Nutrition in Clinical Practice
Volume 34 Number 1
February 2019 156–162

DOI: 10.1002/ncp.10191
wileyonlinelibrary.com
WILEY

Effect of a Probiotic Preparation on Ventilator-Associated Pneumonia in Critically Ill Patients Admitted to the Intensive Care Unit: A Prospective Double-Blind Randomized Controlled Trial

Ata Mahmoodpoor, MD[1]; Hadi Hamishehkar, PharmD[2]; Roghaieh Asghari, MD[3]; Ramin Abri, PhD[4]; Kamran Shadvar, MD[1]; and Sarvin Sanaie, MD, PhD[5]

Abstract

Background: Ventilator-associated pneumonia (VAP) occurs as a life-threatening complication in critically ill mechanically ventilated patients. Probiotic administration may modify the gut microbiota; however, whether this modification could decrease VAP occurrence is not known. *Methods:* In this study, 100 adult critically ill patients undergoing mechanical ventilation for >48 hours were randomly assigned to either the probiotic or the control group. The patients in the probiotic group received 2 capsules of probiotic preparation containing *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* spp., and those in the control group received placebo daily for 14 days. *Results:* The patients in the probiotic group had a lower incidence of statistically microbiologically confirmed VAP. The duration of intensive care unit (ICU) and hospital stay was also lower in the probiotic group ($P < .05$). More than half of the patients in the control group had gastric residuals during ICU stay, compared with only 30% of patients in the probiotic group ($P = .004$). Probiotic usage led to a nonsignificant decrease in diarrhea, gastric and oropharyngeal colonization, and incidence of multidrug-resistant pathogens. The Kaplan-Meier survival curves for time to the first episode of VAP did not show a significant difference between probiotic and control groups (log-rank test = 1.89; $P = .17$). *Conclusions:* The results of probiotic administration for the prevention of VAP remain inconclusive in this trial. However, such an approach can decrease the length of ICU and hospital stay. Well-designed multicenter clinical studies with defined combinations of probiotics and definite end points are necessary in this field. (*Nutr Clin Pract.* 2019;34:156–162)

Keywords

critical illness; intensive care unit; probiotics; ventilator-associated pneumonia

Introduction

Ventilator-associated pneumonia (VAP) is one of the most prevalent and serious complications of mechanical ventilation in critically ill patients admitted to intensive care units (ICUs).[1] The incidence rate of VAP ranges from 6% to 50%, with a crude rate of 1%–3% per day of intubation and mechanical ventilation, and a crude mortality rate of 24%–76%.[2,3] VAP prolongs the duration of mechanical ventilation and the length of ICU and hospital stay.[4] Because the pathogenesis of VAP is so complex, there are several pharmacological and nonpharmacological

From the [1]Department of Anesthesiology, Fellowship of Critical Care Medicine, Evidence-Based Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; [2]Department of Clinical Pharmacy, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran; [3]Anesthesiology Research Team, Tabriz University of Medical Sciences, Tabriz, Iran; [4]Department of Microbiology, Food and Drug Safety Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; and [5]Department of Nutrition, Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Financial disclosure: None declared.

Conflicts of interest: None declared.

This article originally appeared online on August 8, 2018.

Corresponding Author:
Sarvin Sanaie, MD, PhD, Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Golgasht Street, Tabriz, Iran.
Email: sarvin_so2000@yahoo.com

interventions for its prevention, such as antibiotics, semirecumbent position, selective gut decontamination, endotracheal tubes equipped with subglottic secretion drainage, and chlorhexidine mouthwash.[5-7] With the increasing incidence of antibacterial resistance in ICUs and the lack of new antibiotics, strategies that target nonantibiotic interventions need to be developed. Probiotics are living microorganisms that, when administered in adequate amounts, can help maintain the integrity of the intestinal barrier function by modulating the immune response.[8] Such administration has been included in the management of critically ill patients as a therapeutic approach.[9] Probiotic administration can be considered as a nonantibiotic option for the prevention of VAP through various local and systemic mechanisms that minimize the colonization by virulent species or modulate the host immune defense.[10,11] Many studies have shown positive results of the administration of probiotics in critically ill patients toward decreasing VAP[12-15]; however, some studies have reported negative results.[16,17] The main issue regarding the probiotic effect in critically ill patients is the administration of living microcorganisms, which can increase the risk for iatrogenic infection. Two recent meta-analyses reported uncertain results regarding the effect of probiotic administration on VAP incidence and recommended that advanced high-quality trials should be carried out, and that probiotics should be applied to specific populations.[18,19] Another meta-analysis showed no beneficial effect on the decrease of VAP because of the heterogeneity of studies.[20] Thus, the present trial was carried out to show the efficacy and safety of the administration of a probiotic compound in decreasing the incidence of VAP in critically ill patients admitted to the surgical ICU.

