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Protocol and Statistical Analysis Plan for the PROSPECT (Probiotics: Prevention of Severe Pneumonia and Endotracheal Colonization) Trial

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ABSTRACT

Introduction: The PROSPECT Trial (Probiotics: Prevention of Severe Pneumonia and Endotracheal Colonization) aims to determine the impact of the probiotic *Lactobacillus rhamnosus* GG on ventilator associated pneumonia (VAP) and other clinically important outcomes in critically ill patients.

Methods and analysis: PROSPECT is a multicenter, concealed, randomized, stratified, blinded, controlled trial in patients ≥18 years old, anticipated to be mechanically ventilated ≥ 72 hours, in intensive care units (ICUs) in Canada, the United States and Saudi Arabia. Patients receive either 1 x 10^{10} colony forming units of *Lactobacillus rhamnosus* GG twice daily or an identical appearing placebo. Those at increased risk of probiotic infection are excluded. The primary outcome is VAP. Secondary outcomes are other ICU-acquired infections including *Clostridium difficile* infection, diarrhea (including antibiotic-associated diarrhea), antimicrobial use, ICU and hospital length of stay and mortality. The planned sample size of 2650 patients is based on an estimated 15% VAP rate and will provide 80% power to detect a 25% relative risk reduction.

Discussion: This protocol and statistical analysis plan outlines the PROSPECT Trial methodology, primary and secondary analyses, sensitivity analyses and subgroup analyses. The results of PROSPECT will inform practice guidelines worldwide.

Clinical Trial Registration: NCT02462590

Keywords: Critically ill; Infection; Intensive Care; Probiotics; Ventilator associated pneumonia

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Randomized placebo controlled multicenter trial
- Evaluation of the effect of probiotics on pneumonia, other intensive care unit (ICU)-acquired infections and diarrhea
- International enrolment including patients over 65 years of age to enhance the generalizability of the findings
- Characterization of pre-hospital frailty to help understand the relationship between frailty, probiotics and ICU-acquired infections
- Severely immunocompromised patients are excluded for safety reasons

INTRODUCTION

Ventilator associated pneumonia (VAP) is the most common healthcare associated infection in critically ill patients, and is associated with a significant burden of disease [1]. In a systematic review, the pooled incidence of VAP ranged from 10-23%, and VAP conferred a 2-fold attributable-risk of dying in the intensive care unit (ICU), with an attributable cost ranging from USD$10,000-$13,000 per patient [1]. Therefore, preventing VAP is a patient safety priority [2, 3].

Unfortunately, VAP prevention strategies are variably applied in practice [4], which underscores the need for simple, safe, effective and affordable VAP reduction strategies. Probiotics may represent one such novel approach. Probiotics have emerged as a biologically plausible strategy to prevent VAP, through modifying the microbiome, enhancing gut barrier function, and reducing pathogenic bacterial load [5 - 8]. Systematic reviews suggest that probiotics reduce VAP by 25% - 30% when compared to placebo [9, 10, 11]. However, most previous randomized trials were small, single center studies. Meta-analyses of small single center trials often yield implausibly large treatment effects [12, 13]. Hence, the clinical benefits of probiotics may be overestimated, and a large, well-powered multicenter trial is needed.

We recently completed the Probiotics: Prevention of Severe Pneumonia and Endotracheal Colonization (PROSPECT) Trial (PROSPECT) Pilot Trial [NCT01782755][14] in 14 ICUs. The feasibility objectives were related to 1) Recruitment: at least 2 patients per ICU per month; 2) Maximal protocol adherence: ≥90% of prescribed doses are actually administered; 3) Minimal contamination: <5% of patients receive a single dose of open-label probiotics and 4) Outcome incidence: at least 10% of enrolled patients developed VAP. The Pilot Trial met all 4 feasibility outcomes: 1) 150 patients were enrolled over 11 months, with 1.9 patients per ICU per month; 2) Adherence of study product was 97.4%; only 2.6% of doses prescribed were not received; 3)

Contamination did not occur; no patients received a dose of open-label probiotic at any time; and 4) The adjudicated VAP rate was 19% [15]. Therefore we launched PROSPECT - a multicenter randomized concealed stratified blinded parallel-group placebo-controlled superiority trial to determine whether the probiotic *Lactobacillus rhamnosus* GG compared to placebo reduces VAP and other clinically important outcomes in critically ill mechanically ventilated patients [NCT02462590]. In this paper we summarize the protocol [REB-approved version, version 1.0, date: February 27, 2015] and statistical analysis plan [version 2.0, date May 17 2018] for the PROSPECT Trial's primary analysis, reported using both the SPIRIT guidelines which define standard protocol items for clinical trials [16] and recent statistical analysis plan guidelines [17].

METHODS

Trial Population and Eligibility:

Patients will be recruited from 44 ICUs in Canada, United States and Saudi Arabia. The inclusion and exclusion criteria are presented in Table 1. Following completion of the PROSPECT Pilot [14, 15], the exclusion criteria were refined, informed by an extensive literature review focused on the safety or harm of *Lactobacillus* probiotic administration [18], experience with probiotics in the pilot trial [15], and following discussions with the Steering Committee and the Canadian Critical Care Trials Group [19](Table 1).

Consent and Randomization

Research Coordinators screen all mechanically ventilated patients for potential trial enrolment, recording those that meet individual inclusion and exclusion criteria. Once eligibility is confirmed, *a priori* written informed consent or deferred consent is obtained from the patient

or substitute decision maker as per our consent guidelines [20], and according to local ethics approval. The local Study Pharmacist obtains the allocation in a 1:1 ratio by a computer-based random number generator in variable unspecified block sizes, stratified by center and by medical, surgical or trauma admission status.

Blinding

Patients, bedside clinicians, investigators, and research coordinators are blinded to allocation. Study pharmacists at each center are not blinded; they randomize patients and prepare study product for administration without being involved in the day-to-day care of the patients. The biostatisticians will remain blinded until the final analysis is complete.

Interventions & Comparator:

Patients in the intervention group receive $1x10^{10}$ colony forming units of *L. rhamnosus* GG (i-Health, Inc.) in 1 capsule suspended in tap water or sterile water (dependant on local site practices), administered through a nasogastric or orogastric feeding tube. Patients in the placebo group receive an identical capsule containing microcrystalline cellulose. The same dose of microcrystalline cellulose is present in the *L. rhamnosus* GG capsules as well. Patients receive study product post randomization until: 1) ICU discharge or death; or 2) 60 days in the ICU; or 3) isolation of *Lactobacillus* spp. in a culture from a sterile site or if it is the sole or predominant organism in a culture from a non-sterile site.

The intervention is packaged in blister-cards of 10 capsules. For quality assurance purposes, we are performing an independent quality assessment of the study product supplied throughout the trial [21]. One randomly selected capsule from every 10th card of both probiotic

and placebo is cultured in Dr. Michael Surette's laboratory at McMaster University (Hamilton, Ontario), to ensure the dose and integrity of both the study product and placebo, as successfully done in the Pilot Trial [14, 15].

Data Collection

Research Coordinators collect data at baseline (e.g., demographics, illness severity, life support), and daily (e.g., study product administration, VAP prevention strategies and other cointerventions), and all primary and secondary outcomes (Appendix 1) using a secure web-based electronic data capture system (iDataFax, Seattle, Washington).

Outcomes

Primary outcome

The primary outcome is adjudicated VAP. Clinically suspected VAP at participating sites is being centrally adjudicated independently and in duplicate by 2 physicians blinded to allocation and center, informed by the following standardized definition: receiving invasive mechanical ventilation for $\geq$ 48 hours, when there is a new, progressive or persistent radiographic infiltrate on chest radiograph plus any 2 of the following: 1) fever (temperature>38 °C) or hypothermia (temperature <36 °C); 2) relative leukopenia (<3.0 x 10^6/L) or leukocytosis (>10 x 10^6/L) and 3) purulent sputum [23]. As the ACCP definition did not provide thresholds for leukopenia or leukocytosis, the thresholds were obtained from Morrow *et al* [22] as their VAP definition was also based on the American College of Chest Physicians (ACCP) definition [23]. Any disagreement in adjudication will be resolved through discussion and consensus.

Acknowledging that there is no universally accepted gold standard VAP definition [24], we are also collecting data allowing VAP reporting according to several other definitions [25 - 28].

Secondary Outcomes

a) Early VAP, late VAP, and post-extubation pneumonia: We are classifying VAP by early VAP and late VAP, as the etiologic organisms may differ, the antimicrobials prescribed may differ, and the prognosis is often worse for late VAP [29, 30]. Early VAP is defined as pneumonia arising on day 3, 4 or 5 after the initiation of mechanical ventilation. Late VAP is defined as VAP arising on day 6 of mechanical ventilation or later, and including up to 2 days after discontinuation of mechanical ventilation. We are also recording pneumonia arising in the ICU following discontinuation of mechanical ventilation (2 or more days after discontinuation), termed post-extubation pneumonia, to avoid suppressing potentially relevant lung infections that arise in ICU (Figure 1). We will also report a composite outcome of early VAP, late VAP, and post-extubation pneumonia.
b) *Clostridium difficile* in the ICU and in hospital: diarrhea (as defined in [c]) and laboratory confirmation of *C. difficile* or colonoscopic or histopathologic findings demonstrating pseudomembranous colitis [31].
c) Any infection acquired during the ICU stay, defined as respiratory or other infection including bloodstream infection, intravascular catheter-related bloodstream infection, intra-abdominal infection, *Clostridium difficile* infection, urinary tract infection, skin and soft tissue infection, and others. These individual infections are classified using established definitions [26], as adapted in prior studies [25]. We will also report a composite outcome of any infections acquired during the ICU stay. Secondary infectious

outcomes are being centrally adjudicated by 1 physician blinded to allocation and center, based on review of data collected at each participating site.

d) Diarrhea in the ICU: using the WHO definition (>3 loose or watery bowel movements per day [32]), and the Bristol Stool classification (type 6 or 7, > 3 times per day)[33].

e) Antibiotic-associated diarrhea in the ICU: diarrhea (as defined above in [d]) occurring the day of or within 24 hours of any antibiotic [34].

f) Antimicrobial use in ICU: defined as daily doses of therapy (DOT), defined daily dose (DDD) and antimicrobial-free days [35, 36].

g) Duration: mechanical ventilation, ICU stay and hospital stay.

h) ICU mortality and in-hospital mortality.

Serious adverse events (SAEs)

In PROSPECT, an SAE is defined as isolation of *Lactobacillus* spp. in a culture from a sterile site as the sole or predominant organism cultured from a non-sterile site that results in: 1) persistent or significant disability or incapacity; 2) that is life-threatening or 3) that results in death [37]. The rationale for our approach to SAEs accords with our guidelines for academic drug trials in critical care [38]. Any culture obtained by the ICU team and processed by the clinical microbiology laboratory as positive for *Lactobacillus* spp. is recorded. Any such bacterial sample is sent to the Surette Laboratory at McMaster University for strain genotyping to evaluate consistency with the administered *L. rhamnosus* GG strain.

Sample size and power

Based on an estimated 15% VAP rate, 2650 patients will be required to detect a 25% relative risk reduction (RRR) (and absolute risk reduction of 3.75%) with 80% power (alpha 0.05)(Table 2). The estimated 15% VAP rate is based on the PROSPECT Pilot Trial (adjudicated VAP rate of 19% [15]) and the REDOXS trial (14% [25]). The 25% RRR was observed in our meta-analysis of probiotics versus placebo [9] and a 24% RRR was found in a recent meta-analysis [11] and is more conservative than the 30% RRR in a Cochrane analysis [10]. Thus, we will enroll 1325 patients/group (2650 patients). Based on our pilot trial recruitment, we anticipate enrolling approximately 1.9 patients/month/site [15].

Central statistical monitoring

Thrice yearly throughout the trial, we perform central statistical monitoring by analyzing site-specific data receipt and completeness, to help identify and overcome barriers to timely data completion. We also monitor the proportion of non-screening weeks, and number and reasons for eligible non-randomized patients, to identify and remediate potential recruitment challenges.

We monitor and report other types of protocol adherence [39]. For example, protocol adherence regarding non-receipt of study product acknowledges sensible bedside decision-making, according to metrics from our pre-specified taxonomy [40]. We track categories such as admissible protocol deviations for clinically justified reasons (e.g., strict nil per os status for possible bowel perforation) and logistical reasons (e.g., patient discharged early from the ICU so no evening dose given) as distinct from oversights which are protocol violations (e.g., dispensing errors).

Statistical Analysis

Patients randomized in PROSPECT will be analyzed according to the intention-to-treat principle for the main analysis. We will present baseline characteristics of the 2 groups, including demographic and life support characteristics, and all prevalent infections. Infections will be defined as prevalent if pneumonia is present the day of, or diagnosed one day after randomization (the latter presumed to have started the day of randomization). Prevalent infections will not be considered outcomes for the trial because they cannot be modified by probiotics. A CONSORT flow diagram will be generated, representing all randomized patients, their outcomes, the number and reasons for any consent withdrawals or loss to follow-up, as well as the number and reasons for screened eligible non-randomized patients [41].

The main analysis will be a Cox proportional hazards analysis evaluating the primary outcome of VAP. This time-to-event analysis will use all information up to the time of censoring such that patients remain in the denominator and contribute information while they are at risk. The assumption for this analysis is that censoring is uninformative. The Cox model will be stratified by: a) center, and b) medical versus surgical versus trauma admission diagnosis, reflecting the stratification variables for randomization. The only independent variable will be randomized treatment group. We will present Kaplan Meier curves for the primary outcome. We will also report VAP incidence rate, as number of VAP cases per 1,000 ventilator days [42]. We will report exposures during the ICU stay such as advanced life supports and relevant cointerventions.

For the dichotomous secondary outcomes, we will also use time-to-event analyses. Hazard ratios and associated 95% confidential intervals will be estimated using a stratified Cox proportional hazards model.

For continuous outcomes, we will report estimates of the difference, 95% confidence intervals and associated p-values. For the continuous outcomes which are often skewed (e.g., duration of ventilation, ICU stay, and hospital stay), we will first log-transform these variables to see if they become normally distributed; if so, we will use parametric methods on the log-transformed variables to compare between groups. If not, we will compare the 2 groups using a non-parametric approach on the non-transformed variables.

For the main analysis, when there is a statistically significant difference in binary outcomes, we will calculate other metrics. For example, depending on the results, these may be expressed as the number needed to prophylax (NNP) with probiotics to prevent 1 case of pneumonia, or the number needed to harm (NNH) to cause 1 case of iatrogenic infection with *L. rhamnosus* GG.

We will not be missing any covariates for the primary outcome analysis – the only independent variable is treatment versus control, and the stratification variables are captured in the randomization system. We anticipate very little missing outcome data, since most data are collected in the ICU (except hospital vital status and length of stay, and *Clostridium difficile* infection which is also recorded following ICU discharge in the hospital). For any other outcome that is missing for more than 2% of the patients, we will perform multiple imputation analysis [43, 44].

We will use graphics and other relevant methods to examine the residuals to assess model assumptions and goodness-of-fit including the proportional hazards assumption for Cox-regression analyses [45 - 47].

All estimates of effect will be reported to two decimal places. P-values will be reported to three decimal places with those less than 0.001 reported as $p < 0.001$. The criterion for statistical

significance will be set at alpha = 0.05, using 2-sided tests, but adjusted appropriately for the 2 planned interim analyses using the Peto-Haybittle approach [48, 49]; see details under the *Ethics Oversight* section below. Secondary and subgroup analyses will not be adjusted for multiple analyses since these are exploratory [50]. All analyses will be performed using the most up to date version of SAS (Cary, NC).

