ORIGINAL ARTICLE

Susceptibility to rifaximin and other antimicrobials of bacteria isolated in patients with acute gastrointestinal infections in Southeast Mexico

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KEYWORDS
Rifaximin; Bacterial resistance; Bacterial susceptibility; Southeast Mexico; Gastroenteritis; Acute diarrhea

Abstract
Introduction: Enteropathogenic bacteria isolated in Mexico City have shown a high rate of resistance to different antibiotics, with the exception of rifaximin (RIF). RIF is a nonabsorbable antibiotic that reaches high fecal concentrations (\(\approx 8,000\,\mu g/g\)). Susceptibility to antimicrobials can vary in different geographic regions.

Aim: To study the susceptibility to rifaximin and other antimicrobials of enteropathogenic bacteria isolated in patients with acute diarrhea in the southeastern region of Mexico.

Material and methods: A total of 614 strains of bacteria isolated from patients with acute diarrhea from 4 cities in Southeast Mexico were analyzed. An antibiogram with the following antibiotics was created: ampicillin (AMP), trimethoprim/sulfamethoxazole (T-S), neomycin (NEO), furazolidone (FUR), ciprofloxacin (CIP), chloramphenicol (CHL), and fosfomycin (FOS), assessed through the agar diffusion method at the standard concentrations recommended by the Clinical and Laboratory Standards Institute (CLSI) and the American Society for Microbiology (ASM), and RIF, assessed through microdilution at 4 concentrations.

Results: The bacteria were Escherichia coli (55%), as the majority, in all its pathogenic variants, Shigella (16.8%), Salmonella (15.3%), Aeromonas (7.8%), and less than 5% Campylobacter, Yersinia, Vibrio, and Plesiomonas. The accumulated overall susceptibility to RIF was 69.1, 90.8, 98.9, and 100% at concentrations of 100, 200, 400, and 800\,\mu g/ml, respectively. Overall susceptibility to other antibiotics was FOS 82.8%, CHL 76.8%, CIP 73.9%, FUR 64%, T-S 58.7%.

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Susceptibility to rifaximin and other antimicrobials of isolated bacteria

INTRODUCTION

Worldwide, 2 billion cases of acute diarrhea are calculated to occur yearly. This disease is the second cause of child mortality, preceded only by pneumonia. In recent decades, the extended use of oral rehydration solutions, together with improved sanitary conditions, have resulted in a noticeable drop in mortality from this type of diarrhea. Nevertheless, it continues to be one of the main causes of illness and death in children in the developing countries, with children under 5 years of age having a mean 3 diarrheic episodes per year. Infections contribute to childhood malnutrition, given that each episode reduces the nutrients necessary for growth and development. In Mexico, there is evidence that up to 17.8% of children presenting with frequent episodes of diarrhea have clinical signs of malnutrition.

There are numerous etiologies of diarrheic disease. The main origin in the developing countries is bacterial and parasitic infection, as a consequence of exposure to contaminated food and water. In Mexico, acute diarrhea is the fifth cause of death in children between 1 and 4 years of age. Frequency is up to 16% greater in certain geographic zones, mainly in the Southeast (Chiapas, Tabasco, and Yucatán), as opposed to the Northern states of Mexico.

It is difficult to determine the etiology of this disease based solely on clinical symptoms, but in children under 5 years of age it is frequently due to viral infection, whereas in older children and adults the cause is often bacterial infection. Etiologic diagnosis enables treatment to be given that aids in reducing symptoms, shortens the illness, prevents complications, and reduces the spread of pathogens in the population. However, in the majority of cases antimicrobial therapy is empirical, making it necessary to conduct epidemiologic surveillance studies on causal organisms and antimicrobial susceptibility that take local or regional patterns into account. In a previous study, we reported on the antimicrobial susceptibility of the microorganisms causing acute diarrhea in 1,000 patients, children and adults, in Mexico City. That study revealed a high frequency of bacterial resistance to the common antimicrobials, whereas...