Methods

This study was approved by the ethics committee of Tabriz University of Medical Sciences. Informed consent was obtained from all patients or their next of kin. A total of 120 critically ill patients from the surgical ICUs of 2 university-affiliated hospitals (22 beds in all) in northwest Iran were enrolled in the trial from January 2015 to September 2016. Figure 1 shows the flow diagram of the study. The trial is registered in the Iranian Registry of Clinical Trials (IRCT201411182582N10). The inclusion criterion was all critically ill patients >18 years old who were admitted to the ICU and had been undergoing mechanical ventilation for >48 hours. The exclusion criteria were previous history of pneumonia, pregnancy, immunosuppression, prosthetic cardiac valve or valvular graft, history of rheumatic fever, recent gastroesophageal or intestinal injury, placement of tracheostomy, and patient refusal. The primary outcome of the study was VAP occurrence, and secondary outcomes were ICU and hospital length of stay, duration of mechanical ventilation, and complications during the study. After informed consent, patients were randomized in variable unspecified block sizes into 2 groups. Investigators, primary care physicians, nurses, and laboratory personnel were blinded to the study. All patients received routine care, standard precautions for VAP prophylaxis, and antibiotic therapy when needed. These included semirecumbent position, hand washing, suctioning when needed, endotracheal tube with subglottic secretion drainage, using heat and moisture exchanger filter, sedation with a Richmond Agitation Sedation Scale target −1 to +1, oral hygiene with chlorehexidine, maintenance of cuff pressure between 20 and 30 mm Hg, and changing of tubes or ventilator circuit only when needed. Patients in the probiotic group received 2 capsules of probiotic preparation (1 capsule every 12 hours) daily for 14 days. Each capsule contained 10^{10} bacteria consisting of *Lactobacillus* species (*casei*, *acidophilus*, *rhamnosus*, *bulgaricus*), *Bifidobacterium* species (*breve*, *longum*), and *Streptococcus thermophilus*. The probiotics (Lactocare; Zist-Takhmir, Tehran, Iran) were administered via feeding tube separately, not with gavage formula. Patients in the control group received placebo instead of the probiotic. The placebo preparation contained only sterile maize starch powder, which was visually identical. Patients continued to receive probiotic or placebo during the study period or until death. All patients received standard formula (1 kcal/mL; Ensure; Abbott Laboratories, Zwolle, Netherlands) in the total amount of 25 kcal/kg. All patients received enteral feeding with the standard protocol. The method for enteral feeding was via feeding pump with a 2-hour infusion and 1 hour off. The feeding was performed via nasogastric tube with size 16F. Our patients received enteral feeding 7 times a day, as the gut was rested from 2 a.m. until 6 a.m. If the patient did not tolerate the enteral nutrition and we had to support him or her with parenteral nutrition, we excluded the patient from the study. We considered gastric residue when the amount of gastric aspirate before the next administration of enteral formula was >50% of the amount of gavage during the past 6 hours. If this happened, prokinetics such as metoclopramide or domperidone would be administered and the amount of feeding would be decreased to half of the previous rate. Meanwhile, probiotic preparation was given to the patient regardless of gastric residual volume. Baseline data including demographic characteristics, medical history, and Acute Physiology and Chronic Health Evaluation (APACHE) score were recorded for all patients. Primary outcome was VAP frequency, and secondary outcomes were mortality, ICU length of stay, duration of mechanical ventilation, and adverse events of probiotic administration. Based on American Critical Care Physicians clinical criteria, patients with a new or persistent infiltration on chest x-ray with 2 of the following criteria of hyperthermia >38 or hypothermia <36, leukocytosis or leucopenia, or purulent sputum underwent bronchoalveolar