Following the publication of the PROSPECT Trial, the dataset will be used to design observational studies addressing additional hypothesis-driven questions (e.g. predictors of ICU acquired *Clostridium difficile*).

Sensitivity Analyses

We will conduct 4 sensitivity analyses. To the extent that these sensitivity analyses yield similar results to the main analysis, inferences about the primary outcome will be strengthened [51, 52].

1. In case the exact timing of the onset of VAP is uncertain, we will compare the proportion of patients with VAP in the 2 groups using the Mantel-Haenszel Chi square test, stratified by centre and medical versus surgical versus trauma. Thus, in this sensitivity analysis we will not use a time-to-event approach.
2. We will check for competing risks to address the problem that those who die can no longer develop VAP. We will analyze PROSPECT to explicitly account for death as a competing risk using the Fine and Gray proportional sub-distribution hazards model [53, 54]. This analysis will not assume that the censoring of deaths is uninformative; rather, it will assume that deaths could be informative. The rationale for this sensitivity analysis is to assess the robustness of the main findings [51].

3. We will conduct an efficacy analysis of each incident infection, and a composite of all incident infections, restricted to patients who received study product on ≥90% of study days. The rationale for this sensitivity analysis is to investigate the effect of probiotics under conditions of maximal exposure [55].
4. We will include all VAP events that occur after the day of randomization. The rationale for this sensitivity analysis is that pneumonia arising the day after randomization may be less likely to be influenced by study product exposure than pneumonia arising 2 or more days after initial study product exposure.

Subgroup Analyses

We will conduct 5 subgroup analyses based on baseline characteristics. These will evaluate whether these 5 baseline characteristics have an 'effect modification' when the effect of probiotics versus placebo on VAP are compared [56, 57]. Subgroup analyses will only be performed for the primary outcome.

1. We will conduct subgroup analyses among medical versus surgical versus trauma patients (the latter defined as patients cared for by a trauma service). We hypothesize that in medical patients, the treatment effect may be attenuated due to more risk factors for VAP that are non-modifiable [1]. To perform this subgroup analysis, we will run the primary Cox regression analysis except that we will include medical versus combined surgical/trauma as an independent variable instead of stratifying by it. We will also include the interaction term between medical versus combined surgical/trauma and randomized treatment.

2. We will conduct subgroup analyses based on age (>75 years of age versus 65 – 75 years versus <65 years). Although little is known about the effects of probiotics in the elderly [58, 59], we hypothesize that if, overall, probiotics are associated with a lower rate of VAP than placebo, the treatment effect will be attenuated among older patients because immunosenescence renders their risk of infection less modifiable than younger patients. To perform this subgroup analysis, we will add age >75 versus 65 – 75 years versus <65 years as an independent variable as well as its interaction with randomized treatment group to the primary Cox regression model.
3. We will conduct subgroup analyses of the effect of probiotics on VAP after accounting for frailty, defined as a baseline Clinical Frailty Score of ≥5 out of 9 [60]. We hypothesize that if, overall, probiotics are associated with a lower rate of VAP than placebo, the treatment effect will be attenuated among patients who are frail, as their risk of infection may not be modifiable. To perform this subgroup analysis, we will add baseline Clinical Frailty Score of ≥5 as an independent variable as well as its interaction with randomized treatment group to the primary Cox regression model. We did not start measuring frailty data until 483 patients were enrolled in response to a national mandate [61]; thus, rather than imputing frailty status, we will restrict this subgroup analysis to patients enrolled thereafter.
4. We will conduct subgroup analyses among patients who received antibiotics for 2 days prior to randomization and the day of randomization versus patients who did not receive antibiotics for 2 days prior to, or the day of, randomization. We hypothesize that if, overall, probiotics are associated with a lower rate of VAP than placebo, the treatment effect will be attenuated in patients without recent antibiotic exposure when compared to

patients with antibiotic exposure. To perform this subgroup analysis, we will add antibiotic exposure prior to randomization defined as those receiving antibiotics for 2 days prior to randomization and the day of randomization as an independent variable as well as its interaction with randomized treatment group to the primary Cox regression model.

5. We will conduct subgroup analyses on patients with pneumonia at baseline versus no pneumonia at baseline. We hypothesize that if overall, probiotics are associated with a lower rate of VAP than placebo, the treatment effect will be attenuated among patients with pre-randomization pneumonia due to challenges interpreting whether the baseline pneumonia has resolved prior to the development of another pneumonia event. To perform this subgroup analysis, we will add pneumonia at baseline as an independent variable as well as its interaction with randomized treatment group to the primary Cox regression model.

Steering Committee

The PROSPECT Steering Committee is responsible for overseeing the conduct of the trial, for upholding or modifying study procedures as needed, and addressing any challenges with protocol implementation. They advise as necessary on operational issues arising that are clinical, methodologic, biostatistical or ethical. As enrolment ensues, they share new emerging clinical, laboratory or epidemiology information that may impact on the trial. The Steering Committee has discussed and approved the interim statistical analysis plans and final statistical analysis plan, and will assist with data interpretation, and abstract and manuscript preparation. The PROSPECT organizational chart is in Appendix 2.

Data Monitoring Committee

The PROSPECT Data Monitoring Committee (DMC) is independent from other persons involved in PROSPECT, and has the requisite expertise in randomized clinical trial design, epidemiology, biostatistics, warning guides/stopping rules, infectious diseases and critical care (Drs. A. Laupacis, C. Brun-Buisson, R. Roberts). The primary responsibilities of the DMC are to independently review reports prepared at the Methods Center regarding: 1) recruitment (center and patient) and screening, consent and coenrolment rates; 2) protocol procedures (randomization, stratification, protocol adherence including maintaining blinding); and 3) data tables for 2 blinded interim and final analyses (baseline characteristics, primary and secondary outcomes, adverse events and SAEs). After each interim analysis, the DMC will recommend whether to continue, suspend or terminate enrolment.

The roles and responsibilities of the DMC are as outlined and approved in the PROSPECT DMC Charter [62], modeled on the Data Monitoring Committees: Lessons, Ethics, Statistics (DAMOCLES) Study Group charter [63].

Patient and Public Involvement

In the PROSPECT Trial, we are involving patients and their families in the following ways. First, before beginning PROSPECT, we ensured that patients and families were supportive of the use of probiotics in the ICU setting. We conducted a substudy nested within the PROSPECT Pilot Trial [14, 15] whereby patient's surrogate decision makers (SDMs) were interviewed at the time of enrollment to explore their comfort with probiotic use during critical illness [64]. In total, 103 SDMs participated in 8 centers. We found no difference in

characteristics of SDMs who consented versus declined the PROSPECT Pilot Trial for their critically ill loved one. Rather, the rationale for SDM consent was related to personal beliefs regarding possible benefits to the patient, as well as predictions of patient's wishes regarding this trial. These findings gave us confidence that patients and families were supportive of the trial. Second, in accordance with many local ICU research practices, patients who gain capacity after resolution of their critical illness are asked to agree to ongoing participation in the trial. We have no formal patient or single family advisor for the trial. When PROSPECT results are available following trial completion, we will ensure the results are disseminated by having the academic message of each "in press" manuscript translated into press releases for the public. Possible target hospital media include newsletters, emails and intranet bulletins. Possible target public media include newspapers, radio, television, and social media. High citizen awareness and public probiotic consumption predict strong media interest.

Ethical oversight

PROSPECT is approved by Health Canada [#9427-M1133-45C], the Research Ethics Boards of all participating hospitals, and Public Health Ontario. The study is underway in accordance with Good Clinical Practices following the Tri-Council Guidelines [65] and in accordance with ethical principles of the Declaration of Helsinki [66].

The DMC will use conservative 'warning guides' for apparent benefit in PROSPECT; there are no stopping guides for futility. DMC members will evaluate the primary endpoint using the Haybittle-Peto method as a warning guide to apply to each of the 2 interim analyses at one third and two thirds of total enrollment, performed when complete ICU data are available for 883

and 1766 patients, respectively. Two-sided tests will be used, with a fixed conservative α=0.001 for the first and second interim analyses, and α=0.05 for the final analysis [48, 49].

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DISCUSSION

Probiotics may be a simple, cost-effective strategy to prevent VAP [67]. However, despite encouraging findings of efficacy, trials to date have been limited by insufficient power and risk of bias [9 - 11]. Studies of probiotics in the critical care setting have been criticized as difficult to interpret due to differences in populations and heterogeneous probiotics and combination products used [68]. Indeed, experts in the field have emphasized the need for well-powered studies of probiotics in the ICU setting [68]. To address this call, the PROSPECT Trial is a large, international, rigorous multicenter randomized trial that aims to determine whether probiotics are effective, have no benefit, or are harmful in critical illness.

Additional strengths of the PROSPECT trial include representation of persons greater than 65 years of age to enhance the generalizability of the findings, and separate peer-review funding for this population from the Canadian Frailty Network [61]. The efficacy of probiotics may be less in the elderly, as illustrated by a recent rigorous trial that found probiotics did not prevent *C.*

difficile infection in persons ≥65 years admitted to hospital and receiving at least one antibiotic [69]. We are also documenting baseline pre-hospital frailty with the Clinical Frailty Score [70] to further understand the relationship between frailty, immunosenence and critical care-associated infections.

Given a previous meta-analyses suggesting that probiotics may reduce all healthcare associated infections in the ICU [9], we are evaluating all infectious outcomes in PROSPECT. Also, a recent large trial of 2556 neonates and children conducted in India showed that probiotics decrease the risk of sepsis [71]. Given the growing interest in the dysbiosis of critical illness, this trial will advance our understanding of whether microbiome modification with probiotics has any influence on infectious and non-infectious clinically important outcomes [72, 73].

The International Conference for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use [ICH] E9 [74] and SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) [16] guidelines endorse a separate statistical analysis plan for clinical trials. Recommendations [16] include more technical and detailed elaboration of the principal features of the analysis described in the protocol, including procedures for executing the statistical analysis of the primary and secondary variables and other data [74]. We followed these expert recommendations [17] for 55 items in 6 sections: Title and Trial Registration (11 items/subitems); Introduction (2 items); Study Methods (9 items/subitems); Statistical Principles (8 items/subitems); Trial Population (8 items/subitems); and Analysis (17 items/subitems). Dissemination of this document aligns with calls to make statistical analysis plans publically available [75] to aid in the transparent reporting of trial results.

TRIAL STATUS

PROSPECT is supported by a longstanding research consortium (the Canadian Critical Care Trials Group) dedicated to investigator-initiated, peer-review funded studies designed to understand and improve the outcomes of critically ill patients [19]. Recruitment is ongoing, with 73% of the target sample size accrued as of May 2018. At the first interim analysis, the DMC made no suggestions to suspend enrolment. Randomization is anticipated to continue until approximately February 2019. Final data entry, data validation and outcome adjudication will ensue for 6-9 months thereafter, with an anticipated database lock in September 2019, followed by the terminal statistical analyses. PROSPECT results will inform global practice in critical care medicine.

Author Contributions:
Concept and design: J Johnstone, D Heels-Ansdell, L Thabane, D Cook
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Drafting of the manuscript: J Johnstone, D Heels-Ansdell, L Thabane, D Cook
Critical revision of the manuscript for important intellectual content: everyone
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Obtained funding: everyone
Administrative, technical, or material support: D Heels-Ansdell, E Duan, N Zytaruk, L Thabane, D Lamarchee, M Surette
Drs. J Johnstone and D Cook as Co-Principal Investigators take responsibility for the integrity of the data

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REFERENCES

1. Safdar N, Dezfulian C, Collard H, Saint S. Clinical and economic consequences of ventilator associated pneumonia: A systematic review. Crit Care Med 2005; 33: 2184.
2. Muscedere J, Dodek P, Keenan S, et al. Comprehensive evidence-based clinical practice guidelines for ventilator associated pneumonia: Prevention. J Crit Care 2008; 23: 126.
3. http://www.jointcommission.org. Accessed January 11, 2018.
4. Sinuff T, Muscedere J, Cook D, et al. The active observational study of the adoption and transfer of clinical practice guidelines through education, for ventilator associated pneumonia study (ABATE VAP). Crit Care Med 2013; 41: 15.
5. Marshall J. The gastrointestinal flora and its alterations in critical illness. Curr Opin Crit Care 1999; 5: 119.
6. Stappenback T, Hooper L, Gordon J. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proc Natl Acad Sci USA 2002; 99: 15451.
7. Tanoue T, Honda K. Induction of Treg cells in the mouse colonic mucosa: a central mechanism to maintain host-microbiota homeostasis. Semin Immunol 2012; 24: 50.
8. Brenchley J, Douek D. Microbial translocation across the gastrointestinal tract. Annu Rev Immunol 2012; 30: 149.
9. Petrof E, Dhaliwal R, Manzanares W, et al. Probiotics in the critically ill. Crit Care Med 2012; 40: 3290.
10. Bo L, Li J, Tao T, et al. Probiotics for preventing ventilator associated pneumonia. Cochrane Database of Syst Rev 2014; 10: CD009066.
11. Manzanares W, Lemieux M, Langlois PL, Wischmeyer PE. Probiotic and synbiotic therapy in critical illness: a systematic review and meta-analysis. Crit Care. 2016; 19: 262.
12. Nuesch E, Trelle S, Reichenbach S, et al. Small study effects in meta-analyses of osteoarthritis trials: meta-epidemiological study. BMJ 2010; 341: c3515.
13. Dechartres A, Boutron I, Trinquart L, et al. Single-center trials show larger treatment effects than multicenter trials. Ann Intern Med 2011; 155: 39.
14. Johnstone J, Meade M, Marshall J, et al. PROSPECT: Protocol for a feasibility randomized pilot trial. Pilot and Feasibility Studies 2015; 1: 19.
15. Cook D, Johnstone J, Marshall J, et al. Probiotics: Prevention of Severe Pneumonia and Endotracheal Colonization Trial - PROSPECT: A Pilot Trial. Trials 2016; 17: 377.
16. Chan A, Tetzlaff J, Altman D, et al. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med 2013; 158: 200 - 207.
17. Gamble C, Krishan A, Stocken D, et al. Guidelines for the content of statistical analysis plans in clinical trials. JAMA 2017; 318: 2337 – 2343.
18. Duan EH, Johnstone J, Meade MO, et al. Refining exclusion criteria following pilot trials: Lessons from PROSPECT. Canadian Critical Care Forum 2016, Toronto, ON, October 2016 Can Crit Care Forum Abstract Book 2016.
19. Marshall JC, Cook DJ for the Canadian Critical Care Trials Group. Investigator-led clinical research consortia: The Canadian Critical Care Trials Group. Crit Care Med 2009; 36:S165-S172.
20. Smith O, McDonald E, Zytaruk N, et al. Enhancing the informed consent process for critical care research: strategies from a thromboprophylaxis Trial. Intensive and Critical Care Nursing 2013; doi.org/10.1016/j.iccn.2013.04.006.

