

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-554

MICROBIOLOGY REVIEW(S)

MICROBIOLOGY REVIEW
DIVISION OF SPECIAL PATHOGEN AND TRANSPLANT PRODUCTS

NDA #: 21361 (S-011, SDN #100)
and 22554 (S-06, SDN #1)

REVIEWER : Anne Purfield
CORRESPONDENCE DATE : 7-21-09; 6-24-09
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APPLICANT: Salix Pharmaceuticals, Inc.
1700 Perimeter Dr
Morrisville, NC 27560

DRUG CATEGORY: Antibacterial

INDICATION: Treatment of travelers' diarrhea
Treatment of hepatic encephalopathy (NDA #22554 under review in
Division of Gastroenterology Products)

DOSAGE FORM: Tablet for oral administration

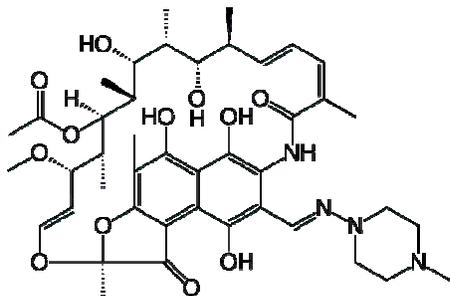
PRODUCT NAMES:

a. **PROPRIETARY:** Xifaxan®

b. **NONPROPRIETARY:** Rifaximin

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

STRUCTURAL FORMULA:



Molecular weight: 785.879
Molecular formula: C₄₃H₅₁N₃O₁₁

SUPPORTING DOCUMENTS: NDA #22554, NDA #21361

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1. Executive Summary

Rifaximin (Xifaxan®) is approved for the treatment of patients (≥ 12 years of age) with travelers' diarrhea caused by noninvasive strains of *Escherichia coli*. In this submission, the applicant has included three nonclinical and two clinical studies to support the above statements. The applicant is seeking approval for changes to the label that include PLR formatting and add additional information to the Microbiology Section 12.4 that include

- rifaximin has a unique mechanism of action which results in a lower rate of pathogen eradication and a lack of alteration of the gut flora in patients treated with rifaximin compared to fluoroquinolones and aminoglycosides.
- rifaximin may alter virulence factors of enteric bacterial pathogens without killing them, which has been seen with subtherapeutic levels of drugs and colonization fimbriae of enterotoxigenic *E. coli*.
- morphological changes are observed when susceptible or resistant bacteria are exposed to low concentrations of rifaximin.
- rifaximin reduces the viability and virulence of resistant bacteria.

Of the 3 nonclinical studies, one study by Debbia *et al.*, 2008³, describe the effects of bacterial exposure to sub-inhibitory rifaximin concentrations in vitro, including induced resistance and virulence mechanisms such as plasmid stability and frequency of plasmid transfer. However, the description of methods and results are inadequate to support the applicant's statements. For example, colonization of fimbriae was not described in this study and appropriate controls for virulence factors and morphological changes were not included. Fimbriae are external structures of Gram negative bacteria which enable the bacteria to adhere to host cells and promotes persistence of infection. The other two studies either did not include testing of rifaximin (Vosbeck *et al.*, 2008) or the method used was not specified (Jiang *et al.*, 2005).

Two clinical trials by Dupont *et al.*, 1998⁴, and 2001⁵, describe the efficacy of rifaximin treatment for traveler's diarrhea. DuPont *et al.*, 1998⁴, show that patients with traveler's diarrhea have a lower rate of treatment failure when treated with rifaximin compared to TMP/SMX; however the duration of diarrhea is not statistically different. However, an aminoglycoside was not used as a comparator in either trial, as the applicant proposes to state in the labeling. Similarly, the other study by Dupont *et al.*, 2001⁵ shows that clinical outcome, microbiologic cure, and the number of treatment failure were not statistically different for patients treated with rifaximin or ciprofloxacin. The two clinical studies (DuPont *et al.*, 1998⁴ and DuPont *et al.*, 2001⁵) did not compare the rate of pathogen eradication between rifaximin and an aminoglycoside, nor did either study correlate such changes with significant alteration of gut flora or describe a unique mechanism of action.

In summary, the referenced publications included for review do not support the applicant's proposed changes to the microbiology section of the rifaximin labeling.

2. Introduction and Background

Rifaximin (Xifaxan®) is approved by FDA for treatment of patients (≥ 12 years of age) with travelers' diarrhea caused by noninvasive strains of *E. coli*. Rifaximin is approved for use in 27 countries, including Mexico and countries in Europe, Northern Africa and Asia. In this submission, the applicant is seeking approval of changes to the label that include PLR formatting and changes to the Microbiology Section 12.4.

3. Preclinical/Nonclinical Microbiology

Preclinical studies were previously reviewed (NDA #21-361 Microbiology Reviews by Mr. Peter Dionne and Dr. Avery Goodwin dated 3/14/02 and 4/13/04, respectively). In this submission, the applicant includes three non-clinical studies (Jiang *et al.*, 2005⁶, Vosbeck *et al.*, 1979⁹, and Debbia *et al.*, 2008³), to support statements made in the label. The studies are summarized below.

3.1. Jiang *et al.*, 2005⁶

The study included a summary of in vitro susceptibility data from multiple studies without including methods (Table 1).