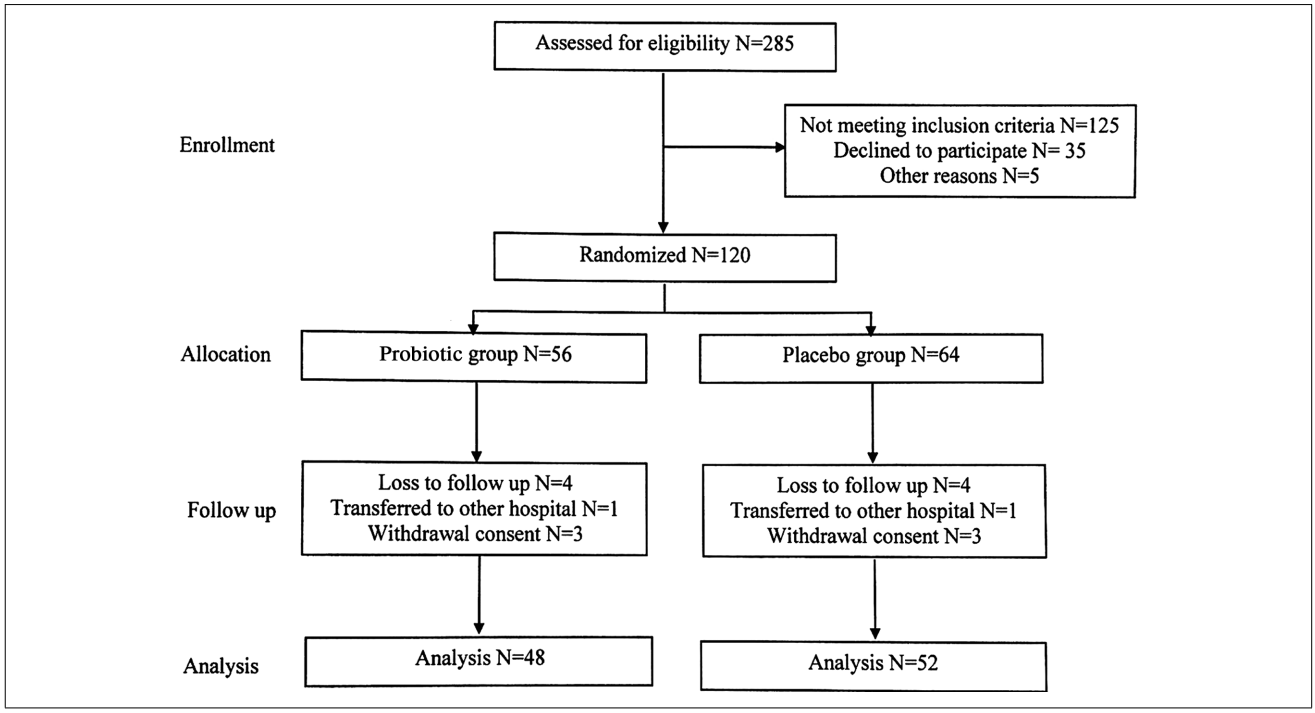

Figure 1. Study overview.

lavage (BAL). Samples were taken blind, and patients were considered VAP-positive if the quantitative BAL culture had at least 10^4 colony-forming units/mL in patients who were mechanically ventilated for >48 hours.

We also noted mortality rate, time to occurrence of VAP, and diarrhea (>3 times in a day, weight >250 g, volume >500 mL). All patients with diarrhea were evaluated for *Clostridium difficile* toxin in stool examination. To evaluate the effect of probiotic administration on gut microbial flora, we performed gastric aspirates on the first, third, and seventh days after the clinical diagnosis of VAP. Multidrug-resistant (MDR) pathogens, defined as acquired nonsusceptibility to at least 1 agent in ≥3 antimicrobial categories, were identified on gastric and BAL and blood cultures.