NEO 55.8%, and AMP 23.8%. Susceptibility to RIF at 400 and 800 μg was significantly greater than with the other antimicrobials (P < .001).

Conclusions: The data of the present study were similar to those of a previous study carried out in Mexico City: susceptibility to RIF in > 98% of the bacterial strains and a high frequency of resistance to several common antimicrobials.

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rifaximin (RIF) demonstrated excellent antibacterial activity. It is a nonabsorbable, broad-spectrum antibiotic that reaches high concentrations in the intestinal lumen. One of the limitations of that study was that it only included a population in Mexico City. Given the high prevalence of diarrheic syndromes reported in other areas of the country, we decided to conduct a similar study on bacterial isolates from patients with acute diarrhea in 4 cities in the Southeast of Mexico.

Materials and methods

Bacterial isolates

A total of 614 bacterial strains were analyzed. They were isolated from feces from the same number of patients of both sexes and all ages that were clinically diagnosed with acute diarrhea. The samples came from 4 private laboratories in the cities of Veracruz, Veracruz (153 cases), Villahermosa, Tabasco (160 cases), Mérida, Yucatán (151 cases) and Tuxtla Gutiérrez, Chiapas (150 cases). The strains were conserved in milk broth and soy broth with glycerol and frozen at -70°C before their biochemical, serologic, and antimicrobial susceptibility identification. The following culture media were utilized in the initial sample isolations performed in each laboratory: MacConkey agar, MacConkey-sorbitol agar, Shigella-Salmonella agar, XLD agar, Campylobacter agar, Yersinia agar, TCBS agar, Brilliant Green agar, and tetrathionate broth.

The isolates were biochemically identified at each laboratory through manual and automatized systems: Autocan 4, Walkaway/ (Microscan), or VITEK 2, with acceptance probability in their identification greater than 95%. Typing and serologic identification of certain bacterial species, such as Escherichia coli (E. coli), Salmonella, and Shigella were done, using different commercial brands of specific antiserums and agglutination or conglutination reagents.

Antimicrobial susceptibility testing

Following the procedures established by the American Microbiology Society and the U.S. Clinical Laboratory Standards Institute (CLSI), the antimicrobial susceptibility testing was carried out using agar diffusion and dilution plating with the following antibiotic concentrations: ampicillin (AMP) 10 μg/ml, chloramphenicol (CHL) 30 μg/ml, ciprofloxacin (CIP) 50 μg/ml, fosfomycin (FOS) 50 μg/ml, furazolidone (FUR) 100 μg/ml, trimethoprim/sulfamethoxazole (T-S) 1.25/23.75 μg/ml, and neomycin (NEO) 30 μg/ml. RIF was tested at 4 different concentrations: 100, 200, 400, and 800 μg/ml. When strains were not susceptible to a given concentration, they were tested with successively higher ones. The minimum inhibitory concentration at which 90% of isolates are inhibited (MIC90) was considered for all the antibiotics, but MIC100 was considered for RIF.

Statistical analysis

Bacterial susceptibility to RIF was compared with that of the other antimicrobials through the Z-test. Statistical significance was set at a p < 0.05. The same test was employed to compare the results of the present analysis with those of the previously published study conducted in Mexico City. The statistical analysis was carried out using Statistica 8.0 and Stata 11 software packages.

Results

A total of 614 bacterial isolates were analyzed that came from the stool cultures of 308 males (50.1%) and 306 females.

![Figure 1](image-url)  
**Figure 1** Frequency of bacterial isolates in 614 patients with acute gastroenteritis in four cities in the Southeast of Mexico.
(49.9%), the majority of whom were adolescents and young adults (65.9%). A total of 0.9% were under 10 years of age and 3.7% were above 70 years of age. Ninety-one percent of the samples were from outpatients and 9% were from hospitalized patients.

**Bacteria.** More than half of the isolates were identified as some serotype of “diarrheagenic” *E. coli*, one third were species of the *Shigella* and *Salmonella* genera, followed by *Aeromonas*, *Campylobacter*, *Yersinia*, *Vibrio*, and *Plesiomonas*, in that order (Figure 1).

