Cássia Righy Shinotsuka[1], Marta Ribeiro Alexandre[2], Cid Marcos Nascimento David[3]

Enteral nutritional therapy with pre, pro and symbiotic and gastrointestinal tract and inferior airway colonization in mechanically ventilated patients

Terapia nutricional enteral associada à pré, pró e simbióticos e colonização do trato gastrintestinal e vias aéreas inferiores de pacientes ventilados mecanicamente

1. Master, Physician from the Neuro-Intensive Care Unit of the Hospital Copa D'Or, Rio de Janeiro (RJ), Brazil.
2. Physician from the Clinical Unit of the Hospital Copa D'Or, Rio de Janeiro (RJ), Brazil.
3. PhD, Adjunct Professor from the Faculdade de Medicina of the Universidade Federal do Rio de Janeiro - UFRJ and Phycisian from the Hospital Universitário Clementino Fraga Filho – UFRJ, Rio de Janeiro (RJ), Brazil.

Received from the Intensive Care Unit of the Hospital Universitário Clementino Fraga Filho (HUCFF) da Universidade Federal do Rio de Janeiro (RJ), Brazil.

Submitted on May 15, 2008
Accepted on July 5, 2008

Address for correspondence:
Cássia Righy Shinotsuka
R. Figueiredo Magalhães, 875 – Copacabana
22031-010 Rio de Janeiro (RJ), Brazil.
Phone (21) 2545-3412
Email: cassiarighy@gmail.com

ABSTRACT

Objectives: Sepsis is the main cause of death in the intensive care unit. New preventive measures for nosocomial infections have been researched, such as pre, pro and symbiotic usage, due to its immunoregulatory properties. The objective was to evaluate the effect of administration of pre, pro and symbiotic on gastrointestinal and inferior airway colonization and on nosocomial infections, particularly ventilator-associated pneumonia.

Methods: Patients who were admitted to the intensive care unit at Hospital Universitário Clementino Fraga Filho between November 2004 and September 2006 and mechanically ventilated were randomized in one of four groups: control (n = 16), prebiotic (n = 10), probiotic (n = 12) or symbiotic (n = 11). Treatment was administered for fourteen days. Outcomes measured were: a) Colonization of the gastrointestinal tract and trachea; b) incidence of nosocomial infections, particularly ventilator associated pneumonia; c) duration of mechanical ventilation, length of stay in the intensive care unit, duration of hospitalization, mortality rates, and d) development of organ dysfunction.

Results: Forty-nine patients were evaluated. intensive care unit's mortality was 34% and in-hospital mortality was 53%, APACHE II median was 20 (13 -25). The groups were matched at admission. There was no difference between the groups in relation to the incidence of ventilator associated pneumonia or nosocomial infection. There was a non-significant increase in the proportion of enterobacteria in the trachea at the seventh day in the pre and probiotic groups compared to control. There was a non-significant decrease in the number of bacteria found in the stomach in the pre, pro and symbiotic group at day 7. No significant difference, in regards to the remaining measured parameters, could be found.

Conclusions: Probiotic therapy was not efficient in the prevention of nosocomial infection but there was a tendency to reduction in tracheal colonization by non-fermenting bacteria.

Keywords: Nutritional therapy; Enteral nutrition; Gastrointestinal tract/metabolism; Pneumonia, ventilator-associated; Probiotics/therapeutic use

INTRODUCTION

Sepsis and its complications are the main cause of death in intensive care units (ICU). It is considered an extremely severe and high cost condition (5 to 16.7 billion dollars per year in the United States of America).[1] The increase of sepsis cases over the years has led to increased consumption of antibiotics and, as a consequence, to appearance of multiresistant bacterial strains.[2] Recently new forms for prevention of nosocomial infections and antimicrobial treatment

such as selective decontamination of the gastrointestinal tract[3] and use of pre, pro and symbiotics have been researched as an alternative to use of antibiotics.

