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PROBIOTIC PROPHYLAXIS OF VENTILATOR-ASSOCIATED PNEUMONIA: A BLINDED, RANDOMIZED, CONTROLLED TRIAL

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ABSTRACT

Rationale: Enteral administration of probiotics may modify the gastrointestinal environment in a manner which preferentially favors growth of minimally virulent species. It is unknown whether probiotic modification of the upper aerodigestive flora can reduce nosocomial infections.

Objectives: To determine whether oropharyngeal and gastric administration of *Lactobacillus rhamnosus* GG can reduce the incidence of ventilator-associated pneumonia (VAP).

Methods: We performed a prospective, randomized, double-blind, placebo-controlled trial of 146 mechanically ventilated patients at high risk of developing VAP. Patients were randomly assigned to receive enteral probiotics (n=68) or an inert inulin-based placebo (n=70) twice a day in addition to routine care

Measurements and Main Results: Patients treated with *Lactobacillus* were significantly less likely to develop microbiologically-confirmed VAP when compared to patients treated with placebo (40.0% vs. 19.1%, P=0.007). Although probiotic treated patients had significantly less *Clostridium difficile* associated diarrhea than placebo treated patients (18.6% vs. 5.8%, P=0.02) the duration of diarrhea per episode was not different between groups (13.2±7.4 days vs. 9.8±4.9 days, P=0.39). The probiotic treated cohort had fewer days of antibiotics prescribed for VAP (8.6±10.3 days vs. 5.6±7.8 days, P=0.05) and for *Clostridium difficile* associated diarrhea (2.1±4.8 SD days vs. 0.5±2.3 days, P=0.02). No adverse events related to probiotic administration were identified.

Conclusions: These pilot data suggest that *Lactobacillus rhamnosus* GG is safe and efficacious in preventing VAP in a select, high-risk ICU population.

INTRODUCTION

It is estimated that ventilator-associated pneumonia (VAP) complicates the care of up to 30% of patients receiving mechanical ventilation (1-4). Patients with VAP have increased morbidity, mortality and hospital costs as well as prolonged intensive care unit (ICU) and hospital lengths of stay, and increased costs (1,5-7). The pathogenesis of VAP is complex but typically involves colonization of the aerodigestive tract with pathogenic bacteria, formation of biofilms, and microaspiration of contaminated secretions (5,8). Accordingly, current effective VAP prevention strategies target modifiable risk factors for colonization and aspiration including elevation of the head of the bed, subglottic secretion draining or silver coated endotracheal tubes, intensive oral care, and minimizing the duration of mechanical ventilation through regular use of sedation vacations and weaning protocols (3,8-14).

In view of these events central to the pathogenesis of VAP, probiotic therapy is an intriguing option as a non-antibiotic strategy for maintenance of the host's aerodigestive microbial balance and VAP prevention. Probiotics are defined by the World Health Organization as living microbial agents of human origin which are able to tolerate the hostile gastrointestinal (GI) environment (acid and bile) such that they ultimately persist in the lower alimentary tract to confer health benefits to the host (15). Probiotics could potentially reduce the incidence of VAP through various local and systemic effects that minimize colonization by more virulent species and/or optimize host immune defenses. These effects include reduced overgrowth of potentially pathogenic microorganisms, improved gut mucosal barrier function, reduced bacterial translocation, and toll-like receptor mediated up-regulation of immune function (16-21). Evidence supporting this theory is limited – but promising (22).

Studies enrolling adult trauma, neurosurgical, liver transplant, and general surgery patients have all demonstrated trends towards reduced rates of infections, including pneumonia, in probiotic treated patients (23-27). However, almost all of these studies included co-administration of prebiotics (non-digestible ingredients that stimulate the growth and activity of

bacteria in the gut), a practice known as synbiotic therapy. Because prebiotics have effects on the intestinal flora that are analogous to those seen with probiotic administration, the precise role of probiotics in reducing infectious complications remains unknown. Such an assessment is critically important as administration of living microbial species to critically ill individuals carries the inherent risk of iatrogenic infection. Therefore we conducted a study with two main goals. Our first goal was to determine the efficacy of isolated probiotic administration for the prophylaxis of VAP. The second goal was to examine the safety of probiotic therapy in a high-risk, critically ill population. Some of the results of these studies have been previously reported in the form of abstracts (28, 29).

Methods

Creighton University's institutional review board approved the study protocol – including written, informed surrogate consent – prior to enrolling any patients. The trial was registered with ClinicalTrials.gov on January 31, 2008 (NCT00613795) to comply with NIH requirements.

All screening was performed daily by the lead investigator (LEM) and/or a dedicated study coordinator. Adults at least 19 years old (the age of majority in Nebraska) were eligible for enrollment if the lead investigator and the treating physician agreed that there was a 95% likelihood that the patient would require mechanical ventilation with an endotracheal tube for at least 72 hours. Exclusion criteria were selected to exclude patient subsets previously described as being at risk for iatrogenic probiotic infection: pregnancy; immunosuppression; prosthetic cardiac valve or vascular graft; cardiac trauma; history of rheumatic fever, endocarditis or congenital cardiac abnormality; gastroesophageal or intestinal injury or foregut surgery during the current admission; oropharyngeal mucosal injury; and placement of a tracheostomy. The rationale underlying each of the many exclusion criteria is included in the online supplement. Patients were also excluded if the investigators were unable to obtain informed written consent and administer the first dose of study drug within 24 hours of intubation. Patients were recruited

from July 2004 to January 2009 at a 325-bed university-based hospital that provides Level 1 trauma services.

