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Research Article

Effect of A Probiotic Preparation on Gut Microbiota in Critically Ill Septic Patients Admitted to Intensive Care Unit: A Pilot Randomized Controlled Trial

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Abstract

Background: Sepsis promotes severe physiologic alterations in patients, and it has been reported to induce profound changes in the gut microbial composition. The decrease of 'health-benefiting' microbes and the increase in dysbiosis in critically ill patients are thought to induce or aggravate sepsis. In this study, we aimed to explore the effect of a probiotic preparation, Lactocare®, on gut microbiota in critically ill septic patients admitted to the intensive care unit (ICU).

Methods: Forty critically ill patients diagnosed with sepsis were assessed in this pilot randomized controlled trial. Patients were randomized into two groups: Lactocare and control groups. Patients in the Lactocare group received two capsules of Lactocare® for 10 days. Fecal samples were taken from all patients on days 1 and 10 for determining the gut microbial pattern. The primary outcome was gut microbial flora, and secondary outcomes were intensive care unit (ICU) length of stay and mortality.

Results: Intragroup changes showed that all microbial flora considerably changed during the study period; the number of microbial flora significantly decreased in the control group and increased in the Lactocare group. Patients in the Lactocare group had a significantly lower incidence of diarrhea and infection with multidrug-resistant organisms. There was no difference in ICU length of stay in the Lactocare group compared to the control group (p= 0.289). The mortality rate was 30% in the control group compared to 20% in the Lactocare group (p: 0.465).

Conclusion: This study showed a remarkable effect of the probiotic preparation on the gut microbiota in critically ill septic patients as it decreased the number of opportunistic pathogens. However, additional clinical research is needed to translate research into clinical practice to refine the clinical indication of the specific probiotic strains.

Introduction

The human gastrointestinal tract contains trillions of bacteria that compose a complex ecosystem known as the gut microbiota; it has an essential role in human health and disease, especially in nosocomial infections.[1] Gut microbiota can outcompete pathogens for space, metabolites, and nutrients, and can inhibit pathogens with the modulation of the host immune response. Perturbation of this mechanism is a common starting point for infection, as well as antibiotic therapy, inflammation, and infection representing as the most common causes of dysbiosis.[2] As sepsis is defined as a dysregulated immune response to infectious insult, many researchers hypothesized that dysbiosis potentially predisposes patients to a state of immunosuppression and thus increases the risk of sepsis. The identification of such microbes and their abundance changes associated with the diseases have been broadly described in the latest years. The changes in the gut microbiota diversity of critically ill patients after intensive care unit (ICU) admission seem to combine a reduction in strict anaerobes and an increase in pathogenic species, thus associated with increased mortality.[3,4] The management of dysbiosis in critically ill septic patients is a current trend of clinical research which could decrease the mortality rates in ICU patients. Interventions like the decontamination of the digestive tract, administration of probiotics and prebiotics alone or in combination (synbiotics), and fecal microbiota transplantation can restore the normal gut microbiota in these patients.[5-7]

The administration of probiotics and synbiotics has

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been demonstrated to reduce infectious complications, and meta-analyses suggest that probiotics are safe and effective at preventing infection in both postoperative and mechanically ventilated patients.[8] In the context of sepsis models and ICU patients, probiotics have been studied and evaluated in terms of sepsis evolution and subsequent outcomes. Probiotics enhance the integrity of the intestinal barrier and can modulate intestinal immunity.[9,10] Agudelo-Agudelo-Ochoa *et al.*[11] evaluated the intestinal microbiota in 155 critically ill patients with and without sepsis and its effect on patients outcomes. They showed a substantial dysbiosis and loss of microbial diversity during ICU stay in patients with sepsis.The dysbiosis is expected given that the gut microbial of sepsis patients are exposed to adverse effects, such as antibiotic administration, altered nutrition delivery, and drastic changes in dietary patterns during lengthened ICU stays.[12] Mahmoodpoor *et al.*[13] in their study on the effect of a probiotic compound (Lactocare®) on the incidence of ventilator associated pnemonia showed a nonsignificant decrease in the mortality and morbidity of critically ill patients. Tang *et al.*[14] evaluated the correlation of gut microbiota with severity markers in COVID-19 patients. They showed that the abundance of butyrate-producing bacteria, such as *Faecalibacterium prausnitzii*, *Clostridium butyricum*, *Clostridium leptum*, and *Eubacterium rectale*, decreased significantly, and this shift in the bacterial community may help discriminate critical patients from general and severe patients.