21. Lamarche D, Rossi L, Shah M, et al. Quality control in the conduct of a probiotic randomized trial. Can Crit Care Forum, Toronto, ON, October 28, 2014.
22. Morrow L, Kollef M, Casale T. Probiotic prophylaxis of ventilator associated pneumonia. Am J Respir Crit Care Med 2010; 182: 1058.
23. Grossman R, Fein A. Evidence-based assessment of diagnostic tests for ventilator associated pneumonia. Chest 2000; 117(4 Suppl 2): 177S – 181S.
24. Muscedere J, Sinuff T, Heyland D, et al. The clinical impact and preventability of ventilator associated conditions in critically ill patients who are mechanically ventilated. Chest 2013; 144: 1453.
25. Heyland D, Muscedere J, Wischmeyer P, et al. A randomized trial of glutamine and antioxidants in critically ill patients. N Engl J Med 2013; 368: 1489.
26. Calandra T, Cohen J for the International Sepsis Forum Definition of Infection in the ICU Consensus Conference. Crit Care Med 2005; 7: 1538.
27. Pugin J, Auckenthaler R, Mill N, et al. Diagnosis of ventilator associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic blind bronchoalveolar lavage fluid. Am Rev Respir Dis 1991; 143: 1121.
28. Horan T, Andrus M, Dudeck M. CDC/NHSN surveillance definition of healthcare associated infections and criteria for specific types of infections in the acute care setting. Am J Infect Control 2008;36:309.
29. Chastre J, Wolff M, Fagon J, et al. Comparison of 8 vs 15 days of antibiotic therapy for ventilator-associated pneumonia in adults: a randomized trial. JAMA 2003; 290: 2588 – 2598.
30. Chastre J and Fagon J. Ventilator associated pneumonia. Am J Respir Crit Care Med 2002; 165: 867–903
31. Cohen S, Gerding D, Johnson S, et al. Clinical practice guidelines for C. difficile infection in adults: 2010. Infect Control Hosp Epidemiol 2010; 31: 431.
32. World Health Organization. http://www.who.int/topics/diarrhoea/en/ Accessed May 14 2018.
33. Heaton K, Lewis S. Stool form scale as a useful guide to intestinal transit time. Scan J Gastroenterol 1997; 32: 920.
34. Thibault R, Graf S Clerc A, et al. Diarrhea in the intensive care unit. Crit Care 2013; 17: R153.
35. Polk R, Fox C, Mahoney A et al. Measurement of adult antibacterial drug use in 130 US hospitals: comparison of defined daily dose and days of therapy. Clin Infect Dis 2007; 44: 664-70.
36. Kuster S, Ruef C, Ledergerber B et al. Quantitative antibiotic use in hospitals: comparison of measurements, literature review, and recommendations for a standard of reporting. Infection 2008; 36:549-59.
37. The Guidance Document for Industry – Reporting Adverse Reactions to Marketed Health Products: www.hc-sc.gc.ca/dhp-mps/pubs/medeff/_guide/2011-guidance-directrice_reporting-notification/index-eng.php
38. Cook D, Lauzier F, Rocha M, et al. Serious adverse events in academic critical care research. CMAJ 2008; 178: 1181.
39. Bhatt A. Protocol deviation and violation. Perspect Clin Res. 2012; 3: 117.
40. Lauzier F, Adhikari N, Seely A, et al. Protocol adherence for continuously titrated interventions in randomized trials: An overview of the current methodology and case study. BMC Medical Research Methodology 2017; 17: 106.

41. Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. Trials 2010, 11: 32.
42. National Healthcare Safety Network. https://www.cdc.gov/nhsn/pdfs/pscmanual/6pscvapcurrent.pdf Accessed May 14 2018.
43. Little R, Rubin D: Statistical analysis with missing data. 2nd edition. Hoboken, NJ: Wiley; 2002; Rubin DB: Multiple imputation for nonresponse in surveys. New York: J Wiley & Sons; 1987.
44. Zhang Y, Alyass A, Vanniyasingam T, et al. A systematic survey of the methods literature on the reporting quality and optimal methods of handling participants with missing outcome data for continuous outcomes in randomized controlled trials. J Clin Epidemiol 2017; 88: 67-80.
45. Schoenfeld D: Partial residuals for the proportional hazards regression model. Biometrika 1982, 69:239–241.
46. Wei LJ: Testing goodness of fit for proportional hazards model with censored observations. J Am Stat Assoc 1984, 79:649–652.
47. Harrell FE, Lee KL: Verifying Assumptions of the Cox Proportional Hazards Model. Proceedings of the Eleventh Annual. SAS User's Group International Conference. Cary, N.C.: SAS Institute, Inc.; 1986:823–828.
48. Haybittle, JL. Repeated assessment of results in clinical trials of cancer treatment. British J Radiology 1971; 44: 793.
49. Peto R, Pike M, Armitage P, et al. Design and analysis of randomized control trials requiring prolonged observations of each patient. British J Cancer 1976; 34: 585.
50. Li G, Taljaard M, Van den Heuvel E, et al. An introduction to multiplicity issues in clinical trials: the what, why, when and how. International Journal of Epidemiology, 2017, 746–755.
51. Thabane L, Mbuagbaw L, Zhang S, et al. A tutorial on sensitivity analyses in clinical trials: the what, why, when and how. BMC Med Res Methodol 2013, 13(1):92.
52. de Souza RJ, Eisen R, Perera S, et al. Best (but oft-forgotten) practices: sensitivity analyses in randomized controlled trials. Am J Clin Nutr 2016; 103: 5-17.
53. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. Journal of the American Statistical Association 1999;94(446):496-509.
54. Bakoyannis G, Touloumi G. Practical methods for competing risks data: A review. Statistical Methods in Medical Research 2012;21(3):257-272.
55. Cramer JA, Roy A, Burrell A, et al. Medication compliance and persistence: terminology and definitions. Value Health. 2008; 11: 44–7.
56. Rothwell P. Subgroup analysis in randomised controlled trials: importance, indications, and interpretation. Lancet 2005; 365: 176–186.
57. Sun X, Briel M, Walter SD, Guyatt GH. Is a subgroup effect believable? Updating criteria to evaluate the credibility of subgroup analyses. BMJ 2010; 340: 85-854.
58. Patel PJ, Singh SK, Panaich S, Cardozo L. The aging gut and the role of prebiotics, probiotics, and synbiotics: a review. J Clin Gerontol Geriatr 2014;5:3e6.
59. Wachholz PA, P Boas. Evidence on the role of prebiotics, probiotics, and synbiotics in gut health and disease prevention in the elderly. J Clin Gerontol Geriatr 2014; 5: 1-2.
60. Bagshaw S, Stelfox T, McDermid R, et al. Association between frailty and short- and long-term outcomes among critically ill patients: a multicentre prospective cohort study. CMAJ 2013; DOI:10.1503/cmaj.130639.
61. Canadian Frailty Network. http://www.cfn-nce.ca/ Accessed May 14, 2018.

62. PROSPECT. www.prospecttrial.com Accessed May 14 2018.
63. DAMOCLES Study Group. A proposed charter for clinical trials data monitoring committees: helping them to do their job well. Lancet 2005; 365: 711-722.
64. Shears M, Clarke F, Mehta S, et al. Inter-Prob:INTERviewing substitute decision makers about PROBiotics. Can Crit Care Forum, Toronto, ON, October 25-28, 2015.
65. Grimes D, Hubacher D, Nanda K, et al. The Good Clinical Practice guideline: a bronze standard for clinical research. Lancet 2005; 366: 172–74
66. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013; 310(20):2191-4.
67. Branch-Elliman W, Wright S, Howell M. Determining the ideal strategy for ventilator associated pneumonia prevention. Am J Respir Crit Care Med 2015; 192: 57.
68. Crooks NH, Snaith C, Webster D, et al. Clinical review: Probiotics in critical care. Critical Care 2012, 16: 237.
69. Allen S, Wareham K, Wang D, et al. Lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhoea and Clostridium difficile diarrhoea in older inpatients (PLACIDE): a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 2013; 382: 1249–1257.
70. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly people. CMAJ 2005; 173: 489–495.
71. Panigrahi P, Parida S, Nanda N, et al. A randomized synbiotic trial to prevent sepsis among infants in rural India. Nature 2017; 548: 407–412.
72. McDonald D, Ackermann G, Khailova L et al. Extreme dysbiosis of the microbiome in critical illness. mSphere 2016; 1: e00199-16.
73. Relman D. The human microbiome and the future practice of medicine. JAMA 2015; 314: 1127-8.
74. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline: Statistical Principles for Clinical Trials E9. London, England: European Medicines Agency; 1998.
75. Finfer S, Bellomo R. Why publish statistical analysis plans? Crit Care Resusc 2009; 11: 5-6.

Table 1: Inclusion and Exclusion Criteria

Inclusion criteria
1. Adults ≥18 years of age admitted to a medical, surgical or trauma ICU 2. Receiving invasive mechanical ventilation, estimated to be required for ≥72 hours
Exclusion criteria
1. Invasively mechanically ventilated >72 hours at the time of screening 2. Potential increased risk of iatrogenic probiotic infection including specific immunocompromised groups: HIV <200 CD4 cells/μL, chronic immunosuppressive medications, previous transplantation at any time, chemotherapy in the last 3 months, absolute neutrophil count <500. Previous or current corticosteroids use is not exclusionary 3. Risk for endovascular infection: rheumatic heart disease, congenital valve disease, surgically repaired congenital heart disease, unrepaired cyanotic congenital heart disease, valvular replacement (mechanical or bio-prosthetic), previous or current endocarditis, permanent endovascular devices (e.g., endovascular grafts, inferior vena cava filters, dialysis vascular grafts), tunneled hemodialysis catheters, pacemakers or defibrillators. These are not exclusions: coronary artery stents or bypass grafts, mitral valve prolapse, bicuspid aortic valve, temporary catheters (central venous, peripherally inserted, extra-corporeal life support-related) or neurovascular coils 4. Primary diagnosis of severe acute pancreatitis 5. Percutaneously inserted feeding tubes in situ, as per Health Canada 6. Strict contraindications or inability to receive enteral medications 7. Intent to withdraw advanced life support 8. Previous enrolment in this trial, or current enrolment in a potentially confounding trial

*Changes from the PROSPECT Pilot Trial are as follows: 1. Omitted radiation therapy as an exclusion criterion; 2. Omitted steroid exposure as an exclusion criterion; 3. Better defined transplant to explicitly exclude all transplant patients (autologous stem cell patients are now excluded); 4. Better defined the cardiac valvular diseases at risk; 5. Removed surgery of esophagus/stomach/small bowel as exclusion criteria and replaced with any strict contraindication or inability to receive enteral medications; 6. Replaced severe acute pancreatitis with organ dysfunction with primary diagnosis of severe acute pancreatitis; 7. Omitted pregnancy as exclusion criterion [18].

Table 2: Determination of the sample size: based on an estimated 15% VAP rate, 2650 patients (n=1325 in each arm) will be required to detect a 25% relative risk reduction with 80% power.

Per Group **Sample Size for 80% power and alpha=0.05, using continuity correction.**

	RRR				
Baseline Risk	**10%**	**15%**	**20%**	**25%**	**30%**
8%	17473	7635	4221	2653	1809
9%	15374	6720	3716	2337	1594
10%	13695	5988	3313	2084	1422
12%	11176	4891	2707	1704	1164
14%	9377	4107	2275	1433	979
15%	8657	3793	2102	**1325**	906
16%	8028	3519	1951	1230	841
18%	6978	3061	1699	1072	734
20%	6139	2695	1497	945	647
22%	5452	2396	1332	842	577
24%	4879	2147	1194	756	518
25%	4627	2037	1134	718	493
30%	3620	1598	892	566	389
35%	2900	1284	719	458	316
40%	2361	1049	589	376	260
50%	1605	719	408	262	183

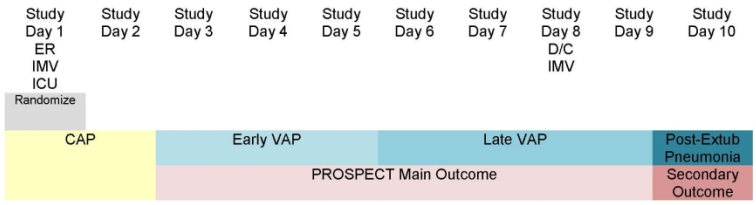
Figure 1: Pneumonia Classification

Legend for Figure 1: These figures illustrate the pneumonia classification that we are using, according to when the lung infection develops in a patient's hospital trajectory. The different classifications over time in each example relate to the day of hospital admission, day of ICU admission, day of initiation of mechanical ventilation (via endotracheal intubation or tracheostomy), day of randomization in the trial, and day of discontinuation of mechanical ventilation. Note that the pneumonia classifications over time do not reflect persistent or progressive lung infections, but rather the pneumonia classification that would be ascribed if a new infection develops on each day shown.

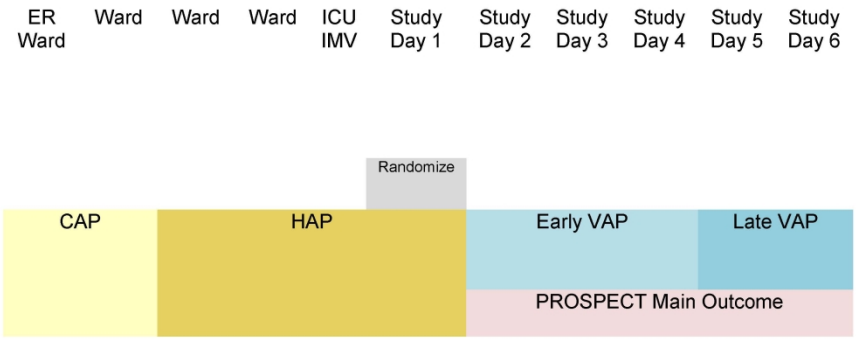
Abbreviations: ER – emergency room; Extub – Extubation; IMV – invasive mechanical ventilation; ICU – intensive care unit; ICUAP – intensive care unit associated pneumonia; Rand – randomization; CAP – community acquired pneumonia; VAP – ventilator associated pneumonia; HAP – hospital acquired pneumonia;

Figure 1a: Pneumonia classifications that could arise in patients who require ICU admission and invasive mechanical ventilation at the time of presentation to the emergency room, and are randomized into PROSPECT that day.

Figure 1b: Pneumonia classifications that could arise in patients who require ICU admission and invasive mechanical ventilation after an initial hospital stay, and are randomized into PROSPECT the day following ICU admission.