Patients were randomly assigned in a 1:1 ratio to treatment groups using permutation blocks (n=4 per block) within three severity of illness strata by Acute Physiology and Chronic Health Evaluation (APACHE) II scores (<18, 18-24, or >24). Investigators, bedside nurses, primary care clinicians, and microbiology laboratory personnel were blinded to group assignments. Patients randomized to probiotic therapy received 2 x 10^9 colony forming units (cfu) of *Lactobacillus rhamnosus* GG on a twice-daily basis: the contents of one capsule containing 10^9 cfu of *Lactobacillus* were suspended in sterile, water-based surgical lubricant and administered as a slurry to the oropharynx while the contents of a second capsule containing 10^9 cfu of *Lactobacillus* were suspended in sterile water and given through the nasogastric tube. The same methods were used to deliver the contents of identical appearing capsules containing the inert plant starch inulin to patients randomized to placebo.

Patients continued to receive active intervention or placebo until extubation, tracheostomy placement, or death. Patients received all routine care – including VAP-preventive measures as per hospital protocols and antibiotic therapy as deemed necessary – under the direction of their admitting physicians throughout the study. Institutional VAP-prevention measures remained unchanged throughout the study period and are described further in the online supplement.

The study protocol-mandated baseline data included demographic information, medical history and the APACHE II score. Additional information collected on a daily basis included chest radiograph findings, clinical signs of VAP, adverse events, lengths of stay in the ICU and hospital, duration of mechanical ventilation, and mortality. If patients were diagnosed with VAP using the American College of Chest Physicians (ACCP) clinical criteria, quantitative cultures of distal airways samples were obtained by non-bronchoscopic bronchoalveolar lavage (BAL) using a protected catheter (Combicath; KOL Biomedical Instruments, Chantilly, Virginia). The

ACCP clinical criteria require a new and persistent infiltrate on chest radiographs with two of three supporting findings: fever (>38.5° C or <35.0° C), leukocytosis (white blood cells >10,000/mm^3 or <3,000/mm^3) and/or purulent sputum.

Non-bronchoscopic BAL was performed using previously described techniques (30). Samples obtained using this technique are collected blindly: they are not specifically collected from the site of radiographic abnormality. However, the diagnostic utility of non-bronchoscopic BAL is comparable to that of specimens obtained bronchoscopically (30).

The primary outcome was microbiologically-confirmed VAP incidence based on quantitative BAL culture with at least 10^4 cfu/mL in patients intubated for 48 hours or longer. Secondary outcomes included: mortality; time to occurrence of VAP; durations of mechanical ventilation, ICU stay, and hospital stay; *Clostridium difficile* associated diarrhea; other ICU-associated diarrhea; antibiotic consumption (total, VAP-specific, and *Clostridium difficile*-specific); and hospital charges. Antibiotic consumption was measured in antibiotic-days, a composite measure incorporating the number and duration of antibiotics prescribed. Antibiotic-days were calculated by summing the number of antibiotics administered across all of the days antibiotics were prescribed. This calculation is discussed further in the online supplemental materials. All patients with diarrhea (three or more loose stools per 24-hour period or placement of a fecal management system for continuous liquid stool) had a *Clostridium difficile* cytotoxin assay sent. Each negative assay was repeated twice in order to minimize the rate of false negative tests. Patients with diarrhea but three negative *Clostridium difficile* cytotoxin assays were classified as having “ICU-associated” diarrhea, presumably due to acute illness, dietary changes, and antibiotic administration.

In order to assess whether probiotic administration resulted in measurable changes in the oropharyngeal flora, patients had an oral swab, gastric aspirate, and non-bronchoscopic BAL collected prior to administration of the first dose of study medication, after 72 hours of study participation (immediately prior to dose 7 of study drug), and with the clinical diagnosis of VAP.

Oral swabs and gastric aspirates were sent for semiquantitative cultures while BAL fluid was sent for quantitative culture.

An independent data and safety monitoring board supervised the study investigation and reviewed interim data after enrolling the first 40 patients and after enrolling the first 80 patients. Board members had no financial relationship wish the sponsor. The board had access to all data and made the final determination whether the study would be continued, terminated or modified based on study enrolment, trends toward futility or inferiority in the primary outcome (VAP), lack of measured effect on colonization, and safety. None of the interim analyses resulted in modifications or termination based on our application of the predefined early stopping rules of O'Brien and Fleming (31).

Statistical Analysis

Sample size calculations assumed a 38% incidence of VAP in the control arm based on historic trends from this ICU, a 50% reduction in VAP caused by the intervention based on existing published data, and a dropout rate of 5%. We calculated that approximately 146 patients should be enrolled to achieve statistical power of 80% with a 2-sided significance level of 0.05. In modified intention-to-treat analyses, patients intubated for 48 hours or longer (those "at-risk" for VAP) were analyzed as the primary efficacy population. All patients enrolled were analyzed for safety.