Based on the previous studies, it seems that the results of probiotic administration for critically ill septic patients remain inconclusive. In this regard, the effect of probiotic supplementation on the gut microbiota in septic patients should be evaluated since the improvement of microbiota by probiotic administration can lead to better clinical outcomes. Moreover, it has been stated in previous studies that performing more probiotic studies using microbiome signatures is needed to characterize critical illness-related dysbiosis and to determine ideal probiotic therapies.[12] So, we decided to evaluate the effect of a probiotic compound containing *Lactobacillus* species (*casei*, *acidophilus*, *rhamnosus*, *bulgaricus*), *Bifidobacterium* species (*breve*, *longum*), and *Streptococcus thermophilus* on the gut microbiota of critically ill patients with sepsis in this study.

Methods

After obtaining approval from the local ethics committee and getting informed consent from patients or their next of kin, 45 critically ill adult patients from the surgical ICUs of two university-affiliated hospitals in northwest Iran were enrolled in this pilot randomized controlled trial from September 2018 to October 2020. Figure 1 shows the flow diagram of the study. Inclusion criteria were all adult patients admitted to two university-affiliated ICUs with the diagnosis of sepsis and fed enterally. The sepsis criterion to discern the study groups was adopted according to the Third International Consensus Definitions for Sepsis and Septic Shock.[15] Exclusion criteria were pregnancy, breastfeeding, age less than 18 or more than 70 years old, previous history of probiotic and corticosteroid use, and surgical operation during the past one month. Patients were randomly assigned into two groups by block randomization: Lactocare and control groups. Initially, the blocks (n=4) with different arrangements of A and B were defined. Considering the different probable arrangements of A and B, blocks were numbered from 1 to 6. To enroll initial 4 patients into the study, one of the arrangements

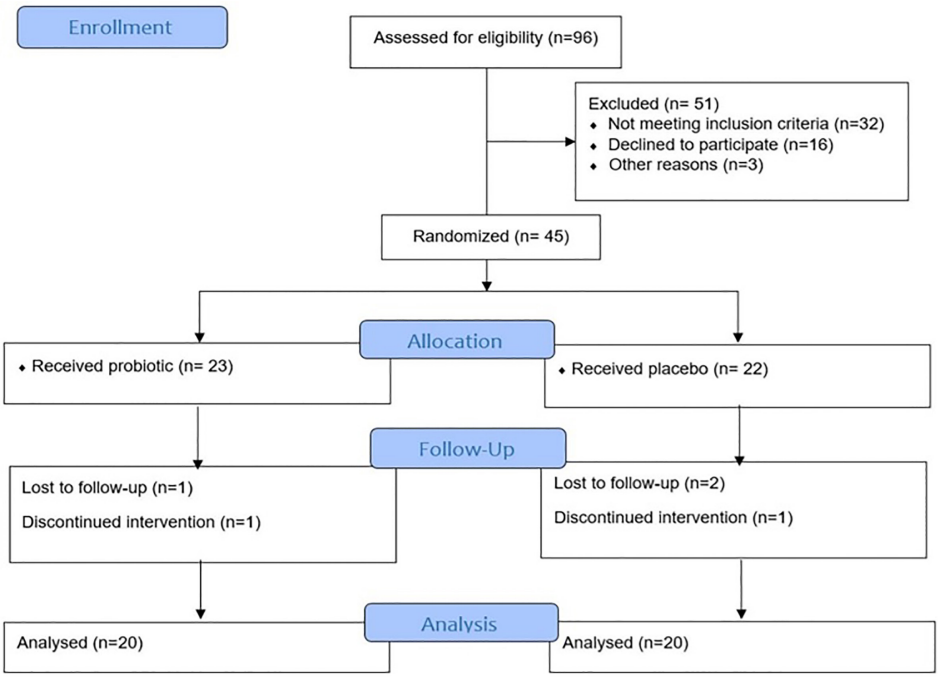

Figure1. CONSORT flow diagram of the study.

