Susceptibility to rifaximin and other antimicrobial agents of bacteria isolated from acute gastrointestinal infections in Mexico

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KEYWORDS
Rifaximin; Bacterial resistance; Bacterial susceptibility; Antibiotics; Gastroenteritis; Small intestinal bacterial overgrowth

Abstract
Background: Bacterial resistance may hamper the antimicrobial management of acute gastroenteritis. Bacterial susceptibility to rifaximin, an antibiotic that achieves high fecal concentrations (up to 8,000 µg/g), has not been evaluated in Mexico.

Objective: To determine the susceptibility to rifaximin and other antimicrobial agents of enteropathogenic bacteria isolated from patients with acute gastroenteritis in Mexico.

Material and methods: Bacterial strains were analyzed in stool samples from 1,000 patients with diagnosis of acute gastroenteritis. The susceptibility to rifaximin (RIF) was tested by microdilution (<100, <200, <400 and <800 µg/ml) and susceptibility to chloramphenicol (CHL), trimethoprim-sulfamethoxazole (T-S), neomycin (NEO), furazolidone (FUR), fosfomycin (FOS), ampicillin (AMP) and ciprofloxacin (CIP) was tested by agar diffusion at the concentrations recommended by the Clinical & Laboratory Standards Institute and the American Society for Microbiology.

Results: Isolated bacteria were: enteropathogenic Escherichia coli (E. coli) (EPEC) 531, Shigella 120, non-Typhi Salmonella 117, Aeromonas spp. 80, enterotoxigenic E. coli (ETEC) 54, Yersinia enterocolítica 20, Campylobacter jejuni 20, Vibrio spp. 20, Plesiomonas shigelloides 20, and enterohemorrhagic E. coli (EHEC 0:157) 18. The overall cumulative susceptibility to RIF at <100, <200, <400, and <800 µg/ml was 70.6, 90.8, 99.3, and 100%, respectively. The overall susceptibility to each antibiotic was: AMP 32.2%, T-S 53.6%, NEO 54.1%, FUR 64.7%, CIP 67.3%.


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Introduction

Acute diarrhea is an important health problem, mainly in the developing countries. Despite the decrease in mortality rates from this disease in the last decade in various countries, including Mexico\(^1\)–\(^3\), acute gastroenteritis continues to be a health problem due to its high morbidity. In Mexico, it is the second most common infectious disease, only preceded by respiratory diseases, with more than 5 million new cases per year\(^4\).

Despite the presence of certain clinical signs, it is difficult to determine the causal agent of acute diarrhea in a patient based solely on clinical findings. Acute gastroenteritis is often due to a viral infection, especially in children under 5 years of age\(^5\), whereas bacterial infection is more habitual in older children and adults. The most frequently detected enteropathogenic bacteria in patients with endemic gastroenteritis are *Escherichia coli* (E. coli) (EPEC, EIEC, EHEC, ETEC), *Campylobacter jejuni* (C. jejuni), *Shigella* spp, *Salmonella* spp, *Yersinia enterocolitica* (Y. enterocolitica)\(^6\)–\(^8\), and less frequently, *Aeromonas* spp, *Vibrios* spp\(^9\)–\(^12\), and *Plesiomonas shigelloides* (P. shigelloides)\(^13\)–\(^15\). The number of cases vary in relation to geographic region, patient age, and the season of the year in which the study was conducted.

The aims of therapeutic management of patients affected with gastroenteritis are to preserve life, relieve symptoms, prevent complications, cut the disease short, and prevent the spread of the pathogenic agents to the population. Oral rehydration is the standard treatment in acute gastroenteritis and antimicrobial agents are indicated in severe or prolonged cases, when shigellosis or cholera

### Susceptibilidad de las bacterias aisladas de infecciones gastrointestinales agudas a la rifaximina y otros agentes antimicrobianos en México

**Resumen**

Antecedentes: La resistencia bacteriana puede dificultar el tratamiento antimicrobiano de las gastroenteritis agudas. La susceptibilidad bacteriana de los enteropatógenos a la rifaximina, un antibiótico que alcanza altas concentraciones fecales (hasta 8,000 μg/g) no se ha evaluado en México.