Probiotics have been studied in a wide range of situations: treatment of acute infectious diarrhea in childhood[4], prevention of Crohn disease recurrence[5] and prevention and treatment of diarrhea associated to use of antibiotics.[6] However, for ICU patients, the major advantage of using probiotics seems to be their possible capability of replacing the pathogenic microbiota by commensal bacteria[7] and their interaction with the immune system.[8]

Several studies were carried out in critically ill patients with conflicting results.[9-12] There have been many explanations for the differences found among results ranging from selection of patients (with positive results in surgical patients), start time of probiotics administration, to the type of probiotic used. Because of such doubts, use of probiotics in the intensive care has been subject to questioning, while waiting for randomized clinical trials.[13]

The objective of this study was to assess the effect of administering pre, pro and symbiotics in gastrointestinal and lower airways colonization of critically ill patients under mechanical ventilation, also the incidence of nosocomial infections.

METHODS

The study was designed in accordance with the Guidelines and Standards Regulating Research Involving Human Beings (Resolution196/1996 of the National Health Council and was approved by the Ethics Committee of Hospital Universitário Clementino Fraga Filho (HUCFF).

By means of a prospective, controlled, randomized and open study, 50 consecutive patients on mechanical ventilation admitted at the ICU of HUCFF, randomly allocated in four groups according to a table, were assessed. Study protocol is described in figure 1.

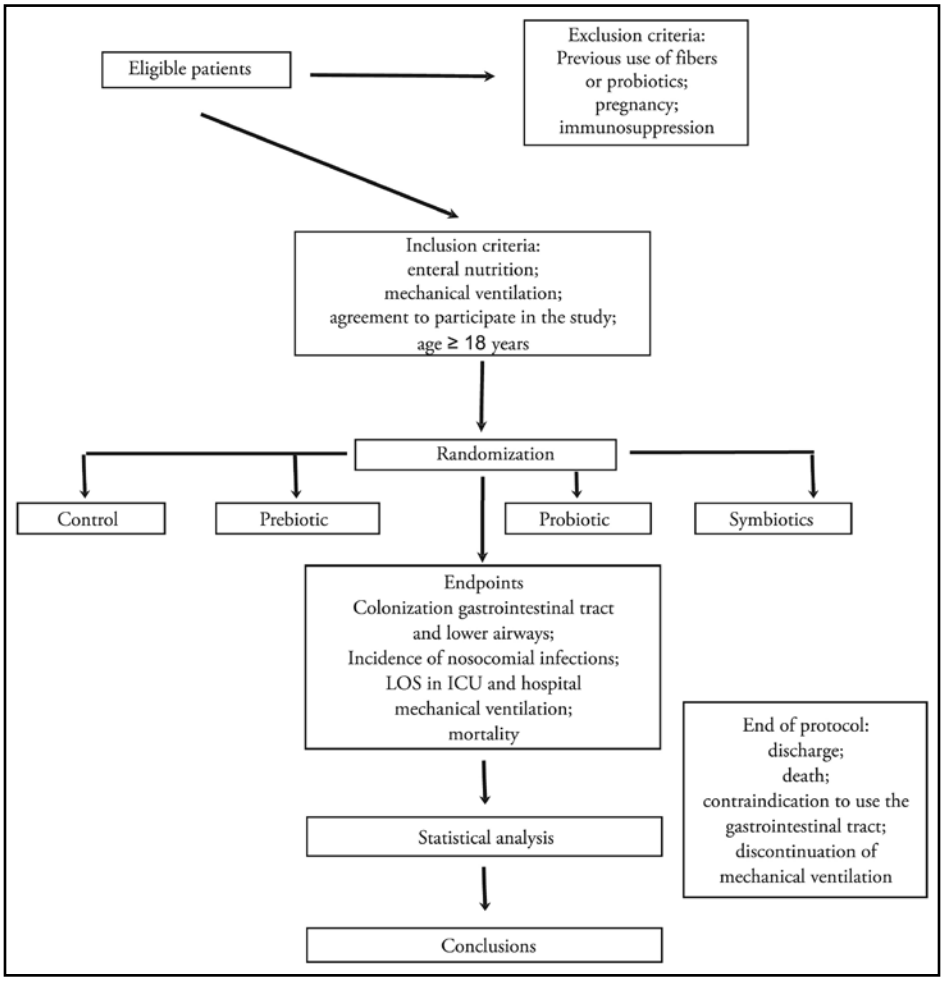

Figure 1 – Study design of intensive care unit patients.
ICU – intensive care unit, LOS – lenght of stay

Group 1 – Control: administration of normocaloric diet (1kcal/mL) polymer, iso-osmolar (315 mOsm/kg) and with 40 g/L of protein in an open system through a nasoenteric tube placed in gastric position.

