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# Effect of Probiotics on Incident Ventilator-Associated Pneumonia in Critically Ill Patients
## A Randomized Clinical Trial

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**IMPORTANCE** Growing interest in microbial dysbiosis during critical illness has raised questions about the therapeutic potential of microbiome modification with probiotics. Prior randomized trials in this population suggest that probiotics reduce infection, particularly ventilator-associated pneumonia (VAP), although probiotic-associated infections have also been reported.

**OBJECTIVE** To evaluate the effect of *Lactobacillus rhamnosus* GG on preventing VAP, additional infections, and other clinically important outcomes in the intensive care unit (ICU).

**DESIGN, SETTING, AND PARTICIPANTS** Randomized placebo-controlled trial in 44 ICUs in Canada, the United States, and Saudi Arabia enrolling adults predicted to require mechanical ventilation for at least 72 hours. A total of 2653 patients were enrolled from October 2013 to March 2019 (final follow-up, October 2020).

**INTERVENTIONS** Enteral *L rhamnosus* GG ($1 \times 10^{10}$ colony-forming units) (n = 1321) or placebo (n = 1332) twice daily in the ICU.

**MAIN OUTCOMES AND MEASURES** The primary outcome was VAP determined by duplicate blinded central adjudication. Secondary outcomes were other ICU-acquired infections including *Clostridioides difficile* infection, diarrhea, antimicrobial use, ICU and hospital length of stay, and mortality.

**RESULTS** Among 2653 randomized patients (mean age, 59.8 years [SD], 16.5 years), 2650 (99.9%) completed the trial (mean age, 59.8 years [SD], 16.5 years; 1063 women [40.1%.] with a mean Acute Physiology and Chronic Health Evaluation II score of 22.0 (SD, 7.8) and received the study product for a median of 9 days (IQR, 5-15 days). VAP developed among 289 of 1318 patients (21.9%) receiving probiotics vs 284 of 1332 controls (21.3%; hazard ratio [HR], 1.03 (95% CI, 0.87-1.22; $P = .73$, absolute difference, 0.6%, 95% CI, −2.5% to 3.7%). None of the 20 prespecified secondary outcomes, including other ICU-acquired infections, diarrhea, antimicrobial use, mortality, or length of stay showed a significant difference. Fifteen patients (1.1%) receiving probiotics vs 1 (0.1%) in the control group experienced the adverse event of *L rhamnosus* in a sterile site or the sole or predominant organism in a nonsterile site (odds ratio, 14.02; 95% CI, 1.79-109.58; $P < .001$).

**CONCLUSIONS AND RELEVANCE** Among critically ill patients requiring mechanical ventilation, administration of the probiotic *L rhamnosus* GG compared with placebo, resulted in no significant difference in the development of ventilator-associated pneumonia. These findings do not support the use of *L rhamnosus* GG in critically ill patients.

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Visual Abstract

Supplemental content

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Probiotics have emerged as a biologically plausible strategy to treat or prevent a wide range of infectious, inflammatory, and autoimmune conditions. Postulated mechanisms of benefit for a broad spectrum of diseases include enhanced gut barrier function, competitive inhibition of pathogenic bacteria, and modulation of the host inflammatory response.[1,2] A recent randomized trial involving 2556 healthy newborns in rural India showed that *Lactobacillus plantarum* and fructooligosaccharide decreased the risk of sepsis and lower respiratory tract infection.[3] Systematic reviews of randomized trials involving adults suggest that probiotics reduce antibiotic-associated diarrhea,[4] but their effect on *Clostridioides difficile* infection appears inconsistent.[5-7] Reports of iatrogenic probiotic-associated infections[8] also highlight the need for evaluation of possible harm associated with their use.[9]

Among critically ill patients, randomized trials suggest that probiotics reduce infection rates by 20%[10] and may decrease the risk of ventilator-associated pneumonia (VAP) by 25% to 30%.[10,11] VAP remains a common, serious, nosocomial infection and an important focus of prevention directives for health care organizations. Economic evaluation suggests the cost-effectiveness of probiotics for VAP prevention.[12] Current guidelines suggest probiotic use for selected medical and surgical intensive care unit (ICU) patients for whom trials have documented safety and benefit.[13] Given the growing interest in microbial dysbiosis in the ICU and the therapeutic potential of microbiome modification,[14,15] probiotics are a promising VAP prevention strategy. This multicenter trial was designed to determine whether *Lactobacillus rhamnosus* GG compared with placebo reduces VAP and other clinically important outcomes for a broad range of critically ill patients.

### Key Points

**Question** Does the probiotic *Lactobacillus rhamnosus* GG prevent ventilator-associated pneumonia (VAP) among critically ill patients?

**Findings** In this randomized trial involving 2650 patients, no significant difference in VAP incidence was found among patients treated with probiotics compared with placebo (21.9% vs 21.3%, respectively; hazard ratio 1.03; 95% CI 0.87-1.22).

