



## Article/Artigo

# The effect of *Lactobacillus casei* and *Bifidobacterium breve* on antibiotic-associated diarrhea treatment: randomized double-blind clinical trial

Efeito de *Lactobacillus casei* e *Bifidobacterium breve* na diarreia associada ao uso de antimicrobiano: estudo clínico randomizado duplo cego

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### ABSTRACT

**Introduction:** Antibiotic-associated diarrhea (AAD) is an important side effect of this specific class of drugs. The objective of this study was to investigate the effect of the use of probiotics in the treatment of AAD. **Methods:** A group of hospitalized patients, who contracted diarrhea during or after 7 days of suspension of antimicrobial medication, was blindly randomized to receive a standardized diet associated with the use of the probiotics (*Lactobacillus casei* and *Bifidobacterium breve*) or its corresponding placebo, three times a day. **Results:** Seventy patients were studied. For the experimental (n=35) and control (n=35) groups, respectively, the average time of treatment was 5.06±2.18 and 5.49±3.17 days (p=0.95), and the average duration of diarrhea, among those who were healed, was 4.87±2.13 and 4.52±2.55 days (p=0.36). Four (11.4%) patients who received probiotics and ten (28.6%) who received the placebo were not cured (p=0.13), and relapse rates were similar between both groups. Seven patients from each group, in addition to diarrhea, presented cases of bloating and/or abdominal cramps and/or vomiting (p=1.00). **Conclusions:** In this light, it is concluded that *L. casei* associated with *B. breve*, in the administered dosage and frequency, has no effect on the antibiotic-associated diarrhea. Similar studies need to be conducted with higher doses of these or other probiotics.

**Keywords:** Probiotics. *Lactobacillus casei*. *Bifidobacterium breve*. Antibiotic-associated diarrhea.

### RESUMO

**Introdução:** A diarreia associada ao uso de antimicrobiano (DAA) é um importante efeito colateral dessa classe de fármacos. O objetivo do presente trabalho é investigar o efeito do uso de probióticos no tratamento da DAA. **Métodos:** Pacientes hospitalizados em um hospital universitário com diarreia, que se desenvolveu durante o uso ou até sete dias após a suspensão de antimicrobianos, foram randomizados, de forma cega, para receberem dieta padronizada associada, três vezes ao dia, ao uso de probiótico (*Lactobacillus casei* e *Bifidobacterium breve*) ou placebo. **Resultados:** Foram estudados um total de setenta pacientes. Para o grupo experimental (n=35) e controle (n=35), respectivamente, o tempo médio de tratamento foi de 5,06 ± 2,18 e 5,49 ± 3,17 dias (p=0,95) e o tempo médio de duração da diarreia, entre aqueles que se curaram, foi de 4,87 ± 2,13 e 4,52 ± 2,55 dias (p=0,36). Quatro (11,4%) pacientes que receberam probióticos e dez (28,6%) que receberam placebo não foram curados (p=0,13) e a frequência de recidiva foi similar entre os grupos. Sete pacientes de cada grupo, além da diarreia, apresentaram distensão e/ou cólica abdominal e/ou vômito (p=1,00). **Conclusões:** *L. casei* associado a *B. breve*, na dosagem e frequência administradas, não demonstraram qualquer efeito no tratamento da diarreia associada a antimicrobiano. Estudos similares merecem ser realizados com doses maiores destes ou de outros probióticos.

**Palavras-chaves:** Probióticos. *Lactobacillus casei*. *Bifidobacterium breve*. Diarreia associada ao uso de antimicrobiano.

### INTRODUCTION

Antibiotic-associated diarrhea (AAD) is characterized by its development after the start of antibiotic therapy or up to six to eight weeks after the completion of the treatment with this group of drugs<sup>1-2</sup>, provided that there is no other agent that causes diarrhea. It occurs in approximately 5% to 25% of patients using antimicrobial agents<sup>3</sup>. The incidence varies, for example, with the types of antibiotics used and also with the definition of diarrhea adopted in different studies<sup>1,4</sup>.

The human gastrointestinal tract is a complex ecosystem of more than 400 different species of microorganisms, predominantly anaerobic bacteria (1,000 anaerobic/1 aerobic)<sup>5-7</sup>, especially in the colon. When these non-pathogenic microorganisms are in a state of equilibrium, the intestinal microbiota plays an important role in controlling the microbial population by means of the colonization resistance mechanism that suppresses the growth of harmful microorganisms to the body, keeping them at lower levels or eliminating them completely<sup>6,8</sup>.