Table 1: In vitro susceptibility (MIC) to rifaximin of enteric pathogens isolated from patients with bacterial diarrhea from multiple areas of the world

Species	Number of isolates	MIC ₅₀ µg/ml	MIC ₉₀ µg/ml	MIC range µg/ml
<i>Aeromonas</i> spp.	27	16	128	16 to >256
<i>Campylobacter jejuni</i>	54	12.5	>100	0.78 to >100
<i>Campylobacter</i> spp.	35	32	128	0.25 to >256
Enteroaggregative <i>E. coli</i>	50	64	128	16 to >256
Enterohemorrhagic <i>E. coli</i>	17	64	>256	32 to >256
Enteroinvasive <i>E. coli</i>	20	64	128	8 to >256
ETEC	153	64	128	8 to 256
ETEC with LT	50	64	256	8 to >256
ETEC with ST	76	64	128	8 to >256
ETEC with ST and LT	27	64	128	32 to >256
<i>Plesiomonas shigelloides</i>	25	64	256	16 to >256
<i>Salmonella</i> spp.	53	64	128	8 to >256
<i>Shigella</i> spp.	88	64	128	32 to >256
<i>Vibrio</i> spp. ¹	25	128	128	8 to 128
<i>Yersinia</i> spp.	91	12.5	25	0.2 to 25

LT = Heat-labile toxin; ST = heat-stable toxin.
¹ *Vibrio* spp. include non-cholera-causing vibrios.

Note: Clinical trials with contributing data: Mathewson *et al.*, unpublished data, Sierra *et al.*, 2001⁸ (previously reviewed by Dr. Avery Goodwin in Microbiology Review dated 4/13/04 for NDA 21-361), and Mignini *et al.*, 1989⁷.

Note: Adapted from Jiang *et al.*, 2005⁶, Table 1

Note: Interpretive criteria for susceptibility have not been established

3.2. Vosbeck *et al.*, 1979⁹

The study did not include testing of effect of sub-inhibitory levels of rifaximin on the adhesive properties of bacteria and therefore was not relevant for this review.

3.3. Debbia *et al.*, 2008³

The effects of exposure to sub-inhibitory concentrations of rifaximin on induced resistance and susceptibility virulence mechanisms, such as plasmid stability and frequency of plasmid transfer, were evaluated for strains with low MIC (8 µg/mL; stated to be rifaximin susceptible) and high MIC (≥ 512 µg/mL; stated to be resistant) in vitro by Debbia *et al.*, 2008³. Rifaximin resistant *E. coli* strains were selected for in vitro and the frequency of spontaneous rifaximin-resistant mutant strains was determined in the presence of rifaximin concentrations below the minimum inhibitory concentration (MIC) (sub-inhibitory). The authors state Clinical Laboratory of Standards Institute (CLSI) standardized methods for MIC determination for enterobacteria using broth microdilution (M2-A8; M100-S15). *E. coli* (ATCC 25922 standard reference strain) cultures were cultured for 18-24 hours in the presence of 0.06x, 0.12x, 0.25x and 0.5x MIC of rifaximin (MIC = 4 mg/L) or ciprofloxacin (MIC = 0.004 mg/L). Approximately 10^7 CFU/mL were seeded onto agar plates containing either 40 µg/mL (10xMIC) rifaximin or 0.04 mg/mL (10xMIC) ciprofloxacin and incubated for 48 hours at 37°C to select for resistant mutants that were then evaluated for in vitro susceptibility.

Table 2 shows that *E. coli* cultured in the presence of rifaximin induces a higher rate of spontaneous mutations than ciprofloxacin or bacteria cultured in the absence of drug. The resulting mutants were resistant to >512 µg/mL rifaximin compared to 8 µg/mL for the sensitive strains. It is unclear if a mutant “strain” was sequenced to show clonality or if “strain” refers to a single colony on the agar plate. Also, the range and or confidence intervals were not included to show experiment to experiment variability for five experiments.

The morphology of the resistant and sensitive strains was assessed microscopically. A change in morphology is observed in rifaximin susceptible (MIC = 4 µg/mL) and resistant (MIC >512 µg/mL) strains in the presence of drug. When either strain was cultured in the presence of very low levels of rifaximin (0.008 x MIC), the morphology appears the same as controls (rods). The “control” was not described. As the concentration of rifaximin is increased, changes in morphology are noted; and the morphologies are described the same for susceptible and resistant strains.

Table 2: The emergence of spontaneous resistant-mutants to rifaximin and ciprofloxacin in drug free medium (control) or sub-inhibitory concentrations of rifaximin and ciprofloxacin.

Antibiotic	MIC (mg/L)	Used dose (xMIC)	Number of spontaneous resistant strains /10 ⁸ CFU/mL
Rifaximin ¹	4	0.5	569
		0.25	1447
		0.125	0
		0.06	0
		Control	27
Ciprofloxacin ²	0.004	0.5	21
		0.25	0
		0.125	0
		0.06	0
		Control	0

¹Selection on plates containing rifaximin (40 mg/L)
²Selection on plates containing ciprofloxacin (0.04 mg/L)
 Average from 5 separate experiments
 Note: Adapted from Debbia *et al.*, 2008³, Table 2

The segregational plasmid stability was evaluated in bacteria carrying plasmids when cultured in sub-inhibitory rifaximin or ciprofloxacin concentrations. Plasmid stability was determined in enterobacteria carrying plasmids encoding antibiotic resistance, including *E. coli*, *Staphylococcus aureus*, *Morganella morganii*, *Citrobacter freundii*, *Proteus mirabilis*, and *Klebsiella pneumoniae*. For this, bacteria (starting inoculum $\leq 10^3$ CFU/mL) were cultured for 20-24 generations at 37°C in the presence of drug. Bacteria were diluted 100-fold with “warm broth” and incubated an additional 90 minutes. It is unclear what “warm broth” refers to (drug-free media or media with drug). Cultures were diluted further and plated on Mueller-Hinton agar plates. The dilution factor or amount of bacteria plated is unclear. Plates were incubated for 18 to 20 hours and colonies were replicated onto medium with antibiotic or without. Bacterial growth on agar containing antibiotic indicated the presence of plasmid and successful transfer of plasmids to daughter cells (stability). The number of colonies was counted and the proportion of cells that lost the plasmid (cells cured) was determined by the number of colonies growing on medium with antibiotic relative to the number grown on antibiotic-free medium. It is not clear if transmission efficiency (plasmid stability in bacteria grown in the absence of antibiotic) was determined. Select bacteria were plated on McConkey’s agar plates and plasmid stability was identified by the use of lactose.