**Meaning** These findings do not support the use of *Lactobacillus rhamnosus* GG for prevention of ventilator-associated pneumonia in critically ill patients requiring mechanical ventilation.

## Methods

Following an internal blinded pilot trial[16] documenting feasibility,[17] the main trial was launched and included patients in pilot phase. The study protocol and statistical analysis plan were published (Supplement 1).[18] Participating hospital institutional review boards approved the trial. Research coordinators obtained a priori written informed consent from eligible patients or substitute decision-makers. Forty-four ICUs participated from Canada (41 ICUs), the United States (2 ICUs), and Saudi Arabia (1 ICU).

Enrolled patients were at least 18 years old, expected to require mechanical ventilation for at least 72 hours as determined by the treating ICU team (**Figure 1**). Excluded patients had already received mechanical ventilation for more than 72 hours; were immunocompromised (HIV with a CD4 cell count <200 cells/μL, chronic immunosuppressive medications, chemotherapy in the last 3 months, prior organ or hematological transplant, or absolute neutrophil count < 500 cells/μL); carried increased risk of endovascular infection[18]; had severe acute pancreatitis; had a percutaneous enteral feeding tube or were unable to receive enteral medication; had plans for palliation; and had previously enrolled in this trial or a related trial.

Concealed 1:1 allocation in this parallel-group trial was stratified by center and admission status (medical, surgical, or trauma), using a web-based randomization system with undisclosed block sizes of 4 or 6. Patients, next of kin, and clinical and research staff remained blinded to allocation. Unblinded study pharmacists randomized patients and prepared the blinded study product.

Patients received $1 \times 10^{10}$ colony forming units of *L rhamnosus* GG (i-Health Inc) or an identical enteral placebo solution (microcrystalline cellulose) twice daily. The study product was administered for up to 60 days or until discharge from the ICU or until *Lactobacillus* species was isolated from a sterile site or cultured as the sole or predominant organism from a nonsterile site. Throughout the trial, every 100th capsule from each site was cultured at the Laboratory for Interdisciplinary Microbiome Research at McMaster University, Hamilton, Ontario,[18,19] to confirm the fidelity of viable probiotic dosing and the integrity of the placebo study product (eText, Supplement 2).

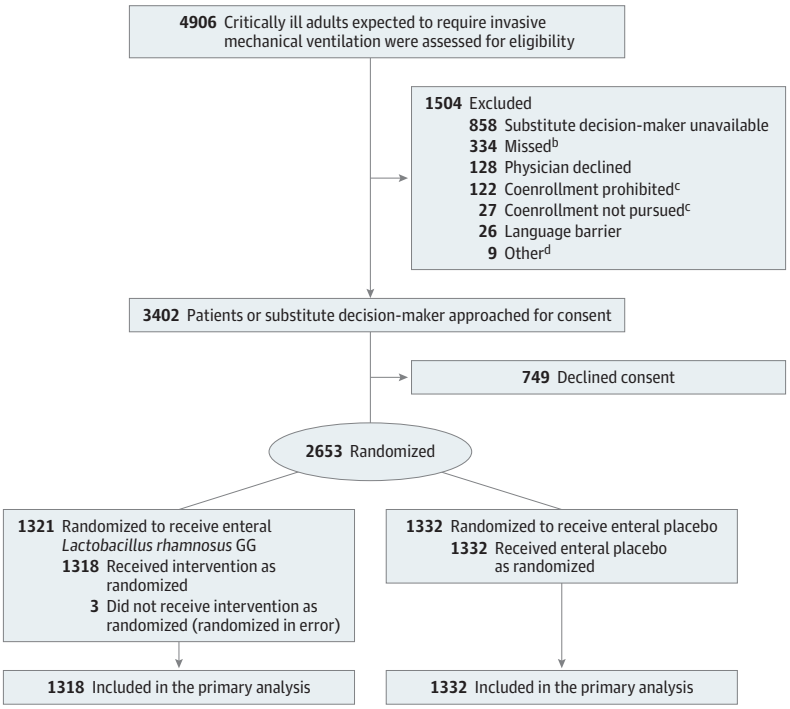
Research coordinators recorded baseline data (eg, demographics, illness severity, life support), daily data (eg, study product administration, pneumonia prevention strategies, and other cointerventions), culture results, infections, diarrhea (documented by bedside nurses), length of stay, and mortality by using a secure web-based system (iDataFax). Relevant anonymized clinical, microbiological, and radiological source data were submitted to the methods center.

