UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS
Programa de Pós-Graduação em Ciência de Alimentos

USE OF PROBIOTICS IN VENTILATOR-ASSOCIATED PNEUMONIA PROPHYLAXIS

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Maringá
2012

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USE OF SYMBIOTICS IN VENTILATOR-ASSOCIATED PNEUMONIA PROPHYLAXIS

Dissertação apresentada ao programa de Pós Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de mestre em Ciência de Alimentos.

Maringá
2012

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BIOGRAFIA

Nestor Alejandro Sainz Rueda nasceu em La Paz, Bolívia. Possui graduação em Medicina pela Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo. Tem título de especialista em Cardiologia pela Sociedade Brasileira de Cardiologia, título de especialista em Terapia Intensiva pela Associação de Medicina Intensiva Brasileira e título de especialista e habilitação em Nutrição Parenteral e Enteral pela Sociedade Brasileira de Nutrição Parenteral Enteral atuando principalmente nos seguintes temas: nutrição enteral e parenteral, probióticos e controle metabólico de pacientes críticos.

Dedico
A minha querida esposa Janaina e ao meu tesouro, Bruno.

AGRADECIMENTOS

À SKL Functional Nutrition e a Healthy Functional Nutrition

Pelo fornecimento dos produtos Fiberfos® e Lactofos® gentilmente doados para este estudo, sem o qual a idéia jamais passaria das pranchetas.

Ao Serviço de Nutrição do Hospital Universitário Regional de Maringá Hospital Universitário

Pela paciência dos meus pedidos e competência no suporte dos pacientes em terapia nutricional.

À assistente social do Hospital Universitário

Pela doação das escovas de dente para possibilitar a higiene oral e administração dos produtos.

À minha esposa e filho

Pelo incomodas horas de minha ausência e pelo incentivo ao desenvolvimento pessoal

À minha orientadora Prof.a. Dra. Jane Martha Graton Mikcha

Pela confiança e orientação

APRESENTAÇÃO

Esta dissertação de mestrado está apresentada na forma de artigo científico

1 Sainz-Rueda NA , Rosa LLB, Mikcha JMG. Use of probiotics in ventilator-associated pneumonia prophylaxis. Submetido ao aceite ao *Journal of Critical Care*. (qualis B1 –área de Ciência de Alimentos).

GENERAL ABSTRACT

INTRODUCTION: Ventilator-associated pneumonia (VAP) is particularly important in the intensive care unit where they increase in morbidity, mortality and cost. **AIMS:** To investigate the effect of a mixture of probiotics and fructooligosaccharides (Lactofos®) on the incidence of ventilator-associated pneumonia in patients in mechanical ventilation compared to a use of fructooligosaccharides (FOS) alone (Fiberfos®) **MATERIAL AND METHODS:** It was a prospective, randomized, double blind, placebo controlled trial performed in a Brazilian teaching hospital. Adult patients expected to be in mechanical ventilation for up to 48 h received probiotics (*Lactobacillus paracasei* Lpc-37, *Lactobacillus rhamnosus* HN001, *Lactobacillus acidophilus* NCFM, *Bifidobacterium lactis* HN019) in gastro-intestinal and oropharyngeal sites plus fibers twice a day or FOS alone. **RESULTS:** The two groups (n=125) were comparable at baseline and received comparables cares except for the use of probiotics. Probiotic group (n=62) was twice less likely to develop VAP than compared to the group that received only prebiotics (41.3% vs. 20.9 %, P = 0.011). Probiotic group also had fewer days of hospitalization (23.1 days vs.18.5 days, P = 0.024). The mortality did not differ significantly between the groups **CONCLUSIONS:** The use of Lactofos® reduced length of hospitalization and ventilator-associated pneumonia incidence compared to Fiberfos® but had no statistically significant impact on mortality.

Key words: Mechanical ventilation, Probiotics, Prevention, Nosocomial infections.