was selected using the random digit table, and the patients were assigned into the A and B groups accordingly. For the following four patients, the arrangement pattern was selected again, and the patients were assigned to the groups; this cycle was repeated to achieve the intended sample size. The unpredictability of assignment and balancing the number of patients across the two groups during or at the end of the study are the main advantages of this method. Numbered opaque envelopes were used for allocation concealment. Treatment allocation was masked from patients, health-care providers, study personnel, and study statisticians. Patients in both groups received routine and standard treatment for critically ill patients consisting of stress ulcer and deep vein thrombosis prophylaxis, enteral nutrition with 25 kcal/kg calories (standard formula with 1 kcal/mL; Ensure; Abbott Laboratories, Zwolle, Netherlands), and sedation with the target of Richmond Agitation-Sedation Scale (RASS) between -1 and 2 for those under mechanical ventilation.[16] Patients in the Lactocare group received two capsules of Lactocare® (Zist-Takhmir, Tehran, Iran) each day (1 capsule/BID) for 10 days via a feeding tube as a separate gavage, not with the formula. Each capsule of Lactocare® contains 10^{10} bacteria consisting of *Lactobacillus* species (*casei*, *acidophilus*, *rhamnosus*, *bulgaricus*), *Bifidobacterium* species (*breve*, *longum*), and *Streptococcus thermophilus*. Patients in the control group received placebo, which consisted of sterile maize starch powder. The probiotic and placebo preparations were visually identical, and both were prepared by the Zist-Takhmir company. Fecal samples were taken from all the patients on days 1 and 10 for determining the gut microbial pattern by plating / count microbial method.[17] Entire fecal samples were taken by the patient's nurse from the diapers with sterile plastic spoons and put in a sterile plastic container with a closing lid. They were immediately sent to the hospital laboratory for microbial investigation. Regarding the culture plate counting method, relevant ranging from 10^{-1} to 10^{-10} were done in triplicates to evaluate the bacterial content on the fecal samples. Of each dilution, 10 µl was spread onto fresh nutrient agar plates and then incubated at 37 °C for 24 h. The total bacteria count of the fecal samples was recorded, and the colony-forming units (CFU)/ml was calculated. Diarrhea was defined as having 3 or more loose or liquid stools per day with stool volume greater than 250 ml/day.[18] Constipation was described as 'failure of the bowel to open for three consecutive days.[19]

The primary outcome was gut microbial flora, and secondary outcomes were ICU length of stay and mortality. Demographic characteristics of all patients, sequential organ failure assessment (SOFA) score, and acute physiologic and chronic health evaluation (APACHE II) score were noted.[20,21] Three patients in the probiotic group and two patients in the control group did not finish the study, and the analysis was performed with 20 patients in each group.

Data were analyzed with SPSS statistical package version 22.0. Results are expressed as frequency and mean± standard deviation. For determining the normal distribution of the data, Shapiro-Wilk test was used. For data analysis, Fisher's exact test, chi-squared, and t-test were used per need. Also for abnormal data, Mann-Whitney U test and Wilcoxon signed-rank test were used. P <0.05 was considered as a significant level.

Results

Forty patients diagnosed with sepsis were finally assessed in this pilot study. Demographic and clinical characteristics of patients are shown in Table 1. There was not a significant difference between the two groups regarding age and sex. The mean score for SOFA and APACHE II did not significantly differ between the two groups. Diarrhea was seen in seven patients (35%) in the control group and none of the patients in the Lactocare group, which showed a significant difference (p= 0.008). Constipation was seen in seven patients (35%) in the control group and six patients (30%) in the Lactocare group (p= 0.736). The cultures results showed a significant difference regarding infection with multidrug-resistant microorganisms (i.e. *Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus* , *Acinetobacter baumannii*) between two groups, which was in favor of the Lactocare group (p= 0.02). The mean ICU length of stay was 12 days in the Lactocare group versus 13 days in the control group (p= 0.289). The mortality rate was 30% in the control group compared to 20% in the Lactocare group (p= 0.465).