Three quarters of the *E. coli* (77%) corresponded to the enteropathogenic *E. coli* (EPEC) serotypes and very few were enterohemorrhagic *E. coli* (O:157). The majority of the *Shigella* were *dysenteriae* and *flexneri*, whereas *Salmonella paratyphi* was the most frequent species of that genus. Table 1 shows the number and proportion of the other genera, species, and serotypes.

**Antimicrobial susceptibility.** Overall, 69.1% of the bacteria were susceptible to RIF at a concentration of 100 µg/ml and 90.8% were susceptible at 200 µg/ml, and almost all at higher concentrations (Figure 2). The bacterial species that more frequently required higher concentrations of RIF were *E. coli* and *Salmonella* (Table 2).

Table 3 shows the susceptibility of the different bacteria to the antibiotics tested. The bacteria that were susceptible > 75% of the time were: EPEC only to RIF and FOS; enterotoxigenic *E. coli* to RIF and CHL; enterohemorrhagic *E. coli* to RIF, CIP, and CHL; *Shigella* to RIF, CIP, and FOS; *Salmonella* to RIF, CIP, FOS, and very close to CHL; and *Aeromonas* to the majority, except AMP, FUR, and FOS. The following were susceptible to almost all the antimicrobials: *Yersinia*, except to NEO and AMP; *Campylobacter*, except to CIP and T-S; *Vibrio*, except to NEO and AMP; and *Plesiomonas*, except to AMP. AMP showed good activity only against *Campylobacter*.

Overall, the susceptibility of the 614 bacterial strains to the antibiotics tested (Figure 3) showed that more than three quarters of the cases were resistant to AMP and almost half were resistant to T-S and NEO. Resistance to FUR occurred in 36% and to CIP in 26%, whereas the majority of the bacteria were susceptible to CHL (77%), FOS (88%), and RIF at 400 µg/ml (98.9%). The accumulated proportion of strains susceptible to RIF < 400 and < 800 was significantly greater than to the other antimicrobials (p < 0.001).

**Discussion**

Susceptibility means that the growth of a given bacterium is inhibited at the concentration of antibiotic that is reached at the infection site with the recommended dose; the concentrations are frequently the serum concentrations. The
Table 2  Bacterial susceptibility to different rifaximin concentrations.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>No.</th>
<th>100 µg/ml</th>
<th>200 µg/ml</th>
<th>400 µg/ml</th>
<th>800 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPEC</td>
<td>261</td>
<td>48.7</td>
<td>26.4</td>
<td>23.7</td>
<td>1.1</td>
</tr>
<tr>
<td>ETEC</td>
<td>67</td>
<td>79.1</td>
<td>9.0</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td>EHEC</td>
<td>10</td>
<td>80.0</td>
<td>20.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shigella</td>
<td>103</td>
<td>97.1</td>
<td>2.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella</td>
<td>94</td>
<td>67.0</td>
<td>31.9</td>
<td>1.1</td>
<td>-</td>
</tr>
<tr>
<td>Yersinia</td>
<td>11</td>
<td>81.8</td>
<td>18.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>11</td>
<td>81.8</td>
<td>18.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vibrio</td>
<td>5</td>
<td>100.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Plesiomonas</td>
<td>4</td>
<td>100.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aeromonas</td>
<td>48</td>
<td>95.8</td>
<td>4.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>614</td>
<td>69.1</td>
<td>18.9</td>
<td>10.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Results expressed in % of the susceptible bacteria.

EHEC: Enterohemorrhagic Escherichia coli; EPEC: Enteropathogenic Escherichia coli; ETEC: Enterotoxigenic Escherichia coli.