Table 3 shows the range of “percent cells cured” (plasmid-free) following bacterial culture in sub-inhibitory drug concentrations. The percent of cured cells was higher in bacteria containing a high molecular weight, low copy plasmid (*Flac*) compared to low molecular weight, high copy plasmids (p507). In many cases, the cells cultured with rifaximin had a wider range of percent cells cured compared to ciprofloxacin; however the drugs elicited a similar percentage of cells

cured (Table 3). The mean, median or statistical significance of this range from five separate experiments was not included, thus it is difficult to interpret the relevance of the results.

Table 3: Plasmid elimination from different bacterial hosts exposed to sub-inhibitory concentrations of rifaximin and ciprofloxacin

Bacterial Host (Plasmid)	Antibiotic	MIC (mg/L)	Sub-MIC Used (mg/L)	Cells Cured* (%)
ATCC25922 (Flac)	rifaximin	4	2	4.5-70
	ciprofloxacin	0.004	0.002	5.7-47.6
ATCC25922 (P507)	rifaximin	4	2	0-18
	ciprofloxacin	0.004	0.002	1.3-10.2
<i>S. aureus</i> I Pen-R Oxa-S	rifaximin	0.004	0.002	8.4-18.2
	ciprofloxacin	0.25	0.125	12.5-33.7
<i>S. aureus</i> II Pen-R Oxa-S	rifaximin	0.008	8	<0.1-18
	ciprofloxacin	0.01	0.005	23-36.2
<i>M. morgani</i> ESBL33	rifaximin	8	4	9.5-58.6
	ciprofloxacin	0.015	0.008	17.4-37.9
<i>C. freundii</i> ESBL33	rifaximin	32	16	10.6-47.1
	ciprofloxacin	0.03	0.015	22.3-40.2
<i>P. mirabilis</i> ESBL33	rifaximin	8	4	2.3-38.7
	ciprofloxacin	0.06	0.03	11.6-61.2
<i>K. pneumoniae</i> ESBL33	rifaximin	8	4	14.3-66.6
	ciprofloxacin	0.06	0.03	31.8-42.6
<i>E. coli</i> ESBL 33	rifaximin	8	4	7.7-43.8
	ciprofloxacin	0.01	0.005	3.4-52.4
<i>E. coli</i> ETEC Mex 264	rifaximin	16	8	22.4-46.1
	ciprofloxacin	0.03	0.015	19.7-55.2

*range from five separate experiments
 Note: Adapted from Debbia *et al.*, 2008³, Table 4
 Note: Reported "Sub MIC" values are only 0.5 x MIC with the exception of "*S. aureus* II" which has a rifaximin Sub-MIC value of MICx1000. It is unclear if this is a typo. Additional "Sub-MIC" concentrations are not included.

The effect of rifaximin on bacterial conjugation, as measured by the frequency of plasmid transfer, was measured. Actively growing donor strain (2×10^3 CFU/mL) and recipient strain (4×10^3 CFU/mL) were mixed in Luria Broth (LB) medium (Table 4). Cells were harvested, diluted and plated on selective media after 90 minutes of incubation. It is unclear when bacteria were exposed to sub-inhibitory concentrations of ciprofloxacin or rifaximin. Control cells were not exposed to ciprofloxacin or rifaximin; however it is unclear if control cells were cultured with selective media. The authors report rifaximin inhibits transfer of genetic material by at least 100-fold when ATCC 29922 (*Flac* TcR) donor and ATCC25922 AzdIR strains are used, and results were comparable to ciprofloxacin. A 20-fold reduction of conjugation was observed with the *E. coli* clinical isolate carrying the conjugative plasmid ESBL33. The results are difficult to interpret because the methods are unclear.

Table 4: Effect of rifaximin and ciprofloxacin on plasmid transfer in *E. coli*

Donator	Recipient	Antibiotic (mg/L)	N. recombinants/10 ⁵ recipients
ATCC29922 (Flac TcR)	ATCC25922 AzdR*	rifaximin(40)	4
		ciprofloxacin(0.2)	3.4
		control	630
ESBL33		rifaximin	21
		ciprofloxacin	12
		control	430

AzdR, Spontaneous sodium azide-resistant (200 mg/L) strain

4. Clinical Microbiology

4.1. Description of clinical studies

The applicant includes two publications to support changes in the label to suggest rifaximin has a unique mechanism of action based on pathogen eradication or alterations in the gut flora.

4.1.1. DuPont *et al.*, 1998⁴

A randomized, prospective, double-blind clinical trial was conducted to determine the efficacy of rifaximin for the treatment of traveler's diarrhea in 72 adults visiting Mexico from the U.S. Participants were randomized to receive rifaximin (200, 400 or 600 mg, t.i.d. for 5 days) or trimethoprim/sulfamethoxazole (160 mg TMP/ 800 mg SMX, b.i.d. for 5 days). Stool was collected pre-treatment and at 24 hours after the end of therapy. Stool was assessed for presence of enteropathogens; however the methods were not included. The test of cure was the passage of formed stool and expressed as "time to last unformed stool" (TLUS) which was defined as the hours elapsed after the first dose of medication until passage of the last unformed stool. Table 5 shows the TLUS for all treatment groups. Participants who were administered the lowest rifaximin dose had the shortest duration of diarrhea. The differences in duration of diarrhea were not statistically significant. Participants were classified as "well" after passage of the last unformed stool. It is unclear if "well" is equivalent to "cure" or considered a successful treatment and whether microbiological cure (pathogen eradication) is included. The rate of reported treatment failure was lower in patients administered rifaximin treatment (6/55, 11%) compared to TMP/SMX (5/17, 29%). Four of six rifaximin treatment failures occurred in the highest dosing arm (600 mg rifaximin, t.i.d.).