Group 2 – Prebiotic: administration of the control diet adding a soybean polysaccharide (14g/L);

Group 3 – Probiotic: administration of control diet adding to the probiotic lactobacillus johnsonii La1 in a 10^9 UFC dose, two times a day;

Group 4 – Symbiotic: administration of the control diet, adding a soybean polysaccharide and the probiotic in the mentioned doses.

Lactobacilli containers were stored for no longer than one month in the ICU refrigerator, following the validity date on the packaging. Containers were taken out at the time of administration and their content aspirated into a 10mL syringe, then administered to the patients by nasoenteric catheter.

Inclusion criteria were: use of enteral nutrition; be under mechanical ventilation; age > 18 years; acceptance to participate in the study by relatives.

Exclusion criteria were: use of fibers or probiotics in the last month; presence of clinically significant immunosuppression (organ transplant, chemotherapy, active hematologic neoplasia, use of corticoids in a dose higher than or equivalent to 1 mg/kg of prednisone in the last three months, acquired immunodeficiency syndrome (AIDS), use of immunosuppressants) and pregnancy.

Criteria for study withdrawn were: impossibility to use the gastrointestinal tract (defined as use of enteral nutrition without reaching 50% of the total energetic value (TEV) or use of parenteral nutrition); discharge from the ICU, death; discontinuation from mechanical ventilation.

As the primary endpoint, colonization of the gastrointestinal tract and trachea by aerobic pathogenic bacteria was assessed upon admission, 7th and 14th day of study. For this purpose, quantitative cultures were collected for analysis of aerobic and anaerobic facultative bacteria of the tracheal secretion, gastric secretion and rectal swab, upon admission, 7th and 14th day of study. Culture samples were collected after a morning interruption of the diet for about three hours, for routine nursing procedures. However, it was not possible to collect samples for microbiological analysis of all patients at all times of the study for a number of reasons, that is to say: removed from study due to discharge, death or discontinuation of mechanical ventilation, impossibility for enteral nutrition, lack of an adequate amount of gastric or tracheal secretion for culture, among others.

As secondary endpoints, incidence of nosocomial infections up to 30 days after admission to the study was assessed, as well as number of days with antibiotic therapy, number of days on mechanical ventilation, length of stay (LOS) in ICU and hospital, in addition to discharge from ICU and hospital.

Furthermore, evolution of organ dysfunction in the first 14 days of admission to the study was analyzed applying the Sequential Organ Failure Assessment (SOFA) score and analysis of the delta SOFA in the first 48 and 72 hours after admission to the study.

Information on the frequency of nosocomial infections developed in the ICU up to one month after inclusion in the study and the causative pathogens was collected in addition to information about use (type, time and doses) of antibiotic therapy. All patients were evaluated according to their severity by the Acute Physiology and Chronic Health Evaluation II (APACHE II)[14] score and Simplified Acute Physiology Score II (SAPS II)[15] upon ICU admission and by applying the SOFA score on the 1st, 2nd, 7th, 10th and 14th day of ICU stay. Comorbidities were assessed using the Charlson index. Infections were determined according to the definitions of the International Sepsis Forum[16], except for nosocomial sinusitis.

All patients were using H_2 receptor blocker or proton pump inhibitor during the entire study period.

Calculation of the sample size was carried out based upon a previous study with critically ill patients [12]. It was estimated that 32 patients were needed in each group to detect an 85% to 50% reduction of gastric colonization with a significant level of 5% and a power of 80%. For this purpose the statistical software Epiinfo® version 3.4.1 was used.

Data were stored in electronic spreadsheets (Microsoft Excel®) and later analyzed with the statistical package SPSS 13.0 (SPSS Inc.). Data were presented as median and interquartile interval. Categorical variables were analyzed by tables crossed with the statistical chi-square test (with Yates continuity correction whenever indicated) or the Fisher exact test. Mann-Whitney test rank sum was used for

comparison of two categorical and non-parametric continuous variables and that of Kruskall-Wallis, for three or more variables. For statistical significance a $p<0.05$ value was adopted.