Regarding gut microbial flora on the first day of the study, the mean colony counts of *C. Butyricum* and *Lactobacillus* were significantly lower in the Lactocare group compared to the control group. Regarding the gut microbiota on the 10^{th} day, the results showed that the colony count of all flora was significantly higher compared to the control group (Table 2). Regarding the microbial changes between day 1 and day 10 between the two groups, a higher number of all microbial flora was seen in the Lactocare group except for *Bifidobacter*, *Atopobium*, and *Enterobacteriaceae* (Figure 2). Intragroup changes in two groups between day 1 and day 10 showed that all microbial flora was altered significantly during the study period and the number of microbial flora significantly decreased in the control group and significantly increased in the Lactocare group. (Table 2).

Discussion

This pilot study showed that administration of a probiotic compound containing *Lactobacillus* species (*casei*, *acidophilus*, *rhamnosus*, *bulgaricus*), *Bifidobacterium* species (*breve*, *longum*), and *Streptococcus thermophilus* had a positive effect on the amount of gut microflora in critically ill septic patients.

Substances from dietary intake like glutamate, histidine, and dietary fiber can be converted to bioactive compounds (histamine, gamma-aminobutyric acid, and short-chain fatty acids) by gut microbiota, having effects

Table 1. Demographic characteristics of patients.

Variable		Groups		P - Value¥
		Lactocare, n = 20 Frequency (percent)	Control, n = 20 Frequency (percent)	
Sex	Male	12 (60)	11 (55)	0.749¥
	Female	8 (40)	9 (45)	
Age		58.35 ± 8.74	60.1 ± 8.73	0.530©
Diagnostic	Pneumonia	6 (30)	5 (25)	1˙
	Catheter infection	2 (10)	3 (15)	
	Meningitis	2 (10)	3 (15)	
	UTI	5 (25)	6 (30)	
	Cellulite	3 (15)	3 (15)	
	Arteritis	1 (5)	0 (0)	
	Osteomyelitis	1 (5)	0 (0)	
SOFA		13 (14.75 – 12)€	13.5 (14.75 – 12)€	0.883ł
APACHE		24.30 ± 5.20£	27.80 ± 3.98£	0.022©
Duration of hospitalization in the ICU		12 (13.75 – 10.25)€	13 (16.25 – 11.25)€	0.289ł
Diarrhea	Yes	0 (0)	7 (35)	0.008˙
	No	20 (100)	13 (65)	
Constipation	Yes	6 (30)	7 (35)	0.736¥
	No	14 (70)	13 (65)	
Infection	Yes	0 (0)	0 (0)	---
	No	100 (100)	100 (100)	
Use of antibiotics	Vancomycin	10 (50)	9 (45)	0.752¥
	Carbapanem	8 (40)	11 (55)	0.342¥
	Floroquinolon	9 (45)	8 (40)	0.749¥
	Aminoglycosid	4 (20)	4 (20)	1˙
	Cephalosporin	8 (40)	5 (25)	0.311¥
	Piperacillin tazobactam	1 (5)	3 (15)	0.605˙
	Colistine	3 (15)	3 (15)	1˙
	Clindamycin	1 (5)	1 (5)	1˙
	Linesolide	4 (20)	6 (30)	0.465¥
Mortality	Yes	4 (20)	6 (30)	0.465¥
	No	16 (80)	14 (70)	

¥Chi –square test; €Median (Interquartile); ˙Fisher Exact test; łMann-Whitney U-test; £ Mean ± SD; ©T-student test; SOFA: Sequential Organ Failure Assessment,; APACHE II: Acute Physiology and Chronic Health Evaluation II

Table 2. Comparison of gut microbiota between two groups.