Descriptive statistics using appropriate tests were used for all baseline characteristics. As determined by the data distribution, the t or Mann-Whitney test was used to compare between-group differences for continuous variables. A χ^2 test was used for categorical variables. The primary outcome, VAP incidence, was analyzed by univariate technique using PASW Statistics 17 (Chicago, IL). Only the initial episode of VAP for each patient was included in the analyses. Kaplan-Meier analyses were performed using product-limit survival estimates with the generalized Wilcoxon test for statistical comparisons. All P values were 2-sided,

significance was set at P<0.05, and the significance level was adjusted for the two interim analyses required by the data and safety monitoring plan.

RESULTS

A total of 2,871 patients were screened (Figure 1); 2,725 were not enrolled because informed surrogate consent could not be obtained during the first 24 hours of mechanical ventilation, patients had exclusion criteria, or patients were unlikely to require intubation for at least 72 hours. Patients were evenly distributed between groups based on demographic and other baseline characteristics (Table 1). Although most VAP risk factors were balanced, the prevalence of chest trauma was higher in the *Lactobacillus* arm of the study (2.9% vs. 19.1%, P=0.002).

Primary Outcome

Among the 138 patients in the modified intention to treat (mITT) analysis, 50 were diagnosed with VAP using the clinical criteria and underwent non-bronchoscopic BAL (33 of 70 placebo patients [47.1% incidence; 95% confidence interval (CI) 35.1-59.1%] vs. 17 of 68 *Lactobacillus* patients [25.0% incidence; 95% CI 14.4-35.6%], P<0.001)(Table 2). Of these, 28 patients receiving placebo had microbiologically-confirmed VAP (40.0% incidence; 95% CI 28.2-51.8%) compared to 13 patients receiving *Lactobacillus* (19.1% incidence; 95% CI 9.4-28.6%, P=0.007).

None of the patients who were randomized but excluded from the mITT analysis (n=8) met the clinical criteria for VAP during their hospitalization. As such, when the intention to treat (ITT) population was analyzed, significant between-group differences remained for clinically-diagnosed VAP (33 placebo cases [45.2% incidence; 95% CI 33.4-57.0%] vs.17 *Lactobacillus* cases [23.3% incidence; 95% CI 13.3-33.3%], P=0.005) and for microbiologically-diagnosed VAP (28 placebo cases [38.4% incidence; 95% CI 27.0-49.8%] vs.13 *Lactobacillus* cases [17.8% incidence; 95% CI 8.8-26.8%], P=0.006).

Secondary Outcomes

In this study cohort, *Lactobacillus* administration resulted in a significant delay in the time to onset of microbiologically-confirmed VAP (P<0.001 by generalized Wilcoxon Test)(Figure 2). Probiotic use led to significant reductions in rates of *Clostridium difficile* cytotoxin assay positive diarrhea (13 patients [18.6%] vs. 4 patients [5.8%], P=0.02). Probiotic-treated patients who tested positive for *Clostridium difficile* did not have fewer days of diarrhea compared to placebo-treated patients who tested positive for Clostridium difficile (9.8±4.9 days vs. 13.2±7.4 days, P=0.39). Diarrhea not caused by *Clostridium difficile* – so called ICU-associated diarrhea – was common in both groups (42 [61.8%] *Lactobacillus* patients vs. 44 [62.9%] placebo patients, P=0.81). The number of days of ICU-associated diarrhea was significantly reduced in patients receiving *Lactobacillus* therapy (4.1±3.7 days vs. 5.9±3.8 days, P=0.03).

Among patients with confirmed VAP, probiotic patients had 16.1±7.9 antibiotic-days for their VAP while placebo patients had 15.3±10.7 antibiotic-days (P=0.81). Among patients with confirmed *Clostridium difficile* infection, probiotic patients had 6.3±5.4 antibiotic-days for their *Clostridium difficile* while placebo patients had 9.5±5.9 antibiotic-days (P=0.35). However, the differences in nosocomial infection rates translated into trends towards reductions in total antibiotic consumption (16.3±14.4 antibiotic-days vs. 13.3±10.4 antibiotic-days, P=0.16) and antibiotics prescribed for VAP (8.6±10.3 antibiotic-days vs. 5.6±7.8 antibiotic-days, P=0.05) in patients randomized to probiotic treatment. There was a significant reduction in antibiotic consumption for *Clostridium difficile* (2.1±4.8 antibiotic-days vs. 0.5±2.3 antibiotic-days, P=0.02) in the group of patients receiving probiotics (Table 3).

Durations of mechanical ventilation, ICU stay, hospital stay, and total charges were not different between groups (Table 3). While mortality was not significantly different between the two study arms (21.4% in the placebo arm vs. 17.6% in the probiotic arm, P=0.42), patients with VAP showed a strong trend towards increased mortality when compared to patients without VAP (23.7% vs. 9.8%, p=0.06). We did not observe any adverse events attributable to probiotic administration. Specifically, no cases of *Lactobacillus* bacteremia or pneumonia were seen in

the intervention arm of the study. We obtained permission for autopsy in three *Lactobacillus* treated patients who died while participating in the study: there was no evidence of *Lactobacillus* infection in any of these patients.