RESULTS

The study did not achieve the expected, planned sample of 128 patients due to low inclusion rate. From November 2004 to August 2006, 50 patients were included in the study. One patient of the symbiotic group was excluded because of consent withdrawal, therefore 49 patients were analyzed. Distribution among the 4 groups was of 16 patients in the control group (Group 1), 10 in the prebiotic (Group 2) 12 in the probiotic (Group 3) and 11 in the symbiotic (Group 4). Mean age was 59 years (46-71) while distribution by gender was 31 (63.3%) males. APACHE II score was 20 (13-25) points, SAPS II was 36 (27-54) points, SOFA on the first day (D1) was 5 (2-8), on the second (D2) was 4 (3-7) and on the third day (D3) 4 (2-7). There was no difference between groups regarding admission characteristics. There were no differences between groups regarding measurement of the delta SOFA on day 1 to day 2 and from day 1 to day 3.

Median of the Charlson index was 3 (1-5) with no difference between groups. The major cause for admission was clinical (37 patients-76%), followed by emergency surgery (7 patients -14%) and finally elective surgeries (5 patients-10%). Hospital LOS was essentially attributed to the neurological system (19 patients-38%), followed by the respiratory (12 patients -24%) and, finally the cardiovascular (9 patients-18%). Demographic data is shown on table 1.

There was no difference between groups regarding the proportion of enterobacteria e non-fermenting bacteria, in the collected samples of rectal swab, gastric secretion and tracheal secretion at admission, 7^{th} and 14^{th} days. In the samples collected from the rectal swab and gastric secretion a prevalence of enterobacteria was found which does not vary over time, while the proportion of enterobacteria and non-fermenting bacteria are balanced in the tracheal secretion sample. Distribution of microbiological samples, on the 7^{th} and 14^{th} days is shown on table 2. There was a non-significant increase in the proportion of enterobacteria in relation to the non-fermenting on the 7^{th} day in the tracheal secretion in the pre and probiotic groups. There was also a non-significant decrease of bacteria isolation and a consequent proportional increase of negative samples of gastric secretion on the 7^{th} day in pre, pro and symbiotic groups when compared to the control group.

There were no differences between groups regarding the number of nosocomial infections (37 episodes) and of mechanical ventilation associated pneumonias (VAP) 20 episodes) as well as time of antibiotic therapy (median of 8 days) (0-11). Principal causes of infectious complications were VAP (20 patients- 35%), infection associated to the catheter (7 patients - 13%) nosocomial sinusitis (4 patients -7%) and primary bacteremia (4 patients -7%). No changes were found between groups regarding number of bacteremias.

Length of stay in the UTI was 24 days (11 to 35) and hospital LOS was 41 days (28 to 60). Time of mechanical ventilation was 20 days (10 – 31). There was no difference between groups regarding these parameters. In the ICU, mortality was 34% and intra-hospital was 53%

No adverse effects related to use of Lactobacillus johnsonii were detected nor any infectious episode/ bacteremia caused by this agent.

Table 1 – Patient characteristics

	Control (n = 16)	Prebiotic (n = 10)	Probiotic (n = 12)	Symbiotic (n = 11)	p value
Male gender	7 (43%)	6 (60%)	9 (75%)	9 (81%)	0.17
Age (years)	60 (32-76)	60 (43-71)	58 (47-71)	59 (52-69)	0.98
APACHE II	20 (12-25)	21 (14-25)	17 (10-26)	18 (15-22)	0.72
SAPS II	34 (28-56)	47 (35-62)	32 (26-46)	43 (24-49)	0.50
Charlson	3 (2-6)	4 (2-6)	2 (0-4)	3 (1-4)	0.39

APACHE – Acute Physiology and Chronic Health Evaluation score, SAPS - Simplified Acute Physiology Score. Results are expressed in median (interquartil), except for gender expressed in number (%)

Table 2 - Colonization of the gastrointestinal tract and lower airways upon admission, seventh and fourteenth day