Table 3  Susceptibility of the bacterial isolates to the antimicrobials tested.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>RIF (400 µg/ml)</th>
<th>FOS</th>
<th>CHL</th>
<th>CIP</th>
<th>FUR</th>
<th>T-S</th>
<th>NEO</th>
<th>AMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPEC</td>
<td>98.9</td>
<td>89.6</td>
<td>72.6</td>
<td>57.6</td>
<td>67.3</td>
<td>55.3</td>
<td>47.6</td>
<td>31.3</td>
</tr>
<tr>
<td>ETEC</td>
<td>94.1</td>
<td>70</td>
<td>79</td>
<td>43</td>
<td>70</td>
<td>46</td>
<td>54</td>
<td>28</td>
</tr>
<tr>
<td>EHEC</td>
<td>100</td>
<td>60</td>
<td>80</td>
<td>100</td>
<td>60</td>
<td>20</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Shigella</td>
<td>100</td>
<td>87.8</td>
<td>71.1</td>
<td>90.2</td>
<td>73.1</td>
<td>75.0</td>
<td>74.1</td>
<td>57.7</td>
</tr>
<tr>
<td>Salmonella</td>
<td>100</td>
<td>87</td>
<td>74.2</td>
<td>99.6</td>
<td>42</td>
<td>32</td>
<td>54.4</td>
<td>41.4</td>
</tr>
<tr>
<td>Yersinia</td>
<td>100</td>
<td>100</td>
<td>82</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>18</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>100</td>
<td>100</td>
<td>55</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vibrio</td>
<td>100</td>
<td>100</td>
<td>75</td>
<td>100</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>Plesiomonas</td>
<td>100</td>
<td>63</td>
<td>79</td>
<td>100</td>
<td>52</td>
<td>92</td>
<td>83</td>
<td>20</td>
</tr>
<tr>
<td>Aeromonas</td>
<td>100</td>
<td>63</td>
<td>79</td>
<td>100</td>
<td>52</td>
<td>92</td>
<td>83</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>98.9</td>
<td>82.8</td>
<td>76.8</td>
<td>73.9</td>
<td>64</td>
<td>58.7</td>
<td>55.8</td>
<td>23.8</td>
</tr>
</tbody>
</table>

Results expressed as % of susceptible bacteria.

Figure 3  Overall susceptibility of the bacteria to the antibiotics tested. The percentage of each antimicrobial is shown at the top of the columns. AMP: ampicillin; CHL: chloramphenicol; CIP: ciprofloxacin; FOS: fosfomycin; FUR: furazolidone; NEO: neomycin; RIF: rifaximin (concentration of 400 µg/ml); T-S: trimethoprim-sulfamethoxazole.

Antimicrobial concentrations for deciding if a bacterium is susceptible or resistant have been defined and published, and are those that have been used in the present study, except for RIF. Its concentration for defining susceptibility has not been established, but other studies have used cutoff points of 32, 128, and 256 µg/ml. Because it is a nonabsorbable antibiotic, RIF reaches very high concentrations in the intestinal lumen. With its recommended dose for the treatment of acute gastrointestinal infections (800 mg daily for 3 days), fecal concentrations of RIF of up to a mean 8,000 µg/g have been reported. Therefore, in the present study we used higher concentrations than those reported by other authors, up to 400 and 800 µg/ml. They have more clinical significance, even though they are 10 to 20 times lower than the mean concentrations found in stools.

Our present findings from the stool cultures analyzed in patients with symptoms of acute diarrhea from 4 cities in the Southeast of Mexico confirm the data from our previous study conducted in Mexico City with the same methodology on stool cultures from 1,000 patients presenting with acute gastroenteritis. The genera and species of the bacterial isolates were similar in the 2 studies, except for a greater proportion of Shigella in the present sample (16.1 vs 12.0% from the previous study), and this difference was statistically significant (Z = 2.27, p < 0.05). In both studies, a low frequency of gastrointestinal bacterial pathogens susceptible to AMP, NEO, T-S, and FUR, more frequent susceptibility to CIP and CHL, and high percentages of susceptibility to FOS and RIF were observed.
Antimicrobial treatment of bacterial infections is useful for reducing symptoms, shortening the illness, and decreasing the transmission of the bacterial pathogen, but the widespread use of antimicrobials favors the appearance of resistant strains and the transmission of resistance to other bacteria.\textsuperscript{12} The appearance of \emph{E. coli} strains and other enterobacteria that produce extended-spectrum beta-lactamases has become a therapeutic problem than can be transmitted from one country to another.\textsuperscript{12} Moreover, in many cases antimicrobials are not indicated, such as in viral respiratory or gastrointestinal infections, or in cases of self-medication, the doses and treatment duration are insufficient. Ever-growing bacterial resistance is a health problem, especially in severe cases. An increase in bacterial resistance in many infections, including gastrointestinal ones, has been observed in other parts of the world.\textsuperscript{16}