### Outcomes

The primary end point was VAP, informed by the presence of a new, progressive, or persistent radiographic infiltrate on chest radiograph after at least 2 days of mechanical ventilation, plus any 2 of the following: (1) fever (core temperature >38 °C) or hypothermia (temperature <36 °C); (2) white blood cell count less than $3.0 \times 10^6$/L or exceeding $10 \times 10^6$/L, and (3) purulent sputum.[20,21]

Secondary end points included different pneumonia classifications (eTable 1 in Supplement 2 for definitions),[22-25] *C difficile* and other infections,[18] and additional clinically important outcomes as detailed below. Early VAP (pneumonia 3-5 days after initiation of mechanical ventilation), was distinguished from late VAP (after ≥ 6 days of mechanical ventilation, including up to 2 days after discontinuing mechanical ventilation), and from postextubation

Figure 1. Screening, Selection, and Flow of Patients in PROSPECT[a]




[a] No data were collected on ineligible patients. Ten patients in the placebo group and 15 in the probiotics group had consent withdrawn for the study product but were followed up for outcomes and were included in the primary analysis.

[b] Missed patients included those admitted to the ICU on weekends or holidays or other times when the research coordinators or pharmacists were unavailable.

[c] Enrollment in an additional study.

[d] Other reasons included nonresidents, incarcerated patients, or family members who were not approached due to extreme stress.

pneumonia (arising ≥3 days after mechanical ventilation discontinuation). A composite outcome incorporated incident early VAP, late VAP, or postextubation pneumonia. All ICU-acquired infections were adjudicated, including bloodstream infections, intra-abdominal infection, *C difficile* infection (requiring diarrhea and laboratory confirmation or colonoscopic or histopathological evidence of pseudomembranous colitis[26]), upper genitourinary tract infection, skin and soft-tissue infection, other infections, adapted from the International Sepsis Forum.[23] A composite outcome incorporated any of the foregoing ICU-acquired infections. Diarrhea was based on the World Health Organization definition (≥3 loose or watery bowel movements per day[27]), and the Bristol Stool Score classification for loose or watery stool (type 6 or 7).[28] Antibiotic-associated diarrhea was defined as occurring any day on which any antibiotic was administered or within 1 day.[29] Antimicrobial use (daily dose of therapy, defined daily dose, and antimicrobial-free days) were recorded in the ICU.[18] Duration of mechanical ventilation, ICU and hospital length of stay, as well as ICU and hospital mortality were documented.

Clinically suspected infections were classified as prevalent if present before randomization, on the day of randomization, or 1 day after randomization; these were not considered trial outcomes and did not include persistent or progressive prevalent pneumonia. Prevalent infections were centrally adjudicated by 1 physician blinded to treatment allocation and center. Incident infections were trial outcomes, occurring 2 or more days after randomization. Clinically suspected incident pneumonia and *C difficile* infection were centrally adjudicated using the clinical notes and by microbiological and radiological source reports, following pilot calibration by 2 independent physicians blinded to allocation and center; disagreement was resolved by discussion or by a third investigator. Other incident infections were adjudicated by 1 physician blinded to allocation and center.

Adverse events were defined as the isolation of *Lactobacillus* species in a culture from a sterile site or as the sole or predominant organism cultured from a nonsterile site. Serious adverse events were those *Lactobacillus* isolates resulting in persistent or significant disability or incapacity or were life-threatening or resulting in death. Any culture obtained by clinicians, processed by the hospital microbiology laboratory as positive for *Lactobacillus* species was documented. The isolate when available underwent strain genotyping at the Microbiome Research Laboratory at McMaster University to analyze whether it was the strain of *L rhamnosus* GG used in the study product.

## Statistical Analysis

Based on an estimated 15% VAP rate,[17,22] 2650 patients were enrolled to detect a 25% relative risk reduction (based on results from prior meta-analyses)[10,30] with 80% power ($\alpha = .05$).

Patients were all analyzed in the group to which they were allocated. Cox proportional hazards analysis used for the primary outcome was stratified by center and admission diagnosis (medical vs surgical vs trauma), and presented using Kaplan-Meier curves. The VAP incidence rate was reported as the number of cases per 1000 ventilator-days. A stratified Cox proportional hazards model, estimating hazard ratios (HRs) and associated 95% CIs was also used

for dichotomous secondary outcomes. Skewed continuous secondary outcomes were log-transformed; if normally distributed, parametric methods were used to compare groups. If the outcome distributions remained skewed after log-transformation, nonparametric methods were used. Graphical approaches were used to examine residuals to assess model assumptions and goodness of fit, including the proportional hazards assumption for Cox-regression analyses. When the assumption of proportional hazards was not met, we compared the proportion of patients with the outcome between groups using the Mantel-Haenszel approach incorporating our stratification variables.

We conducted 4 prespecified sensitivity analyses[18]: (1) The proportion of patients with VAP between groups were compared using the Mantel-Haenszel approach incorporating our stratification variables; (2) VAP results were analyzed accounting for death as a competing risk using the Fine and Gray proportional subdistribution hazards model[31]; (3) A per-protocol analysis of each incident infection and a composite of all ICU-acquired infections among patients receiving the study product for 90% or more of the study days to evaluate maximal probiotic exposure were conducted; and (4) All VAP events were analyzed regardless of when they occurred after randomization.