Table 5: Time to last unformed stool

Drug group	Number	Duration of posttreatment diarrhea, h	
		median	mean
Rif 200	18	26.3	36.9
Rif 400	18	40.5	38.6
Rif 600	19	35.0	53.0
Rif total	55	35.0	43.1
TMP/SMX	17	47.0	55.7
p		N/S	N/S

Rif 200 - Rifaximin 200 mg p.o. t.i.d.; Rif 400 - rifaximin 400 mg p.o. t.i.d.; Rif 600 - rifaximin 600 mg p.o. t.i.d.; Rif total - all rifaximin-treated subjects; TMP/SMX - 160 mg trimethoprim/800 mg sulfamethoxazole p.o. b.i.d.

Adapted from DuPont *et al.*, 1998⁴, Table 1

Results in Table 6 show 27 enteropathogens identified from 26 of the 72 pre-treatment stool samples. In one stool sample, two pathogens were identified, including enteric *E. coli* (ETEC) and *Campylobacter jejuni*. Of the 26 patients, twenty patients were from rifaximin arm and six patients were from TMP/SMX arm. Following treatment, 16 of 20 (80%) pathogens in the rifaximin group were eradicated. Two ETEC isolates in the 400 mg t.i.d. rifaximin group and one *Shigella* isolate and one *Salmonella* isolate in the 600 mg t.i.d. rifaximin group were not eradicated. Seven pathogens identified from pre-treatment stool of 6 patients in the TMP/SMX arm were eradicated. It is unclear if patients with persisting pathogens achieved clinical cure because it is unclear if “cure” (Table 6) refers to microbiological eradication or clinical cure (not defined in methods). From results in Table 6, it is unclear if “cure” refers to microbiological eradication or clinical cure.

Table 6: Enteropathogens identified in pre-treatment samples and eradication during therapy

Drug group	ETEC	Shigella, Salm, Campy	Crypto	Total pathogens	Cure
Rif 200	7	4	0	11	11 (100%)
Rif 400	3	1	1	5	3 (60%)
Rif 600	2	2	0	4	2 (50%)
Rif total	12 ^a	7 ^a	1	20	16 (80%)
TMP/SMX	6	1	0	7	7 (100%)

Salm - *Salmonella* spp; Campy - *C. jejuni*; Crypto - *Cryptosporidium*; Rif 200 - rifaximin 200 mg p.o. t.i.d.; Rif 400 - rifaximin 400 mg p.o. t.i.d.; Rif 600 - rifaximin 600 mg p.o. t.i.d.; Rif total - all rifaximin-treated subjects; TMP/SMX - 160 mg trimethoprim/800 mg sulfamethoxazole p.o. b.i.d.

^a ETEC plus *C. jejuni* on pretreatment sample in 1 subject.

Note: Green boxes surround pathogens that were not eradicated completely

Adapted from DuPont *et al.*, 1998⁴, Table 3

All non-Campylobacter pathogens (n=24) identified from pre-treatment samples were tested for susceptibility to rifaximin and TMP by “dilutional minimum inhibitory concentration (MIC)” testing or “disc testing”, respectively. Further details of the susceptibility testing methods were not included. Table 7 shows the susceptibility of pathogens to both drugs. Note that rifaximin susceptibility breakpoints have not been established. MIC interpretive criteria/breakpoints for trimethoprim are <8 µg/mL for “Susceptible” and >16 µg/mL for “Resistant”².

Table 7: Susceptibility of bacterial enteropathogens to trimethoprim by disc testing and rifaximin by dilutional MIC

Enteropathogen	Number	Trimethoprim susceptible	Median rifaximin MIC (range)
ETEC	18	11 (61%)	12.5 (0.098–25)
<i>Salmonella</i>	4	4 (100%)	18.8 (12.5–50)
<i>Shigella</i>	2	1 (50%)	0.57 (0.39–0.75)

Note: methods are not described in detail and different methods were used to determine susceptibility to trimethoprim and rifaximin

The four enteropathogens that were not eradicated following rifaximin treatment were evaluated for susceptibility to rifaximin. Susceptibility of two ETEC isolates from the 400 mg rifaximin group did not change after treatment (<0.098 and 25 µg/mL). However, the post-treatment *Shigella* isolate from the 600 mg rifaximin group was 0.39 µg/mL before treatment and <0.098 µg/mL post treatment. Likewise, the *Salmonella* isolate, from one patient, in the 600 mg group was 6.25 µg/mL before and 3.125 µg/mL after treatment. It is difficult to interpret the results without knowing the method used.

4.1.2. DuPont *et al.*, 2001⁵

A randomized, double-blind, double-dummy clinical trial was conducted to determine the efficacy of rifaximin for the treatment of traveler’s diarrhea in 187 adults visiting Mexico or Jamaica from the U.S. Participants were randomized to receive rifaximin (200 mg, b.i.d. for three days) or ciprofloxacin (500 mg, b.i.d. for 3 days). The test of cure was determined by consistency of stool and clinical symptoms in a 24 hour (no watery stools and no fever) or 48 hour (no unformed stools and no fever) period. The primary endpoint was resolution of diarrhea and modification of stools. The time to last unformed stool (TLUS) was defined as the interval from initiation of therapy until passage of the last unformed stool, after which subjects were declared healthy. Microbiologic cure was a secondary endpoint and defined by a negative post-treatment sample (pathogen eradication).

Table 8 shows clinical efficacy results as measured by total number unformed stools. Note that Table 8 shows “n=3” in the Rifaximin treatment arm, but the text describes 93 subjects. It is unclear if the data described in Table 7 is calculated from n=3 or n=93. The treatment groups did not differ significantly in the total number of unformed stools or the duration of illness. However, “duration of illness” (Table 8, green box) was not defined. It is unclear if the duration of illness refers to the time to test of cure or time to when patients are declared “healthy” (TLUS); however the duration of illness is longer than the reported TLUS for each treatment

group, which is 25.7 hours (95% CI, 20.9-38.0) for rifaximin-treated participants and 25.0 hours (95% CI, 18.5 – 35.2) for ciprofloxacin treated participants. Eighty-one of 93 (87%) participants that received rifaximin therapy and 83 of 94 (88%) in the ciprofloxacin treatment group were considered cured. Nine (10%) subjects in the rifaximin treatment group and five (6%) subjects in the ciprofloxacin treatment group failed treatment (“did not become healthy”). It is unclear how 3 participants in the rifaximin treatment group and six participants in the ciprofloxacin treatment group were classified.