The present study and the one from Mexico City coincide in demonstrating a high frequency of bacterial resistance to antimicrobials that are used very often in Mexico for different infections, such as AMP and T-S, as well as those that are freely used in cases of acute diarrhea, such as NEO and FUR. Resistance to CIP, an antibiotic that has been in use for “only” a little over 30 years, is growing. CHL is an old antibiotic that was widely used for years, but due to its bone marrow toxicity, has been prescribed almost exclusively in cases of typhoid fever for the last few decades. In our study, the frequency of bacteria susceptible to CHL went beyond 75%. FOS is a broad-spectrum antibiotic that is mainly indicated for urinary infections.\textsuperscript{17} In contrast, almost all the bacterial strains were susceptible to RIF. The low frequency of bacterial resistance to RIF has also been documented in bacteria associated with cases of traveller’s diarrhea coming from Latin America and Asia.\textsuperscript{10-12,18}

There are several reasons that support or explain the low resistance of enteropathogenic bacteria to RIF: \textit{a}) the bacteria that develop resistance to RIF spontaneously disappear from the feces within a few weeks;\textsuperscript{19} \textit{b}) a study reported that the MIC\textsubscript{90} susceptibility measure of intestinal pathogenic bacteria to RIF did not change with respect to bacteria isolated 10 years earlier;\textsuperscript{20} \textit{c}) experiments show that resistance to RIF is not easily induced;\textsuperscript{20} \textit{d}) when resistance appears, it is due to chromosome changes, rather than mediated by plasmids, and therefore it is not easily transmitted to other bacteria;\textsuperscript{20} \textit{e}) when resistant plasmids are induced through sub-inhibitory concentrations, RIF “cures” the plasmids and inhibits the transmission of resistant plasmids, reducing bacterial viability and their virulence, even when they have developed resistance;\textsuperscript{21} and \textit{f}) it has been available in Mexico for fewer than 20 years, but it has been used in European countries for approximately 40 years, with no reports of important resistance.

RIF has been successfully used in acute gastroenteritis in children and adults, including the elderly,\textsuperscript{22,23} in traveller’s diarrhea,\textsuperscript{24,25} and associated with other antimicrobials in recurrent diarrhea associated with \emph{Clostridium difficile}.\textsuperscript{26} It is interesting that in a study on children with acute diarrhea in which RIF significantly shortened diarrhea duration, etiology was not demonstrated in the stool analyses in half of the cases.\textsuperscript{22} Similarly, in 2 studies on patients with traveller’s diarrhea, etiology was not identified in approximately one third of the cases. The patients with “pathogen-negative” diarrhea were analyzed separately and it was shown that rifaximin also shortened diarrhea duration by half.\textsuperscript{27} Even though this may be due to imperfect diagnostic methods, it could also be that other RIF actions, listed in Table 4, are involved.\textsuperscript{28-32}

![Table 4: Rifaximin actions, in addition to the bactericide effect.](image)

When treatment is empiric, bacterial resistance to antibiotics hampers the choice of a useful antibiotic and, in turn, an adequate therapeutic response. According to the data of the present study, if the physician considers indicating empiric treatment with a non-absorbable antibiotic, RIF would be better indicated than NEO. If a gastrointestinal infection with an invasive bacterium (with high fever or bloody diarrhea) is suspected, CIP would be better indicated than FUR. AMP or T-S would not be good choices in any of the cases. Moreover, T-S is the antimicrobial with the greatest frequency of adverse effects.\textsuperscript{33}

We did not separate the data on bacterial frequency according to age groups or on the bacterial resistance in the samples into adults and children, because only a small number of samples were from children under 10 years of age. Nevertheless, together with data from our previous study, this aspect will be the subject of a sub-analysis in the pediatric population.