**Objetivos:** Determinar la susceptibilidad a rifaximina y a otros antimicrobianos de bacterias enteropatógenas aisladas de pacientes con gastroenteritis aguda en México.

**Material y métodos:** Se analizaron las cepas bacterianas en las heces de 1,000 pacientes con diagnostico de gastroenteritis aguda. Se probó la susceptibilidad a la rifaximina (RIF) por microdilución (< 100, < 200, < 400 y < 800 μg/ml), la susceptibilidad a cloranfenicol (CLO), trimetoprim-sulfametoxazol (T-S), neomicina (NEO), furazolidona (FUR), fosfomicina (FOS), ampicilina (AMP) y ciprofloxacino (CIP) se probó por difusión-agar a las concentraciones recomendadas por CLSI y ASM.

**Resultados:** Las bacterias aisladas fueron: *Escherichia coli* (E. coli) enteropatógena (EPEC) 531, *Shigella* 120, *Salmonella* no-typhi 117, *Aeromonas* spp. 80, *E. coli* enterotoxigénica 54, *Yersinia enterocolitica* 20, *Campylobacter jejuni* 20, *Vibrio* spp. 20, *Plesiomonas shigelloides* 20 y *E. coli* enterohemorrágica (EHEC) 0:157) 18. La susceptibilidad global acumulada a RIF < 100, < 200, < 400, < 800 μg/ml fue del 70.6, el 90.8, el 99.3 y el 100%, respectivamente. La susceptibilidad global a cada antibiótico fue: AMP 32.2%, T-S 53.6%, NEO 54.1%, FUR 64.7%, CIP 67.3%, CLO 73%, FOS 81.3%. La susceptibilidad a RIF < 400 y < 800 μg/ml fue significativamente mayor que con los otros antimicrobianos (p < 0.001).

**Conclusiones:** La resistencia de las bacterias enteropatógenas a antimicrobianos utilizados en gastroenteritis es alta. La rifaximina fue activa contra el 99-100% de las bacterias en concentraciones alcanzables en el contenido intestinal con las dosis recomendadas.

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### Conclusiones

Resistance of enteropathogenic bacteria to various antibiotics used in gastrointestinal infections is high. Rifaximin was active against 99-100% of these enteropathogens at reachable concentrations in the intestine with the recommended dose.

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**PALABRAS CLAVE**

Rifaximina; Resistencia bacteriana; Susceptibilidad bacteriana; Antibióticos; Gastroenteritis; Sobreinfección bacteriana intestinal
are suspected, or when the pathogen is known, in order to prevent contagion\textsuperscript{12}. However, in daily clinical practice, when the result of the stool culture is reported, the patient is already recovering or the treatment is delayed. Knowing the susceptibility of the bacteria causing a syndrome to the antimicrobial agents is important in 2 aspects: one is the early therapeutically approach, out of empirical necessity, and the other is the epidemiologic surveillance of bacterial resistance that is useful for taking measures to prevent it.

The empirical use of antimicrobials can be inefficient due to the emergence of bacterial resistance. Therefore the possible therapeutic regimens should be frequently updated, taking the pattern of regional resistance into account\textsuperscript{17-20}. Some clinical studies indicate that quinolones are superior to other antibiotics or to placebo in the empirical treatment of adult patients with diarrhea\textsuperscript{17,20-22}. Other antimicrobial drugs, such as ampicillin (AMP), trimethoprim-sulfamethoxazole (T-S), chloramphenicol (CHL), furazolidone (FUR), and non-absorbable antibiotics, such as neomycin and recently rifaximin (RIF), have been used in children, in whom quinolones are not indicated, and also in adults\textsuperscript{17,23-25}. Nevertheless, recent data on the bacterial susceptibility to these antimicrobial agents is not available in Mexico. RIF is a semisynthetic antibiotic derived from broad spectrum rifamycin S that inhibits the synthesis of bacterial RNA, is not absorbable when taken orally, and reaches a very high concentration in the intestinal lumen (∼8,000 μg/g of feces). It has excellent bactericide activity on enteropathogenic microorganisms and does not cause important alterations in the gut microbiota\textsuperscript{26}. The aim of this study was to investigate the susceptibility of the acute gastroenteritis-causing bacteria to rifaximin and the most widely used antibiotics in the treatment of gastrointestinal infections in Mexico.

