneous resistance in the presence of rifaximin compared to *Clostridia perfringens*. Gram-negative isolates acquired resistance to rifaximin at approximately the same rate under aerobic (2 transfers) and anaerobic conditions (2-3 transfers). Mutation frequencies of 1×10^{-8} to 1.2×10^{-8} was reported for enteropathogenic (EPEC), 1×10^{-7} to 1×10^{-8} for enterohemorrhagic (EHEC), 1×10^{-8} to 3×10^{-8} for enterotoxigenic (ETEC), and 2×10^{-8} for enteroinvasive (EIEC) *E. coli* isolates.

The safety and efficacy of rifaximin (200 mg t.i.d.) was evaluated in the clinical study, RFID3001, in which ciprofloxacin (Cipro) was used as a comparator. The pathogens encountered in the study were predominantly ETEC ST (heat stable toxin), ETEC LT (heat labile toxin),

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ETEC LT/ST, and enteroaggregative (EAEC) *E coli*. However, other pathogens such as *Cryptosporidia*, *Giardia*, *Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas*, *Plesiomonas* species, and *Vibrio cholera* were also isolated from a small number of patients. In the clinical study, the median TLUS were 24.3 hours for rifaximin, 42.8 hours for placebo, and 27.4 hours for Cipro. Of the patients with enteropathogenic *E coli*, 83% were considered clinically cured or well. However, only approximately 64% were clinically cured and free of enteropathogenic *E coli*. The data presented here shows that there is no correlation between clinical cure and microbiologic outcome and the observations reported here are similar to those reviewed in an earlier study (RFID9801, RFID9701 and RFID9601). Rifaximin was not active against *Campylobacter* spp, *Salmonella*, *Shigella*, *Vibrio cholera*, *Aeromonas*, *Plesiomonas*, *Cryptosporidia*, and *Giardia lamblia*.

2 INTRODUCTION AND BACKGROUND

The subject of this NDA resubmission is rifaximin tablets for the treatment of travelers' diarrhea of individuals' ≥ 12 years of age. The FDA recommended that Salix conduct a second clinical trial using 200 mg of rifaximin administered 3 times daily (t.i.d.) compared with Ciprofloxacin (Cipro) and placebo. This new trial should demonstrate a clinically meaningful benefit and a statistically significant reduction in the duration of diarrhea between rifaximin and the placebo groups. Moreover, it was also recommended that Salix should increase the number of patients with pathogens other than *E coli* to assess the clinical and microbiologic efficacy of rifaximin. The sponsor has conducted a second phase 3 clinical study to support the approval of rifaximin for the treatment of travelers' diarrhea.

Travelers' diarrhea is one of the most prevalent health problems affecting people moving from developed to undeveloped countries. The condition is a clinical syndrome and is usually defined by the passage of three or more unformed stools in a 24-hour period plus at least one symptom of enteric disease such as abdominal pain or cramps, nausea, vomiting, or fever. The risk of travelers' diarrhea is approximately 7% in developed countries and as high as 20-50% in the developing world (Ericsson, 2003). The etiologic cause of travelers' diarrhea may be parasitic or viral, however, bacterial organisms such as enterotoxigenic *E coli* (ETEC), enteroaggregative *E coli* (EAEC), *Shigella* species, *Campylobacter jejuni*, *Salmonella* species, *Aeromonas* species, *Yersinia enterocolitica* and non-*cholerae Vibrio* have been shown to cause the majority of illnesses associated with the disease.

Rifaximin, a derivative of rifamycin, is a non-aminoglycoside, semi-synthetic antibiotic. The drug has been shown to be poorly absorbed by the stomach/gastrointestinal tract and exerts its action directly in the lumen (Jiang *et al*, 2000). The result is high fecal concentrations with trace amount of the drug found in the plasma.

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3 PRECLINICAL MICROBIOLOGY**3.1 Mechanism of action**

The mechanism of action was reviewed on March 14, 2002 by Peter Dionne (NDA # 21-361). Briefly, rifaximin exerts its antibiotic activity by binding to the β -subunit of bacterial DNA-dependent RNA polymerase, thus inhibiting protein synthesis and subsequent growth of microorganisms.

3.2 Activity *in vitro*

The *in vitro* activity of rifaximin was reviewed on March 14, 2002 by Peter Dionne (NDA # 21-361). However, additional studies are presented within this review.

3.2.1 Effect on enteric pathogens

The *in vitro* activity of rifaximin and several antimicrobial agents against 177 enteropathogenic isolates was determined by the agar dilution methods according to the NCCLS guidelines (Sierra *et al*, 2001). MICs at which 50 and 90% of the isolates tested were inhibited and the percentages of resistance were calculated for each antimicrobial agent used in the study (Table 1). No information on how percent resistance was determined was provided. The result shows that rifaximin demonstrated MIC₅₀ and MIC₉₀ values of 8-64 μ g/ml against most of the enteric pathogens tested except *C. jejuni* (MIC₅₀ and MIC₉₀ values of 256 and 512 μ g/ml, respectively) and *Y. enterocolitica* (MIC₉₀ 128 μ g/ml). Nalidixic acid and Cipro showed comparatively lower MIC values for all the enteropathogens tested (Table 1).

Table 1 The MICs of several antimicrobial agents for 177 enteropathogens

Microorganism	No of isolates ^a	RFX			RIF			AMP			CHL			TET			NAL			CIP			TMP		
		MIC ₄	MIC ₅₀	MIC ₉₀	MIC ₄	MIC ₅₀	MIC ₉₀	% of resistance	MIC ₄	MIC ₅₀	MIC ₉₀	% of resistance	MIC ₄	MIC ₅₀	MIC ₉₀	% of resistance	MIC ₄	MIC ₅₀	MIC ₉₀	% of resistance	MIC ₄	MIC ₅₀	MIC ₉₀	% of resistance	
EaggEC	28	8	16	8	16	100	>128	>128	89.7	32	>128	71.4	>128	>128	93.4	4	16	3.5	<0.06	<0.06	3.5	16	>128	57.1	
ETEC	38	8	16	8	16	100	>128	>128	89.4	16	64	36.8	>128	>128	77.6	<4	4	2.6	<0.06	<0.06	0	4	>128	47.3	
Salmonella spp	14	4	4	16	16	100	16	128	47.8	8	>128	14.2	\leq 4	128	28.5	4	4	0	0.06	<0.06	0	<1	16	71.4	
<i>C. jejuni</i> ^b	12	256	512	>64	>64		32	>128		8	8		\leq 4	16		16	>256		0.06	32		128	128		
<i>Aeromonas hydrophila</i>	11	8	8	4	16		>128	>128		4	128		8	>128		<4	<4		<0.06	<0.06		4	64		
<i>Y. enterocolitica</i>	10	64	128	16	32	100	>128	>128	100	16	128	40	16	64	4	<4	9	0	<0.06	<0.06	0	16	16	50	
<i>Shigella flexneri</i>	28	4	16	8	8	100	128	>128	53.5	4	128	25	128	128	64.2	<4	4	0	<0.06	<0.06	0	\geq 128	\geq 128	75	
<i>Shigella sonnei</i>	36	8	16	8	16	95	32	>128	86	4	8	25	>128	>128	86.1	<4	8	8.3	<0.06	<0.06	0	128	\geq 128	86	

RFX rifaximin RIF rifampin AMP ampicillin CHL chloramphenicol TET tetracycline NAL nalidixic acid CIP ciprofloxacin TMP trimethoprim EaggEC enteroaggregative *E. coli* ETEC enterotoxigenic *E. coli*

^a The total number of isolates was 177

^b Eleven strains were susceptible to erythromycin (MIC = 1 μ g/ml) for only one was there a MIC of 4 μ g/ml. *Campylobacter* sp. showed 0% of the resistance to erythromycin.

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In another study, 408 *V cholera* clinical isolates from different geographical areas were tested for susceptibility to rifaximin (Scrascia *et al* , 2003) Genetic relationships between isolates were identified by DNA fingerprinting Based on fingerprinting analysis, all isolates were clustered into 23 clones Of the 408 isolates, a total of 359 *V cholera* O1 El Tor isolates were from clinical cases representative of outbreaks that occurred in Africa from 1985 to 1999 These were clustered into 16 clones Twelve isolates from outbreaks in Central and South America and the 32 European isolates were clustered into two clones based on DNA fingerprinting Finally, the three clinical isolates from India were classified in three clones The antibacterial susceptibility pattern of each isolate was determined by the disc diffusion method in accordance with NCCLS guidelines However, no information with respect to MICs was provided for the isolates from India Table 2 shows the distribution of the MICs of rifaximin and tetracycline for the *V cholera* El Tor isolates by geographic area Tetracycline showed lower activity against the African isolates than for those isolated in Europe and America However, there were no differences in the MIC of rifaximin among the three groups

Table 2 The MIC₅₀ and MIC₉₀ (µg/ml) of rifaximin and tetracycline among *V cholera* O1 El Tor strains^a distributed by geographic area

No of strains/ geographic area	Rifaximin		Tetracycline	
	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀
359/Africa	2	4	2	64
32/Europe	4	4	16	16
12/America	2	2	1	2

^aThe MIC range of RFX and TET for the three strains of *V cholerae* O139 and the two strains of *V cholerae* O1 classical biotype was 1–2 mg/L for both antimicrobials

In another study, *in vitro* activity of rifaximin against clinical isolates of *E coli* and *Shigella* species was analyzed by the NCCLS agar dilution method (Sierra *et al* , 2001) The data, provided in Table 3, shows that the MIC₅₀ and MIC₉₀ of the three types of diarrhea causing *E coli* were 8 and 16 µg/ml, respectively The highest MIC observed was 32 µg/ml for enteroaggregative and enterotoxigenic *E coli* isolates The MIC₅₀ and MIC₉₀ for *Shigella flexneri* *Shigella sonnei* and *Shigella dysenteriae* was 4 and 8 µg/ml, respectively, while the MIC₉₀ for *S sonnei* was 16 µg/ml Although some of the clinical isolates of *E coli* showed MICs as high as 32 µg/ml no clinical isolates of *Shigella* species showed MIC > 16 µg/ml

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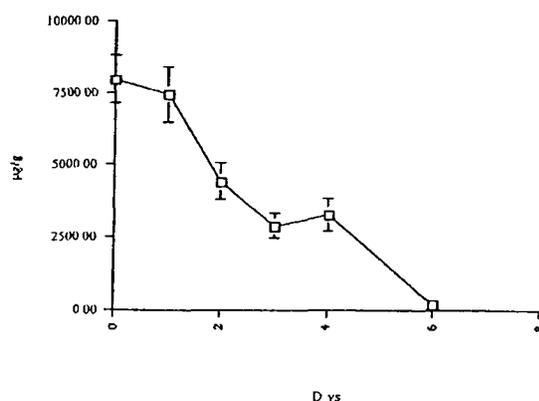
Table 3 Activity of rifaximin against *E. coli* and *Shigella* species