Statistical Analysis

Sample size calculation was performed based on a 40% incidence rate of VAP, a 30% reduction in VAP rate and power of 80%, and a significance level of 5%. We calculated 45 patients in each group and increased to at least 48 patients considering 5% possibility of loss. All values are expressed as mean ± SD. Continuous and categorical data were compared between 2 groups with Student's *t*-test and χ^2 test, respectively. Kaplan-Meier analysis was used for each group, and a log-rank statistic was used to test the null hypothesis that classification into probiotic and control groups does not affect the time of VAP incidence during the 2 weeks of follow-up. All statistical analyses were carried out with the use of the Statistical Package for the Social Sciences software version 16.0 (SPSS, Chicago, IL, USA). The statistician was blinded to the study.

Results

A total of 120 patients were included in the present trial. The demographic characteristics of these patients, including age, sex, primary diagnosis, and severity of illness (APACHE II score), were not significantly different between the probiotic group and the control group (Table 1). Most of the patients suffered from neurological manifestations. Those with intracerebral hemorrhage, hypoxemic encephalopathy, ischemic stroke, and brain tumor were categorized as the cerebrovascular accident group. Those with myasthenia gravis and Guillain-Barré syndrome were included in the neurological events group. Smoking, history of chronic obstructive pulmonary disease, chest trauma, alcohol consumption, and long ICU stay were evaluated as VAP risk factors. Before the trial was carried out, an educational course regarding VAP prevention bundles was established. The course helped us reach a high rate of VAP bundle prevention compliance of around 85%.The 2 groups did not show any significant difference in VAP risk factors. The ICU mortality and duration of mechanical ventilation did not differ between the groups ($P > .05$). The patients who received probiotic administration had a lower, statistically significant VAP incidence (Table 2). The duration of ICU and hospital stay was lower in the probiotic group ($P < .05$).

Table 1. Baseline Demographics and Clinical Information of Patients.

Patient data	Probiotic (n = 48)	Control (n = 54)
Age, mean ± SD (y)	59.1 ± 12.9	57.5 ± 14.5
Male, n (%)	26 (54.2)	29 (53.7)
Admission diagnosis, n (%)		
Cerebrovascular accident	22 (45.8)	16 (29.6)
Postoperation	3 (6.2)	2 (3.7)
Trauma	0	4 (7.4)
Pulmonary emboli	2 (4.2)	2 (3.7)
Fat emboli	2 (4.2)	2 (3.7)
Cancer	12 (25)	17 (31.5)
Neurological events	3 (6.4)	2 (3.7)
Myocardial infarction	1 (2.1)	3 (5.6)
Poisoning	1 (2.1)	3 (5.6)
Acute Physiology and Chronic Health Evaluation II, mean ± SD	24.1 ± 6.2	22.8 ± 4.7
Diabetes mellitus, n (%)	18 (37.5)	18 (33.3)
Ischemic heart disease, n (%)	10 (20.8)	9 (16.7)
Congestive heart failure, n (%)	5 (10.4)	7 (13)
Hypertension, n (%)	21 (43.8)	23 (42.6)

More than half of the patients in the control group had gastric residuals during ICU stay, compared with only 30% of patients in the probiotic group ($P = .004$). Diarrhea as a complication of enteral feeding occurred at a lower rate in the patients who received probiotics; however, this difference was not statistically significant. The gastric and oropharyngeal colonization of hospital pathogens and the incidence of MDR pathogens isolated from patients differed between the probiotic group and the control group (Table 2). Table 3 shows the organisms that caused VAP in this study. The Kaplan-Meier survival curves for time to the first episode of VAP did not show a significant difference between the probiotic and control arms (log-rank test = 1.89; $P = .17$; Figure 2) in the whole group of 100 patients. No serious adverse events related to probiotic usage were observed during our study. The oropharyngeal and gastric colonization was nonsignificantly less in the probiotic group (Table 2). Table 4 shows the results of gastric aspirate cultures.

Table 3. Types of Organism That Caused Ventilator-Associated Pneumonia in This Study.

Organisms	Probiotic Group (7)	Placebo Group (13)
Escherichia coli	1	2
Staphylococcus aureus	1	2
Enterococcus	0	1
Klebsiella spp.	1	2
Pseudomonas	0	2
Acinetobacter	0	1
Citrobacter spp.	1	2
Haemophilus spp.	1	0
Streptococcus spp.	1	0
Serratia spp.	1	1

Discussion

Despite a numerical decrease in VAP occurrence in the probiotic group of mechanically ventilated patients, the present trial failed to detect a significant difference during ICU stay based on the Kaplan-meier analysis. A significant decrease in duration of mechanical ventilation and length of ICU and hospital stay was found in the probiotic group.