Five prespecified subgroup analyses were conducted for the primary outcome of VAP by adding a main effect for the subgroup variable as well as its interaction with randomized treatment to the primary Cox proportional hazards analysis.[18] The test for interaction was the test for significance of the interaction term in this analysis. The subgroup analyses were (1) medical vs surgical vs trauma patients; (2) age (>75 years vs 65-75 years vs <65 years); (3) Baseline Clinical Frailty Score (≥5[32] vs ≤4); (4) patients receiving antibiotics the day of randomization and the 2 preceding days vs other patients; and (5) patients with prevalent pneumonia vs other patients. The hypotheses were that the probiotic effects on the primary outcome would be attenuated in older medical patients due to frailty and immunosenescence, as well as in those who received antibiotics prior to randomization and who had prevalent pneumonia, given that these are pneumonia risk factors that are potentially less likely to be modified by probiotics.

The data monitoring committee independently reviewed blinded interim analyses, with no stopping guides for futility, and conservative warning guides for benefit. Interim analyses occurred at one-third and two-thirds of enrollment using 2-sided tests with a fixed conservative α = .001 for the first and second interim analyses, and an α = .05 for final analyses,[33,34] using SAS version 9.4 (SAS Institute Inc). All analyses used 2-sided testing and an α = .05. Analyses of secondary outcomes as well as sensitivity and subgroup analyses were not adjusted for multiple comparisons and should be interpreted as exploratory.[35] No multiple imputation analyses were needed for missing data because missing data were 0.5%, less than the threshold specified in our statistical analysis plan.[18] In Cox regressions, patients who did not have complete follow-up for outcomes were censored on the final data collection day.

## Results

### Participants

Of the randomized patients included in the primary analysis, 1318 patients received *L rhamnosus* GG (probiotic) and 1332 received placebo (Figure 1).

Of the 2650 participants (mean age, 59.8 years [SD, 16.5 years]; mean Acute Physiology and Chronic Health Evaluation II score, 22.0 [SD, 7.8]), 1063 (40.1%) were women and 2027 (76.5%) had a medical admitting diagnosis. At baseline, all patients were receiving mechanical ventilation, 1621 (61.2%) were receiving inotropes or vasopressors, and 215 (8.1%) were receiving kidney replacement therapy.

On admission, 1877 patients (70.8%) had a prevalent infection, 1576 (59.5%) of whom had pneumonia as a concurrent or primary admitting diagnosis. Antimicrobials were prescribed or ongoing for 2186 patients (82.5%) on the day of randomization. Baseline characteristics between the probiotic and placebo groups were not significantly different (**Table 1**).

Of 2650 patients, 14 (9 in the probiotics and 5 in the placebo group) had consent withdrawn for daily data collection. These patients are represented in all analyses; mortality is documented in each case; for all other outcomes, these patients were censored on their last day of daily data collection.

### Study Product Integrity, Exposure, and Adherence

The study product was administered for a median of 9 days (IQR, 5-15 days) in both groups. Overall, 2630 of 2650 patients (99.2%) received at least 1 dose (identical proportions in both groups). Patients received at least 1 dose on 32 458 of 36 046 study days (90.0%); results were not significantly different in the probiotic group (16 471 of 18 319 [89.9%]) and placebo group (15 987 of 17 727 [90.2%]).

### Primary Outcome

Among 1318 patients receiving *L rhamnosus* GG, 289 (21.9%) developed VAP compared with 284 of 1332 patients (21.3%) receiving placebo (hazard ratio [HR], 1.03; 95% CI, 0.87 to 1.22; *P* = .73; absolute difference, 0.6%; 95% CI, -2.5% to 3.7%; **Table 2**; eFigure in Supplement 2). Sensitivity analyses (eTable 2 in Supplement 2) yielded no significantly different results. Subgroup analyses did not indicate any effect modification based on diagnostic category (medical, surgical, or trauma), age, frailty status, prior receipt of antimicrobials, or prevalent pneumonia at baseline (**Figure 2**).

### Secondary Outcomes

Applying alternative definitions for pneumonia, results were comparable with the primary analysis (eTable 1 in Supplement 2). *C difficile* infection developed in 32 patients (2.4%) receiving probiotics vs 28 (2.1%) receiving placebo (Table 2). Because graphical approaches indicated that the proportional hazards assumption was not met for this infection, we ran a proportions analysis, which yielded an odds ratio (OR) of 1.15 (95% CI, 0.69 to 1.93; *P* = .60; absolute difference, 0.3% (95% CI, -0.8% to 1.5%). No significant difference between