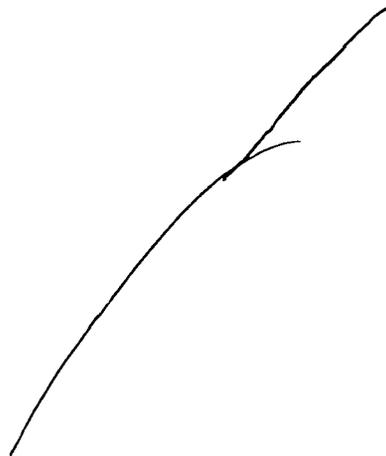
Microorganism	n	range	MIC (mg/L)	
			MIC ₅₀	MIC ₉₀
EaggEC	65	4-32	8	16
ETEC	44	1-32	8	16
EPEC	21	4-16	8	16
<i>S. flexneri</i>	78	2-16	4	8
<i>S. sonnei</i>	4	8-16	4	16
<i>S. dysenteriae</i>	4	4-8	4	8

EaggEC enteroaggregative *E. coli* ETEC enterotoxigenic *E. coli*
 EPEC enteropathogenic *E. coli*

In another study, Jiang *et al* 2000 measured the *in vitro* susceptibility to rifaximin of enteric pathogens, and rifaximin fecal concentrations. Rifaximin concentrations were measured in 39 fecal samples obtained from adult patients following oral administrations of rifaximin (400 mg b i d for 3 days). The authors stated that 120 ETEC isolates, 17 *Shigella* isolates, and 8 *Salmonella* isolates were collected from fecal samples. However, the time of sample collection is not clear. The National Committee for Clinical Laboratory Standards (NCCLS) agar dilution method was used to determine the *in vitro* susceptibility. One gram of stool sample was collected and tested for rifaximin concentration post-treatment. The rifaximin minimum inhibitory concentrations, MIC₅₀ and MIC₉₀, for bacterial isolates were 12.5 µg/ml and 50 µg/ml, respectively. Two ETEC and one *Salmonella* isolates demonstrated MIC's of ≤ 0.098 µg/ml. MICs were in the range of ≤ 0.098-200 µg/ml for the *Salmonella* and ETEC isolates and 1.25-200 µg/ml for the *Shigella* isolates. The result in Figure 1 shows the rifaximin concentration obtained from 39 patients. Drug levels of 7,961 µg/g, were achieved on day one after the first dose followed by a gradual reduction to 154 µg/g on day 6. The concentration on day 6 was higher than the MICs for the bacterial isolates. The study presented here demonstrates that rifaximin is capable of attaining high fecal concentrations following oral administration. However, the correlation between MIC or fecal drug concentrations and clinical outcome was not measured in this study.

Figure 1 Stool concentrations of rifaximin in 39 patients with traveler's diarrhea tested after treatment with 800 mg of rifaximin/day for 3 days





3 3 Activity *in vivo*

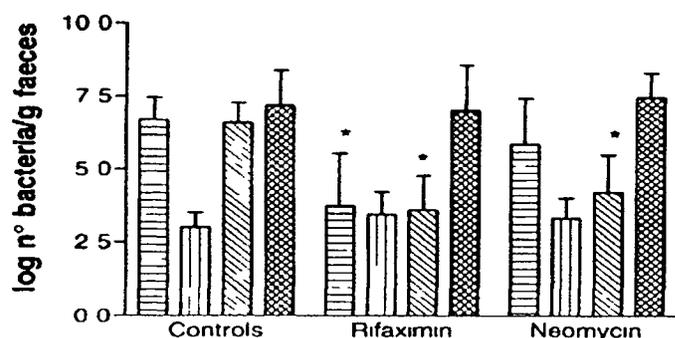
3 3 1 Effect on enteric pathogens

The effect of daily oral administration of rifaximin on fecal aerobic flora was investigated in Male Wistar rats (Migholi *et al* 2001) It is not known if these animals exhibited symptoms of diarrhea Eighteen rats weighing approximately 200 g were divided into three groups (6 in each group), rifaximin, neomycin and placebo Each animal received a dose of 50 mg/kg/day p o once a day in the morning for three days Following the final drug administration, fecal samples were collected for both quantitative and qualitative evaluation of aerobic enteric flora One gram of each specimen was homogenized and 10-fold serial dilutions (from 10^2 to 10^8) were plated and incubated at 37°C overnight on the appropriate media The number of colony forming units (CFU) per gram of specimen was recorded The mean numbers of CFUs are presented in Figure 2 Treatment with rifaximin resulted in a 3-log reduction in the total aerobic bacteria count The number of CFU of *Salmonella* spp also decreased by as much as 3 logs The study shows that rifaximin is capable of decreasing the aerobic fecal bacterial load However, no effect was observed on fecal coliform (anaerobic bacteria)

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Figure 2



Effects of antibiotics on gut rat microflora. Values are expressed as mean (SD). Filling patterns: horizontal lines = total anaerobes, vertical lines = obiforms, oblique lines = Salmonellae, hatched lines = lactobacilli. *Significantly different from control ($P < 0.05$).

3.4 Drug resistance

The development of spontaneous resistance to rifaximin was evaluated by the NCCLS agar dilution and broth dilution methods and resistance rates were compared between gram-positive and gram-negative anaerobic and aerobic bacteria (Marchese *et al* 2000). Organisms were incubated either aerobically or under anaerobic conditions. In the broth dilution methods, bacterial inocula were adjusted to 10^6 CFU per milliliter. Isolates were then incubated under appropriate atmospheric conditions at 37°C for 24 hours with increasing concentrations of the test antibiotic. Aliquots (0.1 ml) of these cultures were transferred from tubes containing the highest concentration that permitted bacterial growth to another series of tubes with 1, 2, 4, and 8 times higher concentration of the same drug. In the agar dilution method, the emergence of spontaneous resistant mutants was determined by plating 0.1 ml of culture (10^8 - 10^{10} CFU/ml) onto three plates containing drugs at concentrations 2, 4, and 8 times the MIC. Samples were then incubated under the appropriate conditions. In addition, the frequency of mutants spontaneously resistant to rifaximin was calculated as the ratio of the number of resistant cells compared to the number of original inocula.

All anaerobic isolates were cultured in broth in the presence of subinhibitory concentrations of rifaximin. Table 5 describes the changes in MIC values obtained following serial transfers from low drug concentration to higher drug concentrations. *Bacteroides* spp and *C. perfringens* resistant clones were observed following 4-5 transfers. The result in Table 6 describes the mutation rates to rifaximin in relation to the MIC. The data shows that *C. difficile* demonstrated relatively low incidences of spontaneous resistance ($<1 \times 10^{-8}$) to rifaximin at 2 x MIC compared to isolates of *C. perfringens* (9.6×10^{-7} to $> 10^{-6}$).

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Table 5 Analysis of the development of mutants of anaerobic bacteria spontaneously resistant to rifaximin

Microorganism	MIC mg/l				
	rifaximin				
	1	2	3	4	5
<i>C. difficile</i> 1	0.008	—	—	—	—
<i>C. difficile</i> 2	0.008	—	—	—	—
<i>C. perfringens</i> 1	0.125	1	16	128	—
<i>C. perfringens</i> 2	0.125	2	32	128	—
<i>F. nucleatum</i> 1	8	128	—	—	—
<i>F. nucleatum</i> 2	4	64	128	—	—
<i>B. distasonis</i> 1	0.03	0.25	2	32	128
<i>B. distasonis</i> 2	0.03	0.5	8	64	128
<i>B. fragilis</i> 1	0.25	4	32	128	—
<i>B. fragilis</i> 2	0.25	4	64	128	—
<i>P. magnus</i> 1	0.25	—	—	—	—
<i>P. magnus</i> 2	0.25	—	—	—	—
<i>P. m. c. os</i> 1	0.25	—	—	—	—
<i>P. m. c. os</i> 2	0.25	—	—	—	—

n 1 = Not tested
1) Number of transfers
0.5 × MIC

Table 6 Rate of emergence of anaerobic mutants spontaneously resistant to rifaximin

Microorganism	Rifaximin	
<i>C. difficile</i> 1	2 × MIC	1×10^{-8}
	4 × MIC	0
	8 × MIC	0
<i>C. difficile</i> 2	2 × MIC	0
	4 × MIC	0
	8 × MIC	0
<i>C. perfringens</i> 1	2 × MIC	$> 10^{-6}$
	4 × MIC	9.3×10^{-7}
	8 × MIC	9.6×10^{-7}
<i>C. perfringens</i> 2	2 × MIC	$> 10^{-6}$
	4 × MIC	7.2×10^{-6}
	8 × MIC	1.3×10^{-7}
<i>F. nucleatum</i> 1	2 × MIC	$> 10^{-6}$
	4 × MIC	9.3×10^{-7}
	8 × MIC	9.6×10^{-7}
<i>F. nucleatum</i> 2	2 × MIC	$> 10^{-6}$
	4 × MIC	7.2×10^{-6}
	8 × MIC	1.3×10^{-7}
<i>B. distasonis</i> 1	2 × MIC	0
	4 × MIC	0
	8 × MIC	0
<i>B. distasonis</i> 2	2 × MIC	0
	4 × MIC	0
	8 × MIC	0
<i>B. fragilis</i> 1	2 × MIC	1.7×10^{-7}
	4 × MIC	1×10^{-8}
	8 × MIC	1×10^{-8}
<i>B. fragilis</i> 2	2 × MIC	5×10^{-8}
	4 × MIC	4×10^{-8}
	8 × MIC	4×10^{-8}

In the Gram-negative organisms, increases in MIC (4-128 µg/ml) were observed following 2-3 transfers (Tables 7 and 8). Under aerobic or anaerobic conditions, isolates of *E. coli* [enteropathogenic (EPEC), enterohemorrhagic (EHEC), enterotoxigenic (ETEC) and enteroinvasive (EIEC)] were shown to acquire resistance following 2-3 transfers (Tables 7 and

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8) The frequency of spontaneous resistance was also determined and the data shows that under aerobic growth conditions, the incidence of resistance for EPEC was 1×10^8 to 1.2×10^8 for EPEC, 1×10^7 to 1×10^8 for EHEC, 1×10^8 to 3×10^8 for ETEC, and 2×10^8 for EIEC (Table 9)

Table 7 Development of mutants spontaneously resistant to rifaximin under aerobic conditions

Microorganism	MIC, mg/l			
	rifaximin			
	1 ¹	2	3	4
<i>C. freundii</i> 1445	64	128	-	-
<i>C. freundii</i> 1539	32	128	-	-
<i>P. rettgeri</i> 141	8	16	128	-
<i>P. rettgeri</i> 187	8	64	128	-
<i>M. morganii</i> 1	32	128	-	-
<i>M. morganii</i> 2	32	128	-	-
<i>P. mirabilis</i> 1	16	64	128	-
<i>P. mirabilis</i> 2	32	128	-	-
<i>P. vulgaris</i> 1	16	128	-	-
<i>P. vulgaris</i> 2	4	64	128	-
<i>E. coli</i> (EPEC) 1	32	128	-	-
<i>E. coli</i> (EPEC) 2	16	128	-	-
<i>E. coli</i> (EHEC) 1	32	128	-	-
<i>E. coli</i> (EHEC) 2	32	128	-	-
<i>E. coli</i> (ETEC) 1	32	128	-	-
<i>E. coli</i> (ETEC) 2	16	64	128	-
<i>E. coli</i> (EIEC) 1	64	128	-	-
<i>E. coli</i> (EIEC) 2	32	128	-	-
<i>S. enteritidis</i> 1	16	128	-	-
<i>S. enteritidis</i> 2	16	128	-	-

nt = Not tested

¹ Number of transfers

Table 8 Development of mutants spontaneously resistant to rifaximin under anaerobic conditions