Surveillance Culture Data

Rates of oral colonization with pathogenic species were not significantly different between study arms at baseline (41.4% for placebo vs. 42.6% for *Lactobacillus*, P=0.88)(Table 4). Rates of gastric colonization were also similar at baseline (31.4% for placebo vs. 32.3% for *Lactobacillus*, P=0.49). After 72 hours of study participation, patients given placebo had significantly higher oral colonization rates compared with patients given placebo (70.0% for placebo vs. 38.2% for *Lactobacillus*, P<0.001). Rates of gastric colonization were also higher at 72 hours in placebo treated patients (45.7% for placebo vs. 32.3% for *Lactobacillus*, P=0.03). Changes in oral colonization were significantly correlated with the development of microbiologically-confirmed VAP (Pearson correlation coefficient 0.22, P=0.009).

DISCUSSION

In this very select, high-risk cohort, probiotic administration was associated with a statistically significant reduction in the incidence of VAP based on rigorous diagnostic criteria requiring microbiological confirmation on invasive lower respiratory tract samples. The estimated number of patients needed to treat with *Lactobacillus* to prevent one case of VAP is approximately 5 (95% CI 3-250) based on the high-risk patients we studied. This novel finding builds on the observations of others who suggest that probiotic therapy is safe for administration in a properly selected, critically ill population. In addition to the reduction of VAP in this cohort, *Lactobacillus* therapy led to statistically significant reductions in *Clostridium difficile* associated diarrhea. Probiotic therapy also showed less utilization of antibiotics for the treatment of *Clostridium difficile* diarrhea. Collectively, these data suggest that *Lactobacillus* may represent a novel, inexpensive (retail price $2.13 per day for a total of four tablets as administered per

protocol), and non-antibiotic approach to prevention of nosocomial infections in properly selected ICU patients.

To date there have been five randomized controlled trials of probiotic therapy as a strategy to prevent VAP (24, 32-35). These studies had a mean sample size of 159 patients (range 50-300) and an average APACHE II score of 17. Three of the studies used double blinding (24, 32, 35) and four analyzed single center data (24, 32-34). While four of the five studies showed trends toward reduced VAP rates in probiotic treated patients, the difference was statistically significant in only two of the studies (34, 35). These studies have significant heterogeneity in their inclusion criteria, populations studied, probiotic agent(s) used, probiotic dosing, route of probiotic administration, and – most importantly – the diagnostic criteria used in establishing VAP. Four of the five studies required only qualitative cultures of tracheal aspirates (24, 33-35): the lone study that used quantitative cultures allowed testing of samples from tracheal aspirate, protected specimen brush, or BAL (33). These studies, when combined using meta-analysis methods, suggest that administration of probiotics results in a 39% reduction in VAP (36). Significant differences were also seen in length of ICU stay and colonization of the respiratory tract with *Pseudomonas aeruginosa*.

Because the presumed mechanisms of probiotic therapy are inherently based on an ability to alter the host flora, the results of these studies must be interpreted within this context. In the three negative VAP prevention trials to date, two were unable to demonstrate significant effects on oropharyngeal colonization (24, 33): the remaining study favorably altered colonization patterns but was underpowered to detect differences in VAP (32). Neither of the positive studies reported data regarding changes in the pathogen colonization rates (34, 35). In the present study probiotic administration significantly reduced both oropharyngeal and gastric colonization. This is a key observation as changes in colonization were significantly correlated with the development of microbiologically-confirmed VAP in the present study.

An interesting – and unexpected – finding was that probiotic administration appeared to have preferential effects on reducing rates of microbiologically-confirmed VAP caused by Gram negative pathogens. While VAP caused by Gram positive organisms did not differ between groups (12.8% vs. 5.8%, P=0.16), VAP caused by Gram negatives were dramatically different (22.8% vs. 8.8%, P<0.02). Our present lack of understanding regarding the mechanisms of probiotics precludes speculation regarding why this observation exists. However, the data on pathogen colonization from the present study implies that changes in the host flora are in some manner relevant. This observation is consistent with two other studies which have showed decreased colonization in patients administered probiotic therapy (32,33). It remains unknown which anatomic sites are the most important targets for modifying the host flora with probiotic therapy.

The present study is unique in that we used lower respiratory tract sampling with quantitative cultures to establish the microbiologic diagnosis of VAP. This study also differs from prior studies in that we intentionally selected a very high risk population as evidenced by the cohort's high APACHE II scores and prolonged duration of mechanical ventilation. The choice of the specific probiotic agent used in this study (*Lactobacillus rhamnosus* GG) was also discrepant from the agent(s) used in existing trials. This particular agent was chosen because it had the most robust safety data as well as cursory data suggesting that it may have preferential activity in the upper airways (37-39). Given the paucity of comparative data in this area, it remains unknown whether other agents would have similar – or superior – results.

