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The Use of Probiotics to Prevent Ventilator-Associated Pneumonia in Adults

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PA 653 Managing Medical Information III: Research Design and Implementation

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### Abstract

**Objective:** To assess the efficacy and safety of preventing the acquisition of ventilator-associated pneumonia with the use of probiotic supplementation, as compared to a placebo, among hospitalized adult men and women receiving more than 24 hours of mechanical ventilation. **Design:** Systematic Literature Review. **Methods:** Systematic searches were conducted through PubMed and Scopus using the search terms “ventilator”, “probiotics”, and “prevention”. Records were excluded from the analysis if they were published before 2015, full text was not available, studies other than randomized control trial or cohort studies, and if the study population was less than 18 years old. **Results:** Of the four studies, only one had statistically significant findings. In the study, incidence of ventilator-associated pneumonia (VAP) was reduced in the probiotic group, probability of remaining VAP-free was significantly higher in the probiotic group, and mean time of onset of VAP after endotracheal intubation was significantly longer in the probiotics group. **Conclusion:** Probiotics are generally safe to administer and may aid in the immune response of the host; however more research and well-designed studies are needed to definitively determine the effectiveness of probiotics in the prevention of VAP in hospitalized mechanically ventilated patients.

### Introduction

Ventilator-associated pneumonia (VAP) is one of the most commonly diagnosed nosocomial bacterial infections in the intensive care unit (ICU), with reported incidences as high as 78%.<sup>1</sup> It is defined as a type of healthcare acquired pneumonia that develops after 48 hours of endotracheal intubation.<sup>2</sup> It is believed that endogenous flora in the oral cavity and upper airway play a significant role in VAP development, with micro-aspiration around the endotracheal tube cuff being the major route of transmission.<sup>3</sup> VAP prolongs the duration of mechanical ventilation, ICU, and hospital stays, with increased medical costs.<sup>1,4</sup> In 2013, the estimated cost of VAP, with risk of complications, was between \$10,000 to \$60,000 USD.<sup>5</sup> Additionally, morbidity and mortality increase with a crude rate of 24-75% with VAP patients.<sup>4</sup> Various pharmacologic and non-pharmacologic techniques are implemented in the ICU to reduce the incidence of VAP. One pharmacologic method involves attenuation of burden of bacterial colonization in the upper digestive tract by antibiotic use.<sup>1</sup> However, with the increasing incidence of antibacterial resistance in ICUs and the lack of new antibiotics, there is significant

concern for development of antibiotic resistant pathogens in this critically ill population. Further prevention strategies that do not involve parenteral antibiotic use should be investigated due to the significant incidence, morbidity, and mortality of VAP.

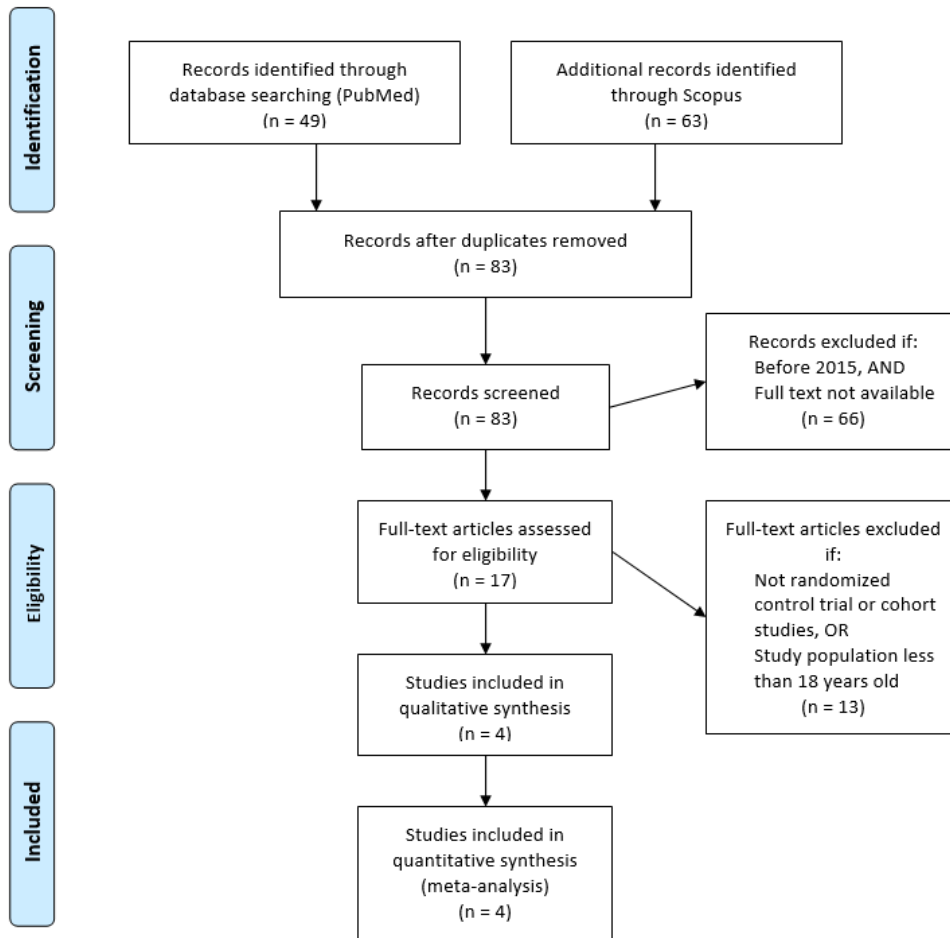
Research is ongoing to identify the potential positive impacts of probiotics in medicine. Probiotics have been projected to exert beneficial effects by enhancing gut barrier function, inhibiting colonization of potentially pathogenic microorganisms, maintaining a normal intestinal milieu, synthesizing antibacterial substances, and stimulating local immunity.<sup>1</sup> Current species of probiotics that are being researched include *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and *Saccharomyces*, among others. In one study, *Lactobacillus casei* (Shirota strain) (LcS) showed inhibitory activity against multi-drug resistant bacteria, including *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, extended spectrum beta-lactamase-producing *Escherichia coli*, *Klebsiella pneumoniae*, and MRSA, resulting in eradication of such organisms at 24 hours in a laboratory-controlled setting.<sup>6</sup> This suggests potential for this strain and others to inhibit common pathogens responsible for VAP, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and other gram-negative bacilli.<sup>2</sup>