RESUMO GERAL

INTRODUÇÃO: Pneumonia associada à ventilação mecânica (PAV) é particularmente importante na unidade de terapia intensiva onde é responsável pelo aumento na morbidade, mortalidade e nos custos. **OBJETIVOS:** Investigar do efeito de uma mistura de probióticos e frutooligossacarídeos (Lactofos®) na incidência de PAV em pacientes sob ventilação mecânica comparado ao uso isolado de frutooligossacarídeos (Fiberfos®) **MATERIAL E METODOS:** Foi realizado estudo prospectivo, randomizado, duplo-cego, placebo controlado em hospital de ensino brasileiro com pacientes com expectativa de ventilação mecânica por mais de 48 h. Os pacientes receberam, por sonda enteral e na orofaringe, os probióticos (*Lactobacillus paracasei* Lpc-37, *Lactobacillus rhamnosus* HN001, *Lactobacillus acidophilus* NCFM, *Bifidobacterium lactis* HN019) com frutooligossacarídeos duas vezes ao dia ou apenas frutooligossacarideos. **RESULTADOS:** Ambos os grupos (n=125) eram comparáveis nas características basais e receberam cuidados semelhantes na prevenção da PAV exceto pelo uso de probióticos. O grupo que recebeu probiótico (n=62) apresentou duas vezes menos chance de desenvolver PAV comparado com o grupo que recebeu apenas prebióticos (41.3% vs. 20.9 %, P = 0.011). O grupo com probióticos também apresentou menor tempo de hospitalização (23.1 dias vs.18.5 dias, P = 0.024). Não houve redução significativa da mortalidade entre os grupos. **CONCLUSÕES:** O uso de Lactofos® reduziu a incidência de PAV e o tempo de hospitalização comparado ao Fiberfos® mas não apresentou impacto significativo na mortalidade.

Palavras chaves: Ventilação mecânica, Probióticos, Prevenção, Infecções nosocomiais.

Use of probiotics in ventilator-associated pneumonia prophylaxis

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☆ Funding: This study was sponsored by department and university funds. Probiotics and fructooligosaccharides (Lactofos®) and fructooligosaccharides (Fiberfos®) were provided by SKL Functional Nutrition and Healthy Functional Nutrition. None of the authors have conflicts of interest to disclose.

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ABSTRACT

Purpose*:** To investigate the effect of a mixture of probiotics and fructooligosaccharides (FOS) compared with FOS alone on the incidence of ventilator-associated pneumonia (VAP) in patients undergoing mechanical ventilation. ***Design: This was a single-center, prospective, randomized, double-blind, placebo-controlled trial. ***Materials and Methods:*** Adult patients expected to be in mechanical ventilation for at least 48 h received probiotics (*Lactobacillus paracasei* Lpc-37, *Lactobacillus rhamnosus* HN001, *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* HN019) plus FOS or FOS alone twice a day. ***Results*:** The two groups (n=125) were comparable at baseline and received standard ICU care, except for the use of probiotics. The probiotic group (n=62) was half as likely to develop VAP compared with the placebo group (41.3% vs. 20.9%, P = 0.011). The probiotic group also had fewer days of hospitalization (23.1 vs.18.5 days, P = 0.024) and fewer pathogens identified in tracheal aspirates (17 vs. 39, P=0.0001). Mortality did not differ significantly between the two groups. ***Conclusions*:** The use of probiotics plus FOS reduced the length of hospitalization and VAP incidence compared with FOS alone, but it had no statistically significant impact on mortality.

Keywords: Mechanical ventilation, Probiotics, Prevention, Nosocomial infections.