We wish to strongly emphasize that these data should be viewed as preliminary in nature and can not be generalized to the general ICU population given the prolonged period of enrolment, the rigorous inclusion criteria, the large number of exclusion criteria, and the small number of patients included. Furthermore, the current study has multiple limitations which merit particular comment. First, these data come from a single center and carry inherent biases related to local practice habits and the population served. Creighton University Medical Center

serves an urban community with limited resources and has a patient population with many risk factors for colonization with healthcare-associated pathogens (frequent readmissions, homelessness, high antibiotic consumption, use of hospital-based clinics and the emergency room for primary care). This is reflected in the high rate of colonization seen in the baseline cultures. Second, in enrolling patients who were very likely to require >72 hours of mechanical ventilation, we selected patients who were very sick (mean APACHE ~ 23) and had prolonged mechanical ventilation (mean duration ~ 10 days), placing these patients at high risk for VAP. The assumptions used in our power calculations and our limited resources required us to aggressively exclude patients who would receive short courses of mechanical ventilation. This was necessary as individuals intubated less than 48 hours can not, by definition, develop VAP and would have an "immortality bias" thereby skewing the results towards the null hypothesis. The anticipated consequence of such selective enrolment was that study completion was prolonged (54 months) and a very large proportion of patients requiring mechanical ventilation who were not included (95%). Coupled with the extensive exclusion criteria, it is imperative to appreciate that the results of this study cannot be generalized to the broader ICU population.

Third, the sample size was not large enough to allow for adequate power when assessing most of our secondary endpoints. Accordingly, the described trends should be interpreted only as observations which merit further investigation. Fourth, like most other existing VAP-prevention strategies, probiotic therapy requires compliance and is inherently susceptible to human error. We rigorously monitored study adherence in real-time, resulting in 97% of doses being administered within strict, protocol-specified time limits. Such compliance may not be obtained in routine practice. Finally, given the concurrent administration of probiotics to two anatomically distinct sites – the oropharynx and the stomach – the critical site of delivery is unknown. Similarly, the lack of samples for mechanistic studies limits further inference regarding mechanisms of action. Furthermore, the data regarding differences in

antibiotic use are limited by the methods used to calculate our composite measure of antibiotic presciption (antibiotic-days).

The potential harms of probiotic therapy also require comment. Historically, the consensus has been that probiotic therapy was of questionable value but was safe. However, this latter assumption was shown to *not* be true in the PROPATRIA trial – a randomized, double-blinded, placebo controlled clinical trial evaluating the efficacy of a novel probiotic combination in preventing infections in predicted severe acute pancreatitis (40). Although the investigators in this multicenter Dutch trial have been heavily criticized regarding the trial's design, execution, and analysis, even detractors agree that probiotic administration led to increased mortality when compared to placebo (15.7% vs. 6.3%, P=0.01) (41). Contrary to the prevailing safety concern – that probiotic administration could lead to iatrogenic infection – the increased mortality seen in the probiotic arm of PROPATRIA was attributed to a significantly higher rate of intestinal ischemia (6.3% vs. 0%, P=0.004). While this unexpected observation may be unique to the novel probiotic preparation used, its specific route of administration, the disease state studied, and/or other unknown factors, this trial should remind us of the risks (potentially unanticipated) of probiotic therapy research and highlights our need for meticulous monitoring of subjects. Although no safety issues have been identified in any of the investigations using probiotics for VAP prevention to date, the findings of the PROPATRIA investigators reinforces the need to view the current study as preliminary observations which merit further investigation and structured safety monitoring.

Probiotic prophylaxis of VAP using *Lactobacillus rhamnosus* GG appears safe and efficacious in a select population of patients with very high risk for VAP. This therapy may also offer an opportunity to prevent related ICU complications such as *Clostridium difficile* and ICU-associated diarrhea. Ultimately, probiotics may fulfill a role in antimicrobial stewardship programs given the reductions in antibiotic consumption. Larger, multi-center clinical trials with more liberal inclusion criteria are needed to further establish efficacy of probiotics and to allow

for extrapolation to a larger at-risk population. It will be critical that such studies include collaboration with basic scientists in order to more rigorously study potential mechanisms of probiotics' effects.

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Table 1. Demographics and Baseline Characteristics for the Intention to Treat Population

	Placebo N=73	*Lactobacillus* GG N=73	P-Value
Female gender	30 (41.1%)	30 (41.1%)	1.00
Age, mean±SD (range) y	54.6±16.3 (21-91)	52.5±19.3 (19-88)	0.47
APACHE II score, mean±SD (range)	23.7±8.0 (8-41)	22.7±7.5 (8-38)	0.45
Race			0.97
Caucasian	58 (79.5%)	57 (78.1%)	
African American	9 (12.3%)	10 (13.7%)	
Hispanic	6 (8.2%)	6 (8.2%)	
VAP risk factors			
Smoker	17 (23.3%)	20 (27.4%)	0.52
COPD	12 (16.4%)	11 (15.1%)	0.82
Chest trauma	2 (2.7%)	13 (17.8%)	0.003
Nursing home resident	4 (5.5%)	10 (13.7%)	0.09
Alcohol abuse	12 (16.4%)	17 (23.3%)	0.30
Reason for ICU admission			0.30
Trauma	23 (31.5%)	31 (42.4%)	
Respiratory failure	20 (27.4%)	16 (21.9%)	
Infection	5 (6.8%)	2 (2.7%)	
Cardiology	8 (11.0%)	6 (8.2%)	
Neurology/Neurosurgery	12 (16.5%)	13 (17.8%)	
Gastrointestinal	4 (5.5%)	3 (4.1%)	
Renal	1 (1.4%)	0 (0.0%)	
Endocrine	0 (0.0%)	2 (2.7%)	

APACHE= Acute Physiology and Chronic Health Evaluation; VAP= ventilator-associated pneumonia; COPD= chronic obstructive pulmonary disease; ICU= intensive care unit.