Table 1. Baseline Characteristics of Randomized Patients

| | No. (%) of patients | |
|---|---|---|
| | *Lactobacillus rhamnosus* GG (n = 1318) | Placebo (n = 1332) |
| Age, mean (SD), y | 60.1 (16.2) | 59.6 (16.8) |
| Women | 541 (41.0) | 522 (39.2) |
| Men | 777 (59.0) | 810 (60.8) |
| Clinical frailty score ≥5, No./total (%)[a] | 240/1084 (22.1) | 232/1098 (21.1) |
| APACHE II score, mean (SD)[b] | 22.3 (7.8) | 21.7 (7.9) |
| Admission category | | |
| Medical | 1006 (76.3) | 1021 (76.7) |
| Trauma | 171 (13.0) | 182 (13.7) |
| Surgical | 141 (10.7) | 129 (9.7) |
| Admitting diagnostic category | | |
| Respiratory | 441 (33.5) | 476 (35.7) |
| Neurological | 227 (17.2) | 242 (18.2) |
| Trauma | 180 (13.7) | 184 (13.8) |
| Sepsis | 179 (13.6) | 147 (11.0) |
| Cardiovascular | 118 (9.0) | 130 (9.8) |
| Other medical | 91 (6.9) | 73 (5.5) |
| Gastrointestinal | 50 (3.8) | 54 (4.1) |
| Other surgical | 32 (2.4) | 26 (2.0) |
| Other medical | | |
| Patient experience prior to randomization, median (IQR) | | |
| ICU admission to randomization, d | 1 (1-2) | 2 (1-2) |
| Intubation to randomization, d | 1 (1-2) | 2 (1-2) |
| Critical care intervention on day 1 | | |
| Invasive mechanical ventilation | 1318 (100.0) | 1332 (100.0) |
| Inotropes or vasopressors | 798 (60.5) | 823 (61.8) |
| Dialysis[c] | 106 (8.0) | 109 (8.2) |
| Enteral and parenteral nutrition on day 1 | | |
| Enteral nutrition on day 1 | 1140 (86.5) | 1151 (86.5) |
| Parenteral nutrition on day 1 | 12 (0.9) | 19 (1.4) |
| Prevalent infections[d] | | |
| Pneumonia[e] | 776 (58.9) | 800 (60.1) |
| Bacteremia | 142 (10.8) | 134 (10.1) |
| Skin or soft-tissue infection | 91 (6.9) | 76 (5.7) |
| Other infections[f] | 79 (6.0) | 77 (5.8) |
| Intra-abdominal infection | 48 (3.6) | 44 (3.3) |
| *Clostridioides difficile* infection | 16 (1.2) | 10 (0.8) |
| Upper urinary tract infection[g] | 9 (0.7) | 12 (0.9) |
| Antibiotic exposure | | |
| Use at randomization | 1095 (83.1) | 1091 (81.9) |
| Use at randomization and the 2 d before | 552 (41.9) | 593 (44.5) |

[a] Degree of fitness and frailty (range, 1-9: 1, very fit; 5, mildly frail; 9, terminally ill).[32] Results were collected for patients randomized on or after May 23, 2016 (not captured retrospectively or for pilot trial patients).

[b] Acute Physiology and Chronic Health Evaluation II (APACHE-II) score measures the severity of illness within the first 24 hours of a patient's admission to an intensive care unit (ICU; range 0 to 71, higher scores indicate more severe disease and a higher risk of death).

[c] Patients with chronic kidney failure who received dialysis prior to the index admission or requiring dialysis.

[d] Prevalent infections are not mutually exclusive, and they include conditions such as the infections listed as well as other infections listed in footnote f.

[e] Any prevalent pneumonia (community acquired, hospital acquired, ventilator associated).

[f] Other infections (eg, meningitis, encephalitis, osteomyelitis, septic arthritis, sinusitis, mediastinitis).

[g] Microbiologically confirmed abscess or other radiographic or surgical evidence of upper urinary tract infection with or without positive urine culture (positive urine culture alone not included).

groups for any infectious outcomes was found (Table 2); per-protocol analyses yielded no significantly different results (eTable 3 in Supplement 2).

Diarrhea occurred in 2156 patients (81.4%) when defined as 1 or more stools of Bristol types 6 or 7. There was no significant difference in diarrhea between patients in the probiotic vs placebo groups using any definition (Table 2). Antibiotic-associated diarrhea was also common; there was no significant difference between groups using any definition (Table 2).

Antimicrobial use was not significantly different between patients receiving probiotics vs placebo, considering metrics of length of therapy, days of therapy, defined daily dose or antimicrobial-free days (all per 1000 patient-days in the ICU; eTable 4 in Supplement).