1. Introduction

Intensivists still struggle to reduce nosocomial infections, particularly in patients requiring mechanical ventilation, despite improvements of care in intensive-care units (ICU). Ventilator-associated pneumonia (VAP) occurs in almost one-third of nosocomial infections, and has worse outcomes with longer ICU and hospital stays.[1] Unfortunately, critically ill patients who develop VAP are twice as likely to die compared with similar patients without VAP. [2] Economically, a single case of VAP could cost as high as US$ 40000.00 attributed to an ICU stay up to six days longer and an increase of more than 50% in prescribed antibiotics.[2,3] A high VAP incidence contributes to the rising number of multidrug-resistant pathogens, making VAP an important focus for risk-reduction strategies.[4]

Many strategies have been developed to reduce VAP, based on the course of its pathogenesis involving colonization of the aerodigestive tract by pathogenic bacteria, development of biofilms and microaspiration of contaminated secretions.[5] Such strategies usually are implemented in care bundles, and the effectiveness of each individual action is currently under investigation.[6,7]

A promising strategy for VAP prevention, using probiotics to maintain the aerodigestive microbial balance has been evaluated. Probiotics are defined by the Food and Agriculture Organization/World Health Organization (FAO/WHO) as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host".[8]

A meta-analysis evaluating the impact of probiotics on the incidence of VAP concluded that they are associated with a lower incidence of VAP compared to controls.[9] After the publication of this meta-analysis, two new trials of probiotics were published, and only one of them showed a reduced incidence of VAP. [10,11] *Lactobacillus rhamnosus* GG was

administered concomitantly in the oropharyngeal and gastric sites and a reduction in the incidence of VAP was found.[10] On the other hand, an enterally administered mixture of probiotics, mostly *Lactobacillus rhamnosus* GG, did not reduce the incidence of ICU-acquired infections, except for catheter-related bloodstream infections.[11] In addition, another study found no advantage of probiotics compared with selective decontamination of the digestive tract (SDD) in preventing ICU infections, including VAP.[12]

Given the increasing rates of antimicrobial resistance, and the continuing doubts about the benefits of the utilization of probiotics in the ICU setting, some investigators have suggested new studies of probiotics administered in the oropharynx, lower digestive tract, or both.[13] Therefore, we conducted a study of VAP prophylaxis utilizing a mixture of probiotics administered oropharyngeally and gastro-intestinally administration compared to a placebo group with similar care bundles, including comparable doses of prebiotics.

2. Methods

2.1 Study design

This was a single-center, randomized, double-blind, placebo-controlled trial of the administration of prophylactic probiotics to mechanically ventilated patients for at least 48 hours. The study was carried out in Maringá University Hospital, Brazil.

The study was approved by the local Institutional Board for Human Studies and the Ethics Committee of the State University of Maringá (Registry Number 03810093000-10).

All patients older 18 years of age and requiring mechanical ventilation (MV) for at least 48 hours were eligible for the study. A written informed consent agreeing to participate in

the study was signed by the patient or the patient's relative.

The following exclusion criteria were used: less than 18 years of age, MV for less than 48 hours, pregnancy, acute pancreatitis, cardiac valvulopathy, immunosuppression, use of probiotics in the month prior hospitalization, moribund patient, use of laxatives in the previous week, intubation after 48 hours of hospital admission, or inability to administer the first dose of probiotics within the first 24 hours of MV.

Patients were recruited from March 2011 to December 2011 in a 123-bed university hospital, which provides tertiary care for the Brazilian Public Health System to a population of about 1,500,000.

2.2 Randomization

After inclusion, eligible patients were randomly assigned by an independent microbiologist to the probiotics or placebo groups using permutation blocks (n = 4 per block) without further stratification. Investigators and all hospital staff were blinded to the group assignments.

2.3 Procedures

Treatment consisted of the administration of a combination of a mixture of probiotics plus fructooligosaccharides (FOS) or FOS alone. As the doses of FOS were the same in the two groups, the only difference was inclusion of probiotics in the intervention group. Therefore, we considered was that the group receiving probiotics was considered active intervention and the group receiving only FOS was placebo.