The use of antibiotics can cause imbalance in this ecosystem, resulting in both decreased fermentation of indigestible carbohydrates, which contributes to the development of osmotic diarrhea, and a negative impact on the mechanism of colonization resistance, creating a favorable environment for the growth of microorganisms that cause diarrhea<sup>2-3</sup>.

*Clostridium difficile* is the pathogen most commonly referenced and is involved in about 20% of all AAD cases in hospitalized individuals. This infection can result in manifestations of uncomplicated diarrhea, ulcerative colitis, or pseudo-membranous colitis<sup>9-11</sup>.

Probiotics are non-pathogenic living microorganisms, which, when ingested in adequate amounts, may colonize the intestinal tract and act beneficially on the host, promoting microbiota restoration<sup>8,12</sup>. *Lactobacillus spp.* and *Bifidobacterium spp.* are commonly used as probiotics and are able

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to survive through the gastrointestinal tract at a rate of 10% and 30%, respectively<sup>13-14</sup>. Thus, probiotics have been indicated for the prevention of AAD, both in the presence or absence of infection by *C. difficile*<sup>15-18</sup>.

The studies published, to date, evaluate the preventive effect of diarrhea with the use of probiotics, together with the antibiotic, and its recurrence with or without the presence of toxins for *C. difficile*<sup>19-21</sup>. However, it is also important for patients, who contract diarrhea while using one or more antibiotics, and the health professionals, who serve them, to be aware of the effect of the probiotics after the onset of AAD.

In this light, the present study was conducted to determine the effect of using the association of *Lactobacillus casei* and *Bifidobacterium breve* for the treatment of clinical manifestations of AAD and the occurrence of relapse.

## METHODS

### Patients and randomization

The study included hospitalized patients aged 18 years or older, in the wards of internal medicine, neurosurgery, traumatology, urology, oncology, and general surgery department, at The Federal University Hospital of Uberlândia. All of them contracted diarrhea during the use or in the next 7 days after the suspension of antimicrobial medication, which had been induced for at least 24h. This occurred with watery or semi-liquid stools on two or more occasions, in the last 24h.

Exclusion criteria were adopted for patients who had a history of chronic diarrhea or presumptive viral etiology, an HIV infection, ulcerative colitis, Crohn's disease, lactose intolerance, an electrolyte disturbance, or short bowel syndrome. The exclusion criteria were also adopted for patients who had been critically ill or were undergoing radiotherapy and chemotherapy; post-gastrectomy and enterectomy or ostomates; in use of enteral nutrition with a concentration greater than 350mOsm/kg; or whose suspected cause of diarrhea was the rapid infusion of the diet (>120ml/h).

For two years and two months, 73 patients were stratified into two groups, one by means of enteral feeding and the other by means of a normal oral diet. Patients from each group were blindly randomized in separate blocks of four, for the constitution of the two groups: experimental and control.

### Treatment

In the experimental group, patients with enteral feeding received industrialized enteral diet and cashew juice, containing 1g of probiotics offered three times a day after the enteral diet. Patients who had an oral diet received a normal diet and cashew juice containing 1g of probiotics, which was offered three times a day. In the control group, patients received the same diets as those from the experimental group; however, instead of the probiotics, a placebo was offered (725mg corn starch). The characteristics of the enteral feeding diet in cases of AAD were: the absence of saccharose, gluten, fiber, and lactose with an osmolality of less than or equal to 350mOsm/kg.

The probiotic, in sachet form (1g), contained  $2 \times 10^7$  to  $10^9$  cells of *Lactobacillus casei* and  $5 \times 10^7$  to  $10^9$  cells of *Bifidobacterium breve*. To certify the number of viable cells, the product underwent laboratory

analysis, whose technique is based on the seeding of the sample or its dilutions in JC Man, M Rogosa and E Sharpe, 1960 (MRS) agar for an enumeration of cells followed by an incubation temperature of  $36 \pm 1^\circ\text{C}$  for 48h according to the Standard Operating Procedure for the enumeration of lactic acid bacteria of the Laboratory for Quality Control and Food Safety of the Faculty of Veterinary Medicine of the Federal University of Uberlândia.

The product sample for analysis was obtained by weighing  $25 \pm 0.2\text{g}$  of the same product, plus 225mL of 0.1% of homogenized saline peptone for about 60 seconds in a *stomacher*, with a  $10^{-1}$  dilution. From this, dilutions were made up to a corresponding amount of  $10^{-8}$  in 0.1% of saline peptone. One milliliter of the dilutions -6, -7, and -8 was plated in duplicate into sterile Petri dishes, plus approximately 15 to 20mL of molten MRS agar, and was kept in bath water up to  $48^\circ\text{C}$ . The samples were homogenized and appropriately placed to solidify on a flat surface<sup>22</sup>. The plates were submitted to inverted incubation at  $36 \pm 1^\circ\text{C}$  for 48h. After this period, counts were performed in the colonies of lactic acid bacteria, resulting in an average amount of  $6.1 \times 10^8$  colony-forming unit/g of total lactic acid bacteria<sup>22</sup>.

