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Major Article

A randomized clinical trial on the effectiveness of a symbiotic product to decolonize patients harboring multidrug-resistant Gram-negative bacilli

***Mariana Correa Coelho Salomão*[1],[2], *Mário Augusto Heluany-Filho*[1], *Mayra Gonçalves Menegueti*[3], *Marlieke Elizabeth Adriana De Kraker*[4], *Roberto Martinez*[2] *and Fernando Bellissimo-Rodrigues*[1],[3]**

[1]. Departamento de Medicina Social, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brasil. [2]. Disciplina de Moléstias Infecciosas e Parasitárias, Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brasil. [3]. Comissão de Controle de Infecção Hospitalar, Hospital das Clínicas, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brasil. [4]. Department of Infection Prevention and Control, University of Geneva Hospitals and Faculty of Medicine, Geneva, Switzerland.

Abstract

Introduction: We aimed to evaluate the effectiveness of a symbiotic product to decolonize the intestinal tract of patients harboring multidrug-resistant (MDR) Gram-negative bacilli and to prevent nosocomial infections. **Methods:** This was a randomized, double blind, placebo-controlled clinical trial, conducted in a tertiary-care university hospital. All adult hospitalized patients with a positive clinical culture and a positive rectal swab for any MDR Gram-negative bacilli were potentially eligible. Exclusion criteria were pregnancy, immunosuppression, and bowel obstruction/perforation. The intervention consisted of administering a symbiotic product (*Lactobacillus bulgaricus*, *Lactobacillus rhamnosus,* and fructo-oligosaccharides) twice a day for seven days via the oral/enteral route. **Results:** Between August 1, 2012 and December 22, 2013, 116 of 275 eligible patients were allocated to treatment (n=57) and placebo (n=59). Overall, 101 patients received at least four doses of the study products and were included in the *modified intention-to-treat* analysis. The primary study outcome, a negative rectal swab for MDR Gram-negative bacilli after treatment, was identified in 16.7% (8/48) and 20.7% (11/53) of patients in the experimental and placebo group, respectively (p=0.60). The secondary outcome, the combined incidence of nosocomial respiratory and urinary tract infections, was 37.5% (18/48) in the experimental group versus 22.6% (12/53) in the control group (adjusted odds ratio: 1.95, 95% confidence interval: 0.69-5.50, p=0.21). Length of stay after the beginning of the intervention, incidence of adverse events, and in-hospital mortality rates were similar in both study groups. **Conclusions:** Under the present study conditions, symbiotic administration was not effective for decolonizing hospitalized patients harboring MDR Gram-negative bacilli.

Keywords: Probiotic. *Lactobacillus.* Symbiotic. Decolonization. Antimicrobial resistance.

INTRODUCTION

In the last decades, the incidence of nosocomial infections caused by multidrug-resistant (MDR) Gram-negative bacilli has risen dramatically worldwide, affecting both developed and developing countries[1][2][3][4][5]. The World Health Organization recently recognized antimicrobial resistance as being a health threat of global concern due to the associated morbidity, mortality, and costs[6]. Currently, antibiotic stewardship, hand hygiene promotion, and contact precautions are the most important measures employed to control transmission of MDR Gram-negative organisms in hospitals. However, these measures are only partially effective and are difficult to implement[7].

One of the major problems regarding the current trends in antimicrobial resistance among Gram-negative bacilli is their ability to colonize the gastro-intestinal tracts of patients for months, or even years[8]. Consequently, colonized patients constitute a huge reservoir of MDR microorganisms, that is constantly expanding within hospitals and extending into the community[9]. Therefore, development of a strategy for the decolonization of these patients could have a great impact on the incidence of MDR infections[10][11].

Considering that probiotic bacteria can effectively colonize the human gastro-intestinal tract and compete with other bacteria for nutrients and space, these therapies could help to decolonize patients harboring MDR Gram-negative bacteria[12]. In fact, a small pilot study at our hospital showed some promising results

***Corresponding author*:** Prof. Fernando Bellissimo-Rodrigues.
e-mail: fbellissimo@fmrp.usp.br
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when using the strains of *Lactobacilli* employed in the present study[13].

We hypothesized that the administration of a symbiotic product to patients colonized by or infected with MDR Gram-negative bacilli could help to decolonize them, thereby preventing nosocomial respiratory and urinary tract infections.