Table 2. Incidence and Microbiology of Ventilator-Associated Pneumonia

	Placebo	*Lactobacillus* GG	P Value
Subjects With At Least One Episode Of Clinically-Diagnosed VAP			
Intention to Treat Analysis	33 of 73 (45.2%)	17 of 73 (23.3%)	0.005
Modified Intention to Treat Analysis	33 of 70 (47.1%)	17 of 68 (25%)	<0.001
Subjects With At Least One Episode Of Microbiologic-Confirmed VAP			
Intention to Treat Analysis	28 of 73 (38.4%)	13 of 73 (17.8%)	0.006
Modified Intention to Treat Analysis	28 of 70 (40.0%)	13 of 68 (19.1%)	0.007
Subjects With Gram Positive Pneumonia	9 of 70 (12.8%)	4 of 68 (5.8%)	0.16
Subjects With Gram Negative Pneumonia	16 of 70 (22.8%)	6 of 68 (8.8%)	0.02
Subjects With Mixed (Gram Positive, Gram Negative, and/or Other) Pneumonia	3 of 70 (4.2%)	3 of 68 (4.4%)	0.97
Microbiology			
Gram Positive Pathogens Isolated	**15**	**10**	
MSSA	8	4	
MRSA	6	4	
Streptococcus species	1	2	
Gram Negative Pathogens Isolated	**31**	**9**	
Pseudomonas	6	0	
Enterobacteriaceae	3	2	
Haemophilus influenza	3	1	
Acinetobacter	2	3	
Klebsiella	3	1	
Proteus	2	0	
E coli	3	0	
Serratia	4	0	
Citrobacter	1	0	
Stenotrophomonas	4	0	
Burkholderia	0	1	
Alcaligenese	0	1	
Other Pathogens Isolated	**1**	**0**	
Yeast	1	0	

VAP=ventilator-associated pneumonia; MSSA=methicillin-susceptible *Staphylococcus aureus*; MRSA=methicillin-resistant *Staphylococcus aureus.*

Table 3. Secondary Outcomes

	Placebo N=70	*Lactobacillus* GG N=68	P Value
Death	15 (21.4%)	12 (17.6%)	0.47
Clostridium difficile diarrhea	13 (18.6%)	4 (5.8%)	0.02
Days of *Clostridium difficile* diarrhea, mean±SD[†]	13.2±7.4	9.8±4.9	0.39
ICU-associated diarrhea	44 (62.9%)	42 (61.8%)	0.81
Days of ICU-associated diarrhea, mean±SD[*]	5.9±3.8	4.1±3.7	0.03
Total antibiotic-days, mean±SD	16.3±14.4	13.3±10.4	0.16
Prescribed for VAP	8.6±10.3	5.6±7.8	0.05
Prescribed for *Clostridium difficile*	2.1±4.8	0.5±2.3	0.02
Hospital length of stay in days, mean±SD	21.7±17.4	21.4±14.9	0.90
ICU length of stay in days, mean±SD	14.6±11.6	14.8±11.8	0.87
Duration of mechanical ventilation in days, mean±SD	9.6±7.2	9.5±6.3	0.91
Hospital charges	$416,446±359,701	$350,847±258,087	0.22

[†]Among patients with a positive *Clostridium difficile* positive cytotoxin assay

[*]Among patients with at least one day of ICU-associated diarrhea

ICU= intensive care unit; VAP= ventilator-associated pneumonia.

Table 4. Surveillance Culture Data

	None	Rare	Few	Moderate	Many	P-Value
Oral Swab Pathogen* Density at Baseline						
Placebo	41	2	7	14	6	0.88
Lactobacillus	39	8	4	7	10	
Gastric Aspirate Pathogen* Density at Baseline						
Placebo	48	4	7	6	5	0.49
Lactobacillus	46	3	3	7	9	
Oral Swab Pathogen Density at 72 Hours						
Placebo	21	4	12	16	17	<0.001
Lactobacillus	42	7	2	5	12	
Gastric Aspirate Pathogen Density at 72 Hours						
Placebo	38	5	6	9	12	0.03
Lactobacillus	46	7	6	4	5	

*Pathogens from oral and gastric aspirates included *Staphylococcus aureus* (including methicillin-resistant strains), *Enterobacteriaceae*, and non-fermenting Gram negative bacteria

None if no growth is seen.

Rare if growth is restricted to only the first quadrant.

Few if growth extends into the second quadrant.

Moderate if growth extends into the third quadrant.

Many if growth extends into the fourth quadrant.

LEGEND

Figure 1. Study participants.

Figure 2. Kaplan-Meier analysis of time to microbiologically-confirmed ventilator-associated pneumonia (VAP). Solid line represents patients receiving Lactobacillus GG and the dashed line represents patients receiving placebo.