Patients received mechanical ventilation for a median of 7 days (IQR, 4-13 days). The median duration of ICU stay of 12 days[8-18] and hospital stay of 22 days (IQR, 13-41 days) were not significantly different between groups. In the ICU, 279

Table 2. Primary and Secondary Outcomes[a]

| | No. (%) of patients | | | | |
|---|---|---|---|---|---|
| | *Lactobacillus rhamnosus* GG (n = 1318) | Placebo (n = 1332) | Absolute difference (95% CI), %[b] | Hazard ratio (95% CI) | *P* value |
| **Primary outcome** | | | | | |
| Ventilator-associated pneumonia at any time[18,20] | 289 (21.9) | 284 (21.3) | 0.6 (-2.5 to 3.7) | 1.03 (0.87 to 1.22) | .73 |
| **Secondary outcomes** | | | | | |
| Pneumonia | | | | | |
| Early ventilator-associated pneumonia[c] | 50 (3.8) | 61 (4.6) | -0.8 (-2.3 to 0.7) | 0.80 (0.55 to 1.17) | .26 |
| Late ventilator-associated pneumonia[d] | 243 (18.4) | 231 (17.3) | 1.1 (-1.8 to 4.0) | 1.09 (0.91 to 1.32) | .35 |
| Postextubation pneumonia[e] | 22 (1.7) | 20 (1.5) | 0.2 (-0.8 to 1.1) | 1.21 (0.63 to 2.32) | .58 |
| Any pneumonia[f] | 307 (23.3) | 300 (22.5) | 0.8 (-2.4 to 4.0) | 1.04 (0.89 to 1.23) | .61 |
| Other infections | | | | | |
| Any infection[g] | 414 (31.4) | 418 (31.4) | 0.0 (-3.5 to 3.6) | 0.97 (0.84 to 1.11) | .64 |
| Positive urine culture | 171 (13.0) | 174 (13.1) | -0.1 (-2.7 to 2.5) | 0.99 (0.79 to 1.24) | .96 |
| Any bacteremia | 106 (8.0) | 101 (7.6) | 0.5 (-1.6 to 2.5) | 1.08 (0.82 to 1.44) | .58 |
| Skin or soft-tissue infection, nonsurgical | 37 (2.8) | 28 (2.1) | 0.7 (-0.5 to 1.9) | 1.11 (0.67 to 1.85) | .68 |
| Any *Clostridioides difficile* infection[h] | 32 (2.4) | 28 (2.1) | 0.3 (-0.8 to 1.5) | 1.15 (0.69 to 1.93) | .60 |
| Other infections[i] | 28 (2.1) | 37 (2.8) | -0.7 (-1.8 to 0.5) | 0.74 (0.45 to 1.22) | .24 |
| Skin or soft-tissue infection, surgical site | 28 (2.1) | 33 (2.5) | -0.4 (-1.5 to 0.8) | 0.80 (0.46 to 1.39) | .43 |
| Intra-abdominal infection | 19 (1.4) | 22 (1.7) | -0.2 (-1.2 to 0.7) | 0.79 (0.41 to 1.50) | .47 |
| Upper urinary tract infection[j] | 2 (0.2) | 3 (0.2) | -0.1 (-0.4 to 0.3) | 1.02 (0.14 to 7.26) | .98 |
| Diarrhea | | | | | |
| ≥ 3 Stools per d | 861 (65.3) | 855 (64.2) | 1.1 (-2.5 to 4.8) | 1.01 (0.91 to 1.11) | .90 |
| ≥1 Stools of Bristol type 6 or 7[k] | 1076 (81.6) | 1080 (81.1) | 0.6 (-2.4 to 3.5) | 1.07 (0.98 to 1.17) | .13 |
| ≥3 Bristol type 6 or 7 stools per d[k] | 756 (57.4) | 731 (54.9) | 2.5 (-1.3 to 6.3) | 1.02 (0.92 to 1.14) | .66 |
| Antibiotic-associated diarrhea | | | | | |
| ≥3 Stools per d | 785 (59.6) | 787 (59.1) | 0.5 (-3.3 to 4.2) | 1.03 (0.93 to 1.14) | .63 |
| ≥1 Stools of Bristol type 6 or 7[k] | 1014 (76.9) | 1016 (76.3) | 0.7 (-2.6 to 3.9) | 1.07 (0.98 to 1.17) | .14 |
| ≥3 Bristol type 6 or 7 stools per d[k] | 691 (52.4) | 671 (50.4) | 2.1 (-1.8 to 5.9) | 1.03 (0.93 to 1.15) | .57 |
| **Other clinical outcomes** | | | | | |
| Mechanical ventilation, median (IQR), d | 7 (4 to 13) | 7 (4 to 13) | | | .81[l] |
| ICU stay, median (IQR), d | 12 (7 to 19) | 12 (8 to 18) | | | .45[m] |
| Hospital stay, median (IQR), d | 22 (13 to 42) | 22 (13 to 40) | | | .42[m] |
| Death in ICU | 279 (21.2) | 296 (22.2) | -1.1 (-4.2 to 2.1) | 0.91 (0.77 to 1.08) | .30 |
| Death in hospital | 363 (27.5) | 381 (28.6) | -1.1 (-4.5 to 2.4) | 0.91 (0.79 to 1.06) | .21 |

Abbreviation: ICU, intensive care unit.