METHODS

Setting

This study was a randomized, double-blind, controlled clinical trial, conducted between August 1, 2012 and December 22, 2013, at the University Hospital of Ribeirão Preto Medical School, a tertiary care public health-care facility. On average, this facility has 700 active beds with an occupancy rate of 73%, admitting 25,000 patients per year. It offers all regular specialties to a community of about 3.32 million inhabitants, and has an on-site microbiology laboratory.

Ethical considerations

The study protocol was submitted and approved by the institutional and national ethics review committees before being implemented. Written informed consent was required and obtained from all participants, or from their relatives in case of unconscious patients.

Patients

All adult patients (aged 18 years or older) admitted to the hospital were considered potentially eligible for the study if they were found to be colonized by or infected with any MDR Gram-negative bacillus[14]. On weekdays, the investigators requested the microbiology laboratory staff to alert them to any cultures, submitted at the instruction of the assistant doctors in charge of the patients, which yielded positive results. Once a candidate patient was identified through this surveillance system, a rectal swab was requested by the investigators to confirm gastrointestinal colonization by MDR Gram-negative bacilli by means of a selective culture, as detailed below. Only patients with a rectal swab positive for MDR Gram-negative bacilli were included in the study. A small percentage of colonized patients was directly identified through surveillance screening using rectal swabs, ordered by the infection control team for isolation purposes. Exclusion criteria consisted of pregnancy; obstructive and/or perforated abdominal pathology; and severe immunosuppressive conditions such as hematological neoplasia, advanced human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) with CD4<200 cells/mm³, or use of immunosuppressive therapy.

Intervention

The pharmacist, involved exclusively in the symbiotic and placebo manufacture, randomized the patients using a computer-generated sequence of numbers. In the experimental group, patients received administration of 10^{10} units of *Lactobacillus bulgaricus* plus 10^{10} units of *Lactobacillus rhamnosus* suspended in fructo-oligosacharide (FOS), preferentially by the oral route, or by nasoenteral tube, twice a day for seven days, beginning immediately after the result of the rectal swab culture was made available. In the control group, patients had a similar regime, but the placebo contained only excipients with color, consistency, and taste indistinguishable from treatment. Patients, their assistant doctors, and the investigators were blinded to the patient's allocation, until the end of data collection. Compliance with the study medications was assessed by checking the patients' medical records and was confirmed with the counting of remaining medication at the end of the intervention period. All included patients that remained in the study for at least two days, having had at least four doses of the study medications, were included in the modified intention-to-treat (MITT) analysis. Those who completed the protocol constituted the *per protocol* subpopulation. Those who prematurely discontinued the intervention in the first 48 hours of the study, due to early hospital discharge or death, were excluded from the analysis because they would not have had enough time to experience decolonization.

Microbiological procedures

Rectal swabs were collected by research assistant nurses at baseline, and within 24 hours after completing the treatment. Patients who were discharged before the end of treatment had their swab collected at discharge. A sterile swab was inserted into the rectum of the patient, turned around, removed, and inspected for the presence of feces. If the swab was still clean, the procedure was repeated until visible feces were retrieved. Specimens were cultured in two different selective media based on tryptic soy broth, one containing ertapenem10µg/mL and the other cefotaxime 30µg/mL. In addition, a control plate without any antibiotic was used to confirm that the sampling was adequate. Any Gram-negative bacillus growing in at least one of the selective media was identified through the Vitek®2 system (Biomerieux).

Study outcomes

All data were collected directly, through physical examination, or from the patient's medical records at inclusion in the study and, subsequently, at least twice a week, until hospital discharge or death. The primary study outcome was the proportion of patients with decolonization of intestinal carriage of MDR Gram-negative bacilli at the end of treatment, defined as a negative culture from the rectal swab at end of treatment. Secondary outcomes included the combined incidence of nosocomial pulmonary and urinary tract infections, since these are mostly related to Gram-negative bacteria, diagnosed according to the current Centers for Disease Control criteria[15]. Other secondary outcomes included length of stay in hospital after starting the intervention, and in-hospital mortality rates. We also evaluated the occurrence of adverse events potentially related to the use of study medications.

Statistics

Data were analyzed using Stata™ statistical software (release 6.0, College Station, TX: Stata Corp.). Descriptive statistics applied Pearson's corrected Chi-squared test, 2-tailed Fisher's exact test, and Wilcoxon/Mann-Whitney test, as appropriate.