### Methods

#### Bacterial isolations

Bacterial strains obtained from the stool samples of 1,000 patients diagnosed with gastroenteritis or acute diarrhea were analyzed in 10 hospital laboratories in Mexico City that attend to hospitalized patients and outpatients. The strains were conserved and frozen before carrying out the biochemical identification, serology, and the antimicrobial susceptibility test in milk broth and soy broth with glycerol at −70°C\textsuperscript{27}. The primary initial isolations from the stool samples were carried out at each particular laboratory, and the following culture media were used: MacConkey Agar, Sorbitol-MacConkey Agar, Salmonella Shigella Agar, XLD Agar, Campylobacter Agar, Yersinia Agar, TCBS Agar, Brilliant Green Agar, and Tetrathionate Broth\textsuperscript{28}.

The isolates were biochemically identified in each laboratory using AutoScan 4, Walkaway (MicroScan), or Vitek 2 (Biomeriux) manual and automated processes and systems\textsuperscript{29-31} with an acceptance probability >95% in the identification; the typing and serologic identification in determined bacterial species, such as \textit{E. coli}, \textit{Salmonella}, and \textit{Shigella}, were then performed using the Bio-Rad\textsuperscript{32}, Phadebact\textsuperscript{33}, Oxoid\textsuperscript{14}, Sanofi-Pasteur\textsuperscript{35}, “O’” Bell\textsuperscript{36}, and ProBact\textsuperscript{37} specific antisera and agglutination or coagglutination reagents. The detected serogroups and serotypes are shown in Table 1.

#### Antimicrobial agents and susceptibility tests

Antimicrobial susceptibility testing (AST) was done through the agar diffusion method (CHL, T-S, neomycin [NEO], FUR, fosfomycin [FOS], AMP, and ciprofloxacin [CIP]), and by