Table 2. Summary of Clinical and Laboratory Parametric Differences Between Groups During Intervention.

Variables	Probiotic (n = 48)	Control (n = 54)	*P*-Value
ICU mortality, n (%)	5 (10.4)	6 (11.1)	.58
VAP incidence[a]	0.66	0.94	.04
Duration of mechanical ventilation, mean ± SD (h)	210 ± 115	290 ± 171	.02
Length of ICU stay, mean ± SD (d)	11.6 ± 8	18.6 ± 6.3	.007
Length of hospital stay, mean ± SD (d)	14.2 ± 8.6	21.1 ± 5.7	.002
Gastric residuals, n (%)[b]	14 (29.2)	31 (57.4)	.004
Diarrhea, n (%)	7 (14.6)	15 (27.8)	.08
Oropharyngeal bacterial colonization, n (%)	23 (48.5)	34 (63)	.11
Gastric bacterial colonization, n (%)	14 (29.2)	20 (37)	.26
Multidrug-resistant pathogen, n (%)[c]	6 (12.5)	10 (18.5)	.29
Use of vasopressors, n (%)	21 (43.8)	17 (31.5)	.14
Prokinetic use, n	11	20	.122

ICU, intensive care unit; VAP, ventilator-associated pneumonia.
[a]VAP incidence is expressed as per 1000 hours of mechanical ventilation.
[b]Gastric residuals numbers are numbers of patients who had 1 gastric residual based on our protocol. Normal gastric volume mentioned as gastric aspirate volume <50% during past 6 hours.
[c]Multidrug resistant was considered as number of patients with a drug-resistant pathogen.

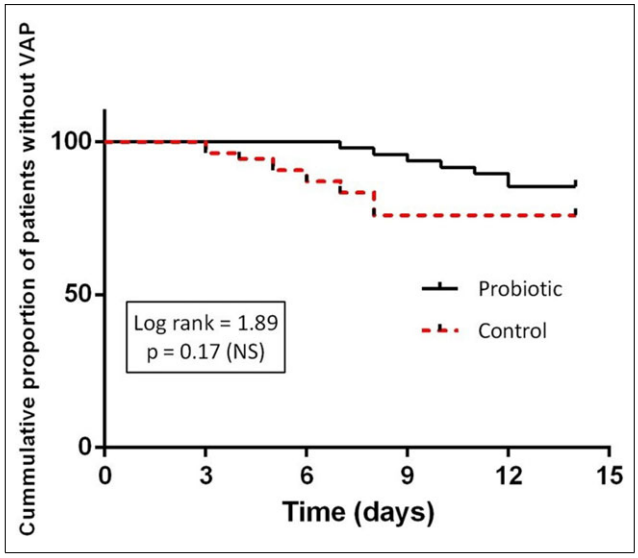

Figure 2. Stratified Kaplan-Meier analysis of time to ventilator-associated pneumonia (VAP) for patients treated with probiotic and those treated without probiotic prescription. The overall differences are not statistically significant (NS).

Table 4. Organisms Responsible for Gastric Bacterial Colonization.

Organisms	Probiotic	Control
Pseudomonas	1	2
Acinetobacter species	1	2
Enterobacteriaceae	3	5
Coagulase-negative *Staphylococcus*	4	4
Staphylococcus aureus	1	2
Streptococcus species	4	5

Previously, 6 different RCTs have shown that the administration of probiotics to mechanically ventilated patients could not prevent VAP occurrence, which is consistent with the present results.[13,17,21-24] In contrast, 5 other RCTs showed that the administration of probiotics to critically ill patients was associated with a statistically significant decrease in the probiotic group.[25-29] The study population in clinical trials that found no significant effect of probiotics on VAP consisted mostly of mechanically ventilated patients, similarly to the present sample. However, other trials have shown that the use of probiotics can decrease the occurrence of infection in traumatic, surgical, and pediatric patients.[30-32] Most of the patients in this trial were surgical cases. Only 9 patients required mechanical ventilation for >3 weeks, which is a major risk factor for VAP.[33] The results indicated that probiotic administration may delay VAP occurrence in critically ill patients; however, because most of the patients had a short duration of mechanical ventilation, the decrease in VAP occurrence was not significant. There are 3 published meta-analyses on the effect of probiotics on the incidence of VAP in critically ill patients. Two of these found no significant effect of probiotic administration in these patients,[18,20] whereas the remaining study showed a statistically significant decrease in nosocomial infection in critically ill patients.[34]