215x279mm (300 x 300 DPI)



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Appendix 1: Objectives, Outcomes, Hypothesis and Methods of Analysis

Objectives	Outcomes	Hypothesis	Methods of Analysis
1) **Primary:** To compare the effects of *Lactobacillus rhamnosus* GG versus placebo on	Ventilator associated pneumonia (VAP)	*Lactobacillus rhamnosus* GG will reduce the risk of the primary outcome	Cox proportional hazards
2) **Secondary:** To compare the effects of *Lactobacillus rhamnosus* GG versus placebo on:	a) Early VAP, late VAP, post-ventilation ICU acquired pneumonia, and a composite of all three.	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	b) Clostridium difficile	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	c) Any infection acquired during the ICU stay	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	d) Diarrhea in the ICU	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	e) Antibiotic-associated diarrhea	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	f) Antimicrobial use	*Lactobacillus rhamnosus* GG will reduce the risk	Independent samples paired t-test Wilcoxon rank sum test
	g) Duration of mechanical ventilation, ICU stay and hospital stay	*Lactobacillus rhamnosus* GG will have no effect	Independent samples paired t-test Wilcoxon rank sum test
	h) ICU mortality and hospital mortality	*Lactobacillus rhamnosus* GG will have no effect	Cox proportional hazards
	i) Serious adverse events	*Lactobacillus rhamnosus* GG will have no effect	Cox proportional hazards
3) **Sensitivity Analyses:**			
i. Compare proportion of patients with VAP in the two groups.	Ventilator associated pneumonia (VAP)	Results remain robust	i. Mantel-Haenszel Chi square test
ii. Check for competing risk of death			ii. Competing risk analysis
iii. Efficacy analysis			iii. Cox proportional hazards
iv. Include all VAP events that occur after the day of randomization			iv. Cox proportional hazards
4) **Subgroup Analyses:**			
i. Medical versus surgical versus trauma patients	Ventilator associated pneumonia (VAP)	Treatment effects may be attenuated in one group compared to another group (see text for details)	Cox proportional hazards with interaction test between each subgroup variable and treatment group evaluating the credibility of subgroup findings using our 11 previously published criteria [Sun 2010]
ii. Older (>75 years versus 65 – 75 years versus <65 years)			
iii. Frail patients versus not frail			
iv. Antibiotics prior to randomization versus no antibiotics prior to randomization			
v. Pneumonia at baseline versus no pneumonia at baseline			

Legend for Appendix 1: In all analyses, results will be expressed as estimate of effect, corresponding 95% and associated p-values. All tests will be two-sided using alpha = 0.05 level of significance in accordance with a superiority hypotheses.

Legend for Appendix 2: PROSPECT Organizational Chart

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier
Funding	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size

Methods: Assignment of interventions (for controlled trials)

Allocation:		
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
✓	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
✓	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
	Ethics and dissemination		
✓	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
N/A	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
✓	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
✓	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
✓	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
✓	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
N/A	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
✓	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
		31b	Authorship eligibility guidelines and any intended use of professional writers
		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

Did not include but can if requested. NA.

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Probiotics in Critically Ill Adults: A Trial Protocol and Statistical Analysis Plan for PROSPECT

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Probiotics in Critically Ill Adults:

A Trial Protocol and Statistical Analysis Plan for PROSPECT

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STRENGTHS AND LIMITATIONS OF THIS STUDY

- Randomized placebo controlled multicenter trial
- Evaluation of the effect of probiotics on pneumonia, other intensive care unit (ICU)-acquired infections and diarrhea
- International enrolment including patients over 65 years of age to enhance the generalizability of the findings
- Characterization of pre-hospital frailty to help understand the relationship between frailty, probiotics and ICU-acquired infections
- Severely immunocompromised patients are excluded for safety reasons

ABSTRACT

Background: PROSPECT (Probiotics: Prevention of Severe Pneumonia and Endotracheal Colonization Trial) aims to determine the impact of the probiotic *Lactobacillus rhamnosus* GG on ventilator-associated pneumonia (VAP) and other clinically important outcomes in critically ill adults.

Methods: PROSPECT is a multicenter, concealed, randomized, stratified, blinded, controlled trial in patients ≥18 years old, anticipated to be mechanically ventilated ≥ 72 hours, in intensive care units (ICUs) in Canada, the United States and Saudi Arabia. Patients receive either 1 x 10^{10} colony forming units of *L. rhamnosus* GG twice daily or an identical appearing placebo. Those at increased risk of probiotic infection are excluded. The primary outcome is VAP. Secondary outcomes are other ICU-acquired infections including *Clostridium difficile* infection, diarrhea (including antibiotic-associated diarrhea), antimicrobial use, ICU and hospital length of stay and mortality. The planned sample size of 2650 patients is based on an estimated 15% VAP rate and will provide 80% power to detect a 25% relative risk reduction.

Ethics and Dissemination: This protocol and statistical analysis plan outlines the methodology, primary and secondary analyses, sensitivity analyses and subgroup analyses. The results of PROSPECT will inform practice guidelines worldwide.

Clinical Trial Registration: www.clinicaltrials.gov NCT02462590

Keywords: Critically ill; Infection; Intensive Care; Probiotics; Ventilator-associated pneumonia

INTRODUCTION

Ventilator-associated pneumonia (VAP) is the most common healthcare associated infection in critically ill patients, and is associated with a significant burden of disease [1]. In a systematic review, the pooled incidence of VAP in patients mechanically ventilated for >48 hours ranged from 10-23%, and VAP conferred a 2-fold attributable-risk of dying in the intensive care unit (ICU), with an attributable cost ranging from USD$10,000-$13,000 per patient [1]. Therefore, preventing VAP is a patient safety priority [2, 3].

Unfortunately, VAP prevention strategies are variably applied in practice [4], which underscores the need for simple, safe, effective and affordable VAP reduction strategies. Probiotics may represent one such novel approach. Probiotics have emerged as a biologically plausible strategy to prevent VAP, through influencing microbiota, enhancing gut barrier function, and reducing pathogenic bacterial load [5-8]. Systematic reviews suggest that probiotics reduce VAP by 25% - 30% when compared to placebo [9-11]. However, most previous randomized trials were small, single center studies. Meta-analyses of small single center trials often yield implausibly large treatment effects [12, 13]. Hence, the clinical benefits of probiotics may be overestimated, and a large, well-powered multicenter trial is needed.

In a recent trial sequential meta-analysis of randomized trials testing the effect of probiotics on VAP during critical illness, 11 of 13 included trials evaluated a *Lactobacillus* species alone or in combination, and 2 of these trials used *Lactobacillus rhamnosus* GG [14], including the most rigorous trial by Morrow *et al* [15]. This high quality trial compared *L. rhamnosus* GG to corresponding placebos in 146 patients and the patients treated with *L. rhamnosus* GG had lower rates of VAP suggesting that *L. rhamnosus* GG, specifically, is a promising probiotic to prevent VAP in a selected high-risk ICU population [15].

We recently completed the PROSPECT pilot [www.clinicaltrials.gov NCT01782755][16] in 14 ICUs which compared *L. rhamnosus* GG to placebo in critically ill mechanically ventilated patients. The feasibility objectives of the pilot trial were related to 1) Recruitment: at least 2 patients per ICU per month; 2) Maximal protocol adherence: ≥90% of prescribed doses are actually administered; 3) Minimal contamination: <5% of patients receive a single dose of open-label probiotics and 4) Outcome incidence: at least 10% of enrolled patients developed VAP. The pilot trial met all 4 feasibility outcomes: 1) 150 patients were enrolled over 11 months, with 1.9 patients per ICU per month; 2) Adherence to study product was 97.4%; only 2.6% of doses prescribed were not received; 3) Contamination did not occur; no patients received a dose of open-label probiotic at any time; and 4) The adjudicated VAP rate was 19% [17]. Therefore we launched PROSPECT - a multicenter randomized concealed stratified blinded parallel-group placebo-controlled superiority trial to determine whether the probiotic *L. rhamnosus* GG compared to placebo reduces VAP and other clinically important outcomes in critically ill mechanically ventilated patients [www.clinicaltrials.gov NCT02462590]. In this paper we summarize the protocol [REB-approved version, version 1.0, date: February 27, 2015] and statistical analysis plan [version 2.0, date May 17 2018] for PROSPECT's primary analysis, reported using both the SPIRIT guidelines which define standard protocol items for clinical trials [18] and recent statistical analysis plan guidelines [19].

METHODS

Trial Population and Eligibility:

Patients will be recruited from 44 ICUs in Canada, the United States and Saudi Arabia (detailed list of study sites available [www.clinicaltrials.gov NCT02462590]). The inclusion and

exclusion criteria are presented in Table 1. Following completion of the PROSPECT pilot [16, 17], the exclusion criteria were refined, informed by an extensive literature review focused on the safety or harm of *Lactobacillus* spp. probiotic administration [20], experience with probiotics in the pilot trial [17], and following discussions with the PROSPECT Steering Committee and the Canadian Critical Care Trials Group [21](Table 1 footnote for details of changes).

Consent and Randomization

Research Coordinators screen all mechanically ventilated patients for potential trial enrolment, recording those that meet individual inclusion and exclusion criteria. Once eligibility is confirmed, *a priori* written informed consent or deferred consent is obtained from the patient or substitute decision maker as per our consent guidelines [22, 23], and according to local ethics approval. The patients are allocated to treatment in a 1:1 ratio via a computer-based random number generator in variable unspecified block sizes, stratified by center and by medical, surgical or trauma admission status.

Blinding

Patients, bedside clinicians, investigators, and research coordinators are blinded to allocation. Study pharmacists at each center are not blinded; they randomize patients and prepare study product for administration without being involved in the day-to-day bedside care of patients. The biostatisticians will remain blinded until the main analysis is complete. Unblinding will not be permissible throughout the trial.

Interventions and Comparator:

Patients in the intervention group receive $1x10^{10}$ colony forming units of *L. rhamnosus* GG (i-Health, Inc.) in 1 capsule suspended in tap water or sterile water (dependant on local site practices), administered through a nasogastric or orogastric feeding tube. Patients in the placebo group receive an identical capsule containing microcrystalline cellulose. The same dose of microcrystalline cellulose is present in the *L. rhamnosus* GG capsules. Patients receive study product post randomization until: 1) ICU discharge or death; or 2) 60 days in the ICU; or 3) isolation of *Lactobacillus* spp. in a culture from a sterile site or if it is the sole or predominant organism in a culture from a non-sterile site.

The intervention is packaged in blister-cards of 10 capsules. For quality assurance purposes, we are performing an independent quality assessment of the study product supplied throughout the trial [24]. One randomly selected capsule from every 10th card of both probiotic and placebo is cultured in the Surette Microbiome Laboratory at McMaster University (Hamilton, Ontario), to ensure the dose and integrity of both the study product and placebo, as successfully done in the pilot trial [16, 17].

Data Collection

Research Coordinators collect data at baseline (e.g. demographics, illness severity, life support using the Acute Physiology and Chronic Health Evaluation [APACHE] II score), and daily (e.g. study product administration, VAP prevention strategies and other cointerventions), and all primary and secondary outcomes (Appendix 1) by completing data collection forms [22] and uploading to a secure web-based electronic data capture system (iDataFax, Seattle, Washington). To protect the personal health information of patients enrolled, all identifying information will be de-linked. Participants will be assigned a unique identification code (study

ID). The code-breaking information will be kept separate from the data extraction files. It will be the responsibility of the site investigators to ensure that the code-breaking information is totally inaccessible to individuals who are not on the research team. Personal health information about enrolled participants will include age, sex and admitting diagnosis, but will be de-identified at the recruiting center and anonymized in the main database over the course of the trial and thereafter.

Outcomes

Primary outcome

The primary outcome is adjudicated VAP. Clinically suspected VAP at participating sites is being centrally adjudicated independently and in duplicate by 2 physicians blinded to allocation and center, informed by the following standardized definition: receiving invasive mechanical ventilation for $\geq$ 2 days, when there is a new, progressive or persistent radiographic infiltrate on chest radiograph plus any 2 of the following: 1) fever (temperature >38 °C) or hypothermia (temperature <36 °C); 2) relative leukopenia (<3.0 x 10^6/L) or leukocytosis (>10 x 10^6/L) and 3) purulent sputum [25]. As the American College of Chest Physicians (ACCP) definition did not provide thresholds for leukopenia or leukocytosis, the thresholds were obtained from Morrow *et al* [15] as their VAP definition was also based on the ACCP definition [25]. Any disagreement in adjudication will be resolved through discussion and consensus. Acknowledging that there is no universally accepted gold standard VAP definition [26], and that in non-immunocompromised patients, routine invasive testing is not associated with improved outcomes [27], we are also collecting data to allow VAP reporting according to several other definitions [28 - 31].

Secondary Outcomes

a) Early VAP, late VAP, and post-extubation pneumonia: We are classifying VAP by early VAP and late VAP, as the etiologic organisms may differ, the antimicrobials prescribed may differ, and the prognosis is often worse for late VAP [32, 33]. Early VAP is defined as pneumonia arising on day 3, 4 or 5 after the initiation of mechanical ventilation. Late VAP is defined as VAP arising on day 6 of mechanical ventilation or later, and including up to 2 days after discontinuation of mechanical ventilation (also relevant for patients with a tracheostomy). We are also recording pneumonia arising in the ICU following discontinuation of mechanical ventilation (3 or more days after discontinuation), labelled post-extubation pneumonia, to avoid suppressing potentially relevant lung infections that arise in ICU (Figure 1 and 2). We will also report a composite outcome of early VAP, late VAP, and post-extubation pneumonia, adjudicated independently and in duplicate by 2 physicians.

b) *Clostridium difficile* in the ICU and prior to discharge from hospital: diarrhea (as defined in [d]) and laboratory confirmation of *C. difficile* or colonoscopic or histopathologic findings demonstrating pseudomembranous colitis [34], which will also be adjudicated independently and in duplicate by 2 physicians.

c) Any infection acquired during the ICU stay, including bloodstream infection, intravascular catheter-related bloodstream infection, intra-abdominal infection, *C. difficile* infection, urinary tract infection, skin and soft tissue infection, and others. These individual infections are classified using definitions adapted from the International Sepsis Forum Consensus Conference on Definitions of Infection in the Intensive Care Unit [29],

as adapted in prior studies [28]. We will also report a composite outcome of any infections (including pneumonia) acquired during the ICU stay. Secondary infectious outcomes (other than pneumonia and *C. difficile*) are being centrally adjudicated by 1 physician blinded to allocation and center, based on review of data collected at each participating site.

d) Diarrhea in the ICU: We will record each bowel movement and define diarrhea incorporating 2 metrics; the World Health Organization definition (≥3 loose or watery bowel movements per day [35]), and the Bristol Stool classification for loose or watery stool (type 6 or 7)[36].

e) Antibiotic-associated diarrhea in the ICU: diarrhea (as defined above in [d]) following the administration of antibiotics, any day antibiotics are administered or within 1 day of any antibiotic [37].

f) Antimicrobial use in ICU: defined as daily doses of therapy (DOT), defined daily dose (DDD) and antimicrobial-free days [38, 39]. Only systemic antimicrobials will be captured (e.g. parenteral, intravenous, oral, enteral) whether prophylactic or therapeutic in intent. Topical creams, eye/ear drops and inhaled antimicrobials will be excluded.

g) Duration: mechanical ventilation, ICU stay and hospital stay.

h) ICU mortality and in-hospital mortality.

Serious adverse events (SAEs)

In PROSPECT, an SAE is defined as isolation of *Lactobacillus* spp. in a culture from a sterile site or as the sole or predominant organism cultured from a non-sterile site and results in: 1) persistent or significant disability or incapacity; 2) that is life-threatening or 3) that results in

death [40]. The rationale for our approach to SAEs accords with our guidelines for academic drug trials in critical care [41]. Any culture obtained by the ICU team and processed by the clinical microbiology laboratory as positive for *Lactobacillus* spp. is recorded. Any such bacterial sample is sent to a McMaster University research laboratory for strain genotyping to evaluate consistency with the administered *L. rhamnosus* GG strain.

Sample size and power

Based on an estimated 15% VAP rate, 2650 patients will be required to detect a 25% relative risk reduction (RRR) (and absolute risk reduction of 3.75%) with 80% power (alpha 0.05)(Table 2). The estimated 15% VAP rate is based on the PROSPECT pilot (adjudicated VAP rate of 19% [17]) and the REDOXS trial (14% [28]). The 25% RRR was observed in our meta-analysis of probiotics versus placebo [9] and a 24% RRR was found in a recent meta-analysis [11] and is more conservative than the 30% RRR in a Cochrane analysis [10]. Thus, we will enroll 1325 patients/group (2650 patients). Based on our pilot trial recruitment, we anticipate enrolling approximately 1.9 patients/month/site [17].