### Table 1  Bacteria isolated from 1,000 patients diagnosed with acute gastroenteritis and their serotypes.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Number</th>
<th>Serotypes involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteropathogenic</td>
<td>531</td>
<td>O127:B8, O111:B4, O55:B5, O26:B6 and other serotypes</td>
</tr>
<tr>
<td>\textit{E. coli} group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A, B, and C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterotoxigenic (LT) \textit{E. coli}</td>
<td>54</td>
<td>Only thermodabile (TL) toxin-producing serotypes</td>
</tr>
<tr>
<td>Enterohemorrhagic \textit{E. coli}</td>
<td>18</td>
<td>Only O:157 serotype</td>
</tr>
<tr>
<td>\textit{Shigella dysenteriae}</td>
<td>24</td>
<td>All the serotypes that are agglutinable with the specific antiserum</td>
</tr>
<tr>
<td>\textit{Shigella flexneri}</td>
<td>24</td>
<td>All the serotypes that are agglutinable with the specific antiserum</td>
</tr>
<tr>
<td>\textit{Shigella boydii}</td>
<td>27</td>
<td>All the serotypes that are agglutinable with the specific antiserum</td>
</tr>
<tr>
<td>\textit{Shigella sonnei}</td>
<td>15</td>
<td>All the serotypes that are agglutinable with the specific antiserum</td>
</tr>
<tr>
<td>\textit{Salmonella} Group A</td>
<td>24</td>
<td>Most likely species: \textit{S. paratyphi}</td>
</tr>
<tr>
<td>\textit{Salmonella} Group B</td>
<td>21</td>
<td>Most likely species: \textit{S. typhimurium}</td>
</tr>
<tr>
<td>\textit{Salmonella} Group C1</td>
<td>24</td>
<td>Most likely species: \textit{S. choleraesuis}</td>
</tr>
<tr>
<td>\textit{Salmonella} Group C2</td>
<td>24</td>
<td>Most likely species: \textit{S. newport}</td>
</tr>
<tr>
<td>\textit{Salmonella} Group D</td>
<td>24</td>
<td>Most likely species: \textit{S. enteritidis}</td>
</tr>
<tr>
<td>\textit{Vibrio} spp.</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>\textit{Yersinia enterocolitica}</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>\textit{Campylobacter jejuni}</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>\textit{Plesiomonas shigelloides}</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>\textit{Aeromonas}</td>
<td>80</td>
<td>–</td>
</tr>
</tbody>
</table>
microdilution (RIF) following the recommendations of the Clinical & Laboratory Standards Institute and the American Society for Microbiology. The concentrations of the antibiotics assayed and AST conditions were: AMP 10 µg/ml, T-S 1.25/23.75 µg/ml, CHL 30 µg/ml, CIP 5 µg/ml, FUR 100 µg/ml, NEO 30 µg/ml, FOS 50 µg/ml. RIF was tested at 100 µg/ml and the strains that were not susceptible at this concentration were successively exposed to concentrations of 200 µg/ml, 400 µg/ml, and 800 µg/ml. Susceptibility to RIF was considered at a minimal inhibitory concentration (MIC) of MIC_{100}, whereas it was MIC_{90} for the other antibiotics.

**Statistical analysis**

Bacterial susceptibility to RIF was compared with the susceptibility to the other antimicrobial agents with the Z test and statistical significance was set at a p < 0.05. The Statistica 8.0 and Stata 11 statistical software were employed.

**Results**

The stool samples from 511 men and 489 women were analyzed. Sixty-five percent of the participants were above 20 years of age. The isolated bacteria were *E. coli* 603 (EPEC 531, ETEC 54, EHEC 18), *Shigella* 120 (*dysenteriae, flexneri, boydii, sonnei*), *Salmonella* 117 (*paratyphi, typhimurium, choleraesuis, newport, enteritidis*), *Vibrio* spp. 20, *Y. enterocolitica* 20, *C. jejuni* 20, *P. shigelloides* 20, and *Aeromonas* spp. 80.

Overall susceptibility of the bacteria to the different concentrations of RIF (MIC_{100}) are shown in Figure 1. The results of accumulated susceptibility patterns in all the strains assayed were at < 100 µg/ml: 70.6%; at < 200 µg/ml: 90.8%; at < 400 µg/ml: 99.3%; and at < 800 µg/ml: 100.0%. Table 2 shows the susceptibility of each group of bacteria to the different RIF concentrations. More than 99% of the strains of *Shigella, Salmonella, Yersinia, Campylobacter, Vibrio, Plesiomonas, and Aeromonas* were susceptible to concentrations < 100 or < 200 µg/ml, whereas 11-15% of the *E. coli* required < 400 or even < 800 µg/ml.

Overall susceptibility of all the bacterial species to each antibiotic was: RIF (< 400 µg/ml) 99.3% and (< 800 µg/ml) 100%, FOS 81.3%, CHL 73.0%, CIP 67.3%, FUR 64.7%, NEO 54.1%, T-S 53.6%, and AMP 32.2%. Overall susceptibility to RIF with < 400 and < 800 µg/ml was superior (p < 0.001) to the overall susceptibility to each of the 8 drugs studied (fig. 2). This was also true in the majority of the cases in each bacterial group alone.

Table 3 shows the susceptibility of each bacterial group to the antibiotics tested.

As can be seen, a large number of strains show a very high proportion of bacterial resistance to NEO, T-S, and AMP, but *Yersinia* continues to be very susceptible to AMP (100%), *Vibrio* to T-S (90%), and *Campylobacter* to NEO.