The probiotic and the placebo, with similar appearance and flavor, were completely dissolved in moderate temperature liquids before being offered to the patient.

The following data were recorded daily: antibiotics used (including for treatment of AAD), time of use, dosage and administration route, number of daily bowel movements, stool consistency and assessment as to the improvement or worsening of symptoms, gastrointestinal symptoms and their evolution after the intervention, the date and reason for the end of the intervention, and relapse of diarrhea after stopping the probiotic or the placebo.

Treatment was initiated after the diagnosis of AAD and suspended after reducing the frequency of bowel movements and when stool consistency was back to normal, or, after four days, if there had not been any degree of improvement. For a recurrence evaluation, characterized by the reappearance of diarrhea in those individuals who met the curing criteria, patients could be visited in their wards, or if they had been discharged from the hospital, they could be contacted by phone calls, until seven days after the end of intervention.

### Statistical analysis

For comparison between the experimental and control groups, the patients who were submitted to the enteral nutrition and oral diets were evaluated together. To detect the difference between the averages of the age, duration of treatment, and healing variables, the Student's *t*-test was used for parametric data and the Mann-Whitney *U*-test was used for non-parametric data. The analysis of proportions between the sex and age of the patients, the period of treatment, and the period of AAD healing, with and without recurrence, as well as cases in which AAD was not healed between groups, was performed using the Chi-square test with the Yates correction. The difference was considered to be significant when the *p*-value was  $< 0.05$ .

### Ethical considerations

All patients were informed about the study and gave their consent, which was documented in writing. The protocol of this study was approved by the Ethics and Research Committee at the Federal University of Uberlândia.

## RESULTS

Out of the 73 patients who met the inclusion criteria, three were subsequently excluded because they died due to causes not related to diarrhea. Out of the 70 remaining patients, 35 were in the experimental group and 35 belonged to the control group; 21 patients from the experimental group and 20 from the control group were under enteral feeding.

There was no significant difference in the average age of the patients ( $p=0.71$ ), in the proportion of male patients ( $p=0.81$ ), and in the proportion of people who were aged 60 years or more ( $p=1.00$ ), among the patients from the experimental and control groups. The antimicrobials most frequently involved with AAD were beta-lactams, particularly cephalosporins and carbapenems, and glycopeptides. Also, it is noteworthy that during the intervention, no antimicrobials were removed or exchanged for others because of diarrhea. The administration of intravenous antibiotics occurred in most patients in the experimental and control groups (Table 1).

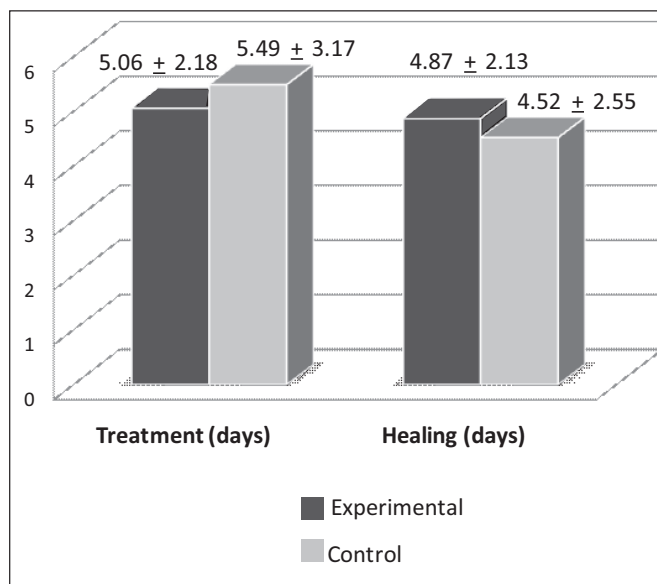
**TABLE 1 - Baseline characteristics of experimental and control groups.**

	Experimental group		Control group	
	n	%	n	%
<b>Sex</b>				
female	18	51.4	20	57.1
male	17	48.6	15	42.9
<b>Age (years)</b>				
mean*	56.17±20.47		54±21.84	
<60	17	48.6	18	51.4
≥60	18	51.4	17	48.6
<b>Antimicrobials</b>				
antifungal	3	5.2	1	2.0
carbapenems (beta-lactams)	10	17.2	9	18.4
cephalosporins (beta-lactams)	15	25.9	17	34.7
other beta-lactams	3	5.2	5	10.2
glycopeptides	14	24.1	9	18.4
lincosamides	4	6.9	2	4.1
metronidazole	5	8.6	2	4.1
polymyxin	1	1.7	0	0.0
quinolones	3	5.2	4	8.2
<b>Administration form</b>				
intravenous	54	93.1	49	100.0
oral/enteral tube feeding	4	6.9	0	0.0

\*Mean ± SD.