Central statistical monitoring

Thrice yearly throughout the trial, we will perform central statistical monitoring by analyzing site-specific data receipt and completeness, to help identify and overcome barriers to timely data completion. We will also monitor the proportion of non-screening weeks, and number and reasons for eligible non-randomized patients, to identify and remediate potential recruitment challenges.

We will monitor and report other types of protocol adherence [42]. We will track categories such as admissible protocol deviations for clinically justified reasons (e.g. strict nil per os status for possible bowel perforation) and logistical reasons (e.g. patient discharged early from the ICU so no evening dose given) as distinct from oversights which are protocol violations (e.g. dispensing errors). Thus, our protocol adherence regarding non-receipt of study product allows for sensible bedside decision-making, according to metrics from our prespecified taxonomy [43].

Statistical Analysis

Patients randomized in PROSPECT will be analyzed according to the intention-to-treat principle for the main analysis. We will present baseline characteristics of the 2 groups, including demographic and life support characteristics, and all prevalent infections. Infections will be defined as prevalent if present the day of, or diagnosed one day after randomization (the latter presumed to have started the day of randomization). For example, prevalent pneumonia could include any patient with pneumonia (community-acquired, healthcare-associated or ventilator-associated) present the day of or the day after randomization; this classification of pneumonia as prevalent relates only to timing of randomization and is independent of timing of intubation. Prevalent infections will not be considered outcomes for the trial because they are present at the time of randomization and are not plausibly modified by probiotics. All prevalent infections will also be centrally adjudicated by 1 physician blinded to allocation and center, based on review of data collected at each participating site. A CONSORT flow diagram will be generated, representing all randomized patients, their outcomes, the number and reasons for any consent withdrawals or loss to follow-up, as well as eligible non-randomized patients [44].

The main analysis will be a Cox proportional hazards analysis evaluating the primary outcome of VAP. This time-to-event analysis will use all information up to the time of censoring such that patients remain in the denominator and contribute information while they are at risk. The assumption for this analysis is that censoring is uninformative. The Cox model will be stratified by: a) center, and b) medical versus surgical versus trauma admission diagnosis, reflecting the stratification variables for randomization. The only independent variable will be randomized treatment group. We will present Kaplan-Meier curves for the primary outcome. We will also report VAP incidence rate, as number of VAP cases per 1,000 ventilator days [45]. We will report exposures during the ICU stay as is customary for critical care trials (e.g., advanced life supports) and cointerventions (e.g., pneumonia prevention strategies) relevant for this research question.

For the dichotomous secondary outcomes, we will also use time-to-event analyses. Hazard ratios and associated 95% confidential intervals will be estimated using a stratified Cox proportional hazards model.

For continuous outcomes, we will report estimates of the difference, 95% confidence intervals and associated p-values. For the continuous outcomes which are often skewed (e.g. duration of ventilation, ICU stay, and hospital stay), we will first log-transform these variables to see if they become normally distributed; if so, we will use parametric methods on the log-transformed variables to compare between groups. If not, we will compare the 2 groups using a nonparametric approach on the non-transformed variables. All secondary analyses will be adjusted for the stratification variables used at randomization (i.e., center and admission diagnostic category [medical, surgical, trauma]).

For the main analysis, when there is a statistically significant difference in binary outcomes, we will calculate other metrics. For example, depending on the results, these may be expressed as the number needed to prophylax (NNP) with probiotics to prevent 1 case of pneumonia, or the number needed to harm (NNH) to cause 1 case of iatrogenic infection with *L. rhamnosus* GG.

We do not anticipate missing any covariates for the primary outcome analysis – the only independent variable is treatment versus control, and the stratification variables are captured in the randomization system. We anticipate very little missing outcome data, since most data are collected in the ICU (except hospital vital status and length of stay, and *C. difficile* infection which is also recorded following ICU discharge in the hospital). For any other outcome that is missing for more than 2% of the patients, we will perform multiple imputation analysis [46, 47].

We will use graphics and other relevant methods to examine the residuals to assess model assumptions and goodness-of-fit including the proportional hazards assumption for Cox-regression analyses [48 - 50].

All estimates of effect will be reported to two decimal places. P-values will be reported to three decimal places with those less than 0.001 reported as $p < 0.001$. The criterion for statistical significance will be set at alpha = 0.05, using 2-sided tests, but adjusted appropriately for the 2 planned interim analyses (baseline characteristics, primary and secondary outcomes, adverse events and SAEs) using the Peto-Haybittle approach [51, 52]; the interim analyses will occur at one third and two thirds of total enrollment, performed when complete ICU data are available for 883 and 1766 patients, respectively. Two-sided tests will be used, with a fixed conservative $\alpha=0.001$ for the first and second interim analyses, and $\alpha=0.05$ for the final analysis [51, 52]. Secondary and subgroup analyses will not be adjusted for multiple analyses since these are

exploratory [53]. All analyses will be performed using the most up-to-date version of SAS (Cary, NC).

Following the publication of PROSPECT, the dataset will be used to design observational studies addressing additional hypothesis-driven questions (e.g. predictors of diarrhea, and ICU-acquired *C. difficile*). Access by other PROSPECT investigators will follow a submitted rationale, analysis plan and approval by relevant REBs in accordance with data sharing policies extant at the time of the request.

Sensitivity Analyses

We will conduct 4 sensitivity analyses. To the extent that these sensitivity analyses yield similar results to the main analysis, inferences about the primary outcome will be strengthened [54, 55].

1. In case the exact timing of the onset of VAP is uncertain, we will compare the proportion of patients with VAP in the 2 groups using the Mantel-Haenszel Chi square test, stratified by centre and medical versus surgical versus trauma. Thus, in this sensitivity analysis we will not use a time-to-event approach.
2. We will check for competing risks to address the problem that those who die can no longer develop VAP. We will analyze PROSPECT to explicitly account for death as a competing risk using the Fine and Gray proportional sub-distribution hazards model [56, 57]. This analysis will not assume that the censoring of deaths is uninformative; rather, it will assume that deaths could be informative. The rationale for this sensitivity analysis is to assess the robustness of the main findings [54].

3. We will conduct an efficacy analysis of each incident infection, and a composite of all incident infections, restricted to patients who received study product on ≥90% of study days. The rationale for this sensitivity analysis is to investigate the effect of probiotics under conditions of maximal exposure [58].
4. We will include all VAP events that occur after the day of randomization. The rationale for this sensitivity analysis is that pneumonia arising the day after randomization may be less likely to be influenced by study product exposure than pneumonia arising 2 or more days after initial study product exposure.

Subgroup Analyses

We will conduct 5 subgroup analyses based on baseline characteristics. These will evaluate whether these 5 baseline characteristics have an 'effect modification' when the effect of probiotics versus placebo on VAP is compared [59, 60]. Subgroup analyses will only be performed for the primary outcome.

1. We will conduct subgroup analyses among medical versus surgical versus trauma patients (the latter defined as patients cared for by a trauma service). We hypothesize that in medical patients, the treatment effect may be attenuated due to more risk factors for VAP that are non-modifiable when compared to surgical or trauma patients [1]. To perform this subgroup analysis, we will run the primary Cox regression analysis except that we will include medical versus surgical versus trauma as an independent variable instead of stratifying by it. We will also include the interaction term between medical versus surgical versus trauma and randomized treatment.

2. We will conduct subgroup analyses based on age (>75 years of age versus 65 – 75 years versus <65 years). Although little is known about the effects of probiotics in the elderly [61, 62], we hypothesize that if, overall, probiotics are associated with a lower rate of VAP than placebo, the treatment effect will be attenuated among older patients because immunosenescence renders their risk of infection less modifiable than younger patients. To perform this subgroup analysis, we will add age >75 versus 65 – 75 years versus <65 years as an independent variable as well as its interaction with randomized treatment group to the primary Cox regression model.
3. We will conduct subgroup analyses of the effect of probiotics on VAP after accounting for frailty, defined as a baseline Clinical Frailty Score of ≥5 out of 9 [63]. We hypothesize that if, overall, probiotics are associated with a lower rate of VAP than placebo, the treatment effect will be attenuated among patients who are frail, as their risk of infection may not be modifiable. To perform this subgroup analysis, we will add baseline Clinical Frailty Score of ≥5 as an independent variable as well as its interaction with randomized treatment group to the primary Cox regression model. We began measuring frailty in response to a Canadian research mandate [64], and did not start documenting frailty until 483 patients were enrolled. Thus, rather than imputing frailty status, we will restrict this subgroup analysis to patients enrolled thereafter.
4. We will conduct subgroup analyses among patients who received antibiotics for 2 days prior to randomization and the day of randomization versus patients who did not receive antibiotics for 2 days prior to, or the day of, randomization. We hypothesize that if, overall, probiotics are associated with a lower rate of VAP than placebo, the treatment effect will be attenuated in patients without recent antibiotic exposure when compared to

patients with antibiotic exposure. To perform this subgroup analysis, we will add antibiotic exposure prior to randomization defined as those receiving antibiotics for 2 days prior to randomization and the day of randomization as an independent variable as well as its interaction with randomized treatment group to the primary Cox regression model.

5. We will conduct subgroup analyses on patients with prevalent pneumonia versus no prevalent pneumonia. We hypothesize that if overall, probiotics are associated with a lower rate of VAP than placebo, the treatment effect will be attenuated among patients with pre-randomization pneumonia due to challenges interpreting whether the prevalent pneumonia has resolved prior to the development of another pneumonia event. To perform this subgroup analysis, we will add prevalent pneumonia as an independent variable as well as its interaction with randomized treatment group to the primary Cox regression model.

Steering Committee

The PROSPECT Steering Committee is responsible for overseeing the conduct of the trial, for upholding or modifying study procedures as needed, and addressing any challenges with protocol implementation. They advise as necessary on operational issues arising that are clinical, methodologic, biostatistical or ethical. The Steering Committee will review any proposed protocol amendment prior to dissemination of the revised protocol to participating centers by email correspondence, and shared on conference calls or webinars. As enrollment ensues, they share new emerging clinical, laboratory or epidemiology information that may impact on the trial. The Steering Committee has discussed and approved the interim statistical analysis plans

and final statistical analysis plan, and will assist with data interpretation, and abstract and manuscript preparation. The PROSPECT organizational chart is in Appendix 2.

Data Monitoring Committee

The PROSPECT Data Monitoring Committee (DMC) is independent from other persons involved in PROSPECT, and has the requisite expertise in randomized clinical trial design, epidemiology, biostatistics, warning guides/stopping rules, infectious diseases and critical care. The primary responsibilities of the DMC are to independently review reports prepared at the Methods Center regarding: 1) recruitment (center and patient) and screening, consent and coenrolment rates; 2) protocol procedures (randomization, stratification, protocol adherence including maintaining blinding); and 3) data tables for 2 blinded interim and final analyses. After each interim analysis, the DMC will recommend whether to continue, suspend or terminate enrollment.

The roles and responsibilities of the DMC are as outlined and approved in the PROSPECT DMC Charter [22], modeled on the Data Monitoring Committees: Lessons, Ethics, Statistics (DAMOCLES) Study Group charter [65].

Patient and Public Involvement

In PROSPECT, we are involving patients and their families in the following ways. First, before beginning PROSPECT, we ensured that patients and families were supportive of the use of probiotics in the ICU setting. We conducted a substudy nested within the PROSPECT Pilot [16, 17] whereby patient's substitute decision makers were interviewed at the time of enrollment to explore their comfort with probiotic use during critical illness [66]. In total, 103 SDMs

participated in 8 centers. We found no difference in characteristics of substitute decision makers who consented versus declined the PROSPECT Pilot for their critically ill loved one. Rather, the rationale for substitute decision maker consent was related to personal beliefs regarding possible benefits to the patient, as well as predictions of patient's wishes regarding this trial. These findings gave us confidence that patients and families were supportive of the trial. Second, in accordance with many local ICU research practices, patients who gain capacity after resolution of their critical illness are asked to agree to ongoing participation in the trial. We have no formal patient or single family advisor for PROSPECT. When PROSPECT results are available following trial completion, we will ensure the results are disseminated by having the academic message of each "in press" manuscript translated into press releases for the public. Possible target hospital media include newsletters, emails and intranet bulletins. High citizen awareness and public probiotic consumption predict strong media interest.

Ethical oversight

PROSPECT is approved by Health Canada [#9427-M1133-45C], the Research Ethics Boards (REBs) of all participating hospitals, and Public Health Ontario. The study is underway in accordance with Good Clinical Practices following the Tri-Council Guidelines [67] and in accordance with ethical principles of the Declaration of Helsinki [68]. Access to the database, study-related files and source documents for scientific or auditing purposes is possible during and after the trial for any PROSPECT Methods Center staff, Health Canada authorities, or REB representatives (local, provincial or central). Participants will not be identified by name, and confidentiality will be maintained unless otherwise regulated. Data will be retained for 25 years as per Health Canada.

The DMC will use conservative 'warning guides' for apparent benefit in PROSPECT; there are no stopping guides for futility.

Funding

PROSPECT is funded by peer-reviewed grants (Canadian Institutes of Health Research, Canadian Frailty Network, Physician Services Incorporated, Hamilton Academic Health Sciences Organization, and Academic Medical Organization of Southwestern Ontario), and funds from St. Joseph's Healthcare Hamilton and McMaster University. The study products are donated by the manufacturers of *L. rhamnosus* GG (i-Health, Inc.) which have no role in the trial conception, design, conduct, oversight analysis or write up.

Knowledge Dissemination

Results of the trial and secondary manuscripts will be communicated through conventional academic channels (e.g., abstracts, posters, peer-review manuscripts), at professional healthcare fora (e.g., grand rounds, teaching sessions, in-services, quality improvement councils), and via media (e.g., newspapers, radio, television, blogs, twitter etc.).

DISCUSSION

Probiotics may be a simple, cost-effective strategy to prevent VAP [69]. However, despite encouraging findings of efficacy, trials to date have been limited by insufficient power and risk of bias [9 - 11]. Studies of probiotics in the critical care setting have been criticized as difficult to interpret due to differences in populations and heterogeneous probiotics and combination products used [70]. Indeed, experts in the field have emphasized the need for well-powered studies of probiotics in the ICU setting [70]. To address this call, PROSPECT is a large,

international, rigorous multicenter randomized trial that aims to determine whether probiotics are effective, have no benefit, or are harmful in critical illness.

Additional strengths of PROSPECT include representation of persons greater than 65 years of age to enhance the generalizability of the findings, and separate peer-review funding for this population from the Canadian Frailty Network [64]. The efficacy of probiotics may be less in the elderly, as illustrated by a recent rigorous trial that found probiotics did not prevent *C. difficile* infection in persons ≥65 years admitted to hospital and receiving at least one antibiotic [71]. We are also documenting baseline pre-hospital frailty with the Clinical Frailty Score [72] to further understand the relationship between frailty, immunosenence and critical care-associated infections.