Another reason for such a controversial result is the existence of different definitions of VAP occurrence. Previous trials have found that the VAP incidence was lower when the microbiologically confirmed diagnosis was used than when the clinical diagnostic criteria were applied.[19] In this study, the cases were confirmed based on the microbiological results, which may explain the negative findings. Two other factors that could influence the results are the sample size and the number of centers enrolled in the study. Most of the previous trials were single-center studies that were not double blind and had a small sample size; this trial is a multicenter double-blind study, which could result in decreased bias. Previous trials have also shown that the sample size has an effect on the occurrence of VAP: the larger the sample size, the higher the VAP occurrence.[35] To overcome the increased emphasis on VAP prevention in the intervention group (unintentional better quality of care), especially in a blind trial, it is recommended that the degree of compliance to the established VAP prevention bundles should be reported. This trial was double blind; however, the high rate of compliance to VAP prevention bundles (85%) decreased the possibility of unintentional biases. The last factor is regarding the different types of probiotic species, which have different colony numbers of microorganisms. In this trial, probiotic administration resulted in a significant decrease in the length of ICU and hospital stay, and a nonsignificant decrease in the duration of mechanical ventilation, which could explain the nonsignificant decrease in VAP. The results showed that probiotic administration nonsignificantly decreased the oropharyngeal and gastric colonization and the MDR organisms during the study. These indicated that probiotic administration could prevent gastric and oropharyngeal colonization by microorganisms but could not eradicate MDR microorganisms, which are among the main risk factors for the occurrence of VAP, as also reported in previous trials.[29,36] Probiotic use also led to a significant decrease in the gastric residual volume in this study. Based on previous trials, gastric residual volume monitoring is recommended for all mechanically ventilated patients; however, it has been recently shown that the absence of gastric volume monitoring was not inferior to routine residual gastric volume monitoring in terms of the development of VAP in adult critically ill patients.[37] Thus, it may be favorable to decrease the value of gastric residual monitoring in VAP occurrence. *Lactobacillus* bacteremia was not found in any of the patients, and immunocompromised patients were excluded to minimize the adverse

effects. The compliance with probiotic administration was high because this was assessed by daily monitoring; the compliance rate remained high with continuous monitoring and the use of a standard checklist.

This study has some limitations. First, it was done in 2 ICUs where most patients were surgical cases; further research in the form of multicenter trials with large sample sizes is necessary to be able to generalize the results to all critically ill patients. Second, the probiotic used was the only available formulary in our hospital; the use of a higher dose or other strains could yield different results. The strengths of this research are its double-blind design and the confirmation of VAP based on microbiological criteria, which decreased the reporting of tracheal colonization and ventilator-associated tracheobronchitis as VAP.

Conclusion

The results showed that probiotic administration was safe but did not have a significant effect on VAP incidence and mortality in mechanically ventilated patients. Probiotic administration decreased the length of ICU and hospital stay, possibly through the prevention of gastric and oropharyngeal colonization, and the decrease of MDR pathogens. Physicians should be aware of the effects of probiotics in different disease states and in various age groups. Thus, additional trials are necessary to clarify the relative significance of strain-specific effects in different situations and populations, as well as the nature of interactions between different types of probiotics.