Probiotics have a high safety profile, have no obvious contraindication or adverse effects, and are cost effective for patients at less than \$2 per day, spurring significant research interest in the medical field.<sup>1,5,7</sup> Probiotics are also easily administered to patients by medical staff with an estimated time of less than five minutes per day. Several studies have been conducted to show probiotic effectiveness in decreasing the length of ICU stays and reducing VAP-related mortality. This literature review will compare four studies since the last meta-analysis publication to determine if probiotic supplementation prevents the acquisition of VAP and decreasing associated rates of morbidity and mortality.

## Methods

Searches were conducted on both PubMed and Scopus in September of 2018 using the search terms “ventilator”, “probiotics”, and “prevention”. Duplicates between the two databases were removed and then screened, excluding those before the last meta-analysis publication date (2015) and if full text was not available. Full text articles were then reviewed for eligibility and were removed if they were not randomized controlled trials or cohort studies, or if the study population was less than 18 years of age. Four promising studies remained and were reviewed.

The PRISMA for this literature review is demonstrated in Figure 1. Study 1 evaluated the incidence of VAP after administration of probiotic *Lactobacillus casei* (Shirota strain) by oral care once daily and enteral feeding once daily. Study 2 evaluated the incidence of VAP after administration of combination probiotic containing *Bacillus subtilis* and *Enterococcus faecilis* by enteral feeding once daily. Study 3 evaluated the incidence of VAP after administration of combination probiotic containing *Lactobacillus acidophilus* (*gasseri*) and *Lactobacillus helveticus* (*bulgaricus*) by tablet twice daily. Study 4 evaluated the incidence of VAP after the administration of combination probiotic containing *Lactobacillus* (*casei*, *acidophilus*, *rhamnosus*, *bulgaricus*), *Bifidobacterium* (*breve*, *longum*) and *Streptococcus thermophiles* species by two capsules, every 12 hours, administered via enteral feeding.



**Figure 1.** PRISMA depicting the algorithm for identifying appropriate studies that utilize probiotics in ventilator-associated pneumonia prevention to be reviewed in this literature review.

## Results

### Study 1

*Randomized Controlled Study of Probiotics Containing Lactobacillus casei (Shirota strain) for Prevention of Ventilator-Associated Pneumonia.* Rongrungruang et al., 2015.

#### **Objective**

To evaluate the efficacy of probiotics, *Lactobacillus casei* (Shirota strain), in reducing the incidence of VAP in medical patients who received mechanical ventilation at Siriraj Hospital in Thailand.

#### **Design**

This study was a prospective, randomized, open-label controlled trial at a 2,300-bed tertiary care university in Bangkok from May 2011 to August 2013. A combined 150 patients were enrolled, with 75 in the probiotics group and 75 in the control. Most study patients were elderly females with comorbidities and severe health problems leading to mechanical ventilation. Baseline characteristics of the patients in both groups were not significantly different. Study patients were included if they were at least 18 years of age and were expected receive at least 72 hours of mechanical ventilation during their hospitalization. Pediatric patients and those had current VAP upon enrollment were excluded.

The study group received 80 ml of commercially-available fermented dairy product (Yakult®) containing  $8 \times 10^9$  colony-forming units (cfu) of LcS for oral care once daily following standard oral care with chlorhexidine. An additional 80 ml was given via enteral feeding once daily for 28 days, or when the patient's endotracheal tube was removed. Probiotics administration was discontinued when diarrhea related to probiotics occurred. The patients in the control group did not receive any additional products.