Admission	Tracheal Secretion				Gastric Secretion				Rectal Swab			
	C	Pre	Pro	Sym	C	Pre	Pro	Sym	C	Pre	Pro	Sym
Enterobacteria	1	5	4	4	10	1	5	7	17	8	11	10
Non fermenting	4	4	3	2	0	0	0	1	1	0	0	1
p value	-	NS	NS	NS	-	NS	NS	NS	-	NS	NS	NS
With no growth	4	1	3	2	5	6	4	1	1	1	1	2
One bacterial species	5	7	7	8	9	2	6	9	15	8	9	8
Múltiple bacterial species	1	2	2	2	1	0	0	1	3	1	2	3
p-value	-	NS	NS	NS	-	NS	NS	NS	-	NS	NS	NS
Collected samples	10	10	12	12	15	8	10	11	19	10	12	13
Seventh Day	Tracheal Secretion				Gastric Secretion				Rectal Swab			
	C	Pre	Pro	Sym	C	Pre	Pro	Sym	C	Pre	Pro	Sym
Enterobacteria	3	5	4	0	4	6	5	1	9	8	6	3
Non fermenting	5	2	2	1	1	0	0	1	3	1	2	2
p value	-	NS	NS	NS	-	NS	NS	NS	-	NS	NS	NS
With no growth	1	0	1	2	1	2	4	2	1	1	0	0
One bacterial species	8	6	6	2	5	4	4	3	9	6	8	5
Multiple bacterial species	0	1	0	0	0	2	1	0	3	3	0	1
p value	-	NS	NS	NS	-	NS	NS	NS	-	NS	NS	NS
Collected samples	9	7	7	4	6	8	9	5	13	10	8	6
Fourteenth Day	Tracheal Secretion				Gastric Secretion				Rectal Swab			
	C	Pre	Pro	Sym	C	Pre	Pro	Sym	C	Pre	Pro	Sym
Enterobacteria	2	2	2	1	3	4	3	3	2	5	5	3
Non fermenting	1	2	0	2	0	0	0	0	0	1	0	0
p value	-	NS	NS	NS	-	NS	NS	NS	-	NS	NS	NS
With no growth	1	0	1	0	1	1	1	0	2	1	0	0
One bacterial species	3	4	2	3	3	4	2	3	2	4	4	3
Multiple bacterial species	0	0	0	0	0	1	1	1	0	2	1	0
p value	-	NS	NS	NS	-	NS	NS	NS	-	NS	NS	NS
Collected samples	4	4	3	3	4	6	3	4	4	7	5	3

C - control, Pre - prebiotic, Pro - probiotic, Sym - symbiotic, NS - not-significant. Numbers represent the absolute values of positive samples collected in the gastrointestinal tract and in the lower airways from the different groups. P values refers to the comparison between the different groups and control group

DISCUSSION

The current study disclosed a non-significant decrease in isolation of bacteria of the gastric content in seven days and a non-significant increase in the proportion of enterobacteria in relation to the non- fermenting on the 7th day in tracheal secretion. Some factors may have contributed for this finding. A study limitation was that the sample planning of 32 patients in each group was not achieved, jeopardizing conclusions reached by the work. As such, this study did not have the power to reach the proposed conclusions and was only capable of pointing out given tendencies that may or may not be confirmed in larger prospective studies.

Another issue seems to be survival of lactobacilli in the gastrointestinal tract. Most probiotics marketed do not seem able to survive to pH and osmolarity variations in sufficient numbers to repopulate the digestive tract lumen. Miettinen et al. showed that, when various strains of lactobacilli were given orally in a concentration of 10^9 ufc/mL, only the strain L plantarum was retrieved upon ileostomy of studied patients in a greater than 10^7 ufc/mL concentration, although non-viable lactobacilli retained their capacity to stimulate the immune system.[17].

Traditionally the gastrointestinal tract microbiota has been analyzed by culture methods. However, it is known that a large part of the gastrointestinal tract microbiota is difficult to cultivate because the growth factors, physiological state and interaction with other components of the microbiota remain unknown. Previous studies suggest

that 60% to 80% of the gastrointestinal tract microbiota have not yet been identified[18] and most of these studies were carried out using molecular techniques for detection, identification and classification of the bacteria based on the nucleotide sequence of the 16S subunit of the rRNA.[19] The current study presented a limitation in the method because it did not use molecular methods to characterize microbial populations inside the digestive tract, as well as to ensure presence and viability of the lactobacilli administered to patients. However, the viability and adherence of the bacilli Lactobacillus johnsonii La1 to the intestinal epithelium has already been described in healthy volunteers, which theoretically would enable their use as probiotic.[20] To date the same description in critically ill patients does not exist.