Table 8: Measurements of efficacy for participants treated with rifaximin or ciprofloxacin

Disease characteristics	Treatment group		P
	Rifaximin (n = 3)	Ciprofloxacin (n = 94)	
Total no. of unformed stools			
Mean ± SD	6.0 ± 3.1	6.1 ± 3.7	.793 ^a
Median (range)	5.0 (3-15)	5.0 (3-23)	
Duration of illness, h			
Mean ± SD	30.4 ± 21.2	27.2 ± 18.3	.466 ^a
Median (range)	27.0 (2.5-71.5)	23.3 (3-68.5)	

^a Determined by analysis of variance
 Note: Rifaximin (n=3) likely a typo from the publication. 93 subjects were evaluated from the Rifaximin treatment group for efficacy.

Stool was collected pre-treatment and at day four or five after initiation of therapy and assessed for presence of enteropathogens. The methods were not described for identification of bacterial species including *Shigella*, *Salmonella*, *Aeromonas*, *Vibrio* spp., and *Plesiomonas* spp., *Campylobacter jejuni*, and *Yersinia enterocolitica*. ELISA was used to identify protozoa, including *Entamoeba histolytica*, *Cryptosporidium* spp., and *Giardia* spp. *E. coli*-like colonies were isolated and transported on peptone stabs to a different laboratory where enterotoxigenic *E. coli* was identified by the production of heat-labile and heat-stable enterotoxin confirmed with a DNA hybridization/probe technique. Enteroaggregative *E. coli* was confirmed using the HEp-2 cell assay for adherence. Details of the methods were not included.

Pathogens were identified from paired pre-treatment and post-treatment samples for participants treated with rifaximin (n=33) or ciprofloxacin (n=30). Table 9 shows the pathogens identified in pre-treatment stool samples and the eradication of those samples after treatment. Pathogens were eradicated in 29 of 39 (74%) participants who received rifaximin and 38 of 43 (88%) in the ciprofloxacin therapy group. ETEC was eradicated in 23 of 33 (70%) rifaximin-treated participants and 28 of 32 (88%) ciprofloxacin-treated participants. A statistical analysis of eradication rates was not included.

Table 9: Pathogens identified pre-treatment from stool of patients administered rifaximin or ciprofloxacin

Pathogen	No. (%) of patients who received					
	Rifaximin treatment			Ciprofloxacin treatment		
	All	Microbiological		All	Microbiological	
	Cure	Failure		Cure	Failure	
ETEC only	30	20 (67)	10 (33)	31	27 (87)	3 (10)
<i>Shigella</i> species	3	3 (100)	0 (0)	5	5 (100)	0 (0)
<i>Salmonella</i> species	2	2 (100)	0 (0)	3	3 (100)	0 (0)
<i>Shigella</i> species and ETEC	1	1 (100)	0 (0)	0	0 (0)	0 (0)
<i>Salmonella</i> species and ETEC	0	0 (0)	0 (0)	2	2 (100)	0 (0)
<i>Campylobacter jejuni</i>	1	1 (100)	0 (0)	0	0 (0)	0 (0)
<i>Cryptosporidium</i> species	0	0 (0)	0 (0)	1	1 (100)	0 (0)
<i>Cryptosporidium</i> species and ETEC	1	1 (100)	0 (0)	1	0 (0)	1 (100)
<i>C. jejuni</i> and ETEC	1	1 (100)	0 (0)	0	0 (0)	0 (0)
In pretreatment stool samples	39	29 (74)	10 (26)	43	38 (88)	4 (9)

NOTE. ETEC, enterotoxigenic *Escherichia coli*.
 Note: only participants who provided a pre-treatment and post-treatment stool sample are included, 33 paired samples from the rifaximin group and 30 paired samples from the ciprofloxacin group
 Adapted from DuPont *et al.*, 2001⁵, Table 2

Bacterial enteropathogens were evaluated for in vitro susceptibility to rifaximin and ciprofloxacin before treatment and after treatment if eradication was not achieved. Standardized agar dilution methods described by the National Committee of Clinical Laboratory Standards (NCCLS) were used to determine the minimal inhibitory concentration (MIC). It is unclear what methods from NCCLS were followed because the authors cite the CLSI method, M27-A¹, which is incorrect. M27-A¹ describes susceptibility testing methods for yeast and not bacteria. All pathogens isolated from pre-treatment stool were evaluated for in vitro susceptibility to both drugs. Table 10 shows the MIC values of bacterial isolates obtained before treatment with rifaximin (n=44) or ciprofloxacin (n=48). The MIC₉₀ was 0.25 to 32 µg/mL for rifaximin and <0.016 to 0.125 µg/mL for ciprofloxacin.

Table 10: MIC values for bacterial isolates from stool samples before treatment with rifamoxicin or ciprofloxacin

Treatment group, isolate	No. of isolates	Rifaximin, µg/mL			Ciprofloxacin, µg/mL		
		MIC ₅₀	MIC ₉₀	MIC range	MIC ₅₀	MIC ₉₀	MIC range
Rifaximin							
ETEC	36	16	32	0.5–128	<0.016	0.016	0.016–0.3125
<i>Shigella</i> species	5	64	64	16–256	<0.016	<0.016	<0.016–32
<i>Salmonella</i> species	3	16	16	16	<0.016	<0.016	<0.016
Ciprofloxacin							
ETEC	36	16	32	8–64	<0.016	<0.016	<0.016
<i>Shigella</i> species	6	32	32	8–64	<0.016	<0.016	<0.016
<i>Salmonella</i> species	6 ^a	32	32	16–64	<0.016	<0.016	<0.016

NOTE. ETEC, enterotoxigenic *Escherichia coli*.
^a One *Salmonella* strain did not grow.