Statement of Authorship

A. Mahmoodpoor and S. Sanaie equally contributed to the conception and design of the research; K. Shadvar contributed to the design of the research; H. Hamishehkar contributed to the acquisition and analysis of the data; S. Sanaie and H. Hamishehkar contributed to the interpretation of the data; and R. Asghari and R. Abri drafted the manuscript; all authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

References

1. Safdar N, Dezfulian C, Collard HR, Saint S. Clinical and economic consequences of ventilator-associated pneumonia: a systematic review. *Crit Care Med*. 2005;33(10):2184-2193.
2. Chastre J, Fagon JY. Ventilator-associated pneumonia. *Am J Respir Crit Care Med*. 2002;165(7):867-903.
3. Gadani H, Vyas A, Kar AK. A study of ventilator-associated pneumonia: incidence, outcome, risk factors and measures to be taken for prevention. *Indian J Anaesth*. 2010;54(6):535-540.
4. Mahmoodpoor A, Peyrovi-far A, Hamishehkar H, et al. Comparison of prophylactic effects of polyurethane cylindrical or tapered cuff and polyvinyl chloride cuff endotracheal tubes on ventilator-associated pneumonia. *Acta Med Iran*. 2013;51(7):461-466.
5. Niederman MS, Craven DE, Bonten MJ, Chastre J, Craig WA; American Thoracic Society; Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med*. 2005;171(4):388-416.
6. Lorente L, Blot S, Rello J. Evidence on measures for the prevention of ventilator-associated pneumonia. *Eur Respir J*. 2007;30(6):1193-1207.
7. Bouza E, Burillo A. Advances in the prevention and management of ventilator-associated pneumonia. *Curr Opin Infect Dis*. 2009;22(4):345-351.
8. Vincent JL, de Souza Barros D, Cianferoni S. Diagnosis, management and prevention of ventilator-associated pneumonia: an update. *Drugs*. 2010;70(15):1927-1944.
9. Manzanares W, Lemieux M, Langlois PL, Wischmeyer PE. Probiotic and synbiotic therapy in critical illness: a systematic review and meta-analysis. *Crit Care*. 2016;19:262.
10. Ghosh S, van Heel D, Playford RJ. Probiotics in inflammatory bowel disease: is it all gut flora modulation? *Gut*. 2004;53(5):620-622.
11. McNaught CE, Woodcock NP, MacFie J, Mitchell CJ. A prospective randomised study of the probiotic Lactobacillus plantarum 299V on indices of gut barrier function in elective surgical patients. *Gut*. 2002;51(6):827-831.
12. Pitsouni E, Alexiou V, Saridakis V, Peppas G, Falagas ME. Does the use of probiotics/synbiotics prevent postoperative infections in patients undergoing abdominal surgery? A meta-analysis of randomized controlled trials. *Eur J Clin Pharmacol*. 2009;65(6):561-570.
13. Knight DJ, Gardiner D, Banks A, et al. Effect of synbiotic therapy on the incidence of ventilator associated pneumonia in critically ill patients: a randomised, double-blind, placebo-controlled trial. *Intensive Care Med*. 2009;35(5):854-861.
14. van Santvoort HC, Besselink MG, Timmerman HM, van Minnen LP, Akkermans LM, Gooszen HG. Probiotics in surgery. *Surgery*. 2008;143(1):1-7.
15. Rayes N, Seehofer D, Theruvath T, et al. Supply of pre- and probiotics reduces bacterial infection rates after liver transplantation—a randomized, double-blind trial. *Am J Transplant*. 2005;5(1):125-130.
16. Watkinson PJ, Barber VS, Dark P, Young JD. The use of pre- pro- and synbiotics in adult intensive care unit patients: systematic review. *Clin Nutr*. 2007;26(2):182-192.
17. Klarin B, Molin G, Jeppsson B, Larsson A. Use of the probiotic Lactobacillus plantarum 299 to reduce pathogenic bacteria in the oropharynx of intubated patients: a randomised controlled open pilot study. *Crit Care*. 2008;12(6):R136.
18. Wang J, Liu KX, Ariani F, Tao LL, Zhang J, Qu JM. Probiotics for preventing ventilator-associated pneumonia: a systematic review and meta-analysis of high-quality randomized controlled trials. *PLoS One*. 2013;8(12):e83934.
19. Bo L, Li J, Tao T, et al. Probiotics for preventing ventilator-associated pneumonia. *Cochrane Database Syst Rev*. 2014;(10):CD009066.
20. Gu WJ, Wei CY, Yin RX. Lack of efficacy of probiotics in preventing ventilator-associated pneumonia probiotics for ventilator-associated pneumonia: a systematic review and meta-analysis of randomized controlled trials. *Chest*. 2012;142(4):859-868.
21. Forestier C, Guelon D, Cluytens V, Gillart T, Sirot J, De Champs C. Oral probiotic and prevention of *Pseudomonas aeruginosa* infections: a randomized, double-blind, placebo-controlled pilot study in intensive care unit patients. *Crit Care*. 2008;12(3):R69.
22. Barraud D, Blard C, Hein F, et al. Probiotics in the critically ill patient: a double blind, randomized, placebo-controlled trial. *Intensive Care Med*. 2010;36(9):1540-1547.
23. Oudhuis GJ, Bergmans DC, Dormans T, et al. Probiotics versus antibiotic decontamination of the digestive tract: infection and mortality. *Intensive Care Med*. 2011;37(1):110-117.