[a] All definitions are detailed in.[18] The number of ventilator-associated pneumonia cases per 1000 ventilator days was 23.3 in the probiotics group and 23.1 in the placebo group.

[b] Unadjusted absolute difference.

[c] Diagnosed on day 3 to 5 after initiation of mechanical ventilation.

[d] Diagnosed on or after day 6 of mechanical ventilation, including up to 2 days after mechanical ventilation discontinued.

[e] Pneumonia arising 3 or more days after mechanical ventilation discontinued.

[f] Composite outcome of incident early ventilator-associated pneumonia, late ventilator-associated pneumonia, or postextubation pneumonia. Rarely, will a patient with an early ventilator-associated pneumonia that resolves (clinically from the perspective of signs and symptoms, microbiologically, and/or radiographically) develop a second ventilator-associated pneumonia 2 weeks later. The rows may therefore not add up to the composite total.

[g] Any of the foregoing infections, not including positive urine cultures alone, considering only the adjudicated pneumonia outcome.

[h] Graphical approaches indicated that the assumption of proportional hazards was not met for *C difficile;* the proportions analysis odds ratio was 1.15 (95% CI, 0.69-1.93; *P* value = .598).

[i] Meningitis, encephalitis, osteomyelitis, septic arthritis, sinusitis, mediastinitis, etc.

[j] Microbiologically confirmed abscess or other radiographic or surgical evidence of upper urinary tract infection with or without positive urine culture (positive urine culture alone not included).

[k] Stool classification system that characterizes each bowel movement (scale range,1-7 ).[28] Types 1 or 2 stool indicates constipation; types 6 and 7 indicate diarrhea.

[l] Wilcoxon rank-sum test.

[m] *t* Test performed on the log-transformed variable.

Figure 2. Subgroup Analyses: Ventilator-Associated Pneumonia

| Subgroup | No./total patients *Lactobacillus rhamnosus* | No./total patients Enteral placebo | Hazard ratio (95% CI) | Interaction *P* value |
|---|---|---|---|---|
| Admission type | | | | |
| Medical | 184/1006 | 199/1021 | 0.94 (0.77-1.16) | |
| Surgical | 44/141 | 29/129 | 1.58 (0.97-2.56) | .14 |
| Trauma | 61/171 | 56/182 | 1.12 (0.77-1.62) | |
| Age, y | | | | |
| <65 | 173/752 | 168/767 | 1.10 (0.88-1.37) | |
| 65-75 | 69/335 | 66/319 | 0.87 (0.61-1.25) | .58 |
| >75 | 47/231 | 50/246 | 1.04 (0.68-1.59) | |
| Clinical frailty score | | | | |
| ≤4 | 188/844 | 191/866 | 1.02 (0.83-1.26) | .38 |
| ≥5 | 37/240 | 40/232 | 0.81 (0.50-1.30) | |
| Received antibiotics on day of randomization and 2 preceding days | | | | |
| No | 193/766 | 181/739 | 1.02 (0.82-1.26) | .92 |
| Yes | 96/552 | 103/593 | 1.00 (0.75-1.34) | |
| Prevalent pneumonia as primary diagnosis or comorbid infection | | | | |
| No | 168/542 | 151/532 | 1.03 (0.81-1.31) | .78 |
| Yes | 121/776 | 133/800 | 0.98 (0.76-1.26) | |

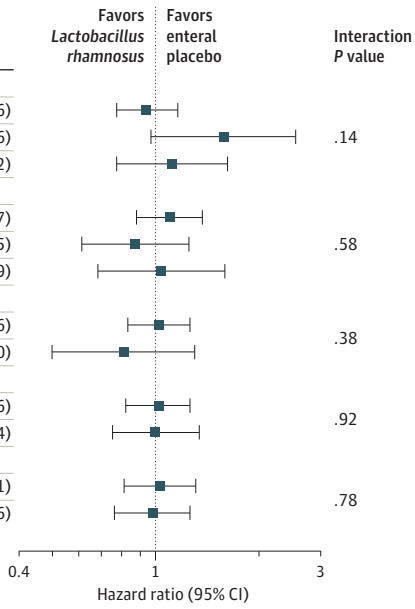



Table 3. Adverse and Serious Adverse Events

| | No. (%) *Lactobacillus rhamnosus* GG (n = 1318) | Placebo (n = 1332) | Odds ratio (95% CI) |
|---|---|---|---|
| Adverse events[a] | 13 (1.0) | 1 (0.1) | |
| Serious adverse events[b] | 2 (0.2) | 0 | |
| Serious adverse events or adverse events | 15 (1.1) | 1 (0.1) | 14.02 (1.79-109.58) |

[a] Defined as the isolation of *Lactobacillus* species in a culture from a sterile site or as the sole or predominant organism cultured from a nonsterile site.