The AAD was initiated, on average, on the tenth day of use of antimicrobials in patients in the experimental group and on the seventh day in the control group ( $p=0.02$ ). Three (8.6%) patients in the experimental group and four (11.4%) in the control group developed AAD only after the discontinuation of the antimicrobial.

The average use of probiotics (treatment period) was  $5.06 \pm 2.18$  days for the experimental group and  $5.49 \pm 3.17$  days for the control group ( $p=0.95$ ), and the duration of diarrhea, (curing time) among those who were healed, was also similar between the two groups (Figure 1). The proportions among those who were cured less



**FIGURE 1 - Average length of treatment and healing between experimental and control groups.**

promptly, more markedly, or did not improve at all over the intervention were similar between the two groups ( $p>0.05$ ). The frequencies of healing and recurrence were proportionally similar between the two groups (Table 2). Seven patients in the experimental group and seven in the control group had bloating and/or abdominal cramps and/or vomiting, in addition to diarrhea ( $p=1.00$ ).

**TABLE 2 - Frequency of healing and recurrence of antibiotic associated diarrhoea in patients taking probiotics and those on placebo.**

Variable	Probiotic		Placebo	
	n	%	n	%
Cure without relapse	19 <sup>a,d</sup>	54.3	17 <sup>a,d</sup>	48.6
Cure with relapse	12 <sup>a,c</sup>	34.3	8 <sup>a,c</sup>	22.8
No cure	4 <sup>b</sup>	11.4	10 <sup>b</sup>	28.6
<b>Total</b>	<b>35</b>	<b>100.0</b>	<b>35</b>	<b>100.0</b>

<sup>a,b</sup> $p=0.13$ , when comparing the probiotic and placebo groups related to the total healing (with or without relapse) and cases that were not cured. <sup>c,d</sup> $p=0.80$ , when comparing the probiotic and placebo groups for relapse.

## DISCUSSION

Although this study has shown the inefficacy of the use of probiotics in the treatment of AAD, some methodological problems limit the extent of generalization of this conclusion. The amount of viable cells offered, the microbial species administered, and the number of patients involved could be the decisive factors in the results.

Some researchers<sup>23-24</sup> have used prophylactically *L. rhamnosus* GG and a multispecies of *Lactobacillus spp.* and *Bifidobacterium spp.* in similar amounts or even superior to those used in this study; these showed no reduction in the incidence of AAD in children, adults, and elderly people in the use of antibiotics, whether hospitalized or not. However, other authors, offering  $2 \times 10^8$  CFU/day of *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus*<sup>25</sup>;  $4.4 \times 10^{10}$  CFU/day of *L. bulgaricus* and *L. immunitas*<sup>20</sup>; or  $2 \times 10^{10}$  CFU/day of *L. acidophilus*<sup>26</sup> demonstrated efficacy in preventing AAD or acute diarrhea.

In a meta-analysis involving a total of 707 patients from 0 to 18 years old in six studies, Johnston et al.<sup>27</sup> found that four of these, offering doses between  $5.5$  and  $40 \times 10^9$  CFU/day of probiotics in different species or strains (*L. casei* GG, *S. boulardii*, and *L. sporogens*), demonstrated the protective effect of probiotics in AAD (RR 0.36, 95% CI 0.25 to 0.53), and in cases in which they used lower doses than  $2 \times 10^9$  CFU/day, the benefit was not achieved. In the present study, the daily dose was  $1.8 \times 10^9$  CFU, which may explain its ineffectiveness. However, it is difficult to attribute the failure of the treatment of AAD only to the quantity of probiotics offered, as, assessing some studies using probiotics to prevent AAD, there is much overlapping of doses among those that showed effectiveness and those that did not. In the majority of those that achieved positive effects, the daily doses were  $2 \times 10^8$  to  $4.4 \times 10^{10}$  CFU<sup>20,25,28-30</sup>, whereas for investigations that obtained negative results, the doses used were  $1 \times 10^9$  to  $4 \times 10^{10}$  CFU<sup>4,11,23-24,31</sup>.