Figure 1. Study Participants

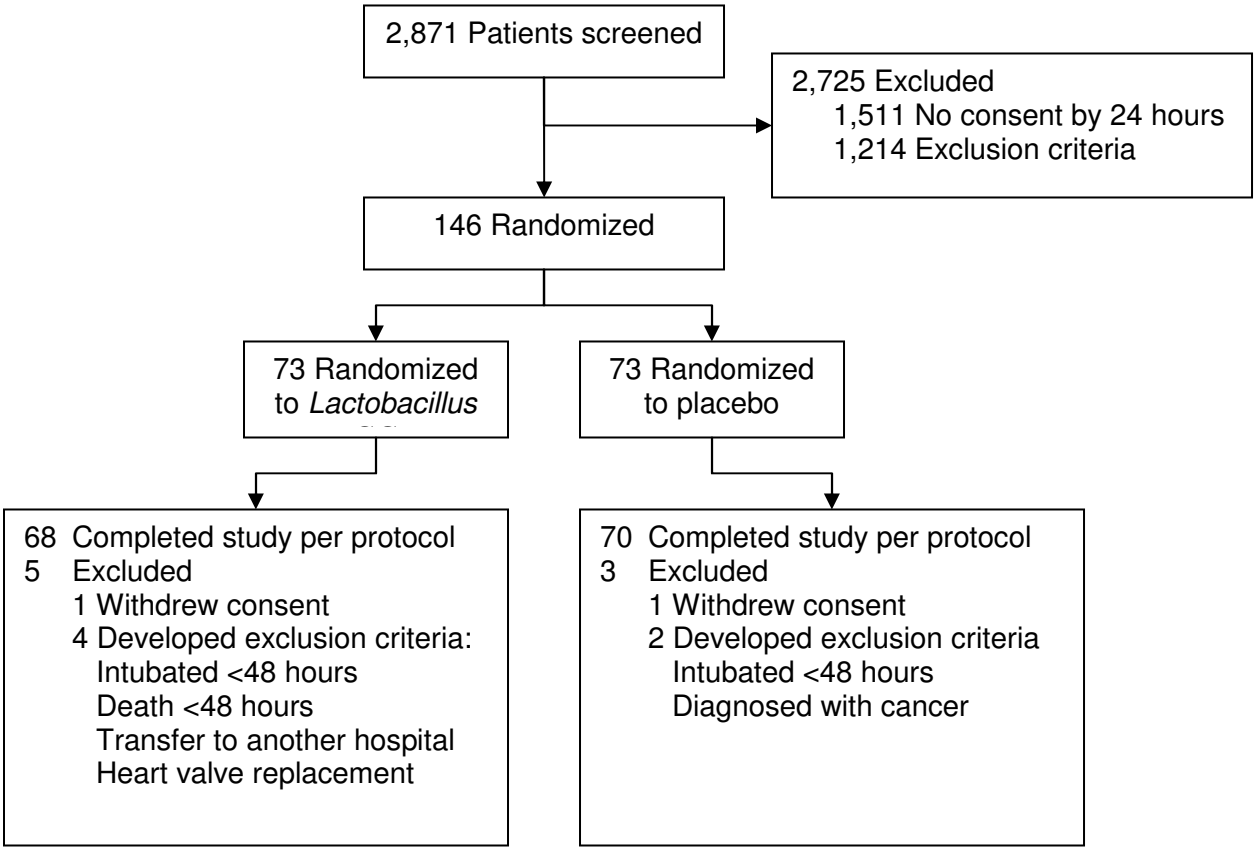
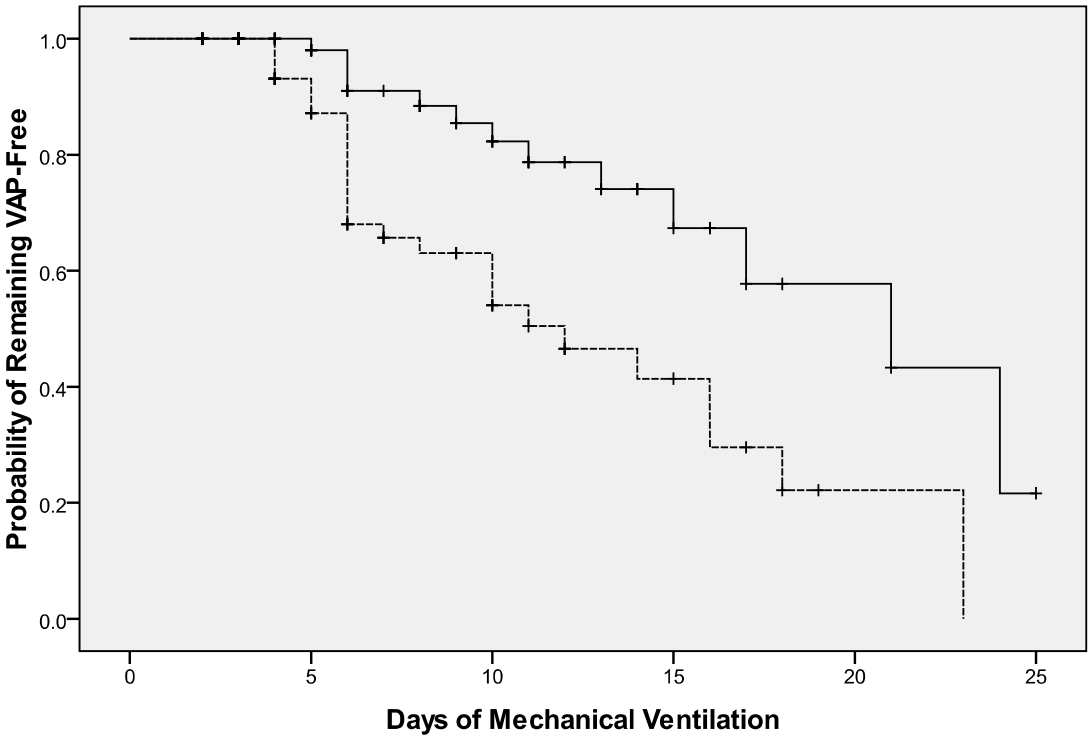