The intervention group received about $1.6x10^9$ colony forming-units (cfu) of a mixture of probiotics (*Lactobacillus paracasei* Lpc-37, *Lactobacillus rhamnosus* HN001,

Lactobacillus acidophilus NCFM and *Bifidobacterium lactis* HN019) and 24 g of FOS in 24 h, divided into 4 sachets. Each patient received a diluted sachet (10 mL) twice-daily through the nasogastric tube and another sachet administered as a fine sludge (10 mL) in the oropharynx, after oral hygiene with toothbrush and a solution of cetylpyridinium chloride. If, exceptionally, the patient was required to fast, all sachets were administered to the oropharynx until the enteral diet was resumed case of exceptional necessity of fasting, all sachets were administered to the oropharynx until the re-introduction of enteral diet. The placebo group received the same care, except for the use of probiotics. Both preparations were identical in appearance, weight, taste, consistency and packaging.

Because fiber administered in enteral diets may function as prebiotic, all patients received enteral nutrition without fiber content as recommended by hospital nutritionist.

Patients continued to receive active intervention or placebo until extubation, death, or the necessity to use laxatives or enteral fiber supplement, according to medical or nutrition staff indication.

Medical, biological and demographic data were collected, including the usual ICU preventive techniques, including maintaining 30-degree head-of-bed elevation during enteral nutrition, radiographic confirmation of feeding-tube placement, maintaining airway-cuff pressure between 20 and 30 cm H_2O, venous thromboembolism and peptic ulcer disease prophylaxis, and daily "sedation vacations" as a part of prophylaxis measures for VAP.[14,15] Silver-coated endotracheal tubes were not used and chlorehexidine mouthwashes, continuous aspiration of subglottic contents and SDD were not used for any patient.

We applied clinical criteria for the diagnosis of VAP. The presence of a new lung infiltrate on chest radiography after 48 h of MV plus at least two of the following criteria: fever > 38 °C, leukocytosis or leukopenia and/or purulent secretions were considered VAP.[14, 16]

All patients with VAP were submitted to quantitative cultures of tracheal aspirates with a threshold of 10^6 cfu/mL.

Patients with diarrhea (three or more loose stools per day) were evaluated with a *Clostridium difficile* cytotoxin assay. The local routine assumes constipation as absence of bowel movements for 3 days, and in case of constipation, laxatives or enemas were prescribed on the fourth day. Considering the effect of some laxatives (e.g. lactulose) as a known prebiotic[17], we interrupted the probiotics, but the case was considered for intention to treat analysis.

2.4 Outcomes

The primary outcome was the occurrence of VAP. Secondary outcomes were mortality, timing of VAP diagnosis, length of stay (LOS) in MV, LOS in ICU, LOS in hospital, number of days of diarrhea or constipation, timing of the first bowel movement number of patients requiring renal replacement therapy and mean glucose levels during mechanical ventilation.

2.5 Sample Size

The required number of patients to achieve sufficient statistical power for analysis was determined assuming the historic trends for the hospital, and a 50% reduction in VAP caused by the use of probiotics, based on published data. For a statistical power of 80% with a 2-sided significance level of 0.05, it was calculated that 120 patients were needed, and 12 additional patients were included to allow for a possible 10 % drop out.

2.6 Statistical analysis

All analysis was done based on an intention-to-treat principle. The primary and secondary end points were compared between the groups. The Shapiro-Wilk test was used to asses whether continuous data were normally distributed. For continuous variables, differences between groups were tested with the Mann-Whitney *U* test for non-normally distributed data. Other data were evaluated in a test of proportions. Kaplan-Meier curves with log-rank test were generated.

3. Results

All patients undergoing mechanical ventilation were screened from March 2011 to December 2011, and after appropriate exclusions, 132 patients were selected and randomly assigned to the groups. Seven additional patients were excluded during the analysis because they were transferred to another hospital and their data were lost.

There were no differences in baseline characteristics between the two groups, as well as no differences in preventive care between the placebo and probiotic groups (Table 1).

The patient population included high proportions of elderly patients (45.6 %), smokers (72.8%), alcohol abusers (40.0%), high gravity scores (median 26 points in APACHE II score) and patients with chronic obstructive pulmonary disease (29.6%), all known risks factors for the development of VAP. Furthermore, 40% of the patients were using corticosteroids, and 81.6 % were taking antibiotics.