All tests were two-tailed with a P-value <0.05 considered statistically significant. To assess differences in the two study arms at baseline, we compared clinical and demographic characteristics. Primary and secondary study outcomes in each study arm were compared at the end of treatment. To correct for possible differences at baseline, separate multivariate logistic regression models were built for the three most important dependent variables: decolonization of intestinal carriage of MDR Gram-negative bacteria, nosocomial infection, and death. We considered age, sex, length of stay prior to inclusion, and exposure to invasive devices as possible confounders. Confounders were included in the final model if they changed the effect estimate in bivariate regression analyses by more than 5%. Collinearity was assessed by generating a correlation coefficient matrix. Model fit was assessed using the Hosmer-Lemeshow test.

Considering spontaneous decolonization to occur in 10% of the patients in the placebo group (unpublished local data), and expecting this particular symbiotic to decolonize at least 35% of the patients, at α=0.05 and with study power of 90%, 54 patients should be included per study arm. Considering a degree of loss-to-follow-up, we decided to include 116 patients.

RESULTS

The flow chart of the inclusion process for this study is shown in **Figure 1**. Between August 2011 and December 2013, of all patients admitted to the clinical, surgical, and intensive care units of the university hospital, 275 had a clinical culture positive for Gram-negative MDR organisms and were thus potentially eligible. In total, 116 patients had a clinical culture and a positive rectal swab and could be included (experimental arm n=57, control arm n=59). Of these, 15 patients (nine in the experimental arm and six in the control arm) died or were discharged in the first two days of treatment, and were excluded from further analysis. The MITT was constituted by the remaining 101 patients.

Compliance with the study medications was higher in the experimental group, among whom 77.1% (37/48) of the allocated patients received the full package of treatment (14 doses), while only 56.6% (30/53) did the same in the control group (p=0.029).

Table 1 describes selected demographic and clinical characteristics of the patients included in the MITT group. A good balance was observed for most of the analyzed characteristics, but we detected an unintended imbalance regarding the proportion of intensive care unit (ICU) admissions (experimental arm: 20/48=41.7%, control arm: 17/53=32.1%), diabetes mellitus (experimental arm: 7/48=14.5%, control arm: 17/53=32.1%), malnutrition (experimental arm: 2/48=4.1%, control arm: 7/53=13.2%), exposure to mechanical ventilation (experimental arm: 14/48=29.1%, control arm: 8/53=15%), and the use of a nasogastric tube (experimental arm: 26/48=54.1%, control arm: 20/53=37.7%). Taken together, these findings suggest that the experimental group was more severely ill at baseline.

Most clinical cultures included urine samples, and *Klebsiella* spp, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* were the most commonly identified bacteria (**Table 2**). More than 70% of the patients included in both study arms experienced a clinical infection associated with their MDR colonizing bacteria. No significant differences were detected between the two study groups regarding the microbiological baseline aspects.

Table 3 describes the primary and secondary outcomes observed according to the study group allocation, in the MITT population, including the incidence of adverse events. No statistically significant differences were observed for any of the outcomes evaluated. Decolonization rates were 16.7% (8/48) in the experimental arm and 20.7% (11/53) in the control arm of the study, according to the MITT analysis (p=0.600). For the *per protocol* analysis, decolonization rates were 18.9% (7/37) in the experimental arm and 23.3% (7/30) in the control arm of the study (p=0.659). In-hospital mortality rates were 27.1% (13/48) in the experimental group and 18.9% (10/53) in the control group (p=0.326).

All adverse events observed were mild to moderate, and none motivated treatment cessation for any study participant. They consisted mainly of diarrhea (3.8% in the placebo group, 2.1% in the experimental group, p=1,000) and nausea/vomiting (0 in the placebo group, 6.2% in the experimental group, p=0.104). No bacteremia due to *Lactobacillus* spp. was detected during the study period.

Table 4 express the results of the three different multivariate analyses performed within the MITT population to identify factors associated with the primary study outcome (decolonization), and with the following secondary outcomes: development of respiratory or urinary tract nosocomial infections, and death. Statistically significant associations were identified between male sex and nosocomial infections (OR: 0.34, 95%CI: 0.12-0.98, p=0.047), exposure to invasive devices and nosocomial infections (OR: 12.73, 95%CI: 3.09-52.33, p<0.001), and exposure to invasive devices and in-hospital death (OR: 23.00, 95%CI: 2.80-190.00, p=0.003). The use of the symbiotic product was demonstrated not to be associated with any of the evaluated outcomes.