Adapted from DuPont *et al.*, 2001⁵, Table 4

Pathogens that were not eradicated were evaluated for susceptibility to rifaximin and ciprofloxacin. Table 11 shows differences in MIC values for paired pre- and post-treatment pathogens. Of 10 microbiological treatment failures in the rifaximin group, the MIC value of one *E. coli* isolate increased by three 2-fold dilutions (0.5 µg/mL to 4 µg/mL). The MIC value decreased by one 2-fold dilution in three and the MIC value was unchanged in five. Of the four microbiological treatment failures in the ciprofloxacin group, one had a lower MIC value in the post-treatment sample (0.5 µg/mL to <0.016 µg/mL) and three were unchanged.

Table 11: MIC values of paired pre- and post-treatment pathogens isolated from the stool of participants treated with rifaximin or ciprofloxacin

Subject number	Treatment group	MIC of rifaximin, $\mu\text{g/mL}$		MIC of ciprofloxacin, $\mu\text{g/mL}$	
		Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
37-021 ^a	Rifaximin	16	8	<0.016	<0.016
37-064	Rifaximin	No growth	8	No growth	<0.016
37-085	Rifaximin	16	16	<0.016	<0.016
37-137 ^a	Rifaximin	32	16	<0.016	<0.016
37-139 ^b	Rifaximin	0.5	4	<0.016	<0.016
37-185	Rifaximin	16	16	<0.016	0.03125
37-194	Rifaximin	16	16	<0.016	<0.016
37-196	Rifaximin	16	16	<0.016	<0.016
37-207	Rifaximin	4	4	<0.016	<0.016
37-209 ^a	Rifaximin	16	8	<0.016	<0.016
37-191	Ciprofloxacin	16	16	<0.016	<0.016
37-094 ^c	Ciprofloxacin	32	32	0.5	<0.016
37-183	Ciprofloxacin	16	16	<0.016	<0.016
37-192	Ciprofloxacin	16	0.25	<0.016	<0.016

^a Rifaximin-treated subjects for whom MICs of rifaximin were lower in the posttreatment samples.
^b Only rifaximin-treated subject for whom the MIC of rifaximin was higher in the post-treatment sample.
^c Ciprofloxacin-treated subject for whom the MIC of ciprofloxacin was lower in the posttreatment sample.

Adapted from DuPont *et al.*, 2001⁵, Table 5

4.2. Interpretive Criteria

Standardized breakpoints for rifaximin have not been established and the applicant does not propose interpretive criteria in this submission.

5. Discussion

The applicant has submitted 3 nonclinical microbiology studies and 2 clinical studies to support changes in the labeling.

The nonclinical study by Debbia *et al.*, 2008³, showed the effects of sub-inhibitory levels of rifaximin on bacterial virulence mechanisms, such as cell morphology, plasmid stability and frequency of plasmid transfer, in bacterial strains with low or high susceptibility to rifaximin. Resistant mutants were selected for and evaluated for susceptibility to rifaximin. Characterization of the mutant's genotype would be helpful to show whether culture with drug induces specific mutations affecting drug transport, DNA repair mechanisms or drug target. Also, the viability of the resulting mutants was not assessed to support the applicant's proposed statement that viability and virulence are reduced with rifaximin exposure.

Morphological changes were observed in both rifaximin resistant and susceptible strains at sub-inhibitory concentrations; however it is unclear if the morphological changes observed are

reversible or affect cell viability. The morphological changes were noted in parallel for rifaximin susceptible and resistant *E. coli* strains as the concentration of drug was increased; however changes in morphology were not correlated with functional changes that may affect virulence or viability. Since both susceptible and resistant strains had similar morphologies, it is unlikely that the changes are associated with drug susceptibility and are, perhaps, a temporal condition in response to environmental stimuli.

Plasmid stability was influenced by sub-inhibitory concentrations of rifaximin. Stability is the successful distribution of at least one plasmid in each daughter cell during division. The development of plasmid free cells can affect bacterial viability and productivity. The authors did not include studies to determine the effects of plasmid loss or viability or drug susceptibility. Also, appropriate controls that show plasmid stability in the absence of drug were not included.

DuPont *et al.*, 1998⁴, show that patients with traveler's diarrhea have a lower rate of treatment failure when treated with rifaximin compared to TMP/SMX; however the duration of diarrhea is not statistically different. The rate of reported treatment failure was lower in patients administered rifaximin treatment (6/55, 11%) compared to TMP/SMX (5/17, 29%). The sample size was too small to show statistical differences between treatment groups. Following treatment, four of 20 pathogens were not eradicated in the rifaximin treatment arm compared to all pathogens (7) in the comparator arm. Pathogens were tested for susceptibility to TMP and rifaximin. The results from the clinical study are inadequate to support the applicant's proposed changes to the label for a comparison of rifaximin to aminoglycosides or fluoroquinolones because the comparator drug in this trial was TMP/SMX (DHFR inhibitor/sulfonamide). Also, no studies were conducted to evaluate a "unique mechanism of action", as cited by the applicant; and the methods for susceptibility testing are not described in detail.

DuPont *et al.*, 2001⁵, show there was no significant difference in the proportion of subjects with traveler's diarrhea that were treated with rifaximin or ciprofloxacin with respect to duration of clinical illness, treatment failure or microbiologic cure. Based on a small number of observations regarding pathogen eradication no reference to alteration in gut flora or eradication rate should be made in the labeling. No studies were conducted to evaluate a unique mechanism of action for rifaximin.