Microorganism	MIC, mg/l				
	rifaximin				
	1 ¹	2	3	4	5
<i>C. freundii</i> 1445	128	-	-	-	-
<i>C. freundii</i> 1539	nt	-	-	-	-
<i>P. rettgeri</i> 141	32	128	-	-	-
<i>P. rettgeri</i> 187	64	128	-	-	-
<i>M. morganii</i> 1	64	128	-	-	-
<i>M. morganii</i> 2	32	128	-	-	-
<i>P. mirabilis</i> 1	4	64	128	-	-
<i>P. mirabilis</i> 2	32	128	-	-	-
<i>P. vulgaris</i> 1	32	128	-	-	-
<i>P. vulgaris</i> 2	16	128	-	-	-
<i>E. coli</i> (EPEC) 1	128	-	-	-	-
<i>E. coli</i> (EPEC) 2	32	128	-	-	-
<i>E. coli</i> (EHEC) 1	128	-	-	-	-
<i>E. coli</i> (EHEC) 2	64	128	-	-	-
<i>E. coli</i> (ETEC) 1	64	128	-	-	-
<i>E. coli</i> (ETEC) 2	32	64	128	-	-
<i>E. coli</i> (EIEC) 1	nt	-	-	-	-
<i>E. coli</i> (EIEC) 2	64	128	-	-	-
<i>S. enteritidis</i> 1	128	-	-	-	-
<i>S. enteritidis</i> 2	16	128	-	-	-

nt = Not tested

¹ Number of transfers

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Table 9 Rate of emergence of spontaneously resistant to rifaximin under aerobic and anaerobic conditions

Microorganism	Rifaximin	Rifaximin	
		aerobic	anaerobic
<i>P. regeri</i> 2	2×MIC	4×10 ⁻	1×10
	4×MIC	3×10 ⁻	0
	8×MIC	1×10 ⁻	0
<i>M. Morganii</i> 1	2×MIC	1×10	2×10
	4×MIC	0	0
	8×MIC	0	0
<i>M. Morganii</i> 2	2×MIC	1.3×10	>10
	4×MIC	3×10	4.2×10
	8×MIC	2×10	3×10
<i>P. mirabilis</i> 1	2×MIC	3.5×10	0
	4×MIC	1.5×10 [†]	0
	8×MIC	9×10	0
<i>P. mirabilis</i> 2	2×MIC	7×10	1.4×10
	4×MIC	7×10	1.3×10
	8×MIC	5×10	1.3×10
<i>P. vulgaris</i> 1	2×MIC	8.3×10	>10
	4×MIC	7×10	4.2×10
	8×MIC	9×10	4×10
<i>P. vulgaris</i> 2	2×MIC	9.7×10	>10
	4×MIC	5×10	1.1×10
	8×MIC	2×10	1.1×10
<i>E. coli</i> O125 (EPEC)	2×MIC	1×10	0
	4×MIC	0	0
	8×MIC	0	0
<i>E. coli</i> O86 (EPEC)	2×MIC	1.2×10	1×10
	4×MIC	0	1×10
	8×MIC	0	1×10
<i>E. coli</i> O157 (EHEC)	2×MIC	1×10	0
	4×MIC	1×10	0
	8×MIC	1×10	0
<i>E. coli</i> O157 (EHEC)	2×MIC	3×0	2×10
	4×MIC	1×10	1×10
	8×MIC	1×10	1×10
<i>E. coli</i> O83 (ETEC)	2×MIC	1×10	0
	4×MIC	0	0
	8×MIC	0	0
<i>E. coli</i> O159 (ETEC)	2×MIC	>10	>10
	4×MIC	3×10	0
	8×MIC	1×0	0
<i>E. coli</i> O28 (EIEC)	2×MIC	2×10	0
	4×MIC	0	0
	8×MIC	0	0
<i>E. coli</i> O24 (EIEC)	2×MIC	2×0	4×0
	4×MIC	0	0
	8×MIC	0	0
<i>S. enteritidis</i>	2×MIC	2.6×0	0
	4×MIC	1.6×10	0
	8×MIC	6×10	0
<i>S. enteritidis</i> 2	2×MIC	3×10	0
	4×MIC	1.2×10	0
	8×MIC	2×10	0

In summary, the frequency of emergence of spontaneous resistance were in the range of $<1 \times 10^9$ to 1.7×10^7 for *C. difficile*. The frequency of spontaneous resistance was in the range of 10^8 to 10^5 for aerobic and anaerobic gram negative organisms. The study demonstrates that rifaximin contributes to the emergence of spontaneous resistance *in vitro* and resistant mutants were more easily selected following pre-incubation of the test bacteria at sub MIC levels of the test drug.

4 Clinical Microbiology

4.1 Study RFID3001

The sponsor conducted a phase 3, randomized, double blind, placebo and active (Cipro, 500 mg, b i d, for 3 days) controlled study (RFID3001) in patients ≥ 18 years of age, to evaluate the safety and efficacy of rifaximin (200 mg orally, t i d, for 3 days) for the treatment of travelers' diarrhea due to enteropathogenic organisms. The study was conducted at 7 sites in 4 countries including India, Mexico, Guatemala, and Peru. Individuals who showed evidence of acute diarrhea (≤ 72 hours), defined by passing 3 or more unformed stools during the 24 hours preceding enrollment, and accompanied by 1 or more of the following qualifying signs and symptoms: abdominal pain

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or cramps, excessive gas/flatulence, nausea, vomiting, fever, (≥ 100 °F), fecal urgency, blood and/or mucus in the stool, were included in the study. Exclusions included evidence of acute diarrhea for ≥ 72 hours, moderate or severe dehydration, females pregnant or breastfeeding. Please refer to the Medical Officer's review for more details.

The study period lasted 4-5 days and included 3 clinic visits. Subjects were asked to maintain a daily diary on stool consistency and clinical symptoms. Stool samples were obtained for microbiologic assessment from each subject at pretreatment (visit 1), 24 hours after the first dose of rifaximin (Day 2 visit 2), and Day 3 or 5 following the initiation of treatment (visit 3). The test of cure was performed at visit 3. However, results of microbiologic assessments at visit 2 were not included. Samples underwent gross observation for blood and mucus, and microscopic assessments were based on speciation, and *in vitro* susceptibility testing. *E. coli* enterotoxin (heat-stable and heat labile) were detected with oligonucleotides labeled by T4 polynucleotide kinase and [32 P]-ATP. Microbiological success was dependent on total eradication of the organism in question. No information was provided on the number of patients experiencing relapse.

The primary clinical endpoint was shortening the time to last unformed stool (TLUS). Efficacy assessments were made based on information recorded in the patient's diary. The secondary efficacy endpoints were the number of unformed stool passed per given time, improving diarrheal symptoms, the number of subjects with wellness, the number of subjects who failed treatment, persistence of clinical symptoms, and microbiologic eradication/persistence. Wellness or clinical cure was defined by the absence of watery stool or no more than 2 soft stools passed within a 24 hour period in conjunction with no other clinical symptoms except for mild excess gas/flatulence, and absence of unformed stool within a 48 hour interval with no fever (≥ 100 °F) with or without other clinical symptoms.

A total of 399 patients with travelers' diarrhea were enrolled (Table 10). Of the 399 subjects, 248 were microbiologically evaluable. The majority of the subjects had *E. coli* as the etiological agent. Bacterial pathogens isolated from stool samples were enterotoxigenic *E. coli* (ETEC) ST, ETEC LT, ETEC LT/ST, *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Aeromonas*, *Plesiomonas* species, and *Vibrio cholera*. In addition, individuals with protozoan parasites such as *Cryptosporidia*, *Giardia lamblia* were also isolated in the study. Non-ETEC bacterial enteric pathogens (*Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas*, and *Vibrio* species) accounted for 49 out of a total of 146 isolates (33%) in the MITT rifaximin treatment arm and 17 out of the 70 (24%) isolates from patients in the placebo arm and 14 out of 66 (21%) in the Cipro treatment arm. Mixed pathogen infection accounted for 36/128 (28%) patients in the rifaximin treatment arm, 18/62 (29%) in the placebo group and 16/58 (27%) in the Cipro group.

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Table 10 Summary of population analysis

Analysis Populations	Number (%) of Subjects		
	Rifaximin (N=197)	Placebo (N=101)	Cipro (N=101)
Total No. Subjects Randomized (ITT Population)			
No. Subjects in MITT Population	128 (65.0%)	62 (61.4%)	58 (57.4%)
No. Subjects Excluded from MITT Population	69 (35.0%)	39 (38.6%)	43 (42.6%)
No. positive pretreatment stool sample	67 (34.0%)	38 (37.6%)	37 (36.6%)
No. posttreatment stool sample	2 (1.0%)	1 (1.0%)	6 (5.9%)

While *E. coli* was the most common pathogen identified at all locations, *Plesimonas* species *Giardia* and *Campylobacter pylori* were more prevalent at site 100 in Calcutta, India (Table 11). Of the 14 clinical isolates of *Campylobacter pylori*, 5 were found to be associated with mixed infections. *Giardia* species was commonly associated with ETEC as mixed infection at site 101 (Goa) followed by site 100 (Calcutta), occurring in 3 and 5 patients, respectively. Mixed infection with *Entamoeba* and ETEC occurred in 3 patients at the Calcutta site. *Vibrio cholera* was identified in association with ETEC only at the Calcutta study site, occurring in 2 patients. *Salmonella* species was identified among mixed infections in the stool samples from the Goa site. Moreover, they were also commonly associated with members of the *Vibrio* spp., *Plesimonas* and *Aeromonas* species.

Table 11 Enteric pathogens identified by site in the rifaximin treatment arm (MITT population)

Identified pathogens	Site 100 (Calcutta)	Site 101 (Goa)	Site 107 (Antigua)	Site 200 (Guadalajara)	Site 242 (Cuernavaca)	Site 269 (Lima)
<i>Aeromonas</i> spp	1	2	0	0	0	0
<i>Campylobacter</i> spp	2	14	4	4	0	0
<i>E. coli</i>	28	18	31	17	3	0
<i>Plesimonas</i> spp	0	5	0	0	0	0
<i>Salmonella</i> spp	0	4	0	0	0	0
<i>Shigella</i> spp	2	2	0	3	0	0
<i>Giardia</i> spp	5	7	0	1	1	1
<i>Entamoeba</i> spp	3	0	0	0	0	0
<i>Cryptosporidia</i> spp	1	2	0	3	0	0
<i>Vibrio cholera</i>	2	0	0	0	0	0

Clinical and microbiological responses at Visit 3 for each treatment arm are presented by pathogen in tables 12, 13 and 14. There were a total of 100 patients with *E. coli* in the rifaximin treatment arm, 52 patients in the placebo, and Cipro treatment arm, respectively. This includes patients with mixed infection at baseline. Rifaximin had a microbiological eradication rate of 75% and a clinical cure rate of 83%. In the placebo arm, the microbiological eradication rate was 71% and the clinical cure rate was 62% while in the Cipro arm, the microbiological eradication, and clinical cure rates were 90% and 81%, respectively.