DISCUSSION

To the best of our knowledge, this is the first clinical trial to study the impact of probiotics on colonization with MDR pathogens in order to find new ways to tackle the worldwide problem of antimicrobial resistance. Considering that probiotic bacteria can effectively colonize the human gastro-intestinal tract and compete with other bacteria for nutrients and space[12], we hypothesized that they could help to decolonize patients harboring MDR Gram-negative bacteria. In fact, in a small pilot study, we found promising results using the strains of *Lactobacilli* administered in this study[13]. However, from the results of the present study, we can conclude that in this setting and in this particular patient population, our symbiotic product was no more effective than placebo for decolonization, or for preventing health-care associated infections or death among hospitalized patients.

Others have used various species of probiotic bacteria in order to prevent health-care associated and recurrent community

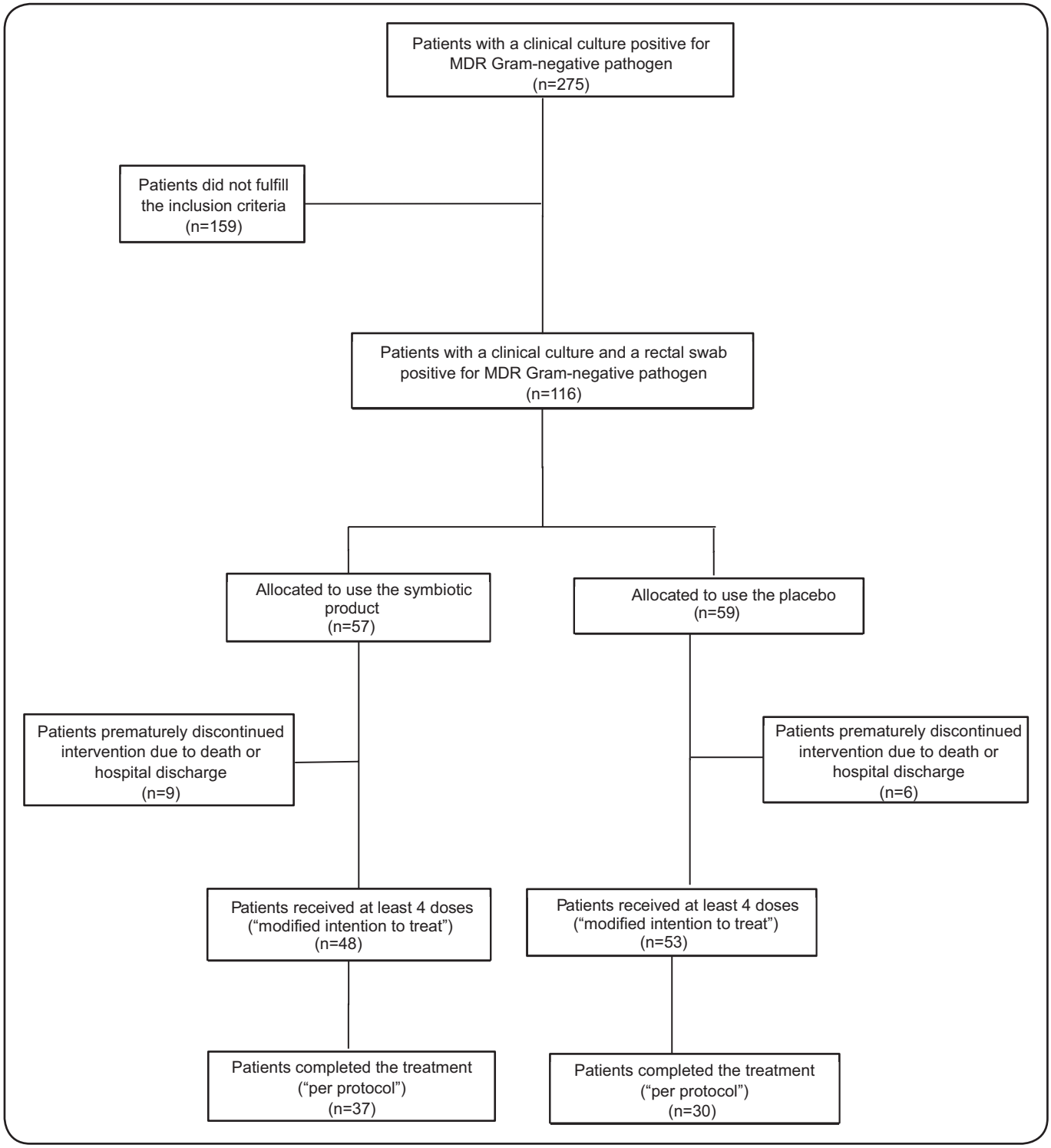

FIGURE 1. Flowchart of inclusion process in the study. **MDR:** multidrug-resistant.