Both groups received similar doses of prebiotics with a median of 114 g of FOS during MV (P=0.823). The sachets were administered as quickly as possible, with a median time of 16 h for the first administration (P=0.412) (Table 1).

VAP was diagnosed in 13 (20.9%) patients receiving probiotics and in 26 (41.3%) treated

with prebiotics (Relative Risk, 2.0; 95% confidence interval, 1.14 to 3.38; P=0.011) (Table 2).

Among the secondary endpoints, probiotics were effective in reducing LOS in hospital (18.5 vs. 23.1 days, P=0.024), number of pathogens in quantitative cultures of tracheal aspirates (17 vs. 39, P=0.0001), number of Gram-negative pathogens (14 vs. 28, P=0.009), and number of Gram-positive pathogens (3 vs 11, P=0.025) (Tables 2 and 3).

A Kaplan-Meier analysis of the probability of remaining free of VAP versus days after onset of MV was constructed (Figure 1), and a difference favoring probiotics was identified with a Log-rank (Mantel-Cox) Test (P=0,0162). Another Kaplan-Meier analysis was done for survival at 90 days after ICU admission and no differences were identified (P=0.98).

Concerning the safety analysis, there were no side effects detected during the study period, including no cases of endocarditis or documented bowel ischemia.

4.0 Discussion

We studied an interesting way to reduce VAP incidence, which affords the advantages of ease of administration, low cost and minimal toxicity. The estimated number of patients needed to treat (NNT) with Lactofos® to prevent one case of VAP was approximately 5, similar to data recently reported elsewhere.[10]

Recent trials studying probiotics administration in VAP patients were methodologically heterogeneous, and it is therefore difficult to make comparisons. There were differences in the type of probiotics, site of administration, time of intervention, and even the definition of VAP. We particularly used Lactofos® because it was already being used in our hospital to control diarrhea. The administration in diet and oropharynx was chosen to enhance a presumed ability of the probiotics to alter local flora, since microaspirations of contaminated secretions from the

aerodigestive tract are part of the pathogenesis of VAP. We utilized clinical diagnostic criteria for VAP because they are currently standard in our hospital, and because quantitative sampling of cultures still did not demonstrate mortality benefits. [19] However, the attending physicians used quantitative cultures as a guide to antibiotic therapy.

The population studied had a high-risk of developing VAP. The risk was associated with high gravity scores and difficulties in providing standard ICU care (i.e. keeping the bed backrest elevated), factors usually linked to high incidences of VAP and mortality. These characteristics were important to enhance the power of the study to demonstrate the reduction of VAP incidence, even with the use of a small sample. Furthermore, the high gravity scores of the patients could explain why a reduction of infections did not affect mortality. These results are in accordance with previous studies that found no effect of probiotics on mortality, despite benefits to VAP prophylaxis. [10,18,20]

The clinical and economic burdens of VAP are still unclear, with a broad range of reported values. Nevertheless, reduction of VAP incidence could lower health-care costs by reducing antibiotic consumption and hospitalization time. In our study, LOS in hospital was indeed reduced. We have not studied hospital charges and total use of antibiotics that would allow us to show a reduction of costs. However, a systematic review showed that VAP increases hospital costs by at least US$ 10,019.00 and significantly lengthens the intensive-care unit LOS (mean = 6.10 days).[5] Although we did not find a reduction in mortality rates using probiotics, the saving in cost achieved by the prevention of 13 VAP cases in our 10-month analysis is worthwhile.

A change in mortality is a difficult goal to reach in a complex disease such as VAP, particularly in small trials. However, some favorable outcomes occurred in the probiotics group, including a significant 5-day reduction in hospital LOS. The duration of MV and ICU LOS

showed only a trend favoring probiotics, suggesting that statistical significance might have been attained if a larger group had been studied. Therefore, considering that hospital LOS is the sum of LOS in ICU and LOS outside ICU, and that LOS in ICU depends directly on the MV duration, it is probable that all these LOS parameters could be affected in future investigations. A meta-analysis of five randomized controlled trials, three of them reporting ICU LOS, evaluated the impact of administration of probiotics on the incidence of VAP.[9] In a fixed model of analysis, patients treated with probiotics remained almost one day less in the ICU.