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In addition, there were a total of 10 patients with *Shigella* in the rifaximin treatment arm. Of that, 7 were isolates of *Shigella sonnei*. Rifaximin had a microbiological eradication rate of 86% and a clinical cure rate of 100%. In the placebo (n=5) and Cipro (n=2) arm, there were very few patients with *Shigella* spp. There were a total of 1 *Shigella sonnei* in the placebo and Cipro treatment arms, respectively. Cipro and placebo had a microbiological cure rate of 100% and 50%, respectively. The median TLUS was 44.8 hours for rifaximin, 120 hours for placebo and 15.6 hours for Cipro.

Table 12 Clinical and microbiological response in patients treated with rifaximin (Study RFID3001)

Pathogen	No with Culture Pre-treatment	Clinical Outcome Cure (%)	Clinical Cure with Micro Eradication (%)	Micro Eradication RFID3001 n/N	Median TLUS in hrs (range)
<i>Giardia lamblia</i>	15	10 (67)	4 (27)	6/15 (40%)	46.5 (0-120)
<i>Entamoeba spp</i>	3	3 (100)	1 (33)	1/3 (33)	61 (6-65.3)
<i>Cryptosporidium spp</i>	6	3 (50)	1 (17)	2/6 (33)	94.8 (24.4-120)
<i>Shigella sonnei</i>	7	7 (100)	6 (86)	6/7 (86%)	44.8 (16-51)
<i>Shigella flexneri</i>	2	2 (100)	2 (100)	2/2 (100%)	55.65 (42.6-68.7)
<i>Shigella boydii</i>	1	1 (100)	1 (100)	1/1 (100%)	25.7 (25.7)
<i>Salmonella spp</i>	4	0	0	2/4 (50%)	120 (71.8-120)
<i>Campylobacter jejuni</i>	24	6 (25)	5 (21)	9/24 (37.5%)	120 (0-120)
<i>Aeromonas spp</i>	3	1 (33)	1 (33)	2/3 (67%)	120 (18.3-120)
<i>Plesiomonas spp</i>	5	2 (40)	1 (20)	4/5 (80%)	71.8 (40.3-120)
<i>Vibrio cholera</i>	2	1 (50)	1 (50)	2/2 (100%)	40.65 (0-81.3)
<i>E coli</i> -LT	35	30 (86)	21 (60)	24/35 (69%)	25.8 (0-120)
<i>E coli</i> -ST/LT	24	17 (71)	13 (54)	19/27 (79%)	32.3 (90-120)
<i>E coli</i> -ST	12	11 (92)	8 (67)	8/12 (67%)	8.1 (0-120)
<i>E coli</i> (EAEC)	29	25 (86)	22 (76)	23/29 (79%)	24 (0-120)

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Table 13 Clinical and microbiological response in patients treated with placebo (Study RFID3001)

Pathogen	No with Culture Pre-treatment	Clinical Outcome Cure (%)	Clinical Cure with Micro Eradication (%)	Micro Eradication (%)	Median TLUS in hrs
<i>Giardia lamblia</i>	9	6 (67)	1 (11)	2 (22)	76 15
<i>Entamoeba spp</i>	1	0	0	0	120
<i>Cryptosporidium spp</i>	4	1 (25)	0	1 (25)	106
<i>Shigella sonnei</i>	1	0	0	1 (100)	120
<i>Shigella flexneri</i>	4	3 (75)	2 (100)	2 (50)	27 85
<i>Shigella boydu</i>	NA	NA	NA	NA	NA
<i>Salmonella spp</i>	1	1(100)	1(100)	1 (100)	58 3
<i>Campylobacter jejuni</i>	10	3 (33)	1 (10)	4 (40)	120
<i>Aeromonas spp</i>	1	1 (100)	1 (100)	1 (100)	67 5
<i>Plesiomonas spp</i>	NA	NA	NA	NA	NA
<i>Vibrio cholera</i>	NA	NA	NA	NA	NA
<i>E coli</i> -LT	13	9 (69)	7 (54)	10/13 (77)	37 9
<i>E coli</i> ST/LT	10	5 (50)	3 (30)	6 (60)	68 75
<i>E coli</i> -ST	7	4 (57)	3 (43)	4 (57)	68 4
<i>E coli</i> (EAEC)	22	14 (64)	11 (50)	17 (77)	42 4

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Table 14 Clinical and microbiological response in patients treated with Cipro (Study RFID3001)

Pathogen	No with Culture Pre-treatment	Clinical Outcome Cure (%)	Clinical Cure with Micro Eradication (%)	Micro Eradication (%)	Median TLUS in hrs
<i>Giardia lamblia</i>	5	3 (60)	2 (40)	3 (60)	39.4
<i>Entamoeba</i> spp	NA	NA	NA	NA	NA
<i>Cryptosporidium</i> spp	6	4 (67)	1 (17)	2 (33)	70.5
<i>Shigella sonnei</i>	1	1 (100)	1 (100)	1 (100)	15.6
<i>Shigella flexneri</i>	1	1 (100)	1 (100)	1 (100)	17.5
<i>Shigella boydu</i>	NA	NA	NA	NA	NA
<i>Salmonella</i> spp	2	2 (100)	2 (100)	2 (100)	13.1
<i>Campylobacter jejuni</i>	9	4 (44)	3 (33)	6 (66)	66.1
<i>Aeromonas</i> spp	1	1 (100)	1 (100)	1 (100)	55.1
<i>Plesiomonas</i> spp	NA	NA	NA	NA	NA
<i>Vibrio cholera</i>	NA	NA	NA	NA	NA
<i>E coli</i> -LT	14	11 (78)	9 (64)	12 (86)	23.4
<i>E coli</i> -ST/LT	15	13 (87)	13 (87)	15 (100)	28.3
<i>E coli</i> -ST	4	4 (100)	4 (100)	4 (100)	0
<i>E coli</i> (EAEC)	19	14 (74)	13 (68)	16 (84)	27.3

In addition, an analysis of the overall microbiological eradication rate, by study site, was conducted. Table 15 (A-D) shows the distributions of different enteropathogens at the different study sites and compared eradication rates of rifaximin, placebo, and Cipro. The data show that rifaximin does not offer any significant benefit over placebo at microbiological eradication. Moreover, at some sites, eradication rates of placebo appear to be superior to that of rifaximin.

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Table 15 Over all microbiological eradication efficacies in the MITT population

A Study site 100
Calcutta

Strain	Rifaximin (# of isolates) % Eradication	Placebo (# of isolates) % Eradication	Cipro (# of isolates) % Eradication
<i>E coli</i>	(28) 78.6	(14) 64.3	(15) 93
<i>C jejuni</i>	(2) 100	(2) 50	(1) 100
<i>Giardia spp</i>	(5) 40	(4) 25	(3) 33
<i>Shigella sonnei</i>	(2) 100	(1) 100	(1) 100
<i>Shigella flexneri</i>	NA	NA	(1) 100
<i>Vibrio cholera</i>	(2) 100	NA	NA
<i>Entamoeba</i>	(3) 33	NA	NA
<i>Cryptosporidium</i>	(1) 0	(1) 0	(1) 100
<i>Aeromonas</i>	(1) 100	NA	NA

B Study site 101
Goa

Strain	Rifaximin (# of isolates) % Eradication	Placebo (# of isolates) % Eradication	Cipro (# of isolates) % Eradication
<i>E coli</i>	(18) 77.8	(11) 91	(18) 94.4
<i>C jejuni</i>	(14) 35.7	(4) 75	(6) 83
<i>Cryptosporidium</i>	(2) 50	NA	(3) 67
<i>Giardia spp</i>	(7) 43	(2) 50	(2) 100
<i>Shigella sonnei</i>	(1) 0	NA	NA
<i>Shigella flexneri</i>	(1) 100	(3) 67	NA
<i>Plesimonas</i>	(5) 100	(1) 0	NA
<i>Salmonella Group C1</i>	(1) 100	NA	NA
<i>Salmonella Group B</i>	(4) 50	NA	NA
<i>Aeromonas</i>	(2) 50	(1) 100	(1) 100

C Study site 107
Antigua

Strain	Rifaximin (# of isolates) % Eradication	Placebo (# of isolates) % Eradication	Cipro (# of isolates) % Eradication
<i>E coli</i>	(31) 71	(16) 75	(8) 75
<i>Shigella flexneri</i>	(3) 100	(1) 0	NA
<i>C jejuni</i>	(4) 25	(1) 0	(1) 0
<i>Salmonella Group C1</i>	NA	(1) 100	(1) 100
<i>Salmonella Group C2</i>	NA	NA	(1) 100

D Study site 200
Guadalajara.

Strain	Rifaximin (# of isolates) % Eradication	Placebo (# of isolates) % Eradication	Cipro (# of isolates) % Eradication
<i>E coli</i>	(17) 88	(6) 33	(9) 100
<i>Shigella sonnei</i>	(3) 100	NA	NA
<i>Giardia spp</i>	(1) 0	NA	NA
<i>C jejuni</i>	(4) 25	(1) 0	(1) 0
<i>Cryptosporidium</i>	(3) 33	(1) 0	(2) 0

C Study site 242
Cuernavaca

Strain	Rifaximin (# of isolates) % Eradication	Placebo (# of isolates) % Eradication	Cipro (# of isolates) % Eradication
<i>E coli</i>	(3) 67	(3) 100	(2) 100
<i>Giardia</i>	(1) 0	NA	NA
<i>C jejuni</i>	NA	(2) 0	NA

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Rifaximin was compared to placebo and Cipro with respect to the number of subjects who were infected with *E coli*, *Shigella flexneri*, or *Shigella sonnei* only (Table 16) TLUS, wellness, and overall microbiological eradication for each patient was analyzed The result shows that the *E coli* microbiological eradication rate for both rifaximin and placebo was 76% and 70%, respectively, while Cipro demonstrated an eradication rate of 94% The clinical cure rate for *E coli* was 84% for rifaximin and approximately 80% for placebo and Cipro, respectively Moreover, the TLUS for rifaximin, placebo, and Cipro was 23.9, 25.4, and 23.4, respectively Such differences appear to be related to the study site (for details please refer to Statistician review) The *Shigella* spp eradication rate was 100% for rifaximin with a median TLUS of 30.6 hours for *S sonnei* and 55.7 hours for *S flexneri* In the placebo arm, the TLUS for *S flexneri* was 75.9 hours and in the Cipro arm the TLUS of the individual infected with *S sonnei* was 15.6 hours

Table 16 Clinical and microbiological response for patients with single infection

Pathogen	Number of patients	Clinical outcome cure	Micro Eradication	Median TLUS (hrs)
Rifaximin				
<i>E coli</i>	55	84%	76%	23.9
<i>S sonnei</i>	5	100%	100%	30.6
<i>S flexneri</i>	2	100%	100%	55.7
Placebo				
<i>E coli</i>	30	80%	70%	25.4
<i>S sonnei</i>	NA	NA	NA	NA
<i>S flexneri</i>	2	50%	50%	75.9
Cipro				
<i>E coli</i>	33	79%	94%	23.4
<i>S sonnei</i>	1	100%	100%	15.6
<i>S flexneri</i>	NA	NA	NA	NA

NA not available

Individuals infected with *C jejuni* showed no relief in TLUS when rifaximin was administered While the median TLUS was 120 hours, microbiological eradication was 37.5% Furthermore, individuals infected with *C jejuni* had a median TLUS of 120 and 66 hours for placebo and Cipro, respectively Both rifaximin and placebo demonstrated similar eradication rates while Cipro had a 66% eradication rate against *C jejuni*