All patients received standard VAP preventive bundle techniques as per the Siriraj Hospital protocol. All patients received oral care four times daily with 2% chlorhexidine oral solution.

Patients were observed for the primary outcomes of VAP incidence, and VAP episodes per 1,000 ventilator days. A diagnosis of VAP was made if the patient had a new, persistent, or

progressive infiltrate visible on chest radiograph in combination with at least three of the following criteria: (1) Body temperature  $>38^{\circ}\text{C}$  or  $<35.5^{\circ}\text{C}$ ; (2) Leukocytosis ( $>10,000$  leukocytes/ $\text{mm}^3$  or leukopenia ( $<3,000$  leukocytes/ $\text{mm}^3$ ); (3) Purulent tracheal aspirate; (4) A semi-quantitative culture of tracheal aspirate samples that was positive for pathogenic bacteria.

## Results

There were no statistically significant differences in primary or secondary outcomes reviewed in this study between the study and control groups ( $p < 0.05$ ). *Acinetobacter baumannii* was the most common cause of VAP in both groups.

## Critique

The study was an open-label design, with the control patients, care team, and patient knowing which patient received which care which allows potential for bias. The sample size of 150 participants was originally hypothesized to be appropriate to show statistical significance between the study and control groups with 5% type I error and 80% power, though this was found to be too small. The population was also predominantly females which does not demonstrate equal efficacy between sexes.

The dose of probiotics by enteral or oral administration may have been too low or administered too infrequently for adequate prophylaxis from VAP. The study did not identify the specific VAP-prevention techniques implemented by Siriraj Hospital other than chlorhexidine four times daily. The authors identify that preceding LcS oral care with chlorhexidine may have caused death of the LcS probiotic and impact its efficacy in preventing VAP.

## Study 2

*Effect of probiotics on the incidence of ventilator-associated pneumonia in critically ill patients: a randomized controlled multicenter trial.* Zeng et al., 2016.

## Objective

To assess the effectiveness of probiotics *Bacillus subtilis* and *Enterococcus faecalis* in the prevention of VAP when administered by nasogastric (NG) tube.

## Design

This study was a prospective, open-label, randomized controlled multicenter study involving 11 participating ICUs in nine Chinese teaching hospitals between May 2010 and April 2015. Patients included were adults at least 18 years of age but less than 80 years of age with an expected need for mechanical ventilation for at least 48 hours. Exclusion criteria were age less than 18 or greater than 80 years of age, severe multiple organ failure (with an Acute Physiology and Chronic Health Evaluation (APACHE) II score of  $\geq 25$ ), mechanical ventilation longer than 72 hours prior to enrollment, failure of enteral feeding, administration of immunosuppressive diseases (e.g. malignant tumor, acquired immune deficiency syndrome, human immunodeficiency virus carriers), and pregnancy or lactation. A total of 234 patients was estimated to have statistical power of 80%, with patients randomized equally into study and control groups.

The study group was administered probiotics three times daily in addition to standard preventive strategies for VAP. Each capsule contained  $4.5 \times 10^9$  cfu/0.25 g *Bacillus subtilis* and  $0.5 \times 10^9$  cfu/0.25 g *Enterococcus faecalis*. The capsule was opened and diluted in 50-80 ml of sterile water and administered as a bolus through NG tube. Researchers recorded compliance to the regimen and considered over 80% adherence of study medication as compliant. The control group did not receive placebo treatment.

Both groups received standard preventive strategies for VAP, including daily screening for weaning from mechanical ventilation potential as soon as possible, hand hygiene, aspiration precautions, and prevention of contamination. All patients were placed in a semi-recumbent position in absence of contraindication. Endotracheal pressure cuff was continuously controlled at around 25 cm H<sub>2</sub>O to prevent regurgitation and aspiration. An endotracheal tube which enabled subglottic secretion aspiration was the first choice and preferred over normal tracheal tube. Until enteral feeding was established, all patients admitted to the ICU received IV proton pump inhibitor (PPI) as stress ulcer prophylaxis. Endotracheal suctioning was performed by the nursing staff if necessary. Tracheotomy was performed when ventilation was anticipated to be necessary for greater than three weeks. Normal oropharyngeal care measures included rinsing the mouth with water and, if possible, brushing the teeth once daily.