6. References

1. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard. M27-A. 1997. National Committee for Clinical Laboratory Standards (NCCLS).
Ref Type: Report
2. Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement. M100-S19. 1-1-2009. Clinical and Laboratory Standards Institute (CLSI).
Ref Type: Report

3. **Debbia, E. A., E. Maioli, S. Roveta, and A. Marchese.** 2008. Effects of rifaximin on bacterial virulence mechanisms at supra- and sub-inhibitory concentrations. *J Chemother.* **20**:186-194.
4. **DuPont, H. L., C. D. Ericsson, J. J. Mathewson, E. Palazzini, M. W. DuPont, Z. D. Jiang, A. Mosavi, and F. J. de la Cabada.** 1998. Rifaximin: a nonabsorbed antimicrobial in the therapy of travelers' diarrhea. *Digestion* **59**:708-714.
5. **DuPont, H. L., Z. D. Jiang, C. D. Ericsson, J. A. Adachi, J. J. Mathewson, M. W. DuPont, E. Palazzini, L. M. Riopel, D. Ashley, and F. Martinez-Sandoval.** 2001. Rifaximin versus ciprofloxacin for the treatment of traveler's diarrhea: a randomized, double-blind clinical trial. *Clin Infect.Dis* **33**:1807-1815.
6. **Jiang, Z. D. and H. L. DuPont.** 2005. Rifaximin: in vitro and in vivo antibacterial activity--a review. *Chemotherapy* **51 Suppl 1**:67-72.
7. **Mignini, F., E. Falcioni, M. Prenna, F. Santacroce, and S. Ripa.** 1989. Antibacterial activity of rifaximin against *Clostridium difficile*, *Campylobacter jejunii* and *Yersinia* spp. *J Chemother.* **1**:220-222.
8. **Sierra, J. M., J. Ruiz, M. M. Navia, M. Vargas, and J. Vila.** 2001. In vitro activity of rifaximin against enteropathogens producing traveler's diarrhea. *Antimicrob Agents Chemother.* **45**:643-644.
9. **Vosbeck, K., H. Handschin, E. B. Menge, and O. Zak.** 1979. Effects of subminimal inhibitory concentrations of antibiotics on adhesiveness of *Escherichia coli* in vitro. *Rev Infect.Dis* **1**:845-851.

7. The Label

7.1. Applicant's version of the label

Additions to the approved label are underlined. This version has been formatted for PLR.

12.1 Mechanism of Action

Rifaximin is an anti-bacterial drug (see 12.4 Microbiology).

12.4 Microbiology

Mechanism of Action

Rifaximin is a non-aminoglycoside semi-synthetic antibiotic derived from rifamycin SV; it is a structural analog of rifampin. The mechanism of action of rifaximin depends on the inhibition of DNA-dependent RNA polymerase of the target microorganisms, leading to the suppression of initiation of chain formation in RNA synthesis.

The lower rate of eradication of fecal pathogens in patients treated with rifaximin compared with fluoroquinolones and aminoglycosides and lack of alteration of gut flora indicate a unique mechanism of action. Rifaximin may alter virulence factors of enteric bacterial pathogens without killing them, as has been seen with subtherapeutic levels of drugs and colonization fimbriae of enterotoxigenic *E. coli*. Rifaximin caused morphological alterations in both susceptible and resistant bacterial strains at concentrations as low as 1/32 of the MIC.¹Rifaximin reduced the viability and virulence of resistant bacteria, suggesting that if *in vivo* pathogens are

exposed to sub-MICs of the drug, not only are their physiological functions compromised, but gene virulence and antibiotic resistance are not fully expressed.

Rifaximin has in vitro antimicrobial activity against numerous Gram-positive and Gram-negative bacteria, such as *Escherichia coli*. Animal and human studies demonstrate negligible systemic rifaximin absorption (< 1%) after oral administration. The negligible systemic absorption of rifaximin from the gastrointestinal tract minimizes the potential adverse events associated with systemically absorbed antibiotics. Rifaximin is delivered at high concentrations to the gastrointestinal tract, which is the therapeutic site of action.

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 pathogen 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-A6⁴². However, the correlation between susceptibility testing and clinical outcome has not been determined.

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.

15 REFERENCES

1. Debbia EA, Maioli E, Roveta S, Marchese A. Effects of rifaximin on bacterial virulence mechanisms at supra- and sub-inhibitory concentrations. *J Chemother.* 2008 Apr;20(2):186-94.
2. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Sixth Edition, Wayne PA. *Approved Standard NCCLS Document M7-A6* January 2003; 23 (2).

7.2. Comments

1. Applicant proposes to add a description of the derivation or rifaximin and structural similarity to rifamycin. This comment is more appropriate for the Chemistry section.
2. Applicant proposes that the unique mechanism of action of rifaximin is supported by evidence that treatment results in a lower rate of pathogen eradication than fluorquinolones and aminoglycosides and a lack of alteration of the gut flora. The applicant cites two studies (DuPont *et al.*, 1998⁴ and DuPont *et al.*, 2001⁵) for supporting this statement. Neither reference compared the rate of pathogen eradication between rifaximin and an aminoglycoside nor did either study correlate these changes with

alteration of gut flora. DuPont *et al.*, 2001, showed that treatment of diarrhea with rifaximin had a lower, yet comparable, rate of pathogen eradication (29 of 39, 74%) compared to ciprofloxacin (38 of 43, 88%) from approximately one-third of patients for who paired pre- and post-treatment stool samples collected. The authors do not address important alterations of gut flora in participants treated with rifaximin or ciprofloxacin as suggested in the proposed labeling; however a review of the study results for enteropathogen eradication suggests it is comparable between treatment groups. DuPont *et al.*, 1998, report that five different enteropathogens were detected in the 20 pre-treatment stool samples of participants receiving rifaximin (200, 400 or 600 mg t.i.d.) and three pathogens (*E. coli*, *Shigella* and *Samonella*) from four participants were detected post-treatment. Two enteropathogens were detected in stool of seven participants treated with TMP/SMX and both pathogens were eradicated. DuPont *et al.*, 2001, reports that six enteropathogens were identified in pre-treatment stool of rifaximin-treated participants and one pathogen, *E. coli*, was identified in post-treatment stool samples. Four enteropathogens were identified in ciprofloxacin pre-treated participants stool, and two pathogens (*E. coli* and *Cryptosporidium*) were identified in post-treatment samples. This evidence does not support the applicant's statement that treatment with rifaximin results in a lack of alteration in gut flora.