Analysis of secondary endpoints was limited because of the small sample size and the short period of the study. These problems possibly occurred in the analysis of diarrhea and constipation occurrence. Perhaps the probiotic administration period of less than one week tested in this study was not enough to demonstrate its potential benefits in reducing constipation and diarrhea in critically ill patients. Even in the general population, the use of probiotics and prebiotics to reduce constipation and diarrhea is still debated. There is some evidence of benefits of probiotics to reduce diarrhea in a general population [21], but the data published to date do not provide sufficient scientific evidence to support a general recommendation about the use of probiotics to treat functional constipation [22]. In addition, there is no conclusive evidence about the benefits of the use of prebiotics and probiotics in critically ill patients. Therefore, neither the European Society for Clinical Nutrition and Metabolism (ESPEN) nor the American Society for Parenteral & Enteral Nutrition (ASPEN) guidelines make recommendations on enteral nutrition in the general ICU population.[23,24]

Unlike other investigators, we were not able to evaluate the benefits of probiotics on *Clostridium difficile*-associated diarrhea because there was no case of positive *Clostridium difficile* toxin assay in our sample.[25,26] Since 2010, our hospital has documented only a single case of positive *Clostridium difficile* toxin (CDT) assay. We believe that the absence of CDT in

this hospital is due to the wide use of probiotics in recent years.

We expected to find better glycemic profiles with less need for glycemia measurements and lower doses of insulin in patients who received probiotics, because of the effect of probiotics in lowering glucose levels.[27,28] Some investigators have shown that probiotics can improve glucose homeostasis in humans.[27,28] We believe that our failure to find any differences resulted from the short period of administration of probiotics and the small sample size.

The need for renal replacement therapy (RRT) was used to evaluate secondary organ damage. Although the number of cases of RRT in the intervention group was almost half that in the placebo group (7 vs 13), this reduction was not statistically significant. Perhaps a larger sample would be necessary to demonstrate benefits of probiotics in reducing the need for RRT.

Under the hypothesis that probiotics could reduce VAP by changing the oropharyngeal flora, we used probiotics simultaneously in the diet and in the oropharynx. To demonstrate this flora change, we compared the results for cultures of tracheal aspirates between groups receiving symbiotics and prebiotics. The reductions in Gram-negative and Gram-positive pathogens resulted in a significant difference between the groups (P=0.0001). Reductions in Gram-negative pathogens were also found in previous trials with probiotics.[10,29] Of particular note, a study comparing probiotics versus antibiotics decontamination of the digestive tract identified lower incidences of Gram–positive cocci and *Pseudomonas aeruginosa* from surveillance cultures in the group that received probiotics.[12]

Future double-blind randomized controlled trials could evaluate individual probiotic strains for a longer period of time, or the use of the same mixture of probiotics offered in a single site (stomach or oropharynx), to determine where the probiotics really act; as well as to include larger samples in multiple centers, to reduce bias arising from local variations in

practices and population heterogeneity.

In conclusion, based on the results of this study, probiotics are feasible and safe agents for preventing VAP and reducing hospital LOS of patients in MV. Also, it seems that probiotics are effective in reducing the number of pathogens identified in tracheal aspirates, but do not reduce mortality in mechanically ventilated patients.