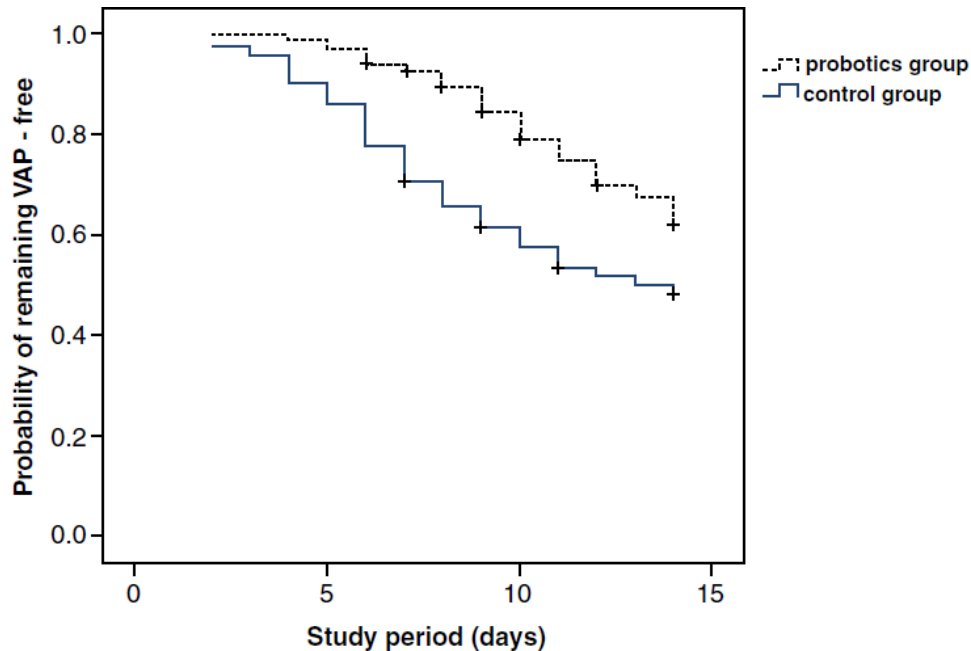


A clinical diagnosis of VAP was based on the presence of new, persistent or progressive infiltrate on chest radiographs that persisted for at least 48 hours (as interpreted by radiologists blinded to the patients' treatment assignments) combined with at least two of the following criteria: (1) temperature  $>38.0^{\circ}\text{C}$  or  $<35.5^{\circ}\text{C}$ ; (2) leukocytosis  $>12,000/\text{mm}^3$  or leukopenia  $<3000/\text{mm}^3$  and/or left shift; (3) purulent tracheal aspirates. All clinical diagnoses of VAP were evaluated and agreed upon by two of the authors.

Primary endpoints were the proportions of eradication of colonization and acquired colonization with potentially pathogenic microorganisms in the oropharynx and stomach, and the incidence of microbiologically-confirmed VAP in patients intubated for at least 48 hours.

## **Results**

Microbiologically-confirmed VAP was significantly reduced in the probiotics (36.4%) compared to the control (50.4%) ( $p = 0.031$ ). The probability of remaining VAP-free was significantly higher in the probiotics group as well by log-rank analysis ( $p = 0.004$ ) (Figure 2). Finally, the mean time of onset of VAP after endotracheal tube intubation was significantly longer in the probiotics group compared to control (10.4 days compared to 7.5 days, respectively;  $p = 0.022$ ). All other primary and secondary endpoints did not demonstrate significant difference.



**Figure 2.** The probability of remaining ventilator-associated pneumonia-free (VAP-free) during the study period in the probiotics and control groups.

Of additional note, there were no statistically significant differences in the pathogens isolated from patients diagnosed with VAP in either the study or control group. The most common gram-negative pathogens isolated were *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The most common gram-positive pathogen isolated was *Staphylococcus aureus*.

### Critique

This was an open-label study which limited blinding. While radiologists interpreting chest radiographs were blinded, the lack of the control group receiving treatment allowed the care teams and patients to be aware of the treatment regimens being administered. This open-label study was notably strict on VAP diagnosis in requiring two of the study's authors to agree upon the clinical diagnosis to be deemed significant. The significant results listed was microbiologically-confirmed VAP though stated clinically diagnosed VAP was not significant. Microbiologically-confirmed VAP was achieved by identifying moderate or heavy growth on cultures of endotracheal aspirate

There was no statistical significance between the pathogens or their respective prevalence between the study and control groups. *P. aeruginosa* was isolated in 13/56 (23.2%) patients with VAP in the study group and 19/67 (28.4%) of the control. *A. baumannii* was isolated in 10/56

(17.9%) and 14/67 (20.9%) in study and control groups, respectively. *S. aureus* was isolated in 12/56 (21.4%) and 16/67 (23.9%) in study and control groups, respectively. Without significant pathogenic difference between the study and control, despite statistical significance in incidence of microbiologically-confirmed VAP, questions are raised as to the mechanism by which probiotics prevent VAP, or the significance of the findings within this study.

### **Study 3**

*Effect of Probiotics on the Incidence of Healthcare-Associated Infections in Mechanically Ventilated Neurocritical Care Patients.* Kenna et al., 2016.

#### **Objective**

The objective of this study was to examine the safety and effectiveness of probiotic administration in the reduction of HAIs, including VAP, among medically ventilated neurocritical care patients.