**Discussion**

The prevalence and incidence of the bacteria that cause gastroenteritis worldwide vary according to the type of population studied, the geographic location of the study, the season of the year in which the microbiologic diagnosis is made, and other clinical and sociodemographic

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**Table 2** Susceptibility of the bacteria to different concentrations of rifaximin.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Number</th>
<th>&lt; 100 µg/ml</th>
<th>&lt; 200 µg/ml</th>
<th>&lt; 400 µg/ml</th>
<th>&lt; 800 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPEC</td>
<td>531</td>
<td>58.95</td>
<td>25.61</td>
<td>14.69</td>
<td>0.75</td>
</tr>
<tr>
<td>ETEC</td>
<td>54</td>
<td>79.63</td>
<td>7.41</td>
<td>7.41</td>
<td>5.56</td>
</tr>
<tr>
<td>EHEC</td>
<td>18</td>
<td>72.22</td>
<td>16.67</td>
<td>11.11</td>
<td>-</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>120</td>
<td>90.00</td>
<td>10.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>117</td>
<td>67.52</td>
<td>31.62</td>
<td>0.85</td>
<td>-</td>
</tr>
<tr>
<td><em>Yersinia</em></td>
<td>20</td>
<td>80.00</td>
<td>20.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>20</td>
<td>70.00</td>
<td>30.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Vibrio</em></td>
<td>20</td>
<td>100.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Plesiomonas</em></td>
<td>20</td>
<td>100.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Aeromonas</em></td>
<td>80</td>
<td>100.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>All</td>
<td>1000</td>
<td>70.6</td>
<td>20.2</td>
<td>8.5</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Results expressed in % of susceptible strains.
Susceptibility of bacteria isolated from acute gastrointestinal infections

Results

Table 3  Susceptibility of each group of bacteria to the antimicrobials tested.

<table>
<thead>
<tr>
<th>Group of bacteria</th>
<th>FOS</th>
<th>CHL</th>
<th>CIP</th>
<th>FUR</th>
<th>NEO</th>
<th>T-S</th>
<th>AMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPEC</td>
<td>7.1</td>
<td>66.6</td>
<td>51.9</td>
<td>66.4</td>
<td>48.5</td>
<td>49.1</td>
<td>27.6</td>
</tr>
<tr>
<td>ETEC</td>
<td>75.9</td>
<td>88.8</td>
<td>50.0</td>
<td>77.7</td>
<td>53.7</td>
<td>50.0</td>
<td>29.6</td>
</tr>
<tr>
<td>EHEC</td>
<td>55.5</td>
<td>83.3</td>
<td>100</td>
<td>50.0</td>
<td>61.1</td>
<td>27.6</td>
<td>16.6</td>
</tr>
<tr>
<td>Shigella</td>
<td>68.3</td>
<td>82.5</td>
<td>97.5</td>
<td>47.5</td>
<td>46.6</td>
<td>16.6</td>
<td>48.3</td>
</tr>
<tr>
<td>Salmonella</td>
<td>77.8</td>
<td>64.1</td>
<td>89.7</td>
<td>64.1</td>
<td>52.1</td>
<td>74.4</td>
<td>50.4</td>
</tr>
<tr>
<td>Yersinia</td>
<td>90.0</td>
<td>90.0</td>
<td>80.0</td>
<td>30.0</td>
<td>2.5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Campylobacter</td>
<td>80.0</td>
<td>95.0</td>
<td>30.0</td>
<td>100</td>
<td>30.0</td>
<td>45.0</td>
<td></td>
</tr>
<tr>
<td>Vibrio</td>
<td>90.0</td>
<td>80.0</td>
<td>100</td>
<td>90.0</td>
<td>70.0</td>
<td>90.0</td>
<td>55.0</td>
</tr>
<tr>
<td>Plesiomonas</td>
<td>100</td>
<td>95.0</td>
<td>80.0</td>
<td>85.0</td>
<td>90.0</td>
<td>90.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Aeromonas</td>
<td>67.5</td>
<td>86.0</td>
<td>87.5</td>
<td>50.0</td>
<td>80.0</td>
<td>78.7</td>
<td>20.0</td>
</tr>
<tr>
<td>All</td>
<td>81.3</td>
<td>73.0</td>
<td>67.3</td>
<td>64.7</td>
<td>54.1</td>
<td>53.6</td>
<td>32.2</td>
</tr>
</tbody>
</table>