Given previous meta-analyses suggesting that probiotics may reduce all healthcare associated infections in the ICU [9], we are evaluating all infectious outcomes in PROSPECT. Also, a recent large trial of 2556 healthy newborns conducted in rural India showed that synbiotics (*Lactobacillus plantarum* plus fructooligosaccharide) decrease the risk of sepsis and lower respiratory tract infections within 60 days [73]. It is unknown whether the benefit was from the *L. plantarum* or the addition of fructooligosaccharide; however, these results suggest that modification of microbiota can reduce infections. Given the growing interest in the dysbiosis of critical illness, this trial will advance our understanding of whether microbiota modification with probiotics has any influence on infectious and non-infectious clinically important outcomes [74 - 76].

The International Conference for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use [ICH] E9 [77] and SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) [18] guidelines endorse a separate statistical analysis

plan for clinical trials. Recommendations [18] include more technical and detailed elaboration of the principal features of the analysis described in the protocol, including procedures for executing the statistical analysis of the primary and secondary variables and other data [77]. We followed these expert recommendations [19] for 55 items in 6 sections: Title and Trial Registration (11 items/subitems); Introduction (2 items); Study Methods (9 items/subitems); Statistical Principles (8 items/subitems); Trial Population (8 items/subitems); and Analysis (17 items/subitems). Dissemination of this document aligns with calls to make statistical analysis plans publicly available [78] to aid in the transparent reporting of trial results.

TRIAL STATUS

PROSPECT is supported by a longstanding research consortium (the Canadian Critical Care Trials Group) dedicated to investigator-initiated, peer-review funded studies designed to understand and improve the outcomes of critically ill patients [21]. Recruitment is ongoing, with 73% of the target sample size accrued as of May 2018. At the first interim analysis, the DMC made no suggestions to suspend enrollment. Randomization is anticipated to continue until approximately April 2019. Final data entry, data validation and outcome adjudication will ensue for 6-9 months thereafter, with an anticipated database lock by December 2019, followed by the terminal statistical analyses. PROSPECT results will inform global practice in critical care medicine.

Author Contributions:
Concept and design: J Johnstone, D Heels-Ansdell, L Thabane, D Cook
Acquisition, analysis, or interpretation of data: J Johnstone, D Heels-Ansdell, L Thabane, M Meade, J Marshall, F Lauzier, EH Duan, N Zytaruk, D Lamarche, M Surette, D Cook
Drafting of the manuscript: J Johnstone, D Heels-Ansdell, L Thabane, D Cook
Critical revision of the manuscript for important intellectual content: everyone
Statistical analysis: D Heels-Ansdell (Trial Biostatistician), L Thabane (Senior Biostatistician), D Lamarche, M Surette
Obtained funding: everyone
Administrative, technical, or material support: D Heels-Ansdell, E Duan, N Zytaruk, L Thabane, D Lamarche, M Surette
Drs. J Johnstone and D Cook as Co-Principal Investigators take responsibility for the integrity of the data

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REFERENCES

1. Safdar N, Dezfulian C, Collard H, Saint S. Clinical and economic consequences of ventilator-associated pneumonia: A systematic review. Crit Care Med 2005; 33: 2184.
2. Muscedere J, Dodek P, Keenan S, et al. Comprehensive evidence-based clinical practice guidelines for ventilator-associated pneumonia: Prevention. J Crit Care 2008; 23: 126.
3. http://www.jointcommission.org. Accessed January 11, 2018.
4. Sinuff T, Muscedere J, Cook D, et al. The active observational study of the adoption and transfer of clinical practice guidelines through education, for ventilator-associated pneumonia study (ABATE VAP). Crit Care Med 2013; 41: 15.
5. Marshall J. The gastrointestinal flora and its alterations in critical illness. Curr Opin Crit Care 1999; 5: 119.
6. Stappenback T, Hooper L, Gordon J. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proc Natl Acad Sci USA 2002; 99: 15451.
7. Tanoue T, Honda K. Induction of Treg cells in the mouse colonic mucosa: a central mechanism to maintain host-microbiota homeostasis. Semin Immunol 2012; 24: 50.
8. Brenchley J, Douek D. Microbial translocation across the gastrointestinal tract. Annu Rev Immunol 2012; 30: 149.
9. Petrof E, Dhaliwal R, Manzanares W, et al. Probiotics in the critically ill. Crit Care Med 2012; 40: 3290.
10. Bo L, Li J, Tao T, et al. Probiotics for preventing ventilator-associated pneumonia. Cochrane Database of Syst Rev 2014; 10: CD009066.
11. Manzanares W, Lemieux M, Langlois PL, Wischmeyer PE. Probiotic and synbiotic therapy in critical illness: a systematic review and meta-analysis. Crit Care. 2016; 19: 262.
12. Nuesch E, Trelle S, Reichenbach S, et al. Small study effects in meta-analyses of osteoarthritis trials: meta-epidemiological study. BMJ 2010; 341: c3515.
13. Dechartres A, Boutron I, Trinquart L, et al. Single-center trials show larger treatment effects than multicenter trials. Ann Intern Med 2011; 155: 39.
14. Weng H, Li JG, Mao Z, et al. Probiotics for preventing ventilator-associated pneumonia in mechanically ventilated patients: A meta-analysis with trial sequential analysis. Front Pharmacol 2017; 8: 717.
15. Morrow L, Kollef M, Casale T. Probiotic prophylaxis of ventilator-associated pneumonia. Am J Respir Crit Care Med 2010; 182: 1058.
16. Johnstone J, Meade M, Marshall J, et al. PROSPECT: Protocol for a feasibility randomized pilot trial. Pilot and Feasibility Studies 2015; 1: 19.
17. Cook D, Johnstone J, Marshall J, et al. Probiotics: Prevention of Severe Pneumonia and Endotracheal Colonization Trial - PROSPECT: A Pilot Trial. Trials 2016; 17: 377.
18. Chan A, Tetzlaff J, Altman D, et al. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med 2013; 158: 200 - 207.
19. Gamble C, Krishan A, Stocken D, et al. Guidelines for the content of statistical analysis plans in clinical trials. JAMA 2017; 318: 2337 – 2343.
20. Duan EH, Johnstone J, Meade MO, et al. Refining exclusion criteria following pilot trials: Lessons from PROSPECT. Canadian Critical Care Forum 2016, Toronto, ON, October 2016 Can Crit Care Forum Abstract Book 2016.

21. Marshall JC, Cook DJ for the Canadian Critical Care Trials Group. Investigator-led clinical research consortia: The Canadian Critical Care Trials Group. Crit Care Med 2009; 36:S165-S172.
22. PROSPECT. www.prospecttrial.com Accessed May 14 2018.
23. Smith O, McDonald E, Zytaruk N, et al. Enhancing the informed consent process for critical care research: strategies from a thromboprophylaxis Trial. Intensive and Critical Care Nursing 2013; doi.org/10.1016/j.iccn.2013.04.006.
24. Lamarche D, Rossi L, Shah M, et al. Quality control in the conduct of a probiotic randomized trial. Can Crit Care Forum, Toronto, ON, October 28, 2014.
25. Grossman R, Fein A. Evidence-based assessment of diagnostic tests for ventilator-associated pneumonia. Chest 2000; 117(4 Suppl 2): 177S – 181S.
26. Muscedere J, Sinuff T, Heyland D, et al. The clinical impact and preventability of ventilator-associated conditions in critically ill patients who are mechanically ventilated. Chest 2013; 144: 1453.
27. Canadian Critical Care Trials Group. A randomized trial of diagnostic techniques and empiric broad-spectrum antibiotics for suspected ventilator-associated pneumonia. N Engl J Med 2006; 355(25):2619-2630
28. Heyland D, Muscedere J, Wischmeyer P, et al. A randomized trial of glutamine and antioxidants in critically ill patients. N Engl J Med 2013; 368: 1489.
29. Calandra T, Cohen J for the International Sepsis Forum Definition of Infection in the ICU Consensus Conference. Crit Care Med 2005; 7: 1538.
30. Pugin J, Auckenthaler R, Mill N, et al. Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic blind bronchoalveolar lavage fluid. Am Rev Respir Dis 1991; 143: 1121.
31. Horan T, Andrus M, Dudeck M. CDC/NHSN surveillance definition of healthcare associated infections and criteria for specific types of infections in the acute care setting. Am J Infect Control 2008;36:309.
32. Chastre J, Wolff M, Fagon J, et al. Comparison of 8 vs 15 days of antibiotic therapy for ventilator-associated pneumonia in adults: a randomized trial. JAMA 2003; 290: 2588 – 2598.
33. Chastre J and Fagon J. Ventilator-associated pneumonia. Am J Respir Crit Care Med 2002; 165: 867–903
34. Cohen S, Gerding D, Johnson S, et al. Clinical practice guidelines for C. difficile infection in adults: 2010. Infect Control Hosp Epidemiol 2010; 31: 431.
35. World Health Organization. http://www.who.int/topics/diarrhoea/en/ Accessed May 14 2018.
36. Heaton K, Lewis S. Stool form scale as a useful guide to intestinal transit time. Scan J Gastroenterol 1997; 32: 920.
37. Thibault R, Graf S Clerc A, et al. Diarrhea in the intensive care unit. Crit Care 2013; 17: R153.
38. Polk R, Fox C, Mahoney A et al. Measurement of adult antibacterial drug use in 130 US hospitals: comparison of defined daily dose and days of therapy. Clin Infect Dis 2007; 44: 664-70.
39. Kuster S, Ruef C, Ledergerber B et al. Quantitative antibiotic use in hospitals: comparison of measurements, literature review, and recommendations for a standard of reporting. Infection 2008; 36:549-59.

40. The Guidance Document for Industry – Reporting Adverse Reactions to Marketed Health Products: www.hc-sc.gc.ca/dhp-mps/pubs/medeff/_guide/2011-guidance-directrice_reporting-notification/index-eng.php
41. Cook D, Lauzier F, Rocha M, et al. Serious adverse events in academic critical care research. CMAJ 2008; 178: 1181.
42. Bhatt A. Protocol deviation and violation. Perspect Clin Res. 2012; 3: 117.
43. Lauzier F, Adhikari N, Seely A, et al. Protocol adherence for continuously titrated interventions in randomized trials: An overview of the current methodology and case study. BMC Medical Research Methodology 2017; 17: 106.
44. Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. Trials 2010, 11: 32.
45. National Healthcare Safety Network. https://www.cdc.gov/nhsn/pdfs/pscmanual/6pscvapcurrent.pdf Accessed May 14 2018.
46. Little R, Rubin D: Statistical analysis with missing data. 2nd edition. Hoboken, NJ: Wiley; 2002; Rubin DB: Multiple imputation for nonresponse in surveys. New York: J Wiley & Sons; 1987.
47. Zhang Y, Alyass A, Vanniyasingam T, et al. A systematic survey of the methods literature on the reporting quality and optimal methods of handling participants with missing outcome data for continuous outcomes in randomized controlled trials. J Clin Epidemiol 2017; 88: 67-80.
48. Schoenfeld D: Partial residuals for the proportional hazards regression model. Biometrika 1982, 69:239–241.
49. Wei LJ: Testing goodness of fit for proportional hazards model with censored observations. J Am Stat Assoc 1984, 79:649–652.
50. Harrell FE, Lee KL: Verifying Assumptions of the Cox Proportional Hazards Model. Proceedings of the Eleventh Annual. SAS User's Group International Conference. Cary, N.C.: SAS Institute, Inc.; 1986:823–828.
51. Haybittle, JL. Repeated assessment of results in clinical trials of cancer treatment. British J Radiology 1971; 44: 793.
52. Peto R, Pike M, Armitage P, et al. Design and analysis of randomized control trials requiring prolonged observations of each patient. British J Cancer 1976; 34: 585.
53. Li G, Taljaard M, Van den Heuvel E, et al. An introduction to multiplicity issues in clinical trials: the what, why, when and how. International Journal of Epidemiology, 2017, 746–755.
54. Thabane L, Mbuagbaw L, Zhang S, et al. A tutorial on sensitivity analyses in clinical trials: the what, why, when and how. BMC Med Res Methodol 2013, 13(1):92.
55. de Souza RJ, Eisen R, Perera S, et al. Best (but oft-forgotten) practices: sensitivity analyses in randomized controlled trials. Am J Clin Nutr 2016; 103: 5-17.
56. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. Journal of the American Statistical Association 1999;94(446):496-509.
57. Bakoyannis G, Touloumi G. Practical methods for competing risks data: A review. Statistical Methods in Medical Research 2012;21(3):257-272.
58. Cramer JA, Roy A, Burrell A, et al. Medication compliance and persistence: terminology and definitions. Value Health. 2008; 11: 44–7.
59. Rothwell P. Subgroup analysis in randomised controlled trials: importance, indications, and interpretation. Lancet 2005; 365: 176–186.

60. Sun X, Briel M, Walter SD, Guyatt GH. Is a subgroup effect believable? Updating criteria to evaluate the credibility of subgroup analyses. BMJ 2010; 340: 85-854.
61. Patel PJ, Singh SK, Panaich S, Cardozo L. The aging gut and the role of prebiotics, probiotics, and synbiotics: a review. J Clin Gerontol Geriatr 2014;5:3e6.
62. Wachholz PA, P Boas. Evidence on the role of prebiotics, probiotics, and synbiotics in gut health and disease prevention in the elderly. J Clin Gerontol Geriatr 2014; 5: 1-2.
63. Bagshaw S, Stelfox T, McDermid R, et al. Association between frailty and short- and long-term outcomes among critically ill patients: a multicentre prospective cohort study. CMAJ 2013; DOI:10.1503/cmaj.130639.
64. Canadian Frailty Network. http://www.cfn-nce.ca/ Accessed May 14, 2018.
65. DAMOCLES Study Group. A proposed charter for clinical trials data monitoring committees: helping them to do their job well. Lancet 2005; 365: 711-722.
66. Shears M, Clarke F, Mehta S, et al. Inter-Prob:INTERviewing substitute decision makers about PROBiotics. Can Crit Care Forum, Toronto, ON, October 25-28, 2015.
67. Grimes D, Hubacher D, Nanda K, et al. The Good Clinical Practice guideline: a bronze standard for clinical research. Lancet 2005; 366: 172–74
68. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013; 310(20):2191-4.
69. Branch-Elliman W, Wright S, Howell M. Determining the ideal strategy for ventilator-associated pneumonia prevention. Am J Respir Crit Care Med 2015; 192: 57.
70. Crooks NH, Snaith C, Webster D, et al. Clinical review: Probiotics in critical care. Critical Care 2012, 16: 237.
71. Allen S, Wareham K, Wang D, et al. Lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhoea and Clostridium difficile diarrhoea in older inpatients (PLACIDE): a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 2013; 382: 1249–1257.
72. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly people. CMAJ 2005; 173: 489–495.
73. Panigrahi P, Parida S, Nanda N, et al. A randomized synbiotic trial to prevent sepsis among infants in rural India. Nature 2017; 548: 407–412.
74. McDonald D, Ackermann G, Khailova L et al. Extreme dysbiosis of the microbiome in critical illness. mSphere 2016; 1: e00199-16.
75. Relman D. The human microbiome and the future practice of medicine. JAMA 2015; 314: 1127-8.
76. Lamarche D, Johnstone J, Zytaruk N, et al. Microbial dysbiosis and mortality during mechanical ventilation: a prospective observational study. Respir Res 2018; 19:245.
77. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline: Statistical Principles for Clinical Trials E9. London, England: European Medicines Agency; 1998.
78. Finfer S, Bellomo R. Why publish statistical analysis plans? Crit Care Resusc 2009; 11: 5-6.