#### **Design**

This study design was assembled into two retrospective cohorts and took place in a 12-bed NCCU tertiary care academic medical center. A total of 167 patients were included in the study. 80 of those patients were assigned to the pre-intervention cohort that took place from July 1, 2011 to December 31, 2011. This cohort included the supplementation of probiotics via one packet (100,000,000 cfu/packet) or four tablets (1,000,000 cfu/tablet) of *Lactobacillus acidophilus (gasseri)* and *Lactobacillus helveticus (bulgaricus)* administered twice daily by nursing staff. The additional 87 patients were assigned to the post-intervention cohort from July 1, 2012 to December 31, 2012. Probiotic supplementation was not utilized in the post-intervention cohort.

Patient criteria included mechanically ventilated individuals who were admitted to the tertiary care academic medical center. Exclusion criteria applied to patients who were immunocompromised or had a lactose allergy. Researchers defined immunocompromised patients as those with a history of human immunodeficiency virus, current chemotherapy, transplantation, or based on neurocritical care attending's discretion. Furthermore, vulnerable populations were excluded from the study to improve probiotic compliance after a phase-in

period in June 2012. These populations included children under 18 years old, pregnant women, and prisoners.

Ultimately, the study measured the incidence of HAIs (primary outcome) and the number of antibiotic days, ventilator days, length of ICU stay, in-hospital mortality, and discharge status (secondary outcomes). HAIs are defined as central line-associated bloodstream infection, catheter-associated urinary tract infection, ventilator-associated pneumonia, catheter-associated ventriculitis, and *Clostridium difficile* infection. Categorical variables were analyzed using Fisher's exact test. Non-normally distributed continuous variables were analyzed using the Wilcoxon rank sum test. Statistical analysis was based on intention to treat and conducted using Stata SE versions 10 and 13. Statistical significance was defined as  $p < 0.05$ .

## Results

Of the 167 patients who participated in the study, baseline characteristics were overall similar between the pre- and post-intervention retrospective cohorts. There were no significant clinical differences between the study population and the control population

Median age (59 years vs 62 years) and percent male (45% vs 48%) were similar between pre- and post- cohorts. Majority of patients were admitted for traumatic brain injury, intracranial hemorrhage, and subarachnoid hemorrhage ( $p = 0.17$ ). Use of enteral nutrition (UN), steroids and antibiotics were similar between groups, however patients in the probiotic group received more antibiotics. No patients in the pre-intervention group received probiotics. Eighty-five (98%) patients in the pre-intervention group received probiotics for an average of 10 days. The two patients who did not receive probiotics had a change in care to advanced comfort measures.

When comparing patient outcomes, there were 14 (18%) HAIs in the pre-intervention group and 8 (9%) HAIs in the post-intervention group ( $p = 0.17$ ). Ventilator days, ICU days, lengths of stay, in-hospital mortality, and discharge disposition were similar between cohorts. There were no adverse events from use of *Lactobacillus* supplementation.

The authors of the study were able to conclude probiotics are safe to administer in neurocritical patients, however there are no significant decreases in HAIs or secondary outcomes associated with probiotics.

## **Critique**

This study was able to demonstrate probiotics are safe in neuro-critically ill patients. In addition to safety, compliance with probiotic administration was ensured with 98% of patients receiving intervention. Adherence to protocol was achieved through daily rounds by the neurocritical care team.

There are several limitations to this study. The location of the tertiary care academic medical center was not specified in the article. This study was broadly focused on HAIs, with VAP incidence being only one of the outcomes reviewed. This could be a limitation in our systemic analysis by overgeneralizing the study outcomes to HAIs. Due to the retrospective nature, the authors were unable to account for bias from unmeasured changes in care over the study time period. However, selection bias was minimized by including patients from a pre-selected time frame with complete data. The smaller sample size of 167 patients restricted the ability to show real significance. This may be a reason for the lack of association between the reduction of VAP in neurocritical patients with the use of probiotics.

The authors described the study as “unpowered” without further description. In order to detect an 8% difference with 80% power, there needed to be 530 patients in total, making 265 patients per treatment group. The study also included unmeasured or unknown variables that may have influenced the overall outcome. It was not stated how probiotics were administered and the type of enteral nutrition each patient received was not recorded, which may have had unknown interactions with the probiotics. Additionally, the ideal dosing, duration, and type of probiotics were unknown to the authors when creating the study and were chosen based off the availability in the medical center.

## **Study 4**

*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.*  
Mahmoodpoor et al., 2018.

## Objective

The objective of this study was to examine the safety and effectiveness of probiotic administration in decreasing the incidence of VAP in critically ill patients admitted to the surgical ICU.