In true epidemiologic spirit for the prevention of bacterial resistance, the Mexican guidelines on the diagnosis and treatment of acute diarrhea in pediatric clinical practice suggest not using antimicrobials initially, unless there are clinical signs suggestive of sepsis, there is a bacterial pathogen reported in a stool culture, or the diarrhea occurs in a day-care setting and spread must be prevented. These guidelines take the high frequency of viral diarrhea into account in infants and preschoolers, as well as the fact that in the case of bacterial etiology, the diarrhea resolves in 7 to 14 days, even without antimicrobials.\textsuperscript{34} In adults, antimicrobials are recommended if the diarrhea persists after 24 to 48 h, if it is severe, or if the pathogenic agent has been identified.\textsuperscript{35} Other measures can contribute to preventing bacterial resistance to antibiotics, such as strengthening medical education in relation to antimicrobial therapy, avoiding the unrestricted sale of antibiotics, suppressing the indiscriminate use of antibiotics in livestock, and periodically carrying out studies on antimicrobial susceptibility. Epidemiologic surveillance is essential.\textsuperscript{36,37}

Treatment with antimicrobials can be a valuable instrument in the control of gastrointestinal infections. It reduces the duration and intensity of the illness, prevents complications that can be severe, and it can reduce disease transmission. The periodic determination of local, national, and international patterns of antimicrobial susceptibility can contribute to a more rational use of antimicrobial

\begin{table}
\centering
\caption{Rifaximin actions, in addition to the bactericide effect.}
\begin{tabular}{ll}
\hline
Action & Description \\
\hline
\hline
↑ Lactobacilli population & \hline
↓ Bacterial adherence to epithelial cells, \hline
↓ Internalization & of NFKB \\
↓ Bacterial translocation & \hline
Activation of pregnane X receptor (↓ of NFKB) & \hline
↓ Intestinal expression of proinflammatory cytokines & \hline
↓ Endotoxia in cirrhotic patients & \hline
↓ Urease production by enterobacteria and \hline
↓ Exotoxins and virulence factors at sub-inhibitory concentrations & \hline
\end{tabular}
\end{table}
therapy. Epidemiology can differ from one region to another, especially in a country as large as Mexico. For example, the frequency of Shigella was greater in the Southeast of the country than in Mexico City. Evaluating other regions far from the center of Mexico could provide interesting data.

In conclusion, the data contained in the present study was similar to that found in our previous study on patients with acute gastroenteritis in Mexico City: more than 98% of the enteropathogenic bacterial strains were susceptible to RIF and there was a high frequency of resistance to the most commonly used antimicrobials, such as AMP, T-S, NEO, and FUR.

Study limitations. This was not an epidemiologic study on the cause of acute gastroenteritis, and so the frequency of bacterial diarrhea could not be determined in relation to the total number of causes of acute diarrhea. Even though the study was not designed to investigate the prevalence of the different bacteria causing acute gastroenteritis, the bacteria included in the study were dependent on the frequency with which the local laboratories detected them, thus reflecting local prevalence to a certain degree.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Financial disclosure

The study was financed by Alfa Wassermann S.A. de C.V.

Conflict of interest

Alberto C. Frati is currently the medical Director of Alfa Wassermann S.A. de C.V., the distributor of rifaximin in Mexico. He participated in the planning of the project and manuscript revision, but did not intervene in any of the study phases, in the enumeration of results, or in the statistical analysis. The rest of the authors declare that they have no conflict of interest.

References