Another issue to be addressed is probiotic capacity to recolonize the gastrointestinal tract in the presence of systemic antibiotic therapy. Data found in literature are conflicting, Sullivan et al. studied 24 healthy volunteers that used clindamicine for 7 days and were randomly allocated to receive a combination of L acidophilus NCFB 1748, B lactis Bb 12 and L paracasei paracasei F19 (Arla Foods, Stockholm, Sweden) in a dose of 10^8 ufc/mL during 14 days. These researchers found that the number of bacterioids in the probiotic group was maintained, while it decreased in the placebo group[21], which was contested by other authors.[22] Manley et al. in a recent study showed that the enterococcus resistant to vancomicine was eradicated from the rectal swab of interned patients who had ingested L. rhamnosus GG[7], even if they were using systemic antibiotic therapy.

It is noteworthy that the different species of probiotics presented different characteristics regarding their viability in the gastrointestinal tract, interaction with the microbiota, fiber fermentation and immunomodulating effect. Viability of the L plantarum and of L johnsonii La1 has been formerly discussed. Müller et al. studied 712 strains of lactobacilli and concluded that only four were able to ferment the inulin fiber: L plantarum (various strains), L. paracasei paracasei, L. brevis and Pediococcus pentosaceus [23]. Various studies showed that L johnsonii La1 has antibacterial[24] and immunomodulating[25] activities in healthy volunteers, while their role in critically ill patients is not well defined.

However, actions of probiotics are not restricted to substitution of the pathogenic microbiota. Even without a significant change in colonization of the gastrointestinal tract, use of probiotics has an immune regulatory function[10] which, ultimately may lead to prevention of nosocomial infections in the critically ill patient.[26] Nevertheless, other studies were not able to show differences in relation to nosocomial infection or time of antibiotic therapy.[18] A recent study carried out in a critically ill pediatric population, not only failed to show a difference regarding the number of infections, but also disclosed a non-significant increase of their incidence in the group using probiotics.[27] A possible explanation for this divergence is that the majority of positive studies, that is to say, those that showed benefit, were performed with patients at postoperative of major surgeries[12] whereas negative studies were made with clinical patients.[10] In surgical patients, time of wound and therefore, the best timing for immunomodulation therapy is well known, while this is not true with clinical patients. Such a dichotomy was already perceived in other studies with critically ill patients, for instance regarding glycemic control in clinical and surgical patients.[28,29]

There is a great controversy in literature about the capability of commonly commercialized probiotics to replace pathogenic microbiota of critically ill patients and to act positively in the prevention of infection and multiple organ dysfunction. Results of this study suggest a decreased colonization of the lower airways by non-fermenting bacteria, which may lead to fewer nosocomial infections. For this reason it is suggested that this study be expanded with an analysis of the microbiota by molecular biology techniques for diagnosis as well as with a larger number of patients.

CONCLUSION

In critically ill patients submitted to mechanical ventilation, administration of pre, pro and symbiotics did not efficiently reduce incidence of nosocomial infections, VAP, LOS in ICU or hospital, or mortality. However, our data point to a tendency of bacterial reduction in the stomach and in the proportion of non-fermenting bacteria in the tracheal secretion of patients who used symbiotics. Substitution of the microbiota may prove beneficial in patients with nosocomial infection. New clinical trials will therefore be required to better define the role of probiotic therapy in the prevention and treatment of nosocomial infections.

ACNOWLEDGMENTS

Our thanks to all nursing, physiotherapy and nutrition teams of the ICU of HUCFF for their support in the performance of the work.

To Dr. André Miguel Japiassú for the encouragement, incentive and help in the review of the article.

RESUMO

Objetivos: A sepse é a principal causa de morte nas unidades de terapia intensiva. Recentemente, têm sido pesquisadas novas formas de prevenção e tratamento de infecção nosocomial, tais como o uso de pré e pró e simbióticos, devido as suas propriedades imunomoduladoras. O objetivo deste estudo foi avaliar o efeito da administração de pré, pro e simbióticos sobre a colonização de trato gastrintestinal e vias aéreas inferiores e sobre a incidência de infecções nosocomiais, particularmente pneumonia associada à ventilação mecânica.

Métodos: Pacientes em ventilação mecânica, internados na unidade de terapia intensiva do Hospital Universitário Clementino Fraga Filho entre novembro de 2004 e agosto de 2006, foram aleatorizados em quatro grupos: controle (n = 16), prebiótico (n = 10), probiótico (n = 12) e simbiótico (n = 11). O tratamento foi administrado por 14 dias. Foram avaliados: a) colonização do trato gastrintestinal e traquéia; b) incidência de infecções nosocomiais, principalmente pneumonia associada a ventilação mecânica; c) tempo de terapia antibiótica, ventilação mecânica, internação e letalidade na terapia intensiva e hospitalar; d) incidência de disfunções orgânicas.