## Design

This study was a randomized control trial approved by the ethics committee of Tabriz University of Medical Sciences. It was coordinated in two university-affiliated hospitals in northwest Iran from January 2015 to September 2016. Investigators consisted of primary care physicians, nurses, and laboratory employees who were blinded to the study. A total of 120 critically ill patients in the surgical ICUs were enrolled in the study, who were then randomized into two groups. One group received probiotics and the other received placebo during the whole study period, excluded from the study or until death. Patients in the probiotic group received two capsules, every 12 hours for 14 days, administered through enteral feeding. The method for enteral feeding was performed via nasogastric tube with size 16F. Feedings were administered seven times a day. The supplements contained  $1 \times 10^{10}$  cfu consisting of *Lactobacillus (casei, acidophilus, rhamnosus, bulgaricus)* *Bifidobacterium (breve, longum)* and *Streptococcus thermophiles* species. The placebo contained sterile maize starch, which looked identical to the probiotics. Supplementation was given via feeding tube. If the patient could not tolerate enteral nutrition, the patients were excluded from the study.

All patients received the same routine care involving standard precautions for VAP prophylaxis, such as hand washing, suctioning, endotracheal tube with subglottic secretion drainage, heat and moisture filtering, sedation, oral hygiene, changing ventilator circuit, and use of antibiotics. Patient inclusion criteria for the study consisted of critically ill patients 18 years of age or older who were admitted to the ICU and had been undergoing mechanical ventilation for a minimum of 48 hours. Patient exclusion criteria included 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. Furthermore, outcomes being measured were defined as primary or secondary. Primary outcomes were VAP occurrence, and secondary outcomes were mortality, ICU length of stay, duration of mechanical ventilation, and adverse events of probiotic use. Probiotic effectiveness

was analyzed through gut microbial flora in gastric aspirates on the first, third, and seventh days after clinical diagnosis of VAP. All patients with associated diarrhea were also evaluated for *Clostridium difficile*.

## Results

Of the 120 patients involved in the study, the baseline characteristics were very similar between probiotic (n=48) and placebo (n=54) groups. The cerebrovascular accident group comprised those with intracerebral hemorrhage, hypoxemic encephalopathy, ischemic stroke, and brain tumor. The neurological event group covered patients with myasthenia gravis and Guillain-Barré syndrome.

The two groups did not show significant difference between VAP risk factors. Smoking, chronic obstructive pulmonary disease, chest trauma, alcohol consumption, and prolonged duration of ICU stay were considered risk factors for VAP. There were no adverse effects from *Lactobacillus* species, *Bifidobacterium* species and *Streptococcus thermophiles* probiotics. ICU mortality and length of mechanical ventilation did not significantly differ between groups. Patients receiving probiotics did show lower incidence of microbiologically-confirmed VAP and the duration of ICU or hospital stay was lower. However, after applying the Kaplan-Meier survival curve to compare time to the first episode of VAP, there was no statistical significance between control and probiotic groups.

## Critique

Strengths of the study include the double-blind design and the confirmation of VAP based on microbiological criteria. Unintentional biases were also minimized by the rate of compliance to VAP prevention bundles (85%).

Limitations of this study include the small sample size of 120 and the limited number of hospitals enrolled. Most patients involved were surgical cases where the patients were mechanically ventilated for a short duration of time making VAP occurrence rare. Lastly, the ideal dosing, duration, and probiotic strains used were based on the availability in the two ICU centers, not based on the ideal strand for VAP.

## Discussion

Ventilator-associated pneumonia in the United States is most commonly due to *S. aureus* (MSSA, MRSA), *P. aeruginosa*, *Klebsiella* spp, *Enterobacter* spp, *Acinetobacter baumannii*, *E. coli*, *Stenotrophomonas maltophilia*, and other microorganisms.<sup>8,9</sup> Clinical benefit of probiotic use is still uncertain, with studies adjusting specific strains, combinations, or concentrations for a variety of health conditions. Some probiotic strains utilized in this analysis show evidence of protection versus the potentially pathogenic specimens that most commonly cause VAP. As previously mentioned, Tiengrim and Thamlikitkul found significant protective potential against many of the VAP-associated organisms by use of LcS.<sup>6</sup> They proposed that LcS inhibits the growth of the organisms by the products it creates by fermenting the milk, notably lactic acid.<sup>6</sup> The study by Tiengrim and Thamlikitkul was of significant clinical interest and motivation behind the pursuit of Study 1 by Rongrungruang et al. Study 1 was unique in its composition as it provided a fermentable medium for LcS, with the idea that the fermentable product of lactic acid may add a gastrointestinal protective agent. While Study 1 did not reach statistical significance, the fermentable medium used brings a unique idea emphasizing the potential complexity to the clinical utilization of probiotics.