*Lactobacillus* spp. and *Bifidobacterium* spp. showed a loss of viability when exposed to the secretions of stomach acid and bile salts<sup>32</sup>. However, an increase of CFU was observed in these bacteria in the stools of individuals who ingested them, which could act beneficially in patients<sup>13-14,33</sup>.

Also, it is known that antimicrobial therapy reduces the anaerobic bacteria of the intestinal flora, especially bifidobacteria<sup>34-35</sup>. Seki et al.<sup>36</sup> investigated the behavior of the microbiota in the use of antimicrobials with or without the use of probiotics. They classified 110 children and teenagers between 1 month and 15 years of age, according to three groups: those who received only antimicrobial treatment, those who initiated the use of probiotics during antibiotic treatment, and those who combined probiotic and antimicrobial from the beginning of the treatment. In the first group, there was a reduction of anaerobic bacteria and over 40% of the amount of intestinal bifidobacteria. In the second group, a similar situation occurred on the third day of antimicrobials. Yet, when the use of probiotics was initiated, an elevation and normalization of the quantity of microorganisms present occurred. In the third group, the levels of colony forming units remained constant. Therefore, the replacement with these intestinal microorganisms can restore normal levels, which would be the basis for a therapeutic effect on AAD. In the present study, treatment was initiated as soon as diarrhea developed, between the seventh and tenth day of the use of antimicrobials, in other words, when the intestinal microbiota was probably already seriously unbalanced. Therefore, it is reasonable to suppose that the amount of probiotics necessary for the therapeutic effect would have to be greater than what was actually used.

Another possibility to explain the ineffectiveness of probiotics in the treatment of AAD is the species used or the combination of both. This does not seem very likely, as, according to Timmerman et al.<sup>37</sup>, there is a greater possibility of effectiveness when using more than one species or more than one strain of probiotic bacteria. They described studies in animals and humans where there was not only the colonization of the species *Lactobacillus* spp., *Bifidobacterium* spp., *Saccharomyces boulardii*, and *Enterococcus faecium* at concentrations ranging from  $0.8 \times 10^8$  CFU/g to  $2.4 \times 10^9$  CFU/g, but also the increased production of IgA, especially by strains *L. casei* and *L. casei* Shirota. The authors tried to explain the greater effectiveness of using more than one species, due to the possibility that one can create an environment more suitable for colonization and adherence than the other in the intestinal epithelium.

As the number of patients involved in this study was modest, there might have been a type-II statistical error, which means that an existing effect might not have been demonstrated, especially if it was small.

In studies with probiotics in preventing AAD<sup>19,30</sup> for children between 6 months and 10 years of age receiving antimicrobial associated with *L. casei* GG, *B. lactis*, and *S. thermophilus*, no differences were found in the duration of diarrhea, with an average of 4.7 days compared with 5.8 days in the placebo group ( $p > 0.05$ ) in one study, and 3.92 days compared with 5.00 days for the placebo group ( $p > 0.05$ ) in another. Thus, in general, the main symptom of the AAD appears to continue for a period of approximately five days, which is consistent with the data from this study.

Although beta-lactam antibiotics were the most commonly used antimicrobials in patients with AAD (55% of all antimicrobials used in both groups) and were common to multiple antimicrobial therapy (more than 50% of patients, both in the experimental group and the placebo group), the study design was not adequate to assess the ability of each antimicrobial to cause AAD, and the prevalence might be due only to the layouts of most antimicrobials used in the Federal University Hospital of Uberlândia, and not due to a lower or higher capacity to cause AAD.

As the other possible gastrointestinal manifestations, which have been caused by the use of probiotics, occurred in a percentage similar to that found in the control group, we can infer that the use of the product is, in this aspect, safe. Thomas et al.<sup>24</sup> and McFarland et al.<sup>29</sup>, while assessing events such as nausea, abdominal cramps, flatulence, and fever, also found them in similar frequencies or even higher in the placebo group than in the experimental group. As such, another study found no significant difference in the incidence of flatulence and bloating in the group receiving *Lactobacillus* GG, along with the antimicrobial agent for preventing recurrence of *Clostridium difficile*-associated diarrhea, compared with the group that received the placebo<sup>38</sup>.

Hence, we conclude that the data from this study showed no efficacy with the use of probiotics *L. casei* and *B. breve* in the dosage and frequency administered, as for the treatment of AAD and its recovery. Similar studies, however, still need to be conducted, but with higher doses than the ones used herein or other probiotics.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. The Yakult® pharmacy did not have any participation in the elaboration of the project or did not make any restrictions in the design of the paper. The reason for the partnership was only to obtain the product, which was provided without restriction on publication, whatever the results.

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