Resultados: Foram avaliados 49 pacientes. A letalidade na terapia intensiva foi de 34%, intra-hospitalar de 53% e a mediana do APACHE II de 20 (13 - 25). Os grupos foram comparáveis na admissão. Houve aumento não significativo da proporção de enterobactérias em relação à de não fermentadores no sétimo dia na secreção traqueal nos grupos pré e probiótico e diminuição não-significativa do número de amostras no estômago nos grupos pré, pró e simbiótico no sétimo dia. Não houve diferença na incidência de pneumonia associada a ventilação mecânica, infecção nosocomial ou nos demais parâmetros.

Conclusões: O uso de pré, pró e simbióticos não foi eficaz na prevenção de infecções nosocomiais, porém houve uma tendência de redução da colonização da secreção traqueal por bactérias não fermentadoras.

Descritores: Terapia nutricional; Nutrição enteral; Trato gastrointestinal/metabolismo; Pneumonia associada à ventilação mecânica; Probióticos/uso terapêutico

REFERENCES

01. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med. 2001; 29(7):1303-10. Comment in: Crit Care Med. 2001; 29(7):1472-4.
02. Neuhauser MM, Weinstein RA, Rydman R, Danziger LH, Karam G, Quinn JP. Antibiotic resistance among gram-negative bacilli in US intensive care units: implications for fluoroquinolone use. JAMA. 2003; 289(7):885-8.
03. de La Cal MA, Cerdá E, García-Hierro P, van Saene HK, Gómez-Santos D, Negro E, Lorente JA. Survival benefit in critically ill burned patients receiving selective decontamination of the digestive tract: a randomized, placebo-controlled, double-blind trial. Ann Surg. 2005; 241(3):424-30. Comment in: Ann Surg. 2006; 244(2):325-6; author reply 326-7.
04. Canani RB, Cirillo P, Terrin G, Cesarano L, Spagnuolo MI, De Vincenzo A, et al. Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations. BMJ. 2007; 335(7615):340. Comment in: BMJ. 2007;335(7617):414. BMJ. 2007; 335(7617):414. J Pediatr. 2008; 152(1):142.
05. Van Gossum A, Dewit O, Louis E, de Hertogh G, Baert F, Fontaine F, et al. Multicenter randomized-controlled clinical trial of probiotics (Lactobacillus johnsonii, LA1) on early endoscopic recurrence of Crohn's disease after lleocaecal resection. Inflamm Bowel Dis. 2007; 13(2):135-42. Comment in: Inflamm Bowel Dis. 2007; 13(10):1314.
06. Hickson M, D'Souza AL, Muthu N, Rogers TR, Want S, Rajkumar C, Bulpitt CJ. Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. BMJ. 2007; 335(7610):80. Comment in: BMJ. 2007; 335(7612):171. BMJ. 2007; 335(7612):171. BMJ. 2007; 335(7612):171. Evid Based Med. 2008; 13(2):46. Evid Based Nurs. 2008; 11(2):57.
07. Manley KJ, Fraenkel MB, Mayall BC, Power DA. Probiotic treatment of vancomycin-resistant enterococci: a randomised controlled trial. Med J Aust. 2007; 186(9):454-7.
08. Foligne B, Zoumpopoulou G, Dewulf J, Ben Younes A, Chareyre F, Sirard JC, et al. A key role of dendritic cells in probiotic functionality. PLoS ONE. 2007; 2(3):e313.
09. Rayes N, Seehofer D, Hansen S, Boucsein K, Müller AR, Serke S, et al. Early enteral supply of lactobacillus and fiber versus selective bowel decontamination: a controlled trial in liver transplant recipients. Transplantation. 2002; 74(1):123-7.
10. McNaught CE, Woodcock NP, Anderson AD, MacFie J. A prospective randomised trial of probiotics in critically ill patients. Clin Nutr. 2005; 24(2):211-9.
11. Rayes N, Hansen S, Seehofer D, Müller AR, Serke S, Bengmark S, Neuhaus P. Early enteral supply of fiber and Lactobacilli versus conventional nutrition: a controlled trial in patients with major abdominal surgery. Nutrition. 2002; 18(7-8):609-15.
12. Rayes N, Seehofer D, Müller AR, Hansen S, Bengmark S,

Neuhaus P. Influence of probiotics and fibre on the incidence of bacterial infections following major abdominal surgery - results of a prospective trial. Z Gastroenterol. 2002; 40(10):869-76. German.