Table 1: Inclusion and Exclusion Criteria

Inclusion criteria
1. Adults ≥18 years of age admitted to a medical, surgical or trauma ICU 2. Receiving invasive mechanical ventilation, estimated to be required for ≥72 hours
Exclusion criteria
1. Invasively mechanically ventilated >72 hours at the time of screening 2. Potential increased risk of iatrogenic probiotic infection including specific immunocompromised groups: HIV <200 CD4 cells/μL, chronic immunosuppressive medications, previous transplantation at any time, chemotherapy in the last 3 months, absolute neutrophil count <500. Previous or current corticosteroids use is not exclusionary 3. Risk for endovascular infection: rheumatic heart disease, congenital valve disease, surgically repaired congenital heart disease, unrepaired cyanotic congenital heart disease, valvular replacement (mechanical or bio-prosthetic), previous or current endocarditis, permanent endovascular devices (e.g., endovascular grafts, inferior vena cava filters, dialysis vascular grafts), tunneled hemodialysis catheters, pacemakers or defibrillators. These are not exclusions: coronary artery stents or bypass grafts, mitral valve prolapse, bicuspid aortic valve, temporary catheters (central venous, peripherally inserted, extra-corporeal life support-related) or neurovascular coils 4. Primary diagnosis of severe acute pancreatitis 5. Percutaneously inserted feeding tubes in situ, as per Health Canada 6. Strict contraindications or inability to receive enteral medications 7. Intent to withdraw advanced life support 8. Previous enrollment in this trial, or current enrollment in a potentially confounding trial

*Changes from the PROSPECT pilot are as follows: 1. Omitted radiation therapy as an exclusion criterion; 2. Omitted steroid exposure as an exclusion criterion; 3. Better defined transplant to explicitly exclude all transplant patients (autologous stem cell patients are now excluded); 4. Better defined the cardiac valvular diseases at risk; 5. Removed surgery of esophagus/stomach/small bowel as exclusion criteria and replaced with any strict contraindication or inability to receive enteral medications; 6. Replaced severe acute pancreatitis with organ dysfunction with primary diagnosis of severe acute pancreatitis; 7. Omitted pregnancy as exclusion criterion [20].

Table 2: Determination of the sample size: based on an estimated 15% VAP rate, 2650 patients (n=1325 in each arm) will be required to detect a 25% relative risk reduction with 80% power.

***Per Group* Sample Size for 80% power and alpha=0.05, using continuity correction.**

	RRR				
Baseline Risk	**10%**	**15%**	**20%**	**25%**	**30%**
8%	17473	7635	4221	2653	1809
9%	15374	6720	3716	2337	1594
10%	13695	5988	3313	2084	1422
12%	11176	4891	2707	1704	1164
14%	9377	4107	2275	1433	979
15%	8657	3793	2102	**1325**	906
16%	8028	3519	1951	1230	841
18%	6978	3061	1699	1072	734
20%	6139	2695	1497	945	647
22%	5452	2396	1332	842	577
24%	4879	2147	1194	756	518
25%	4627	2037	1134	718	493
30%	3620	1598	892	566	389
35%	2900	1284	719	458	316
40%	2361	1049	589	376	260
50%	1605	719	408	262	183

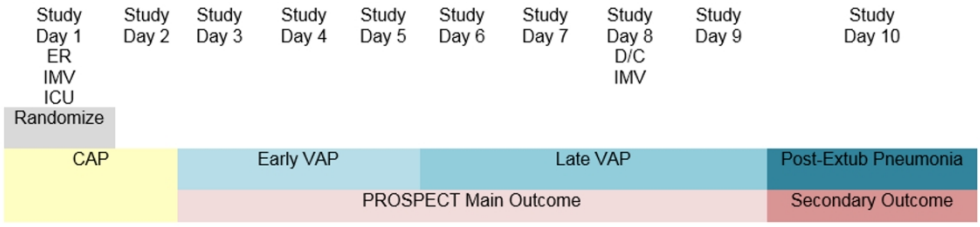
Figure 1 and 2: Pneumonia Classification

Legend for Figure 1 and 2: These figures illustrate the pneumonia classification that we are using, according to when the lung infection develops in a patient's hospital trajectory. The different classifications over time in each example relate to the day of hospital admission, day of ICU admission, day of initiation of mechanical ventilation (via endotracheal intubation or tracheostomy), day of randomization in the trial, and day of discontinuation of mechanical ventilation. Note that the pneumonia classifications over time do not reflect persistent or progressive lung infections, but rather the pneumonia classification that would be ascribed if a new infection develops on each day shown.

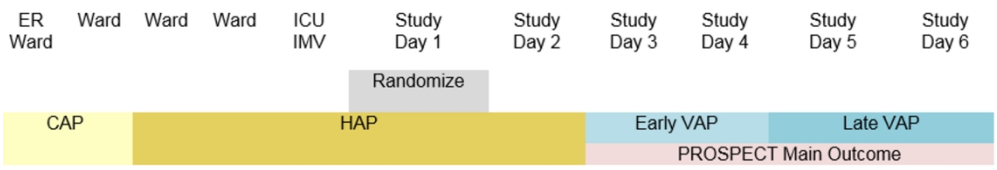
Abbreviations: ER – emergency room; Extub – Extubation; IMV – invasive mechanical ventilation; ICU – intensive care unit; ICUAP – intensive care unit associated pneumonia; Rand – randomization; CAP – community-acquired pneumonia; VAP – ventilator-associated pneumonia; HAP – hospital-acquired pneumonia;

Figure 1: Pneumonia classifications that could arise in patients who require ICU admission and invasive mechanical ventilation at the time of presentation to the emergency room, and are randomized into PROSPECT that day. The primary outcome is adjudicated VAP (any, including early or late), arising on study day 3 or later. Secondary outcomes illustrated include early VAP, late VAP and post-extubation pneumonia.

Figure 2: Pneumonia classifications that could arise in patients who require ICU admission and invasive mechanical ventilation after an initial hospital stay, and are randomized into PROSPECT the day following ICU admission. The primary outcome is adjudicated VAP (any, including early or late), arising on study day 3 or later.



362x90mm (300 x 300 DPI)



165x28mm (300 x 300 DPI)

Appendix 1: Objectives, Outcomes, Hypothesis and Methods of Analysis

Objectives	Outcomes	Hypothesis	Methods of Analysis
1) **Primary:** To compare the effects of *Lactobacillus rhamnosus* GG versus placebo on:	Ventilator-associated pneumonia (VAP)	*Lactobacillus rhamnosus* GG will reduce the risk of the primary outcome	Cox proportional hazards
2) **Secondary:** To compare the effects of *Lactobacillus rhamnosus* GG versus placebo on:	a) Early VAP, late VAP, post-ventilation ICU-acquired pneumonia, and a composite of all three.	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	b) Clostridium difficile	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	c) Any infection acquired during the ICU stay	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	d) Diarrhea in the ICU	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	e) Antibiotic-associated diarrhea	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	f) Antimicrobial use	*Lactobacillus rhamnosus* GG will reduce the risk	Independent samples paired t-test or Wilcoxon rank sum test
	g) Duration of mechanical ventilation, ICU stay and hospital stay	*Lactobacillus rhamnosus* GG will have no effect	Independent samples paired t-test or Wilcoxon rank sum test
	h) ICU mortality and hospital mortality	*Lactobacillus rhamnosus* GG will have no effect	Cox proportional hazards
	i) Serious adverse events	*Lactobacillus rhamnosus* GG will have no effect	Cox proportional hazards
3) **Sensitivity Analyses:**			
i. Compare proportion of patients with VAP in the two groups.	VAP	Results remain robust	i. Mantel-Haenszel Chi square test ii. Competing risk

ii. Check for competing risk of death				analysis
iii. Efficacy analysis				ii. Cox proportional hazards
iv. Include all VAP events that occur after the day of randomization				iii. Cox proportional hazards
4) **Subgroup Analyses:**				Cox proportional hazards with interaction test between each subgroup variable and treatment group evaluating the credibility of subgroup findings using our 11 previously published criteria [60]
i. Medical vs surgical vs trauma patients		VAP	Treatment effects may be attenuated in one group compared to another group (see text for details)	
ii. >75 years vs 65 – 75 years vs <65 years old				
iii. Frail patients versus not frail patients				
iv. Antibiotics prior to randomization vs no antibiotics prior to randomization				
v. Pneumonia at baseline vs no pneumonia				

Legend for Appendix 1: In all analyses, results will be expressed as estimate of effect, corresponding 95% and associated p-values. All tests will be two-sided using alpha = 0.05 level of significance in accordance with a superiority hypothesis.

Appendix 2: PROSPECT Organizational Chart

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym p.1.
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry p.3 (Abstract), p5. (Intro).
	2b	All items from the World Health Organization Trial Registration Data Set N/A (it is on the Registry website)
Protocol version	3	Date and version identifier p.5 (Intro).
Funding	4	Sources and types of financial, material, and other support p20. (Methods
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors p1, 24
	5b	Name and contact information for the trial sponsor p.1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities p20 (Method
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) p18+19 (Me
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention p4+5
	6b	Explanation for choice of comparators p4+5
Objectives	7	Specific objectives or hypotheses p4+5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) p5+6

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained p5+6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) p5+6 + Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered p6+7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) p.7.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) p.11.
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial p. 13.
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended p. 8 - 10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) Table 1, Figure 1+2
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations p. 11.
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size p.5.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions p. 6.

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned p. 6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions p. 6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how p. 6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial p. 6
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol p. 7+8, 11+12
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols p. 14 (very little missing da
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol p. 7, 8, 11, 12
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol p12 - 15
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) p. 15 - 18
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) p. 12, 14
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed p. 18

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial p. 14., 19
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct p10+11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor p11+12
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval p20.
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) p. 18
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) p.6.
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable N IA.
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial p.20., 7+8
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site p24
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators p14+15.
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation N/A.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions p21.
	31b	Authorship eligibility guidelines and any intended use of professional writers p24 (no use of a medical writer).
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code N/A.

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates p 6.
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable N/A.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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Evaluating Probiotics for the Prevention of Ventilator Associated Pneumonia: A Randomized Placebo Controlled Multicenter Trial Protocol and Statistical Analysis Plan for PROSPECT

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Evaluating Probiotics for the Prevention of Ventilator Associated Pneumonia:

A Randomized Placebo Controlled Multicenter Trial Protocol and Statistical Analysis Plan for PROSPECT

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STRENGTHS AND LIMITATIONS OF THIS STUDY

- Randomized placebo controlled multicenter trial
- Evaluation of the effect of probiotics on pneumonia, other intensive care unit (ICU)-acquired infections and diarrhea in a large, adequately powered trial
- International enrolment including patients over 65 years of age to enhance the generalizability of the findings
- Characterization of pre-hospital frailty to help understand the relationship between frailty, probiotics and ICU-acquired infections
- Severely immunocompromised patients are excluded for safety reasons

ABSTRACT

Introduction: Ventilator-associated pneumonia (VAP) is the most common healthcare-associated infection in critically ill patients. Prior studies suggest that probiotics may reduce VAP and other infections in critically ill patients; however, most previous randomized trials were small, single center studies. PROSPECT (the Probiotics: Prevention of Severe Pneumonia and Endotracheal Colonization Trial) aims to determine the impact of the probiotic *Lactobacillus rhamnosus* GG on VAP and other clinically important outcomes in critically ill adults.

Methods: PROSPECT is a multicenter, concealed, randomized, stratified, blinded, controlled trial in patients ≥18 years old, anticipated to be mechanically ventilated ≥ 72 hours, in intensive care units (ICUs) in Canada, the United States and Saudi Arabia. Patients receive either 1 x 10^{10} colony forming units of *L. rhamnosus* GG twice daily or an identical appearing placebo. Those at increased risk of probiotic infection are excluded. The primary outcome is VAP. Secondary outcomes are other ICU-acquired infections including *Clostridioides difficile* infection, diarrhea (including antibiotic-associated diarrhea), antimicrobial use, ICU and hospital length of stay and mortality. The planned sample size of 2650 patients is based on an estimated 15% VAP rate and will provide 80% power to detect a 25% relative risk reduction.

Ethics and Dissemination: This protocol and statistical analysis plan outlines the methodology, primary and secondary analyses, sensitivity analyses and subgroup analyses. PROSPECT is approved by Health Canada [#9427-M1133-45C], the Research Ethics Boards (REBs) of all participating hospitals, and Public Health Ontario. Results will be disseminated via academic channels (peer reviewed journal publications, professional healthcare fora including international conferences) and conventional and social media. The results of PROSPECT will inform practice guidelines worldwide.

Clinical Trial Registration: www.clinicaltrials.gov NCT02462590

Keywords: Critically ill; Infection; Intensive Care; Probiotics; Ventilator-associated pneumonia

INTRODUCTION

Ventilator-associated pneumonia (VAP) is the most common healthcare-associated infection in critically ill patients, and is associated with a significant burden of disease [1]. In a systematic review, the pooled incidence of VAP in patients mechanically ventilated for >48 hours ranged from 10-23%, and VAP conferred a 2-fold attributable-risk of dying in the intensive care unit (ICU), with an attributable cost ranging from USD$10,000-$13,000 per patient [1]. Therefore, preventing VAP is a patient safety priority [2, 3].

Unfortunately, VAP prevention strategies are variably applied in practice [4], which underscores the need for simple, safe, effective and affordable VAP reduction strategies. Probiotics may represent one such novel approach. Probiotics have emerged as a biologically plausible strategy to prevent VAP, through influencing microbiota, enhancing gut barrier function, and reducing pathogenic bacterial load [5-8]. Systematic reviews suggest that probiotics reduce VAP by 25% - 30% when compared to placebo [9-11]. However, most previous randomized trials were small, single center studies. Meta-analyses of small single center trials often yield implausibly large treatment effects [12, 13]. Hence, the clinical benefits of probiotics may be overestimated, and a large, well-powered multicenter trial is needed.

In a recent trial sequential meta-analysis of randomized trials testing the effect of probiotics on VAP during critical illness, 11 of 13 included trials evaluated a *Lactobacillus* species alone or in combination, and 2 of these trials used *Lactobacillus rhamnosus* GG [14], including the most rigorous trial by Morrow *et al* [15]. This high quality trial compared *L. rhamnosus* GG to corresponding placebos in 146 patients and the patients treated with *L. rhamnosus* GG had lower rates of VAP suggesting that *L. rhamnosus* GG, specifically, is a promising probiotic to prevent VAP in a selected high-risk ICU population [15].

We recently completed the PROSPECT pilot [www.clinicaltrials.gov NCT01782755][16] in 14 ICUs which compared *L. rhamnosus* GG to placebo in critically ill mechanically ventilated patients. The feasibility objectives of the pilot trial were related to 1) Recruitment: at least 2 patients per ICU per month; 2) Maximal protocol adherence: ≥90% of prescribed doses are actually administered; 3) Minimal contamination: <5% of patients receive a single dose of open-label probiotics and 4) Outcome incidence: at least 10% of enrolled patients developed VAP. The pilot trial met all 4 feasibility outcomes: 1) 150 patients were enrolled over 11 months, with 1.9 patients per ICU per month; 2) Adherence to study product was 97.4%; only 2.6% of doses prescribed were not received; 3) Contamination did not occur; no patients received a dose of open-label probiotic at any time; and 4) The adjudicated VAP rate was 19% [17]. Therefore we launched PROSPECT - a multicenter randomized concealed stratified blinded parallel-group placebo-controlled superiority trial to determine whether the probiotic *L. rhamnosus* GG compared to placebo reduces VAP and other clinically important outcomes in critically ill mechanically ventilated patients [www.clinicaltrials.gov NCT02462590]. In this paper we summarize the protocol [REB-approved version, version 1.0, date: February 27, 2015] and statistical analysis plan [version 2.0, date May 17 2018] for PROSPECT's primary analysis, reported using both the SPIRIT guidelines which define standard protocol items for clinical trials [18] and recent statistical analysis plan guidelines [19].