24. Rongrungruang Y, Krajangwittaya D, Pholtawornkulchai K, Tiengrim S, Thamlikitkul V. Randomized controlled study of probiotics containing Lactobacillus casei (Shirota strain) for prevention of ventilator-associated pneumonia. *J Med Assoc Thai*. 2015;98(3): 253-259.
25. Giamarellos-Bourboulis EJ, Bengmark S, Kanellakopoulou K, Kotzampassi K. Pro- and synbiotics to control inflammation and infection in patients with multiple injuries. *J Trauma*. 2009;67(4): 815-821.
26. Kotzampassi K, Giamarellos-Bourboulis EJ, Voudouris A, Kazamias P, Eleftheriadis E. Benefits of a synbiotic formula (Synbiotic 2000Forte) in critically Ill trauma patients: early results of a randomized controlled trial. *World J Surg*. 2006;30(10): 1848-1855.
27. 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):119-126.
28. Banupriya B, Biswal N, Srinivasaraghavan R, Narayanan P, Mandal J. Probiotic prophylaxis to prevent ventilator associated pneumonia (VAP) in children on mechanical ventilation: an open-label randomized controlled trial. *Intensive Care Med*. 2015;41(4):677-685.
29. Morrow LE, Kollef MH, Casale TB. Probiotic prophylaxis of ventilator-associated pneumonia: a blinded, randomized, controlled trial. *Am J Respir Crit Care Med*. 2010;182(8):1058-1064.
30. Sommacal HM, Bersch VP, Vitola SP, Osvaldt AB. Perioperative synbiotics decrease postoperative complications in periampullary neoplasms: a randomized, double-blind clinical trial. *Nutr Cancer*. 2015;67(3):457-462.
31. Falcão de Arruda IS, de Aguilar-Nascimento JE. Benefits of early enteral nutrition with glutamine and probiotics in brain injury patients. *Clin Sci (Lond)*. 2004;106(3):287-292.
32. Araujo GV, Oliveira Junior MH, Peixoto DM, Sarinho ES. Probiotics for the treatment of upper and lower respiratory-tract infections in children: systematic review based on randomized clinical trials. *J Pediatr (Rio J)*. 2015;91(5):413-427.
33. Lone NI, Walsh TS. Prolonged mechanical ventilation in critically ill patients: epidemiology, outcomes and modelling the potential cost consequences of establishing a regional weaning unit. *Crit Care*. 2011;15(2):R102.
34. Liu KX, Zhu YG, Zhang J, et al. Probiotics' effects on the incidence of nosocomial pneumonia in critically ill patients: a systematic review and meta-analysis. *Crit Care*. 2012;16(3):R109.
35. Hurley JC. Profound effect of study design factors on ventilator-associated pneumonia incidence of prevention studies: benchmarking the literature experience. *J Antimicrob Chemother*. 2008;61(5):1154-1161.
36. Jain PK, McNaught CE, Anderson AD, MacFie J, Mitchell CJ. Influence of synbiotic containing *Lactobacillus acidophilus* La5, *Bifidobacterium lactis* Bb 12, *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and oligofructose on gut barrier function and sepsis in critically ill patients: a randomised controlled trial. *Clin Nutr*. 2004;23(4): 467-475.
37. Reignier J, Mercier E, Le Gouge A, et al; Clinical Research in Intensive Care and Sepsis (CRICS) Group. Effect of not monitoring residual gastric volume on risk of ventilator-associated pneumonia in adults receiving mechanical ventilation and early enteral feeding: a randomized controlled trial. *JAMA*. 2013;309(3):249-256.