13. Isakow W, Morrow LE, Kollef MH. Probiotics for preventing and treating nosocomial infections: review of current evidence and recommendations. Chest. 2007;132(1):286-94.
14. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med. 1985; 13(10):818-29.
15. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA. 1993; 270(24):2957-63. Erratum in: JAMA 1994; 271(17):1321.
16. Calandra T, Cohen J; International Sepsis Forum Definition of Infection in the ICU Consensus Conference. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. Crit Care Med. 2005; 33(7):1538-48.
17. Miettinen M, Alander M, von Wright A, Vuopio-Varkila J, Marteau P, Veld JH, Mattila-Sandholm T. The survival of and cytokine induction by lactic acid bacteria after passage through a gastrointestinal model. Microb Ecol Health Dis. 1999; 10(3-4):141-7.
18. Langendijk PS, Schut F, Jansen GJ, Raangs GC, Kamphuis GR, Wilkinson MH, Welling GW. Quantitative fluorescence in situ hybridization of Bifidobacterium spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. Appl Environ Microbiol. 1995; 61(8):3069-75.
19. Muyzer G, Smalla K. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie Van Leeuwenhoek. 1998; 73(1):127-41.
20. Yamano T, Iino H, Takada M, Blum S, Rochat F, Fukushima Y. Improvement of the human intestinal flora by ingestion of the probiotic strain Lactobacillus johnsonii La1. Br J Nutr. 2006; 95(2):303-12.
21. Sullivan A, Barkholt L, Nord CE. Lactobacillus acidophilus, Bifidobacterium lactis and Lactobacillus F19 prevent antibiotic-associated ecological disturbances of Bacteroides fragilis in the intestine. J Antimicrob Chemother. 2003; 52(2):308-11.
22. Jernberg C, Sullivan A, Edlund C, Jansson JK. Monitoring of antibiotic-induced alterations in the human intestinal microflora and detection of probiotic strains by use of terminal restriction fragment length polymorphism. Appl Environ Microbiol. 2005; 71(1):501-6.
23. Müller M, Lier D. Fermentation of fructans by epiphytic lactic acid bacteria. J Appl Bacteriol. 1994; 76(4):406-11.
24. Bernet-Camard MF, Liévin V, Brassart D, Neeser JR, Servin AL, Hudault S. The human Lactobacillus acidophilus strain LA1 secretes a nonbacteriocin antibacterial substance(s) active in vitro and in vivo. Appl Environ Microbiol. 1997; 63(7):2747-53.
25. Link-Amster H, Rochat F, Saudan KY, Mignot O, Aeschlimann JM. Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. FEMS Immunol Med Microbiol. 1994; 10(1):55-63. Erratum in: FEMS Immunol Med Microbiol 1995; 12(3-4):273. FEMS Immunol Med Microbiol 1995; 12(1):83.
26. Spindler-Vesel A, Bengmark S, Vovk I, Cerovic O, Kompan L. Synbiotics, prebiotics, glutamine, or peptide in early enteral nutrition: a randomized study in trauma patients. JPEN J Parenter Enteral Nutr. 2007; 31(2):119-26.
27. Honeycutt TC, El Khashab M, Wardrop RM 3rd, McNeal-Trice K, Honeycutt AL, Christy CG, et al. Probiotic administration and the incidence of nosocomial infection in pediatric intensive care: a randomized placebo-controlled trial. Pediatr Crit Care Med. 2007; 8(5):452-8; quiz 464. Comment in: Pediatr Crit Care Med. 2007; 8(5):499-501.
28. Van den Berghe G, Wilmer A, Hermans G, Meersseman W, Wouters PJ, Milants I, et al. Intensive insulin therapy in the medical ICU. N Engl J Med. 2006; 354(5):449-61.
29. van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, et al. Intensive insulin therapy in the critically ill patients. N Engl J Med. 2001; 345(19):1359-67.