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Table 1. Comparison of probiotic versus placebo groups based on baseline characteristics and routine care

	Probiotic[a] (n = 62)	Placebo[b] (n = 63)
Baseline characteristics		
Age (years)	62.4 (21.9 – 86.7)	65.9 (20.9 – 87.4)
Sex (male), n	41 (66.1)	46 (73.1)
Weight (Kg)	64 (40 -100)	65 (42 -120)
NRS 2002[c] score at admission	4 (3 – 7)	4 (3- 7)
APACHE II[d] score at admission	26 (15 -56)	26 (13 -55)
Smokers, n	44 (70.9)	47 (74.6)
Diabetes mellitus, n	13 (20.9)	14 (22.3)
Chronic obstructive pulmonary disease, n	18 (29.1)	19(30.2)
Alcohol abusers, n	24 (38.7)	26 (41.2)
Number of teeth	10 (0 -32)	9,5 (10 -30)
Antibiotics during treatment, n	51 (82.3)	51(82.2)
Recommended calories per day (kcal)	1700 (1200 -2300)	1750 (1200 – 2600)
Use of corticosteroids during treatment, n	25 (40.3)	25 (39,7)
Routine care and interventions		
Number of sachets administered per patient	19 (4 - 48)	19 (7 -44)
Timing of first intervention (hours)	16 (2 -30)	16 (2 -30)
Days of intervention	5 (1 -14)	5 (2 -12)
Total fiber offered (grams)	114 (24 -288)	114 (42 -264)
Adequacy of bed backrest	49.1	51.2
Inclination of bed backrest (degrees)	23.1 (12- 38)	24 (12 -35)
Radiographic confirmation of enteral tube placement, n	57 (91.9)	58 (92.1)
Postpyloric feeding,; n	4 (0.06)	5 (0.08)
Adequacy of airway cuff pressure	84.1	87.5
Venous thromboembolism prophylaxis	95.1	97.7
Peptic ulcer disease prophylaxis	96.5	97.7
Daily "sedation vacations"[e]	46.8	48.3

Timing of enteral nutrition onset (hours)	24 (6 - 40)	24 (6 -72)
Daily calories offered (kcal)	1300 (110 – 2000)	1350 (340 – 2100)

Date given as number (% of total) or median ± range

[a b] Probiotic group received Lactofos®. Placebo group received Fiberfos®

[cd] NRS 2002 Nutritional Risk Screening, APACHE II Acute Physiology and Chronic Health Evaluation II

[e] Adequacy are in percentage, daily "sedation vacations" correspond to the percentage of days when the sedation was reduced or suspended by the physician

All comparisons with $p > 0.05$

Table 2 Results for primary and secondary outcomes

	Probiotic* (n = 62)	PlaceboΘ (n = 63)
VAP	13 (20.9) ***a***	26 (41.3) ***a***
VAP/ 1000 days of mechanical ventilation	22,4	35,3
Timing of VAP diagnosis (days)±SD	11.0±7.5	8.2±7.2
Mortality at 30 days	28 (45.2)	28 (44.4)
Mortality at 120 days	32 (51.6)	33 (52,4)
LOS in ICU (n days)	13.7	15.9
LOS in hospital (n days)	18.5 ***b***	23.1 ***b***
LOS in MV (n days)	9.4	11.7
Number of constipated days	234	304
Number of diarrhea days	57	85
Positive *Clostridium difficile* cytotoxin assay	0	0
Day of first bowel movement	4.8	5.7
Mean glucose (mg/dl) ±SD	138.5±37.3	143.3±36,7
Total glucose measurements per patient	2383	3075
Total dose of insulin (units) per patient	81.9	123.0
Number of patients in renal replacement therapy	7	13