When rifaximin was administered to individuals infected with *Salmonella* spp, a median TLUS of 120 hours and a 50% eradication rate was reported A median TLUS of 58 and 13 hours was reported for placebo and Cipro, respectively Moreover, based on clinical result, and microbiological eradication data, rifaximin appears ineffective against *Giardia lamblia*, *Entamoeba* spp, *Cryptosporidium* spp, *Aeromonas* spp, and *Plesiomonas* spp (Table 12) The MIC was determined on all available isolates at baseline and at test of cure (visit 3) using the NCCLS agar dilution method Bacterial colonies from each stool sample were transferred to

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peptone stabs and shipped to the ' —

— *E coli* clinical isolates were identified by production of heat labile (LT) or heat stable (ST) enterotoxin Protozoan parasites, including *Giardia lamblia*, *Cryptosporidia* species, and *Entamoeba histolytica* were identified by use of enzyme immunoassays — A summary of the pretreatment *in vitro* MICs for all pathogens isolated from the clinical study is presented in Table 17 The MIC values for *E coli* ranged from 0.0625 to > 1024 µg/ml for ETEC and 0.5-32 µg/ml for EAEC However, there was no correlation between baseline MICs and the clinical or the microbiological outcome in the study

Table 17 Pretreatment Rifaximin MIC's for pathogen isolates from clinical trials

Pathogen	Number of Isolates	MIC µg/mL	
		Median	Range
Enterotoxigenic <i>E coli</i>	347	16	0.0625 to >1024
Enteroaggregative <i>E coli</i>	74	4	0.50-32
<i>S sonnei</i>	21	8	0.50-256
<i>Shigella flexneri</i> ^a	17	8	0.0625-64
<i>Plesiomonas shigelloides</i> ^a	7	4	2-16
<i>Aeromonas hydrophilia</i> ^a	6	4	0.25-16

^a = Clinical effectiveness has not been established for these pathogens

There were 10 patients that had an increase (4-256 folds) in their post-treatment rifaximin MIC compared to baseline MIC All isolates were identified as *E coli* that persisted following treatment and of these 10, two had mixed infection at base line (Table 18) The data shows that the median TLUS was 29.65 hours in 9 out of 10 patients reporting wellness More importantly, 6 out of 10 patients had a TLUS of less than 48 hours Increases in post-treatment MIC were only observed in the rifaximin arm, not in the placebo or the Cipro treatment arm

Table 18 Effect of MIC increase on clinical outcome

Pathogen	Pretreatment MIC	Post treatment MIC	TLUS	Wellness	Micro response
ETEC ST	2	8	0	Yes	P
ETEC LT	8	> 1024	22	Yes	P
ETEC ST/LT	4	256	9.3	Yes	P
ETEC/LT	4	> 1024	47.5	Yes	P
ETEC LT	4	16	25.8	Yes	P
ETEC ST/LT	4	> 1024	90.8	Yes	P
ETEC LT	4	> 1024	33.5	Yes	P
ETEC LT	4	> 1024	92.5	Yes	P
*ETEC-LT	2	16	14.3	Yes	P
*ETEC LT	4	> 1024	120	No	P
			Median 29.65		

* Mixed infection at baseline

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Overall, the study RDID3001 shows rifaximin was effective in reducing the TLUS as compared to placebo in patients with *E coli*. The clinical efficacy of the drug appears to be similar to that of Cipro. However, there was no correlation between clinical and microbiological outcome. This study supports the findings observed in studies reviewed earlier (RFID9801, RFID9701 and RFID9601, see Table 19)

Table 19 Clinical and microbiological response in patients treated with rifaximin (Study RFID3001)

Pathogen	Micro Eradication		Median TLUS in hrs	
	RFID3001	RFID9801, RFID9701 and RFID9601	RFID3001	RFID9801, RFID9701 and RFID9601
<i>Giardia lamblia</i>	6/15 (40%)	5/9 (55.5%)	46.5 (0-120)	32.5
<i>Entamoeba</i> spp	1/3 (33%)	3/5 (60%)	61 (6-65.3)	NA
<i>Cryptosporidium</i> spp	2/6 (33%)	18/34 (53%)	94.8 (24.4-120)	41.25
<i>Shigella sonnei</i>	7/7 (86%)	8/9 (89%)	44.8 (16-51)	30
<i>Shigella flexneri</i>	2/2 (100%)	2/4 (50%)	55.65 (42.6-68.7)	18.9
<i>Shigella boydii</i>	1/1 (100%)	NA	25.7 (25.7)	NA
<i>Salmonella</i> spp	2/4 (50%)	9/15 (60%)	120 (71.8-120)	NA
<i>Campylobacter jejuni</i>	9/24 (37.5%)	5/6 (83%)	120 (0-120)	53.5
<i>Aeromonas</i> spp	2/3 (67%)	1/1 (100%)	120 (18.3-120)	NA
<i>Plesiomonas</i> spp	4/5 (80%)	1/1 (100%)	71.8 (40.3-120)	0
<i>Vibrio cholera</i>	2/2 (100%)	NA	40.65 (0-81.3)	NA
<i>E coli</i> -LT	24/35 (69%)	23/28 (82.1%)	25.8 (0-120)	25.25
<i>E coli</i> -ST/LT	19/24 (79%)	32/39 (82.1%)	32.3 (90-120)	32.5
<i>E coli</i> -ST	8/12 (67%)	40/61 (65.5%)	8.1 (0-120)	30.25
<i>E coli</i> (EAEC)	23/29 (79%)	NA	24 (0-120)	NA
<i>E coli</i>	75/100 (75%)	99/143 (69%)	24.9 (8.1-32.3)	30.25

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Salix Pharmaceuticals

5 CONCLUSION

Rifaximin demonstrated *in vitro* activity against enteropathogenic organisms with the exception of *C jejuni*, where MIC values of > 256 µg/ml was reported. In another *in vitro* study, the emergence of spontaneous resistant mutants was observed in some Gram-positive and Gram-negative organisms under aerobic and anaerobic conditions following 2-3 transfers. Furthermore, an increased mutation rate was observed in enteropathogenic isolates of *E coli*.

Rifaximin was shown to reduce the total number of fecal aerobic bacteria in rats. However, no effect on fecal anaerobic coliform bacteria was observed.

The clinical study (RFID3001) measured the clinical efficacy of rifaximin at 7 sites in 4 countries. The primary efficacy endpoint was the reduction of TLUS in patients receiving rifaximin compared with placebo and Cipro. The majority of the isolates were *E coli* in origin. However, some patients had mixed infection at baseline with pathogens other than *E coli*. Of these patients who were infected with *E coli*, 83% were considered clinically cured. However, only 64% were clinically cured with microbiological eradication. The clinical cure rate for those colonized with *Shigella sonnei* was 100%, and 86% were considered clinically cured with microbiological eradication. A median TLUS of 24.9 hours for those patients with *E coli* and 44.8 hours for those with *Shigella sonnei* was reported. Rifaximin also appeared less effective than Cipro against *Campylobacter jejuni*. Individuals who were infected with *C jejuni* had a TLUS of 120 hours.

There is no correlation between clinical outcome and microbiological eradication and from a microbiological judgment, rifaximin does not show any significant increase in efficacy compared to placebo against enteropathogens. The lack of correlation may be due to limitations of the stool examination procedure. In study RFID3001, *E coli* enterotoxin production was detected by oligonucleotide labeled probes, however, the sensitivity of this technique is limited due to the short half life of some bacterial toxins.

The study consisted primarily of *E coli* isolates, few (n=11) *Shigella* isolates, of which two *Shigella flexneri* and 7 *Shigella sonnei* isolates were in the rifaximin treatment arm. Therefore, there were not enough *Shigella* isolates to make a conclusive statement concerning clinical efficacy of rifaximin against *Shigella* spp. The data obtained in this study corroborated those results of the previously reviewed Phase III study (RFID9801, RFID9701 and RFID9601).

Rifaximin

Salix Pharmaceuticals

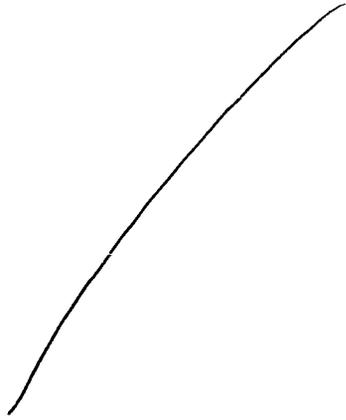
6 LABEL

6.1 Sponsor's proposed labeling

Microbiology

Rifaximin acts by binding to the beta-subunit of bacterial DNA-dependent RNA polymerase resulting in inhibition of bacterial RNA synthesis. Rifaximin has been shown to be active against the following — in clinical studies of infectious diarrhea as described in the INDICATIONS AND USAGE section: *E. coli* (enterotoxigenic and enteroaggregative strains) —

Susceptibility Tests



INDICATIONS AND USAGE

LUMESTAT™ Tablets are indicated for the treatment of patients (≥12 years of age) with travelers' diarrhea caused by —

6.2 COMMENTS

The sponsor states that Rifaximin has been shown to be active against the following pathogens in clinical studies of infectious diarrhea as described in the INDICATIONS AND USAGE section: *E. coli* (enterotoxigenic and enteroaggregative strains) — There is sufficient isolates to make a clinical claim with respect to *E. coli* —

/

6.3 FDA's version of the label

Microbiology

Rifaximin acts by binding to the beta-subunit of bacterial DNA-dependent RNA polymerase resulting in inhibition of bacterial RNA synthesis

Escherichia coli has been shown to develop resistance to rifaximin *in vitro*. However, the clinical significance of such an effect has not been studied.

Rifaximin is a structural analog of rifampin. Organisms with high rifaximin minimum inhibitory concentration (MIC) values also have elevated MIC values against rifampin. Cross resistance between rifaximin and other classes of antimicrobials has not been studied.

Rifaximin has been shown to be active against the following pathogens in clinical studies of infectious diarrhea as described in the **INDICATIONS AND USAGE** section

Escherichia coli (enterotoxigenic and enteroaggregative strains)

Susceptibility Tests

In vitro susceptibility testing was performed according to the National Committee for Clinical Laboratory Standards (NCCLS) agar dilution method M7-A6. However, the correlation between susceptibility testing and clinical outcome has not been determined.