Results expressed in % of susceptible strains.
The low proportion of bacteria that are susceptible to AMP, T-S, neomycin, FUR, and CIP, from 32.2 to 67.3% in that order, coincides with studies conducted in other regions of the world\textsuperscript{5,6} and can be explained by the induction of bacterial resistance due to the widespread use or abuse of those antimicrobial agents. In this respect, it is striking that CHL, which conserves an acceptable general susceptibility of 73%, has been restricted, after its widespread use in Mexico, to treating typhoid fever for the last 30 years. And comparatively, in our study we introduced the test of susceptibility to FOS. This antibiotic is used very little in Mexico, and mainly in urinary infections. The general susceptibility of FOS was greater than that of the other antimicrobial agents, just below rifaximin.

Neomycin is a poorly absorbed antibiotic that is widely used in Mexico and does not require a prescription. According to our data, it is clear that AMP, T-S, neomycin, and FUR would not be good therapeutic options. RIF has been successfully used for treating acute infectious diarrhea in children and adults, as well as for traveler's diarrhea, with excellent tolerance\textsuperscript{5,6}. RIF would appear to be the best option, given that it has a high therapeutic index, combining high efficacy with a low frequency of adverse effects\textsuperscript{6,10}. Adverse effects are frequently attributed to other antibiotics, especially to beta-lactams and T-S mainly due to allergies, and the sulfonamides and CIP are known to be neurotoxic\textsuperscript{11}.

Treatment with antimicrobial agents is a valuable tool in the control of several gastrointestinal infections, given that the length of time and intensity of the disease is reduced, potentially severe complications are prevented, and disease transmission is decreased. Unfortunately, for several decades, enteropathogenic bacteria strains have been selected that are resistant to the commonly used antimicrobial agents and to those that once were considered first choice\textsuperscript{12}. The selection and dissemination of antimicrobial resistance among the different bacterial species that cause gastroenteritis is a growing health problem that complicates the therapeutic management of the severe cases. Studies conducted in many parts of the world have revealed an important increase in antimicrobial-resistant bacteria in many infectious diseases, including gastroenteritis\textsuperscript{13,14}. To reduce the appearance of antimicrobial-resistant bacterial strains, it would seem prudent to avoid the indiscriminate use of antibiotics in cattle, periodically determine local bacterial resistance patterns, reduce the easy access to antimicrobial drugs (for example: prevent self-medication with antimicrobials), and strengthen the medical education on this topic.

Study limitations. The stool cultures were obtained from 10 laboratories that sent positive stool cultures with enteropathogenic bacteria from patients diagnosed with acute gastroenteritis or acute diarrhea. Therefore, it was not possible to obtain the clinical data, such as the length of time of symptom progression, stool characteristics, the symptoms accompanying the diarrhea, and the intensity or severity of the clinical symptoms that would enable their correlation with the genus and species of the bacterial isolates. The stool cultures were from Mexico City and do not necessarily reflect those from the rest of the country.

Ethical responsibilities

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

Data confidentiality. 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

This study was financially supported by Alfa Wassermann S.A. de C.V.

Conflict of interest

Alberto Frati is presently the medical director of Alfa Wassermann S.A. de C.V., which produces rifaximin. He participated in the planning of the project and manuscript revision, but did not intervene in any phase of the study, the enumeration of the results, or the statistical analysis. The other authors declare that there is no conflict of interest.

References

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