TABLE 1

Baseline clinical and demographic characteristics of patients included in the *modified intention-to-treat* study population.

Baseline characteristics	Placebo group (n= 53)	Symbiotic group (n=48)
Demographic		
female sex	17 (32.0)	23 (47.9)
age, median (IQR), years	59 (49–73)	62 (45.5–71.5)
length of stay prior to inclusion, median (IQR), days	33 (15–42)	29 (17.5–47.0)
Clinical:		
diabetes mellitus	17 (32.0)	7 (14.5)
malnutrition*	7 (13.2)	2 (4.1)
COPD	7 (13.2)	6 (12.5)
hypertension	31 (58.4)	24 (50.0)
coronary disease	10 (18.8)	7 (14.5)
autoimmune disease	1 (1.8)	4 (8.3)
renal failure	28 (52.8)	24 (50.0)
obesity**	8 (15.0)	9 (18.7)
cancer	13 (24.5)	11 (22.9)
Clinical management:		
admission to the intensive care unit	17 (32.1)	20 (41.7)
use of systemic antibiotics	43 (81.1)	35 (72.9)
mechanical ventilation	8 (15.0)	14 (29.1)
nasogastric tube	20 (37.7)	26 (54.1)
indwelling foley catheter	13 (24.5)	14 (29.1)

IQR: interquartile range; **COPD:** chronic obstructive pulmonary disease. *****Malnutrition was defined as a body mass index <18.5kg/m^2. ******Obesity was defined as a body mass index ≥30kg/m^2. **Note:** data are n (%) of patients.

TABLE 2

Distribution of the MDR microorganisms isolated from clinical cultures before inclusion, and their source, according to the study group allocation in the *modified intention-to-treat* study population.

Microbiological data	Placebo group (n=53)	Symbiotic group (n=48)
Identified species*		
Klebsiella spp.	21 (39.6)	22 (45.8)
Acinetobacter baumannii	17 (32.0)	12 (25.0)
Pseudomonas aeruginosa	8 (15.0)	6 (12.5)
Escherichia coli	6 (11.3)	4 (8.3)
Enterobacter spp.	4 (7.5)	3 (6.2)
Proteus mirabilis	1 (1.9)	1 (2.1)
Burkholderia cepacia	1 (1.9)	1 (2.1)
Serratia marscescens	0 (0)	1 (2.1)
Infectious disease due to the MDR clinically documented	38 (71.6)	35 (72.9)
Source of positive cultures*		
urine	26 (49.0)	25 (52.0)
surgical wound	13 (24.5)	12 (25.0)
blood	9 (16.9)	3 (6.2)
respiratory tract	7 (13.2)	6 (12.5)
rectal swab	3 (5.6)	3 (6.2)
peritoneal fluid	0	1 (2.0)

MDR: multidrug-resistant. *Each patient could contribute with more than one species and more than one source. **Note:** data are n (%) of patients.

TABLE 3

Primary and secondary outcomes observed according to the study group allocation, in the *modified intention-to-treat* population.

Outcome*	Placebo group (n=53)	Symbiotic group (n=48)	P**
Decolonization	11 (20.7)	8 (16.7)	0.600
Nosocomial respiratory tract infection	8 (15.0)	8 (16.6)	1.000
Nosocomial urinary tract infection	6 (11.3)	13 (27.0)	0.073
In-hospital death	10 (18.9)	13 (27.1)	0.326
Length of stay after the start of intervention	17.0 (9.0–39.0)	31.0 (17.0–56.5)	0.078
Mild to moderate adverse events	4 (7.5)	3 (6.2)	1.000

*Data are n (%) of patients for the categorical variables, and median (interquartile range) for the length of stay after the start of the intervention. **Pearson's corrected chi-squared test for categorical variables and Wilcoxon/Mann-Whitney for continuous variables.