*ΘProbiotic group received Lactofos®. Placebo group received Fiberfos®

Data represent number (% of total) or mean

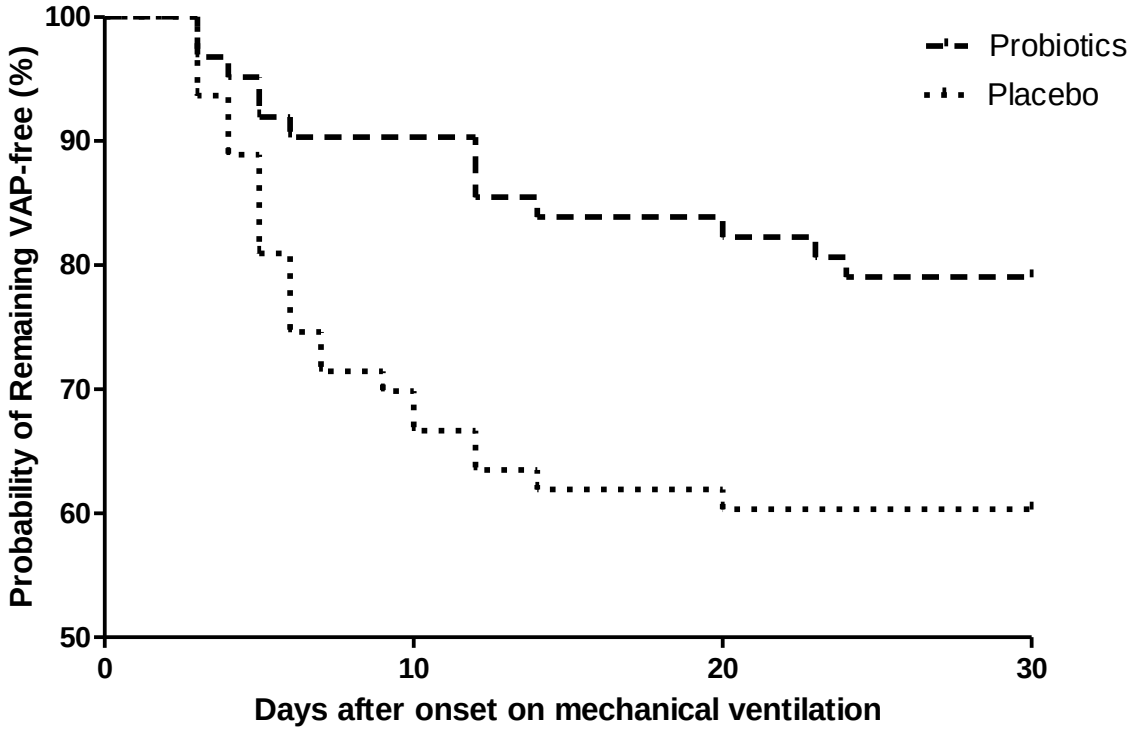
All comparisons with $p > 0.05$ except for ***a*** (P=0.011) and ***b*** (P=0.024)

Table 3 Quantitative cultures of tracheal aspirates with threshold of 10^6 cfu/mL.

	Probiotic*	PlaceboΘ
Gram-negative pathogens isolated	14 [a]	28 [a]
Acinetobacter baumannii	2	4
Burkholderia cepacia	1	0
Enterobacter aerogenes	0	1
Enterobacter cloacae	0	4
Escherichia coli	1	2
Klebsiella oxytoca	0	1
Klebsiella pneumoniae	1	2
Kluyvera ascorbata	1	0
Moraxella catarrhalis	0	1
Morganella morganii	0	1
Providencia stuartti	1	0
Pseudomonas aeruginosa	4	9
Serratia marcescens	2	1
Stenotrophomonas maltophilia	1	2
Gram-positive pathogens isolated	3 [b]	11 [b]
Staphylococcus aureus (MRSA)	1	6
Staphylococcus aureus (MSSA)	1	2
Staphylococcus epidermidis	1	0
Streptococcus anginosus	0	1
Streptococcus mitis	0	1
Enterococcus faecium	0	1
Total bacteria isolated	17 [c]	39 [c]

*ΘProbiotic group received Lactofos®. Placebo group received Fiberfos®

All comparisons with $p>0.05$ except for Gram-negative pathogens isolated [a] (P=0.009), Gram-positive pathogens isolated [b] (P = 0.025), and total bacteria isolates [c] (P=0.0001)



Log-rank (Mantel-Cox) Test, Chi square 5,782, df=1, P=0,0162

Figure 1. Kaplan-Meier analysis of time to confirmed VAP

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Revised February 2012