3. The applicant proposes that "Rifaximin may alter virulence factors of enteric bacterial pathogens without killing them, as has been seen with subtherapeutic levels of drugs and colonization fimbriae of enterotoxigenic *E. coli*." The applicant cites Vosbeck *et al.*, 1979⁹, and Debbia *et al.*, 2008³, to support this statement. Rifaximin was not used in the Vosbeck *et al.*, 1979, study and therefore this study was not included in this review. Debbia *et al.*, 2008, did not investigate colonization fimbriae of *E. coli* when exposed to rifaximin. The methods to support experiments that evaluated virulence factors, such as the source of isolates, methods for culture with drug to select for resistance and morphological changes, and susceptibility testing were not included with appropriate detail to support the applicant's claims.
4. Applicant proposes to state that morphological changes are observed when susceptible or resistant bacteria are exposed to low concentrations of rifaximin. The normal morphology of *E. coli* are rods. Debbia *et al.*, 2008, report the morphology is altered as observed by microscopy following exposure to sub-inhibitory concentrations of rifaximin for 18 to 24 hours and then grown on selective agar containing 10x MIC rifaximin (40 mg/L). As the concentration of drug increases (0.008x MIC to 0.5x MIC), the morphohology is reported to be rods → short rods mixed with rods → short rods → very rare filaments mixed with short rods → rare filaments mixed with short rods → filaments mixed with short rods. The same morphological descriptions are provided for *E. coli* with rifaximin MIC values of 4 µg/mL or >512 µg/mL. This suggests that the morphological changes may not be related to susceptibility. The methods were not adequately described for an independent review and inclusion in labeling. Additional studies to determine the functional effect or reversibility of the morphological changes were not included.

5. Applicant states rifaximin reduces the viability and virulence of resistant bacteria. Debbia *et al.*, 2008³, show frequency of plasmid transfer and plasmid stability is reduced in the presence of sub-inhibitory levels of rifaximin. The frequency of plasmid transfer and plasmid stability are considered virulence factors because (1) they increase the genetic variability of bacteria and (2) genes encoding resistance to antibiotics are frequently encoded on plasmids. The methods and results included in the publication are unclear and inadequate for an independent review and therefore do not support inclusion of this statement in the label. In addition, the clinical relevance of such an effect is not known.
6. The applicant includes a statement that describes rifaximin activity in vitro against broad spectrum bacteria. Rifaximin is approved for treatment of *E. coli* and inclusion of such a statement may be misleading.
7. The applicant includes several statements regarding systemic absorption, adverse events associated with systemic absorption and drug concentrations in the gut. These statements are inappropriate for Section 12.4.
8. The applicant includes a statement regarding cross resistance with other antimicrobial agents. This sentence was relocated to coincide with rifaximin resistance.
9. The applicant includes repeated statements about resistance in the last paragraph of Section 12.4. To avoid redundancy, these statements should be deleted.

7.3. FDA's version of the label

Additions to the applicant's proposed label are double underlined, deletions are struck through.

12.1 Mechanism of Action

Rifaximin is an anti-bacterial drug [see Clinical Pharmacology (12.4)].

12.2 Microbiology

Mechanism of Action

~~— Rifaximin is a non-aminoglycoside semi-synthetic antibiotic derived from rifamycin SV; it is a structural analog of rifampin. The mechanism of action of rifaximin depends on the inhibition of DNA-dependent RNA polymerase of the target microorganisms, leading to the suppression of initiation of chain formation in RNA synthesis.~~

~~The lower rate of eradication of fecal pathogens in patients treated with rifaximin compared with fluoroquinolones and aminoglycosides and lack of alteration of gut flora indicate a unique mechanism of action. Rifaximin may alter virulence factors of enteric bacterial pathogens without killing them, as has been seen with subtherapeutic levels of drugs and colonization fimbriae of enterotoxigenic *E. coli*.⁷ Rifaximin caused morphological alterations in both susceptible and resistant bacterial strains at concentrations as low as 1/32 of the MIC.⁴ Rifaximin reduced the viability and virulence of resistant bacteria, suggesting that if *in vivo* pathogens are exposed to sub-MICs of the drug, not only are their physiological functions compromised, but gene virulence and antibiotic resistance are not fully expressed.~~

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~~potential adverse events associated with systemically absorbed antibiotics. Rifaximin is delivered at high concentrations to the gastrointestinal tract, which is the therapeutic site of action.~~

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Susceptibility Tests

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~~*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.~~

15 REFERENCES

1. ~~Debbia EA, Maioli E, Roveta S, Marchese A. Effects of rifaximin on bacterial virulence mechanisms at supra and sub inhibitory concentrations. J Chemother. 2008 Apr;20(2):186-94.~~

1-2. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Sixth Edition, Wayne PA. *Approved Standard NCCLS Document M7-A6* January 2003; 23 (2).

8. Recommendations:

The citations included for review do not support the applicant's proposed changes to the microbiology section of the rifaximin labeling.

Anne Purfield
Anne Purfield, Ph.D.
Microbiologist, DSPTP

CONCURRENCES:

DSPTP /Microbiology Team Leader Shukal Bala Signature 12/8/09 Date
CC:

DSPTP/Original NDA
DSPTP/PM/June Germain

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-21361	SUPPL-11	SALIX PHARMACEUTICA LS INC	XIFAXAN
NDA-22554	ORIG-1	SALIX PHARMACEUTICA LS INC	XIFAXAN

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/s/

ANNE E PURFIELD
12/08/2009

SHUKAL BALA
12/08/2009