INDICATIONS AND USAGE

Xifaxan™ Tablets are indicated for the treatment of patients (≥ 12 years of age) with travelers' diarrhea caused by noninvasive strains of *Escherichia coli* (see Microbiology, CLINICAL STUDIES and WARNINGS)

7 REFERENCES

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Marchese A *et al* (2000) *In vitro* activity of rifaximin, metronidazole and vancomycin against *C difficile* and the rate of selection of spontaneously resistant mutants against representative anaerobic and aerobic bacteria, including ammonia-producing species *Chemotherapy* **46** 253-266

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Approved Standard NCCLS document M7-A6, Vol 23 No 2, NCCLS, Wayne, PA, January 2003

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Rifaximin

Salix Pharmaceuticals

Sierra *et al*, (2001) In vitro activity of rifaximin against enteropathogens producing travelers' diarrhea *Antimicrobial Agents and Chemotherapy* 45(20) 643-644

Riley, T V (1994) The epidemiology of *Clostridium difficile*-associated diarrhea *Reviews in Medical Microbiology* 5 117-22

8 RECOMMENDATIONS

This NDA is approvable with respect to the microbiology pending an accepted version of the label

Microbiologist, HFD-590

CONCURRENCES

HFD-590/Deputy Dir _____ Signature _____ Date _____

HFD-590/Micro TL _____ Signature _____ Date _____

CC

HFD-590/Original IND

HFD-590/Division File

HFD-590/MO

HFD-590/Pharm

HFD-590/Chem

HFD-590/Review Micro

HFD-590/CSO

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature**

/s/

Avery Goodwin
5/20/04 02 55 08 PM
MICROBIOLOGIST

Shukal Bala
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5/21/04 08 13 05 AM
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MICROBIOLOGY REVIEW
DIVISION OF SPECIAL PATHOGEN AND IMMUNOLOGIC DRUG PRODUCTS
(HFD-590)

NDA# 21-361

REVIEWER	Peter A Dionne
CORRESPONDENCE DATE	21-DEC-01
CDER DATE	26-DEC-01
REVIEW ASSIGN DATE	31-DEC-01
REVIEW COMPLETE DATE	14-MAR-02

SPONSOR

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SUBMISSION REVIEWED Original NDA Submission

DRUG CATEGORY Antimicrobial Rifamycin

INDICATIONS — Diarrhea in Travelers

DOSAGE FORM 200 mg Tablet

DRUG PRODUCT NAME

PROPRIETARY

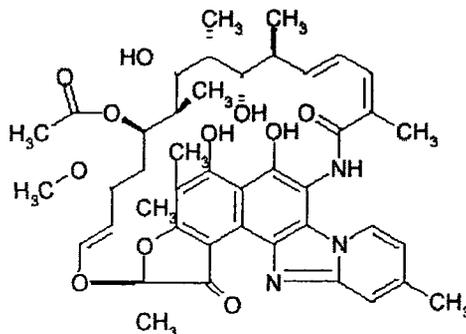
Lumenax™

NONPROPRIETARY/USAN

Rifaximin

CHEMICAL NAME [2S-[2R*,16Z,18E,20R*,21R*,22S*,23S*,24S*,25R*-
26S*,27R*,28E)]-25-(Acetyloxy)-5,6,21,23-tetrahydroxy-27-
methoxy-2,4,11,16,20,22,24,26-octamethyl-7-
[epoxypentadeca[1,11,13]-trienimino]benzofuro
[4,5-e]pyrido[1,2-a]benzimidazole-1,15(2H)-dione

STRUCTURAL FORMULA



Molecular Formula
Molecular Weight

C₄₃H₅₁N₃O₁₁
785.89

SUPPORTING DOCUMENTS

IND 52,980—Salix IND for rifaximin for → diarrhea

REMARKS/COMMENTS

This original New Drug Application is for Lumenax (rifaximin) tablets. Rifaximin is a semi-synthetic antimicrobial derived from rifamycin SV. Rifaximin acts by binding to the beta-subunit of the bacterial DNA-dependent RNA polymerase, resulting in inhibition of bacterial RNA synthesis. There is negligible absorption of rifaximin from the gastrointestinal tract following oral dosing. Fecal concentrations of rifaximin after 200 mg oral dosing have been established at about 8,000 µg/g feces.

The sponsor is requesting an indication of traveler's diarrhea caused by *Escherichia coli*.

Two randomized comparative controlled Phase 3 studies, RFID9701 and RFID9801, provide the primary support for the clinical efficacy of rifaximin for the treatment of infectious diarrhea in travelers. RFID9701 compared the clinical efficacy and safety of rifaximin to a standard regimen of ciprofloxacin. RFID9801 is a placebo-controlled study that investigated the superiority of rifaximin to placebo. In each study medication was taken for three days with one to two days of additional observation after the end of the study. In study RFID9701 rifaximin was dosed at 400 mg twice a day. In study RFID9801 rifaximin was dosed at 200 mg and 400 mg three times a day.

Supportive information is also provided by one dose-comparison Phase 2 study, RFID9601. In this study three dose regimens of rifaximin (200 mg, 400 mg, and 600 mg three times a day) were compared to a standard regimen of trimethoprim/sulfamethoxazole. Study medications were taken for 5 days.

The sponsor is asking for a dosing regimen of 200 mg three times a day for 3 days. Only study RFID9801 used this dosing.

CONCLUSIONS & RECOMMENDATIONS

From the microbiological viewpoint it appears that this application is not approvable.

There also appears to be no correlation between bacterial eradication and clinical efficacy (time to last unformed stool). Placebo eradicated organisms from patients stool samples as well as rifaximin did.

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EXECUTIVE SUMMARY

This application is for a new drug rifaximin. The application provides data to support an indication of acute diarrhea in travelers. Rifaximin is a semi-synthetic antimicrobial derived from rifamycin SV. Rifaximin acts by binding to the beta-subunit of the bacterial DNA-dependent RNA polymerase, resulting in inhibition of RNA synthesis.

From the microbiology viewpoint this application should not be approved. Eradication rates for placebo were equivalent to those for rifaximin. The only organism that was treated with the proposed dose in adequate numbers to draw any conclusions was *Escherichia coli*.

There is negligible absorption of rifaximin from the gastrointestinal tract following oral dosing. Fecal concentrations of rifaximin after 200 mg oral dosing have been established at about 8,000 µg/gram of feces.

Data have been provided that show the *in vitro* activity of rifaximin against pathogens that cause diarrhea. These data are shown in TABLE A.

TABLE A—*In Vitro* Activity of Rifaximin

Organism	No of Strains	µg/mL		MIC Range
		MIC ₅₀	MIC ₉₀	
<i>Aeromonas</i> spp	27	12.5	100	12.5->200
<i>Aeromonas</i> spp	17	16	64	16-512
<i>Aeromonas</i> spp	3	16	16	8-16
<i>Campylobacter</i> spp	35	>200	>200	0.195->200
<i>Campylobacter jejuni</i>	24	128	256	0.25-512
<i>Campylobacter</i> spp	11	32	64	8-64
<i>Clostridium difficile</i>	93	0.004	2	0.004 – 128
<i>Clostridium</i> spp	38	0.001	0.005	<0.0001-2
Enteroaggregative <i>E. coli</i>	65	8	16	4-32
Enteroaggregative <i>E. coli</i>	62	64	128	8-128
Enterotoxigenic <i>E. coli</i>	44	8	16	1-32
Enterotoxigenic <i>E. coli</i>	153	50	100	6.25->200
Enterotoxigenic <i>E. coli</i>	77	64	64	4-1024
Enterotoxigenic <i>E. coli</i>	347	32	64	0.098-512
<i>Plesiomonas shigelloides</i>	25	50	200	12.5->200
<i>Plesiomonas shigelloides</i>	30	32	128	16-256
<i>Plesiomonas shigelloides</i>	2	4	8	4-8
<i>Salmonella</i> spp	53	50	100	6.25->200
<i>Salmonella</i> spp	47	64	128	32-256
<i>Salmonella</i> spp	32	32	50	6.25-64
<i>Shigella dysenteriae</i>	4	4	8	4-8
<i>Shigella flexneri</i>	78	4	8	2-16
<i>Shigella sonnei</i>	4	4	16	8-16
<i>Shigella</i> spp	88	50	100	25->200
<i>Shigella</i> spp	65	64	128	2-256
<i>Shigella</i> spp	27	32	64	0.098-256
<i>Vibrio cholerae</i>	25	100	100	6.25-100
<i>Vibrio</i> spp	18	32	128	2-128
<i>Vibrio</i> spp	5	16	32	8-32

These data demonstrate that rifaximin's MIC₉₀ against *Escherichia coli* is around 32-64 µg/mL. *Campylobacter* species, especially *C jejuni*, has a higher MIC₉₀ of about 256 µg/mL. *Salmonella* species have MIC₉₀ values of 64-128 µg/mL. *Shigella* species have somewhat lower MIC₉₀ values of 8-64 µg/mL.

TABLE B compares the *in vitro* activity of rifaximin with that of other agents

TABLE B
Distribution of MIC₉₀s of Eleven Agents Against Enteropathogens

Antimicrobial Agent	MIC ₉₀ (µg/mL) (Number of isolates tested)					
	ETEC (N=97)	EAEC (N=75)	<i>Salmonella</i> (N=46)	<i>Shigella</i> (N=35)	<i>Campylobacter</i> (N=9)	Others ^a (N=21)
Rifaximin	32	32	64	64	32	4
Amdinocillin	8	16	2	16	4	1
Ampicillin	>1024	>1024	4	512	64	512
Azithromycin	≤0.015	≤0.015	1	0.5	0.25	Not tested
Ceftraxone	≤0.015	0.03	0.125	0.03	2	Not tested
Ciprofloxacin	0.25	0.25	0.03	0.03	0.06	≤0.015
Doxycycline	64	64	128	128	64	4
Levofloxacin	1	1	0.25	0.25	0.25	0.06
Nalidixic acid	256	128	16	8	4	32
Trimethoprim	1024	>1024	1024	1024	64	128

^a Others include non-cholera-causing vibrios, *P. shigelloides* and *Aeromonas* species
 ETEC = Enterotoxigenic *E. coli*; EAEC = Enteroaggregative *E. coli*

These data show that rifaximin has higher MIC₉₀ values against *Campylobacter* species compared to other enteropathogens. Rifaximin's activity is better than or comparable to most other agents tested except for the fluoroquinolones. The fluoroquinolones, especially ciprofloxacin, have a much lower MIC values for most enteropathogens when compared to rifaximin.

Development of resistance to rifaximin is probably similar to that of rifampin. Rifampin resistance is primarily due to chromosomal one-step alteration in the drug target, DNA-dependent RNA polymerase.

The rate of selection of spontaneous rifaximin-resistant mutants was correlated to the drug concentration employed and to the bacterial species tested. Spontaneous mutations can easily be detected when a low concentration of drug is present. At the highest dose used (8 x MIC), the frequency of emergence of spontaneous mutants ranged from <1 x 10⁻⁹ to 1.6 x 10⁻⁸ for Gram-positive aerobic and anaerobic cocci. For Gram-negative bacteria the range was <1 x 10⁻⁹ to 1.7 x 10⁻⁷. In comparison to Gram-positive cocci, drug-resistant mutants of Gram-negative bacteria usually emerged with a slightly lower incidence. Rates were lower under anaerobic conditions. These values are higher than those seen with most fluoroquinolones. When grown in sub-inhibitory concentrations of rifaximin all organisms showed a rapid increase in MIC values. Rifaximin, which is similar to rifampin in structure and mode of action, probably has rifampin's tendency to select resistant strains with treatment. This drug is going to be used for diarrhea, however. The proposed oral dosing leads to extremely high intraluminal concentrations of the drug, which should prevent the development of resistance.

Incubation with sub-inhibitory concentrations of rifaximin does not seem to increase rifampin MIC values for *Mycobacterium tuberculosis*. Oral treatment of guinea pigs infected with *Mycobacterium tuberculosis* with rifaximin for 90 days did not increase

rifaximin or rifampin MIC for *Mycobacterium tuberculosis*. The 3 days of treatment used in the submitted clinical trials did not lead to an increase in rifaximin MICs for any of the detected pathogens.

Rifaximin given orally was ineffective in a *Mycobacterium tuberculosis* guinea pig model of infection. Rifampin was effective in this model. Rifaximin was also ineffective in mice infected with *Staphylococcus aureus* when given orally, but was effective when subcutaneously dosed. Oral gentamicin was also ineffective in this mouse model.