*Bacillus* spp have been shown to produce antimicrobial substances, enhance epithelial gut barrier functions, and stimulate cytokine and systemic immunoglobulin A (SIgA) release in humans.<sup>10</sup> SIgA is the predominant immunoglobulin class in human external secretions and is essential in the maintenance of gut microbiota homeostasis and in the protection of gastrointestinal and respiratory tract pathogens.<sup>10</sup> In a randomized, double-blind, placebo-controlled study, *B. Subtilis* CU1 strain was found to be an immune system stimulator in the elderly during a common infectious disease period.<sup>10</sup> The study found that *B. subtilis* CU1 significantly increased the levels of SIgA detected by saliva and stool analysis.<sup>10</sup> The authors note the importance of this finding because production of SIgA at mucosal surfaces decreases with age and can lead to an increased risk of infection.<sup>10</sup>

Study 2 by Zeng et al. reviewed a probiotic regimen of 90% *B. subtilis* combined with 10% *E. Faecalis*. The data from the study show a significant decrease in microbiologically-confirmed VAP incidence in the study group compared to the control. Though SIgA was not measured as a primary or secondary outcome in the study, previous data supports the role of *B.*



*subtilis* in increased SIgA which could explain the seemingly nonspecific reduction in incidence of VAP. Recall that the organisms isolated did not statically differ between the study and control, however, with the most predominant organisms being *P. aeruginosa*, *A. baumannii*, and *S. aureus*. The patients in the study did not significantly vary in their reason for intubation or the nature of their admission (medical, elective or emergent surgical), however it cannot be stated that there is no significant difference in the stressors experienced by the patients stratified into each group based on their medical condition. This is important to note because SIgA secretion appears to be modulated by stress.<sup>10, 11</sup> While Study 2 was open-label and did not blind its patient, healthcare team, or researchers, the significance of the results may be clinically important when considering sequelae of probiotic administration.

*Enterococcus faecalis* was paired with *B. subtilis* in Study 2. *E. faecalis* is a part of normal gut flora, however it also is of concern for opportunistic pathogenicity.<sup>12</sup> The reasoning for the choice of probiotic strains by the authors in Study 2 is not explicitly stated. Additionally, it is unclear if the authors recognize the potential pathogenicity of *Enterococcus* spp. The authors identify that administration of a probiotic yogurt containing *Lactobacillus rhamnosus* GG was associated with significant reduction in gastrointestinal carriage of vancomycin resistant enterococci.<sup>1</sup> Having a low supplemental dosage in the significantly higher presence of non-pathogenic *B. subtilis* may promote the maintenance of normal gut microbiome, though there is concern for *Enterococcus* spp VAP.<sup>8,9</sup>

There are multiple variables that were inconsistent across the studies. Briefly mentioned was the concentration of probiotics used per administration, use of a medium within which the probiotics were diluted (milk product, sterile water, tablet), route (oral cleanse, naso- or orogastric tube), and frequency of administration. Each of these variables could be altered and the incidence of VAP be observed since there is no accepted standard for any variable at this time. Study 2 was the single study in this review to identify significant difference between VAP incidence. The total probiotics administered amounted to  $5.0 \times 10^9$  cfu administered by enteral feeding once daily. Other studies reviewed all had higher concentrations of cfu administered, were administered multiple times daily, and Study 1 had the added oral care administration of their study strain of LcS. This variability with lack of significance suggests strains may be more important compared to amount of cfu, frequency, or route of administration.

The diagnostic requirements from each study varied (Table 1). Study 3 focused on all HAIs with having incidence of VAP as one of the infections reviewed. Due to this, it cannot be compared to the requirements of other studies. Studies 1, 2, and 4 have similar criteria required, with some slight differences in temperature and leukocyte count or bandemia requirements. As previously mentioned, Study 2 split the primary outcomes into clinically diagnosed VAP (Table 1) and microbiologically-diagnosed VAP (described in the critique for Study 2). The statistically significant differences of these two outcomes varied, with only microbiologically-diagnosed VAP being significant. By separating clinical from microbiological, there may be added benefit in requiring microbiology to confirm VAP diagnosis. However, this could also show that there was added scrutiny in Study 2, an open-label randomized controlled trial. Two of the authors had to agree upon the clinical diagnosis of VAP which may be added scrutiny that could bias the results (Table 1).

**Table 1.** The ventilator-associated pneumonia diagnostic requirements respective to each study reviewed.

VAP = ventilator-associated pneumonia; CXR = chest X-ray radiograph; cfu = colony-forming units

	<b>Study 1</b>	<b>Study 2*</b>	<b>Study 3</b>	<b>Study 4</b>
<b>Diagnostic criteria</b>	Presence of new, persistent, or progressive infiltrate on chest radiograph in combination with at least 3 of the following criteria:  1) Temperature >38.0°C or <35.5°C  2) Leukocytosis >10,000/mm <sup>3</sup> or leukopenia <3,000/mm <sup>3</sup>  3) Purulent tracheal aspirate  4) A semi-quantitative culture of tracheal aspirate samples that was positive for pathogenic bacteria	Presence of new, persistent, or progressive infiltrate on chest radiographs that persisted for ≥48 hours, combined with 2 of the following criteria:  1) Temperature of >38.0°C or <35.5°C  2) Leukocytosis >12,000/mm <sup>3</sup> or leukopenia <3,000/mm <sup>3</sup> and/or left shift  3) Purulent tracheal aspirate  <i>*All clinical diagnoses of VAP were evaluated and agreed upon by two of the authors</i>	Not specified in this study	Presence of new or persistent infiltration on CXR with 2 of the following criteria:  1) Temperature >38.0°C or <36.0°C  2) Leukocytosis or leukopenia (ranges not specified)  3) Bronchoalveolar lavage with at least 10 <sup>4</sup> cfu/mL