TABLE 4

Multivariate logistic regression of the impact of symbiotic therapy and possible confounders on three different outcomes: decolonization, respiratory and/or urinary tract nosocomial infection, and death, in the *modified intention-to-treat* population.

Risk factors	Decolonization OR (95%CI)	p	Nosocomial infections OR (95%CI)	p	In-hospital death OR (95%CI)	p
Male sex	1.34 (0.44–4.12)	0.598	0.34 (0.12–0.98)	0.047	0.51 (0.16–1.55)	0.237
Age	0.98 (0.95–1.01)	0.463	1.01 (0.98–1.04)	0.349	1.01 (0.98–1.05)	0.226
Length of stay before intervention	0.98 (0.95–1.00)	0.195	1.00 (0.98–1.02)	0.497	1.00 (0.98–1.02)	0.669
Exposure to invasive devices	0.75 (0.26–2.20)	0.611	12.73 (3.09–52.33)	<0.001	23.00 (2.80–190.00)	0.003
Symbiotic use	0.71 (0.24–2.07)	0.538	1.94 (0.68–5.49)	0.208	1.33 (0.44–4.00)	0.604
Number of symbiotic or placebo doses	1.19 (0.89–1.60)	0.229	0.89 (0.71–1.12)	0.337	0.97 (0.75–1.25)	0.832

OR: odds ratio; **CI:** confidence interval.

infections, generally with positive results[16][17][18][19][20][21][22][23][24][25]. Probiotic or symbiotic products have been demonstrated to be capable of preventing neonatal enterocolitis[23], recurrent urinary tract infections[19], antibiotic-associated diarrhea[25], and health-care associated pneumonia[17][18]. However, none of these studies found probiotic administration to have a significant impact on mortality rates among hospitalized patients[22][23][24].

Many hypotheses could be elicited to explain our negative findings. Perhaps one or two species of probiotics were insufficient to produce the desired results. Maybe a more varied group of endogenous species, more representative of the natural human microbiome, could produce better results, similar to the positive results obtained with fecal transplantation for patients colonized/infected by *Clostridium difficile*[26].

Another possible explanation is the high proportion of patients receiving systemic antibiotics during the intervention period (72.9% experimental arm, 81.1% control arm), possibly limiting the viability and thus the colonization-power of the *Lactobacilli*. Future studies should focus on colonized rather than infected patients, in order to achieve better decolonization rates.

Another point to be discussed is the potential role of a conditioning phase pre-intervention based on the use of non-absorbable antibiotics, intended to reduce the concentration of the MDR Gram-negative bacilli in the gastro-intestinal tract before administration of the symbiotic. This might facilitate the colonization process of the *Lactobacilli*[11][27].

Our study has some limitations. First, loss-to-follow-up and rates of decolonization in the placebo arm were both more frequent than expected, compromising the study power to identify potential benefits of the symbiotic administration. Second, we used rectal swabs instead of the gold standard method, selective stool culture, for identifying MDR Gram-negative bacilli in the gastro-intestinal tract. Rectal swabs were used mainly because their collection does not depend on a patient's defecation, which could compromise the compliance with the study endpoints, since many hospitalized patients experience obstipation. However, rates of agreement between rectal swabs and stool culture are over 90% and samples before and after the intervention were treated in the same way[28]. Third, due to our limited sample size, the study populations were not adequately balanced; the population allocated to use

the symbiotic product was more severely ill than the control group at the time of admission, making the interpretation of the study outcomes difficult. We tried to overcome this limitation by using multivariate statistical analysis.

In conclusion, the use of 10^{10} units of *Lactobacillus rhamnosus* and10^{10} units of *Lactobacillus bulgaricus* in FOS two times daily for seven days did not improve decolonization in patients harboring MDR Gram-negative bacilli in their gastrointestinal tract. As a consequence, the incidence of nosocomial respiratory and urinary tract infections among patients in the experimental and control arm were equal. We do believe symbiotics can play a role in decolonization and would recommend that future studies use a larger diversity of probiotic species, improve therapy compliance, focus on patients not receiving systemic antibiotics, and consider including a conditioning phase. We trust that these types of studies can lead the way to successful implementation of symbiotic therapy to reduce carriage of MDR Gram-negative bacilli in the gastro-intestinal tract.

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Conflict of interest

The authors declare that there is no conflict of interest.

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