[b] Defined as *Lactobacillus* isolates that resulted in persistent or significant disability or incapacity, were life-threatening, or resulted in death.

patients (21.2%) in the probiotics group and 296 patients (22.2%) in the placebo group died (HR, 0.91; 95% CI, 0.77 to 1.08; *P* = .30; absolute difference, -1.1%; 95% CI, −4.2% to 2.1%). Death in the hospital occurred in 363 patients (27.5%) in the probiotics group and 381 (28.6%) in the placebo group (HR, 0.91; 95% CI, 0.79 to 1.06; *P* = .21; absolute difference, -1.1%; 95% CI, -4.5% to 2.4%; Table 2).

### Adverse Events and Serious Adverse Events

Of the 16 patients with an adverse event (isolation of *Lactobacillus* species in a culture from a sterile site or as the sole or predominant organism in a nonsterile site) or serious adverse event during the trial, 12 *Lactobacillus* isolates were available to sequence; 12 were confirmed as *L rhamnosus* GG, which were all in the probiotic group (**Table 3**). The sources included 10 blood, 1 blood and hepatic abscess, 1 intra-abdominal abscess, 1 peritoneal fluid, 1 pleural fluid, and 2 urine. Fifteen patients (1.1%) receiving probiotics compared with 1 patient (1.1%) receiving placebo experienced either an adverse event or a serious adverse event (OR, 14.02; 95% CI, 1.79-109.58; *P* = .001). Both patients who had a serious adverse event died. (eTable 5 in Supplement 2).

## Discussion

In this trial involving critically ill patients, the probiotic *L rhamnosus* GG did not significantly reduce the risk of VAP, *C difficile,* or other infections. Furthermore, no effects on diarrhea, antimicrobial use, length of stay or mortality were identified. In this broad population of ICU patients with high illness severity, life support dependence, antimicrobial exposure, and propensity for ICU-acquired infection, *L rhamnosus* GG did not confer any other benefits.

These results differ from meta-analyses of previous small, predominantly single-center studies, suggesting decreased VAP rates associated with probiotics during critical illness, including this strain.[10,11] However, findings from this trial do accord with a trial showing no effect of a *Lactobacillus acidophilus* and *Bifidobacterium* preparation on *C difficile* infection in older hospitalized patients receiving antibiotics.[7] Furthermore, the increased risk of adverse events observed among patients receiving probiotics aligns with a recent report of *L rhamnosus* GG bacteremia in critically ill children prescribed this probiotic.[36] These results indicate that,

although critically ill patients exhibit loss of commensal microbiota, overgrowth of potential pathogens and thus highly perturbed microbial communities,[14,15,37] probiotics may not improve clinically important outcomes associated with dysbiosis in this setting. Rigorous probiotics trials with neutral results enhance clinical decision-making, inform resource allocation, and ensure balanced systematic reviews and guidelines.

In this trial population, central genomic analyses of clinical specimens allowed distinction between endogenous or environmental strains of *Lactobacillus* species and the study product. Isolation of the probiotic *Lactobacillus* species in sterile sites such as blood may reflect impaired gut integrity, despite excluding patients at risk of increased gut permeability and withholding the study product if this developed in enrolled patients. Some bloodstream isolates may represent contamination during clinical testing in patients receiving the study product, although strict infection prevention protocols guided capsule handling. *Lactobacillus* species bacteremia may have clinical significance, increasing the risk of death when serious underlying comorbidities coexist.[38]

This randomized, concealed, blinded trial had high protocol adherence and no loss to follow-up. Probiotic capsule integrity was independently documented,[19] aligning with calls for larger rigorous trials of probiotics in a range of human conditions.[8,9,36] All infectious outcomes underwent blinded adjudication. Analyses were prespecified, and findings were consistent in unadjusted, adjusted, prespecified sensitivity and subgroup analyses.[39] Participation of 44 centers in 3 countries over 4 years enhances the generalizability of results for this population. The findings have implications for practice and policy,[13] suggesting circumspect prescribing of probiotics during serious illness.[40]

### Limitations

This study has several limitations. First, in the absences of direct comparative studies, *L rhamnosus* GG was the probiotic evaluated, given that it is the most common intervention tested in this setting that had shown initial promise.[21] However, results may have differed using an alternate dose, genus, species, or strain or if studied in specialized populations such as patients who experienced trauma or were of low surgical risk with lower antimicrobial exposure or lower infectious risk. Second, it was not possible to examine pulmonary microbiota over time or between groups, or probiotic gastrointestinal colonization in this international trial. Third, there are inherent limitations of each VAP definition and no universal reference standard; however, our analyses were strengthened by protocolized data collection and use of several definitions.[18]

## Conclusions

Among critically ill patients requiring mechanical ventilation, administration of the probiotic *L rhamnosus* GG compared with placebo resulted in no significant difference in the development of ventilator-associated pneumonia. These findings do not support the use of *L rhamnosus* GG for prevention of ventilator-associated pneumonia or other clinically important outcomes in critically ill patients.

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**Data Sharing Statement:** See Supplement 4.

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