Measures		Group		P-Value
		Lactocare, n = 20 Frequency (range)	Control, n = 20 Frequency (range)	
First day	*Lactobacilus*	2150 (1625-2500)	2250 (2000- 4450)	0.045
	Bifidobacter	445000 (382500-470000)	450000 (332500-560000)	0.355
	F. prausnitzii	15500 (12000-18000)	16500 (30000 -15000)	0.090
	C. butyricum	23500 (19000-26750)	25500 (23250-38000)	0.040
	C. leptum	662000 ± 43961.7	678500 ± 108204.2	0.533
	E. rectale	8550 (8100-8700)	8400 (7600-9100)	1
	Enterobateriaceae	46000 (41250-48000)	42000 (37250-48750)	0.289
	Atopobium	8250 (8325-9075)	7900 (2000-8800)	0.065
After 10 days	*Lactobacilus*	838 (712.5-972.5)	172 (127.5-197.5)	<0.0001
	Bifidobacter	20500 (11000-46750)	9500(8300-11000)	<0.0001
	F. prausnitzii	1850 (1500-1850)	225 (181.25-270)	<0.0001
	C. butyricum	3000 (1572-7975)	850 (625-1000)	<0.0001
	C. leptum	49515 ± 32820.1	1124.5 ± 400.2	<0.0001
	E. rectale	1200 (905-1572)	70 (50-75)	<0.0001
	Enterobateriaceae	2500 (1350-7500)	190 (172.50-242)	<0.0001
	Atopobium	1450 (925-2100)	175 (160-207.5)	<0.0001

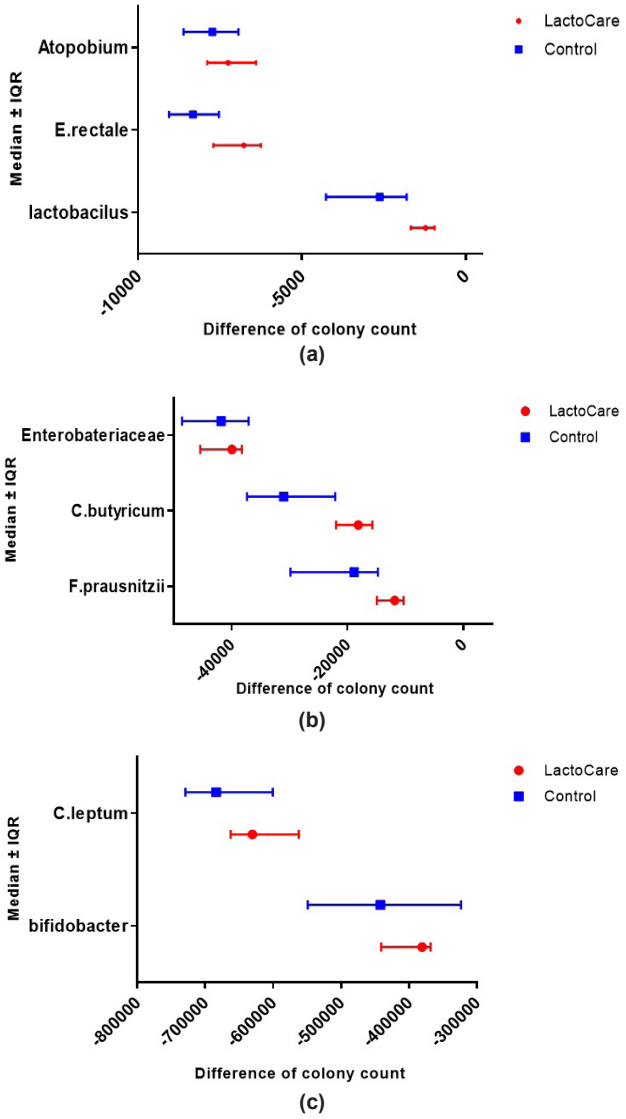

Figure 2. Microbioal flora a) *Lactobacilus*, *E. Rectale* and *Atopobium, b) Enterobacteriaceae*, *C. Butyricum* and *F. Prausnitzii*) and c) *Bifidobacter* and *C. Leptum,* between two groups during the study. IQR: Interquartile range.