Sample size of the study population is an important factor when determining its clinical relevance. Studies reviewed in this analysis had population sizes ranging from 100 to 235 patients (Table 2). The small sample sizes increase Type 2 error risk and are of concern in clinical efficacy. The largest sample size was seen in Study 2, which was the single study that showed statistical significance in VAP incidence in this review (Table 2). Sample sizes of this range are not ideal due to the increased risk of Type 2 error. In other words, there is a higher chance of accepting a false hypothesis, which does not contribute to clinical practice. A larger sample size in the four studies analyzed would be an appropriate way to render research more efficient and reliable concerning the effectiveness of probiotics in the prevention of VAP in hospitalized patients.

**Table 2.** An overview of each article reviewed in this literature, including study title, type, year published, number of participants in the study, the composition of the probiotics used and their concentrations if stated, the primary outcomes, and statistically significant findings.

Cfu = colony-forming units; VAP = ventilator-associated pneumonia

	Study 1	Study 2	Study 3	Study 4
<b>Study title</b>	Randomized Controlled Study of Probiotics Containing <i>Lactobacillus casei</i> (Shirota strain) for Prevention of Ventilator-Associated Pneumonia	Effect of probiotics on the incidence of ventilator-associated pneumonia in critically ill patients: a randomized controlled multicenter trial	Effect of Probiotics on the Incidence of Healthcare-Associated Infections in Mechanically Ventilated Neurocritical Care Patients	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
<b>Study type</b>	Open-label randomized controlled trial	Open-label randomized controlled trial	Prospective cohort study	Double-blind randomized controlled trial
<b>Year published</b>	2015	2016	2016	2018
<b>Number of participants</b>	150	235	167	100
<b>Probiotic composition</b>	8x10 <sup>9</sup> cfu <i>Lactobacillus casei</i> (Shirota strain)	4.5x10 <sup>9</sup> cfu <i>Bacillus subtilis</i> 0.5x10 <sup>9</sup> cfu <i>Enterococcus faecalis</i>	1.0x10 <sup>8</sup> cfu <i>Lactobacillus acidophilus</i> (gaserry) 1.0x10 <sup>8</sup> cfu <i>Lactobacillus helveticus</i> (bulgaricus)	1.0x10 <sup>10</sup> cfu containing <i>Lactobacillus casei</i> , <i>acidophilus</i> , <i>rhamnosus</i> , <i>bulgaricus</i> , <i>Bifidobacterium (breve, longum)</i> , <i>Streptococcus thermophiles</i> spp
<b>Primary outcomes</b>	Incidence of VAP episodes per 1,000 ventilator-days	VAP incidence  Eradication of colonization with potentially pathogenic microorganisms in the oropharynx and stomach	The incidence of hospital-associated infections	VAP occurrence
<b>Statistically significant findings</b>	None	Microbiologically confirmed VAP reduced in the probiotics group (36.4%) compared to control (50.4%) (p = 0.031)  Probability of remaining VAP-free was higher in the probiotics group (p = 0.004)  Mean time of VAP onset after endotracheal intubation was longer in the probiotic group (10.4 days) compared to control (7.5 days) (p = 0.022)	None	None

### Conclusion

In reviewing the literature, data regarding probiotic use and VAP prevention vary between meta-analyses. With the high incidence and mortality of VAP, as well as the increasing rates of antibiotic resistance with the relative deficiency of new antibiotics, additional VAP prevention options must be pursued. Probiotics are generally of no pathogenic concern and have the potential to aid in immune responses to decrease infectious complications in mechanically-ventilated patients. However, as discussed, only one of the four studies reviewed was able to show significantly reduced microbiologically-confirmed VAP with the supplementation of probiotics. The three remaining studies were inconclusive in demonstrating efficacy of probiotics.

There is significant variance among strains, concentrations, administration routes, administration frequency, and VAP-prevention bundles used throughout these studies. Additionally, there is variance between clinical diagnostic requirements of VAP. These

differences make comparisons between studies and resultant clinical applications difficult to determine. Recommendations for future research into VAP prevention would be a double-blinded randomized controlled trial involving the use of *B. subtilis* and *E. faecalis* at the same concentrations (Table 2) and diagnostic requirements (Table 1) as specified in Study 2.

Additional interest involves the use of LcS as specified in Study 1 though without the oral care of chlorhexidine. In general, further research is needed to determine the efficacy of probiotics in the clinical care and prevention of VAP.

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