Figure 2. Kaplan-Meier Analysis of Time to Microbiologically-Confirmed VAP



Log Rank Statistic 10.861, df=1, P=0.001

Appendix : Information for Online Supplement

1. Rationale for exclusion criteria

The study's exclusion criteria were selected to ensure that subsets of patients who were theoretically at increased risk for iatrogenic probiotic infection would not be enrolled into the study. These at risk populations were selected based on published case reports and the concerns of the Creighton University Institutional Review Board. Protocol-defined exclusion criteria included: pregnancy; immunosuppression; prosthetic cardiac valve or vascular graft; cardiac trauma; history of rheumatic fever, endocarditis or congenital cardiac abnormality; gastroesophageal or intestinal injury or foregut surgery during the current admission; oropharyngeal mucosal injury; and placement of a tracheostomy.

Epidemiologic data from Finland showed that after *Lactobacillus* GG products were placed on the commercial market in that country, there was a surge in consumption to some three million kilograms within four years (1). During that time, eight cases of *Lactobacillus* bacteremia were identified (0.24% of all blood stream infections). Risk factors for *Lactobacillus* bacteremia – none of which were actually *Lactobacillus* GG by molecular analysis – included structural heart abnormalities such as rheumatic valvular disease, cardiac valve prosthesis, congenital cardiac malformation, prior bacterial endocarditis, or surgical cardiac procedures.

Lactobacillus infections have been documented in immunosuppressed patients including patients with acquired immune deficiency syndrome (AIDS), after lung transplantation, and after liver transplantation (2-7). Although the normal GI flora was presumably the source of infection in these cases, administration of probiotic agents to these individuals is not justified until further investigations assure its safety. *Lactobacillus* VAP has only been reported once (8).

There are two published reports of cases highlighting infections with *Lactobacillus* GG in patients taking this organism as a probiotic (9,10). In the first report, a 74-year-old diabetic woman developed a liver abscess which required percutaneous drainage and two months of antibiotic therapy (9). Molecular analysis of the isolated organism confirmed that it was

genetically identical to concurrently tested *Lactobacillus* GG, ATCC 53103. The authors attributed the infection to ingested *Lactobacillus* GG with caution, noting a published report of confirmed identification of this organism from the fecal flora of an infant with no exposure to *Lactobacillus* GG, ATCC 5310 (11). In the second report, the authors present two cases of *Lactobacillus* sepsis in pediatric patients receiving probiotic therapy (10). While the first patient had a documented risk factor – a congenital cardiac defect – the second patient did not. In each case molecular analysis of the isolated organism confirmed that it was genetically identical to the *Lactobacillus* species being administered.

Anatomic and mucosal defects of the digestive tract have been proposed as another possible route for iatrogenic infection (12,13). Accordingly, all patients with gastroesophageal or intestinal injury, foregut surgery, and/or oropharyngeal mucosal injury were excluded from the present study. Given the potential for probiotic-laden secretions to contaminate a tracheal surgical wound, patients requiring placement of a tracheostomy were also excluded from the present study.

In order to address concerns of the Creighton University Institutional Review Board regarding the lack of adequate safety data regarding probiotic *Lactobacillus* use in pregnant women, these patients were also excluded from study participation.

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2. Calculation of Antibiotic-Days

Antibiotic consumption was calculated as antibiotic-days for each patient. This entailed summing the number of antibiotics administered daily across all days where antibiotics were prescribed. As an example, a patient receiving three antibiotics on days 1-2, two antibiotics on days 3-5, and 1 antibiotic on days 6-7 has a total of 14 antibiotic-days (3+3+2+2+2+1+1) despite only receiving therapy for 7 days. Incomplete dose administration was recorded as a fraction of the prescribed daily dose (i.e. if a patient receives two injections of a TID drug this was recorded as 0.66 for that day).

3. Protocols for the Prevention of VAP

Institutional protocols for VAP prevention were uniformly employed for all mechanically ventilated patients throughout the duration of the present study. These measures remained unchanged following study initiation and included: routine elevation of the head of the bed 30-45 degrees; oral care with commercially available 1.5% hydrogen peroxide every 4 hours (Sage Products, Cary, IL); withholding sedation daily to achieve a Richmond Agitation-Sedation Scale (RASS) score of 0; use of a post-operative ventilator weaning protocol; and standardized, evidence-based order sets for venous thromboembolism and stress ulcer prophylaxis. Compliance with each of these measures was prospectively assessed and did not differ between groups.