Data from two Phase III and one Phase II studies have been submitted in this application. In the Phase II study, RFID9601, rifaximin was dosed at 200 mg, 400 mg, or 600 mg twice a day for 5 days. Trimethoprim/sulfamethoxazole was used as a comparator. TABLE C shows the pathogen eradication rate for this study.

TABLE C
Microbiological Cure Rate by Pathogen (Study RFID9601)

Pathogen	200 mg tid Rifaximin		400 mg tid Rifaximin		600 mg tid Rifaximin		TMP/SMX	
	No	No Eradicated (%)	No	No Eradicated (%)	No	No Eradicated (%)	No	No Eradicated (%)
<i>Escherichia coli</i>	7	7/7 (100 0%)	3	1/3 (33 3%)	2	2/2 (100 0%)	6	6/6 (100 0%)
<i>Shigella sonnei</i>	1	1/1 (100 0%)	0	---	1	0/1 (00 0%)	0	---
<i>Salmonella</i> Group C1	1	1/1 (100 0%)	0	---	1	0/1 (00 0%)	1	1/1 (100 0%)
<i>Salmonella</i> Group C2	0	---	1	1/1 (100 0%)	0	---	0	---
<i>Campylobacter jejuni</i>	2	2/2 (100 0%)	0	---	0	---	0	---
<i>Cryptosporidium parvum</i>	0	---	1	1/1 (100 0%)	0	---	0	---
TOTAL	11	11/11 (100%)	5	3/5 (60%)	4	2/4 (50%)	7	7/7 (100%)

The data in the above Table indicate that there were too few isolates of any species to draw any reliable conclusions about the eradication rate.

In study RFID9701, 400 mg rifaximin taken twice a day was compared to ciprofloxacin taken twice a day for treatment of infectious diarrhea in travelers. Treatment was for 3 days. TABLE D shows the pathogen eradication rate for this study.

TABLE D
Microbiological Cure Rate by Pathogen (Study RFID9701)

Pathogen	Rifaximin 400 mg bid		Ciprofloxacin 500 mg bid	
	No	No Eradicated (%)	No	No Eradicated (%)
<i>Escherichia coli</i>	35	24/35 (68 6%)	36	30/36 (83 3%)
<i>Shigella sonnei</i>	4	3/4 (75 0%)	1	1/1 (100 0%)
<i>Shigella flexneri</i>	1	1/1 (100 0%)	5	4/5 (80 0%)
<i>Salmonella</i> species	0	---	1	1/1 (100 0%)
<i>Salmonella</i> Group C1	2	1/2 (50 0%)	3	2/3 (66 6%)
<i>Salmonella</i> Group C2	1	1/1 (100 0%)	2	2/2 (100 0%)
<i>Campylobacter jejuni</i>	2	2/2 (100 0%)	0	---
<i>Entamoeba histolytica</i>	1	0/1 (0 0%)	0	---
<i>Giardia Lamblia</i>	0	---	1	0/1 (0 0%)
<i>Cryptosporidium parvum</i>	1	1/1 (100 0%)	2	1/2 (50 0%)
TOTAL	47	33/47 (70 2%)	51	41/51 (80 3%)

From the above TABLE it can be seen that there were very few of any organisms other than *Escherichia coli*. The dosage regimen in this study was not the one proposed for the product in this application (200 mg tid for 3 days). It appears that the eradication rate for rifaximin is not as good as for ciprofloxacin.

Study RFID9801 compared rifaximin doses of 200 mg and 400 mg taken three times a day with placebo. Dosing was for 3 days in this study. This was the only study that included the proposed dose regime of 200 mg rifaximin taken three times a day for three days. TABLE E shows the pathogen eradication rate for this study.

TABLE E
Microbiological Cure Rate by Pathogen (Study RFID9801)

Pathogen	Placebo		Rifaximin 200 mg tid		Rifaximin 400 mg tid	
	No	No Eradicated (%)	No	No Eradicated (%)	No	No Eradicated (%)
<i>Escherichia coli</i>	54	40/54 (74.1%)	54	38/54 (70.4%)	41	27/41 (65.9%)
<i>Shigella</i> species	0	---	0	---	1	1/1 (100.0%)
<i>Shigella sonnei</i>	2	2/2 (100.0%)	2	2/2 (100.0%)	1	1/1 (100.0%)
<i>Shigella flexneri</i>	0	---	2	1/2 (50.0%)	1	0/1 (0.0%)
<i>Salmonella</i> Group C1	1	1/1 (100.0%)	2	1/2 (50.0%)	4	3/4 (75.0%)
<i>Salmonella</i> Group C2	1	1/1 (100.0%)	0	---	3	1/3 (33.3%)
<i>Campylobacter jejuni</i>	1	0/1 (0.0%)	2	1/2 (50.0%)	0	---
<i>Campylobacter coli</i>	1	1/1 (100.0%)	0	---	0	---
<i>Aeromonas sobria</i>	1	1/1 (100.0%)	0	---	0	---
<i>Aeromonas hydrophila</i>	0	---	0	---	1	1/1 (100.0%)
<i>Entamoeba histolytica</i>	1	1/1 (100.0%)	1	1/1 (100.0%)	3	2/3 (66.6%)
<i>Giardia lamblia</i>	4	3/4 (75.0%)	6	4/6 (66.6%)	3	1/3 (33.3%)
<i>Cryptosporidium parvum</i>	11	7/11 (63.6%)	18	12/18 (66.6%)	14	4/14 (28.6%)
<i>Plesiomonas shigelloides</i>	1	1/1 (100.0%)	0	---	1	1/1 (100.0%)
<i>Vibrio fluvialis</i>	0	---	1	1/1 (100.0%)	1	0/1 (0.0%)
<i>Vibrio parahaemolyticus</i>	1	1/1 (100.0%)	0	---	1	1/1 (100.0%)
TOTAL	79	59/79 (74.7%)	88	61/88 (69.3%)	75	43/75 (57.3%)

From the above TABLE it can be seen that there were very few of any organisms other than *Escherichia coli*. Only one arm in this study used the proposed dosage regimen (200 mg tid for 3 days). Both rifaximin dosage regimens had about the same eradication rate with the lower dose giving slightly better eradication. The eradication rate for placebo was as good as or better than that for the drug. Only about half the patients had an organism detected pre-treatment. There were only four *Shigella* species and two *Salmonella* species treated with the proposed dose.

The data from all these studies combined indicate that rifaximin is no better than placebo at eradicating pathogens. Only about half the patients in these studies had a pre-treatment pathogen detected. Rifaximin appears to be slightly less effective in eradicating pathogens than is ciprofloxacin but the difference may not be significant.

Almost all the *Cryptosporidium parvum* patients were from Kenya. Many of them had another pathogen along with the *Cryptosporidium parvum*. These other organisms may be the cause of the diarrhea.

PRECLINICAL EFFICACY (IN VITRO)

MECHANISM OF ACTION

Rifaximin is a structural analogue of rifampin and, therefore, would be expected to have a mechanism of action that is similar to that of rifampin. Rifampin inhibits DNA-dependent RNA polymerase activity in susceptible cells. Rifampin interacts with bacterial RNA polymerase but does not inhibit the mammalian enzyme. The inhibition potential of rifaximin compared to rifampin on DNA, RNA, and protein synthesis in *Escherichia coli* was studied (1). In this study the effect of rifaximin, rifampin, and chloramphenicol on the incorporation of ³⁵S-methionine, ³H-uridine, and ³H-thymidine by *E. coli* (ATCC 8739) was examined. Aliquots of the radiolabeled material were added to *E. coli* cultures. The antibiotics were added to the cultures and samples were taken at 7 to 29 minute intervals after addition. Samples were measured for incorporation of the radiolabeled compounds. Rifaximin and rifampin did not affect ³⁵S-methionine incorporation. At rifaximin and rifampin concentrations of 30 µg/mL ³H-uridine incorporation was depressed indicating that PNA synthesis was affected. Rifaximin and rifampin did not affect ³H-thymidine incorporation. Similar to rifampin, rifaximin selectively inhibited RNA synthesis in *E. coli*.

ANTIMICROBIAL SPECTRUM OF ACTIVITY

ANTIMICROBIAL ACTIVITY—SPONSOR STUDIES

Six studies were conducted to evaluate the antimicrobial activity of rifaximin against various bacterial isolates from clinical trials. TABLE 1 summarizes rifaximin MIC₅₀, MIC₉₀, and MIC ranges for pathogens associated with infectious diarrhea from these studies (2,3,4,5,6,7). One study (4) only tested a upper rifaximin concentration of 200 µg/mL, which was too low to establish MICs for many of the isolates.

For the 1,607 clinical isolates tested, the highest MIC was 1024 µg/mL. *Clostridium* species were found to be the most sensitive organisms tested with an MIC₉₀ range of 0.005-2.0 µg/mL. The MIC₅₀ and MIC₉₀ ranges for *Escherichia coli* were 8-64 µg/mL and 16-128 µg/mL, respectively. *Campylobacter* species have a higher MIC₉₀ value of >100 µg/mL to about 256 µg/mL in most studies. The MIC₉₀ value for *Salmonella* species varies from around 8.0 µg/mL to about 128 µg/mL. *Shigella* species seem to have a MIC₉₀ range similar to that for *Salmonella* species.

TABLE 1
In Vitro Activity of Rifaximin—Sponsor Studies^a

Organism	No of Strains	µg/mL		MIC Range ^b	Study No
		MIC ₅₀	MIC ₉₀		
<i>Aeromonas</i> spp	27	12.5	100	12.5->200	4
<i>Aeromonas</i> spp	17	16	64	16-512	5
<i>Aeromonas</i> spp	3	16	16	8-16	6
<i>Campylobacter</i> spp	35	>200	>200	0.195->200	4
<i>Campylobacter jejuni</i>	24	128	256	0.25-512	5
<i>Campylobacter</i> spp	11	32	64	8-64	6
<i>Clostridium difficile</i>	93	0.004	2	0.004 – 128	3
<i>Clostridium</i> spp	38	0.001	0.005	<0.0001-2	7
Enter aggregative <i>E. coli</i>	65	8	16	4-32	2
Enter aggregative <i>E. coli</i>	62	64	128	8-128	5
Enterohemorrhagic <i>E. coli</i>	17	50	>200	25->200	4
Enteroinvasive <i>E. coli</i>	20	50	100	6.25->200	4
Enteropathogenic <i>E. coli</i>	21	8	16	4-16	2
Enterotoxigenic <i>E. coli</i>	44	8	16	1-32	2
Enterotoxigenic <i>E. coli</i>	153	50	100	6.25->200	4
Enterotoxigenic <i>E. coli</i>	77	64	64	4-1024	5
Enterotoxigenic <i>E. coli</i>	347	32	64	0.098-512	6
Hep-2 adherent <i>E. coli</i>	50	50	100	12.5->200	4
<i>Plesiomonas shigelloides</i>	25	50	200	12.5->200	4
<i>Plesiomonas shigelloides</i>	30	32	128	16-256	5
<i>Plesiomonas shigelloides</i>	2	4	8	4-8	6
<i>Salmonella</i> spp	53	50	100	6.25->200	4
<i>Salmonella</i> spp	47	64	128	32-256	5
<i>Salmonella</i> spp	32	32	50	6.25-64	6
<i>Shigella dysenteriae</i>	4	4	8	4-8	2
<i>Shigella flexneri</i>	78	4	8	2-16	2
<i>Shigella sonnei</i>	4	4	16	8-16	2
<i>Shigella</i> spp	88	50	100	25->200	4
<i>Shigella</i> spp	65	64	128	2-256	5
<i>Shigella</i> spp	27	32	64	0.098-256	6
<i>Vibrio cholerae</i>	25	100	100	6.25-100	4
<i>Vibrio</i> spp	18	32	128	2-128	5
<i>Vibrio</i> spp	5	16	32	8-32	6
Total or Range ^c	1607	0.001-128	0.005-256	<0.001-1024	