on immnomodulation, nutrition, epithelial maintenance, and pain perception.[22] The homeostasis between gut microbiota and the host immune system is impaired by sepsis, antibiotic use, and inflammation.[23,24] There were significant changes in the intestinal microbiota in our study of septic patients, including an increase in the number of *Lactobacillus*, *Bifidobacterium*, *C. Butolynum*, and a decrease of opportunistic pathogens such as *C. leptum*, *E. rectale*, and *Enterobacteriaceae* which are involved in disease severity. Opportunistic pathogens can enter the bloodstream through the intestinal barrier and cause an infection or increase the disease severity.[25,26] Cernada *et al.*[27] showed that sepsis in preterm infants could induce inflammation in the gastrointestinal tract and changes in gut microbiota. They also support the relevance of oxidative stress in sepsis, which may cause the reduction of *Bifidobacterium* spp. and other beneficial anaerobes in the gut lumen.They hypothesized the potential role of human breast milk acting as a "gut protector" and antioxidant.[28]

Our results showed a significant decrease in the incidence of gastrointestinal complications like diarrhea after probiotic administration, which is similar to previous studies and emphasizes the effect of probiotics on the gut epithelium function and permeability.[29] Our results also showed a decrease in the incidence of infection with multidrug-resistant microorganisms with probiotic use, which is very important in this population. Kwon *et al.*[30] could not show a significant difference in the emergence of multidrug-resistant organisms with probiotic usage. These results may be due to the type of probiotic used by them (only one species), the dosage of the drug, and different populations with lower severity of the disease. The mortality and ICU length of stay did not significantly differ between the two groups in our study. A meta-analysis conducted by Barraud *et al.*[31] reported that the administration of probiotics in critically ill patients did not significantly reduce ICU mortality but could reduce ICU length of stay.Nevertheless, various clinical trials analyzed in this study had shown different results in this regard. However, to achieve desirable results in these clinical outcomes, a longer duration of probiotic administration might be needed.

This study has some limitations. First, it was a pilot RCT with small sample size; this sample may not fully represent the ICU patient population. Second, for severe and critical patients, antibiotic use was high because of the need to prevent and control secondary infections, which can interfere with the results of the study. Third, the duration of the study was only 10 days. However, previous studies showed a significant difference between the two groups after 4 days and suggested that 7 days may be sufficient to see the effects of probiotics.[32-34] Fourth, we used the plating/count microbial method. Although it has some advantages, such as being convenient and repeatable, non-invasive, and inexpensive, it cannot accurately reveal gut microbiota changes and uneven distribution of bacteria within feces result in bias when homogenizing fecal samples.

Microbiota-targeted therapies in the early stay may help to decrease the disease severity but have not currently received enough attention in the acute setting for critically ill patients. New types of probiotics or medicinal compounds derived from the microbiome may be used as future strategies to promote health, prevent disease, and treat different disorders. It seems that OMICs can help to define the interactions between probiotics, gut microbiota, and the mammalian gastrointestinal tract. Modulating the gut microbiota by using probiotics constitutes a future perspective for the development of either nutritional or pharmaceutical tools to maintain health in critically ill patients.

Conclusion

This study showed a significant effect of the probiotic compound, Lactocare®, on the gut microbiota in critically ill

septic patients as it decreased the number of opportunistic pathogens. However, additional clinical research is needed to translate research into clinical practice to refine the clinical indication of the specific probiotic strain.

Ethical Issues

The study started after obtaining approval from the ethics committee of Tabriz University of Medical Sciences and getting informed consent from patients or their next of kin. This trial was registered in Iranian Registry of Clinical Trials (IRCT) (IRCT20091012002582N19, www.IRCT.ir).

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We appreciate the ICU staff that committed to the achievement of this study and agreed to support the study.

Data Sharing

Applicants can obtain data by contacting the corresponding author.

Author Contributions

AM and SS designed, directed and supervised the study. RG, KS, and SS conducted the study and collected the data. AM and SR performed data analysis. SS, SR, AS drafted and revised the manuscript. All authors read and confirmed the manuscript. All authors read and approved the manuscript.

Conflict of Interest

The authors have declared that they have no conflicts of interest.

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