^a Including patients from Mexico, Zambia, Egypt, India, Jamaica, Kenya, Zambia and Guatemala between 1992-2000

^b MIC values reported as greater than were above the highest tested dilution concentration

^c Excludes MIC values that were not determined, e.g. >200 µg/mL

Antimicrobial susceptibility testing was also performed against anaerobic bacteria (7) Results are shown in TABLE 2

TABLE 2
***In Vitro* Activity of Rifaximin against Strict Anaerobic Bacteria**

Organism	Number of Isolates	µg/mL		
		MIC ₅₀	MIC ₉₀	Range
<i>Bacteroides</i>	32	0.2	1	0.1 - > 256
<i>Bacteroides distaeonis</i>	5			0.1 - 32
<i>Bacteroides fragilis</i>	8			0.2 - >256
<i>Bacteroides ovatus</i>	4			0.2 - 1
<i>Bacteroides thetaiotamicron</i>	9			0.2
<i>Bacteroides uniformis</i>	2			0.2
<i>Bacteroides vulgatus</i>	4			0.2 - 4
<i>Bifidobacterium</i>	18	2	4	0.2 - 8
<i>Bifidobacterium adolescentis</i>	5			0.5 - 4
<i>Bifidobacterium longum</i>	2			0.2 - 2
<i>Bifidobacterium angulatum</i>	1			0.2
<i>Bifidobacterium erksonii</i>	4			2 - 8
<i>Bifidobacterium dentium</i>	3			2 - 4
<i>Bifidobacterium magnum</i>	1			4
<i>Bifidobacterium bifidum</i>	2			2 - 4
<i>Clostridium</i>	38	0.001	0.005	<0.0001-2
<i>Clostridium barati</i>	5			0.0002-0.0005
<i>Clostridium bifermentans</i>	1			<0.0001
<i>Clostridium difficile</i>	11			<0.0001-0.001
<i>Clostridium paraputrificum</i>	1			0.005
<i>Clostridium perfringens</i>	14			0.002-0.01
<i>Clostridium sordellii</i>	3			<0.0001-0.002
<i>Clostridium sporogenes</i>	3			0.5-2
<i>Fusobacterium</i>	4	2	16	2-16
<i>Fusobacterium necrophorum</i>	1			16
<i>Fusobacterium nucleatum</i>	3			2-8
<i>Peptostreptococcus</i>	7	2	256	2-256
<i>Peptostreptococcus anaerobius</i>	1			16
<i>Peptostreptococcus magnus</i>	4			2-256
<i>Peptostreptococcus micros</i>	1			2
<i>Peptostreptococcus productus</i>	1			16
<i>Prevotella</i>	4	2	4	1-4
<i>Prevotella bivia</i>	3			1-4
<i>Prevotella buccae</i>	1			2

ANTIMICROBIAL ACTIVITY—LITERATURE STUDIES

Tables 3 summarize the antimicrobial activity of rifaximin against various clinical isolates. This table was compiled from studies found in the literature.

TABLE 3
In Vitro Activity of Rifaximin (Literature Studies)

Organism	No of Isolates	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	MIC Range ^a (µg/mL)	Reference
<i>Acinetobacter</i> species	10	2	4	0.06-4	8
<i>Bacillus cereus</i>	7	0.06	---	0.03-0.12	8
<i>Campylobacter jejuni</i>	54	12.5	>100	0.78->100	9
<i>Citrobacter</i> species	4	---	>25	12->25	10
<i>Clostridium difficile</i>	59	0.39	>100	0.10->100	9
<i>Staphylococcus</i> coagulase-negative	20	<0.015	<0.015	<0.015	8
<i>Enterobacter</i> species	6	12	12	12	10
<i>Enterobacter agglomerans</i>	10	4	8	1-8	8
<i>Enterococcus faecalis</i>	21	2	8	0.5->8	8
<i>Enterococcus</i> species	10	0.25	2	≤0.015->4	8
<i>Escherichia coli</i>	15	6.25	12.5	0.7->25	10
<i>Haemophilus influenzae</i>	58	0.25	2	≤0.03-2	8
<i>Moraxella catarrhalis</i>	20	<0.03	<0.03	<0.03-0.06	8
<i>Neisseria</i> species	16	0.5	2	≤0.03-2	8
<i>Proteus</i> species	26	6.25	25	3.12->50	10
<i>Proteus mirabilis</i>	20	4	4	1-4	8
<i>Proteus vulgaris</i>	10	4	4	2->8	8
<i>Providencia stuartii</i>	10	4	4	2-4	8
<i>Salmonella</i> species	56	3.12	6.25	0.78->25	10
<i>Salmonella enteritidis</i>	10	2	8	2->8	8
<i>Serratia</i> species	10	25	>50	12->50	10
<i>Shigella</i> species	10	4	8	2->8	8
<i>Staphylococcus aureus</i>	30	0.03	1.0	≤0.005-3.0	10
<i>Staphylococcus aureus</i> (Methicillin-S)	40	<0.015	<0.015	<0.015-0.03	8
<i>Staphylococcus epidermidis</i>	20	<0.015	<0.015	<0.015	8
<i>Staphylococcus haemolyticus</i>	10	<0.015	<0.015	<0.015->8	8
<i>Streptococcus</i> Group A	19	0.12	0.25	<0.03-0.25	8
<i>Streptococcus</i> Group B	20	0.12	0.25	0.06-0.25	8
<i>Streptococcus</i> Group C F G	14	<0.03	0.06	<0.03-0.5	8
<i>Streptococcus pneumoniae</i>	30	<0.03	0.06	<0.03->4	8
<i>Stenotrophomonas maltophilia</i>	10	8	<8	≤0.015-<8	8
<i>Yersinia enterocolitica</i>	74	6.25	12.5	0.2-12.5	9
<i>Yersinia kristensenii</i>	12	1.56	12.5	0.39-12.5	9
<i>Yersinia frederiksenii</i>	2	---	---	3.12	9
<i>Yersinia pseudotuberculosis</i>	1	---	---	6.25	9
<i>Yersinia intermedia</i>	2	---	---	1.56-3.12	9

^a MIC values reported as greater than were above the highest tested dilution

IN VITRO COMPARISON WITH OTHER AGENTS

Table 4 is from a study (11), which looked at the *in vitro* susceptibility of enteropathogens causing traveler's diarrhea in four geographic regions

TABLE 4
Distribution of MIC₉₀s of Eleven Agents Against Enteropathogens

Antimicrobial Agent	MIC ₉₀ (µg/mL) (Number of isolates tested)					
	ETEC (N=97)	EAEC (N=75)	Salmonella (N=46)	Shigella (N=35)	Campylobacter (N=9)	Others ^a (N=21)
Rifaximin	32	32	64	64	32	4
Amdinocillin	8	16	2	16	4	1
Ampicillin	>1024	>1024	4	512	64	512
Azithromycin	≤0.015	≤0.015	1	0.5	0.25	Not tested
Ceftriaxone	≤0.015	0.03	0.125	0.03	2	Not tested
Ciprofloxacin	0.25	0.25	0.03	0.03	0.06	≤0.015
Doxycycline	64	64	128	128	64	4
Levofloxacin	1	1	0.25	0.25	0.25	0.06
Nalidixic acid	256	128	16	8	4	32
Trimethoprim	1024	>1024	1024	1024	64	128

^a Others include non-cholera-causing vibrios, *P. shigelloides*, and *Aeromonas* species
ETEC = Enterotoxigenic *E. coli*, EAEC = Enteroaggregative *E. coli*

Table 5 summarizes the antimicrobial activity of rifaximin compared to eight standard agents. This table is compiled from data from two sponsor studies (2, 3). One study compared activity against enteropathogens (2) and the other study only tested activity against *Clostridium difficile* (3).

These data show that rifaximin has the highest MIC₉₀ value against *Campylobacter* species compared to other enteropathogens. Rifaximin's activity is better than or comparable to most other agents tested except for the fluoroquinolones. The fluoroquinolones, especially ciprofloxacin, have much lower MIC values for most enteropathogens when compared to rifaximin.

In another study (12) done by the sponsor, the activity of rifaximin and tetracycline were evaluated against 111 strains of *Vibrio cholerae*. Rifaximin and tetracycline were found to have MIC ranges of 0.5-2 µg/mL and 0.5-64 µg/mL, respectively.

TABLE 5
Comparative Activity (MIC₉₀) of Rifaximin and Eight Other Agents

Antimicrobial Agent	MIC ₉₀ (µg/mL) (Number of Isolates Tested)									Reference
	EAEC (N=28)	ETEC (N=38)	<i>Salmonella</i> species (N=14)	<i>Campylobacter</i> <i>Jejuni</i> (N=12)	<i>Aeromonas hydrophila</i> (N=11)	<i>Yersinia enterocolitica</i> (N=10)	<i>Shigella flexneri</i> (N=28)	<i>Shigella sonnei</i> (N=36)	<i>Clostridium difficile</i> (N=93)	
Rifaximin	16	16	4	512	8	128	16	16	2	2,3
Ampicillin	>128	>128	>128	>128	>128	>128	>128	>128	---	2
Chloramphenicol	>128	>128	64	>128	8	128	128	128	---	2
Tetracycline	>128	>128	128	16	>128	64	>128	>128	---	2
Nalidixic acid	16	4	4	>256	<4	8	<4	8	---	2
Ciprofloxacin	<0.06	<0.06	<0.06	32	<0.06	<0.06	<0.06	<0.06	---	2
Trimethoprim	>128	>128	16	128	64	16	>128	>128	---	2
Metronidazole									0.25	3
Vancomycin									2	3

ETEC = Enterotoxigenic *E. coli*, EAEC = Enteroaggregative *E. coli*

ASSESSMENT OF BACTERIAL RESISTANCE

Development of resistance to rifaximin is probably similar to that of rifampin. Rifampin resistance is primarily due to a chromosomal one-step alteration in the drug target, DNA-dependent RNA polymerase.

SPONTANEOUS FREQUENCY OF MUTATION

The spontaneous mutation frequency of rifaximin for a number of organisms was determined by the sponsor (13) and compared to the rate for other agents. In these experiments a high bacterial inocula (10^8 - 10^{10} cfu/mL) were added to agar containing each antibiotic at various concentrations above the MIC (2, 4, and 8 times). Two isolates of each species were analyzed. Other representative strains were also included.

The frequency of emergence of spontaneous resistant mutants to rifaximin and vancomycin in anaerobic gram-positive strains was determined by using Wilkins-Chalgren agar plates. After incubation at 37 C for 48 hours colonies were counted and the mutation frequency was calculated. Results are shown in TABLE 6.