METHODS

Trial Population and Eligibility:

Patients will be recruited from 44 ICUs in Canada, the United States and Saudi Arabia (detailed list of study sites available [www.clinicaltrials.gov NCT02462590]). The inclusion and

exclusion criteria are presented in Table 1. Following completion of the PROSPECT pilot [16, 17], the exclusion criteria were refined, informed by an extensive literature review focused on the safety or harm of *Lactobacillus* spp. probiotic administration [20], experience with probiotics in the pilot trial [17], and following discussions with the PROSPECT Steering Committee and the Canadian Critical Care Trials Group [21](Table 1 footnote for details of changes).

Consent and Randomization

Research Coordinators screen all mechanically ventilated patients for potential trial enrolment, recording those that meet individual inclusion and exclusion criteria. Once eligibility is confirmed, *a priori* written informed consent or deferred consent is obtained from the patient or substitute decision maker as per our consent guidelines [22, 23], and according to local ethics approval. The patients are allocated to treatment in a 1:1 ratio via a computer-based random number generator in variable unspecified block sizes, stratified by center and by medical, surgical or trauma admission status.

Blinding

Patients, bedside clinicians, investigators, and research coordinators are blinded to allocation. Study pharmacists at each center are not blinded; they randomize patients and prepare study product for administration without being involved in the day-to-day bedside care of patients. The biostatisticians will remain blinded until the main analysis is complete. Unblinding will not be permissible throughout the trial.

Interventions and Comparator:

Patients in the intervention group receive $1x10^{10}$ colony forming units of *L. rhamnosus* GG (i-Health, Inc.) in 1 capsule suspended in tap water or sterile water (dependant on local practices), administered through a nasogastric or orogastric feeding tube. Patients in the placebo group receive an identical capsule containing microcrystalline cellulose. The same dose of microcrystalline cellulose is present in the *L. rhamnosus* GG capsules. Patients receive study product post randomization until: 1) ICU discharge or death; or 2) 60 days in the ICU; or 3) isolation of *Lactobacillus* spp. in a culture from a sterile site or if it is the sole or predominant organism in a culture from a non-sterile site.

The intervention is packaged in blister-cards of 10 capsules. For quality assurance purposes, we are performing an independent quality assessment of the study product supplied throughout the trial [24]. One randomly selected capsule from every 10th card of both probiotic and placebo is cultured in the Surette Microbiome Laboratory at McMaster University (Hamilton, Ontario), to ensure the dose and integrity of both the study product and placebo, as successfully done in the pilot trial [16, 17].

Data Collection

Research Coordinators collect data at baseline (e.g. demographics, illness severity, life support using the Acute Physiology and Chronic Health Evaluation [APACHE] II score), and daily (e.g. study product administration, VAP prevention strategies and other cointerventions), and all primary and secondary outcomes (Appendix 1) by completing data collection forms [22] and uploading to a secure web-based electronic data capture system (iDataFax, Seattle, Washington). To protect the personal health information of patients enrolled, all identifying information will be de-linked. Participants will be assigned a unique identification code (study

ID). The code-breaking information will be kept separate from the data extraction files. It will be the responsibility of the site investigators to ensure that the code-breaking information is totally inaccessible to individuals who are not on the research team. Personal health information about enrolled participants will include age, sex and admitting diagnosis, but will be de-identified at the recruiting center and anonymized in the main database over the course of the trial and thereafter.

Outcomes

Primary outcome

The primary outcome is adjudicated VAP. Clinically suspected VAP at participating sites is being centrally adjudicated independently and in duplicate by 2 physicians blinded to allocation and center, informed by the following standardized definition: receiving invasive mechanical ventilation for $\geq$ 2 days, when there is a new, progressive or persistent radiographic infiltrate on chest radiograph plus any 2 of the following: 1) fever (temperature >38 °C) or hypothermia (temperature <36 °C); 2) relative leukopenia (<3.0 x 10^6/L) or leukocytosis (>10 x 10^6/L) and 3) purulent sputum [25]. As the American College of Chest Physicians (ACCP) definition did not provide thresholds for leukopenia or leukocytosis, the thresholds were obtained from Morrow *et al* [15] as their VAP definition was also based on the ACCP definition [25]. Any disagreement in adjudication will be resolved through discussion and consensus. Acknowledging that there is no universally accepted gold standard VAP definition [26], and that in non-immunocompromised patients, routine invasive testing is not associated with improved outcomes [27], we are also collecting data to allow VAP reporting according to several other definitions [28 - 31].

Secondary Outcomes

a) Early VAP, late VAP, and post-extubation pneumonia: We are classifying VAP by early VAP and late VAP, as the etiologic organisms may differ, the antimicrobials prescribed may differ, and the prognosis is often worse for late VAP [32, 33]. Early VAP is defined as pneumonia arising on day 3, 4 or 5 after the initiation of mechanical ventilation. Late VAP is defined as VAP arising on day 6 of mechanical ventilation or later, and including up to 2 days after discontinuation of mechanical ventilation (also relevant for patients with a tracheostomy). We are also recording pneumonia arising in the ICU following discontinuation of mechanical ventilation (3 or more days after discontinuation), labelled post-extubation pneumonia, to avoid suppressing potentially relevant lung infections that arise in ICU (Figure 1 and 2). We will also report a composite outcome of early VAP, late VAP, and post-extubation pneumonia, adjudicated independently and in duplicate by 2 physicians. For the timing of all pneumonia outcomes, we use days rather than hours to inform the classification.

b) *Clostridioides difficile* in the ICU and prior to discharge from hospital: diarrhea (as defined in [d]) and laboratory confirmation of *C. difficile* or colonoscopic or histopathologic findings demonstrating pseudomembranous colitis [34], which will also be adjudicated independently and in duplicate by 2 physicians.

c) Any infection acquired during the ICU stay, including bloodstream infection, intravascular catheter-related bloodstream infection, intra-abdominal infection, *C. difficile* infection, urinary tract infection, skin and soft tissue infection, and others. These individual infections are classified using definitions adapted from the International Sepsis

Forum Consensus Conference on Definitions of Infection in the Intensive Care Unit [29], as adapted in prior studies [28]. We will also report a composite outcome of any infections (including pneumonia) acquired during the ICU stay. Secondary infectious outcomes (other than pneumonia and *C. difficile*) are being centrally adjudicated by 1 physician blinded to allocation and center, based on review of data collected at each participating site.

d) Diarrhea in the ICU: We will record each bowel movement and define diarrhea incorporating 2 metrics; the World Health Organization definition (≥3 loose or watery bowel movements per day [35]), and the Bristol Stool classification for loose or watery stool (type 6 or 7)[36].

e) Antibiotic-associated diarrhea in the ICU: diarrhea (as defined above in [d]) following the administration of antibiotics, any day antibiotics are administered or within 1 day after starting any antibiotic [37].

f) Antimicrobial use in ICU: defined as daily doses of therapy (DOT), defined daily dose (DDD) and antimicrobial-free days [38, 39]. Only systemic antimicrobials will be captured (e.g. parenteral, intravenous, oral, enteral) whether prophylactic or therapeutic in intent. Topical creams, eye/ear drops and inhaled antimicrobials will be excluded.

g) Duration: mechanical ventilation, ICU stay and hospital stay.

h) ICU mortality and in-hospital mortality.

Serious adverse events (SAEs)

In PROSPECT, an SAE is defined as isolation of *Lactobacillus* spp. in a culture from a sterile site or as the sole or predominant organism cultured from a non-sterile site and results in:

1) persistent or significant disability or incapacity; 2) that is life-threatening or 3) that results in death [40]. The rationale for our approach to SAEs accords with our guidelines for academic drug trials in critical care [41]. Any culture obtained by the ICU team and processed by the clinical microbiology laboratory as positive for *Lactobacillus* spp. is recorded. Any such bacterial sample is sent to a McMaster University research laboratory for strain genotyping to evaluate consistency with the administered *L. rhamnosus* GG strain.

Sample size and power

Based on an estimated 15% VAP rate, 2650 patients will be required to detect a 25% relative risk reduction (RRR) (and absolute risk reduction of 3.75%) with 80% power (alpha 0.05)(Table 2). The estimated 15% VAP rate is based on the PROSPECT pilot (adjudicated VAP rate of 19% [17]) and the REDOXS trial (14% [28]). The 25% RRR was observed in our meta-analysis of probiotics versus placebo [9] and a 24% RRR was found in a recent meta-analysis [11] and is more conservative than the 30% RRR in a Cochrane analysis [10]. Thus, we will enrol 1325 patients/group (2650 patients). Based on our pilot trial recruitment, we anticipate enrolling approximately 1.9 patients/month/site [17].

Central statistical monitoring

Thrice yearly throughout the trial, we will perform central statistical monitoring by analyzing site-specific data receipt and completeness, to help identify and overcome barriers to timely data completion. We will also monitor the proportion of non-screening weeks, and number and reasons for eligible non-randomized patients, to identify and remediate potential recruitment challenges.

We will monitor and report other types of protocol adherence [42]. We will track categories such as admissible protocol deviations for clinically justified reasons (e.g. strict nil per os status for possible bowel perforation) and logistical reasons (e.g. patient discharged early from the ICU so no evening dose given) as distinct from oversights which are protocol violations (e.g. dispensing errors). Thus, our protocol adherence regarding non-receipt of study product allows for sensible bedside decision-making, according to metrics from our prespecified taxonomy [43].

Statistical Analysis

Patients randomized in PROSPECT will be analyzed according to the intention-to-treat principle for the main analysis. We will present baseline characteristics of the 2 groups, including demographic and life support characteristics, and all prevalent infections. Infections will be defined as prevalent if present the day of, or diagnosed one day after randomization (the latter presumed to have started the day of randomization). For example, prevalent pneumonia could include any patient with pneumonia (community-acquired, healthcare-associated or ventilator-associated) present the day of or the day after randomization; this classification of pneumonia as prevalent relates only to timing of randomization and is independent of timing of intubation. Prevalent infections will not be considered outcomes for the trial because they are present at the time of randomization and are not plausibly modified by probiotics. All prevalent infections will also be centrally adjudicated by 1 physician blinded to allocation and center, based on review of data collected at each participating site. A CONSORT flow diagram will be generated, representing all randomized patients, their outcomes, the number and reasons for any consent withdrawals or loss to follow-up, as well as eligible non-randomized patients [44].

The main analysis will be a Cox proportional hazards analysis evaluating the primary outcome of VAP. This time-to-event analysis will use all information up to the time of censoring such that patients remain in the denominator and contribute information while they are at risk. The assumption for this analysis is that censoring is uninformative. The Cox model will be stratified by: a) center, and b) medical versus surgical versus trauma admission diagnosis, reflecting the stratification variables for randomization. The only independent variable will be randomized treatment group. We will present Kaplan-Meier curves for the primary outcome. We will also report VAP incidence rate, as number of VAP cases per 1,000 ventilator days [45]. We will report exposures during the ICU stay as is customary for critical care trials (e.g., advanced life supports) and cointerventions (e.g., pneumonia prevention strategies) relevant for this research question.

For the dichotomous secondary outcomes, we will also use time-to-event analyses. Hazard ratios and associated 95% confidential intervals will be estimated using a stratified Cox proportional hazards model.

For continuous outcomes, we will report estimates of the difference, 95% confidence intervals and associated p-values. For the continuous outcomes which are often skewed (e.g. duration of ventilation, ICU stay, and hospital stay), we will first log-transform these variables to see if they become normally distributed; if so, we will use parametric methods on the log-transformed variables to compare between groups. If not, we will compare the 2 groups using a nonparametric approach on the non-transformed variables. All secondary analyses will be adjusted for the stratification variables used at randomization (i.e., center and admission diagnostic category [medical, surgical, trauma]).

For the main analysis, when there is a statistically significant difference in binary outcomes, we will calculate other metrics. For example, depending on the results, these may be expressed as the number needed to prophylax (NNP) with probiotics to prevent 1 case of pneumonia, or the number needed to harm (NNH) to cause 1 case of iatrogenic infection with *L. rhamnosus* GG.

We do not anticipate missing any covariates for the primary outcome analysis – the only independent variable is treatment versus control, and the stratification variables are captured in the randomization system. We anticipate very little missing outcome data, since most data are collected in the ICU (except hospital vital status and length of stay, and *C. difficile* infection which is also recorded following ICU discharge in the hospital). For any other outcome that is missing for more than 2% of the patients, we will perform multiple imputation analysis [46, 47].

We will use graphics and other relevant methods to examine the residuals to assess model assumptions and goodness-of-fit including the proportional hazards assumption for Cox-regression analyses [48 - 50].

All estimates of effect will be reported to two decimal places. P-values will be reported to three decimal places with those less than 0.001 reported as $p < 0.001$. The criterion for statistical significance will be set at alpha = 0.05, using 2-sided tests, but adjusted appropriately for the 2 planned interim analyses (baseline characteristics, primary and secondary outcomes, adverse events and SAEs) using the Peto-Haybittle approach [51, 52]; the interim analyses will occur at one third and two thirds of total enrolment, performed when complete ICU data are available for 883 and 1766 patients, respectively. Two-sided tests will be used, with a fixed conservative $\alpha=0.001$ for the first and second interim analyses, and $\alpha=0.05$ for the final analysis [51, 52]. Secondary and subgroup analyses will not be adjusted for multiple analyses since these are

exploratory [53]. All analyses will be performed using the most up-to-date version of SAS (Cary, NC).

Following the publication of PROSPECT, the dataset will be used to design observational studies addressing additional hypothesis-driven questions (e.g. predictors of diarrhea, and ICU-acquired *C. difficile*). Access by other PROSPECT investigators will follow a submitted rationale, analysis plan and approval by relevant REBs in accordance with data sharing policies extant at the time of the request.

Sensitivity Analyses

We will conduct 4 sensitivity analyses. To the extent that these sensitivity analyses yield similar results to the main analysis, inferences about the primary outcome will be strengthened [54, 55].

1. In case the exact timing of the onset of VAP is uncertain, we will compare the proportion of patients with VAP in the 2 groups using the Mantel-Haenszel Chi square test, stratified by centre and medical versus surgical versus trauma. Thus, in this sensitivity analysis we will not use a time-to-event approach.
2. We will check for competing risks to address the problem that those who die can no longer develop VAP. We will analyze PROSPECT to explicitly account for death as a competing risk using the Fine and Gray proportional sub-distribution hazards model [56, 57]. This analysis will not assume that the censoring of deaths is uninformative; rather, it will assume that deaths could be informative. The rationale for this sensitivity analysis is to assess the robustness of the main findings [54].

3. We will conduct an efficacy analysis of each incident infection, and a composite of all incident infections, restricted to patients who received study product on ≥90% of study days. The rationale for this sensitivity analysis is to investigate the effect of probiotics under conditions of maximal exposure [58].
4. We will include all VAP events that occur after the day of randomization. The rationale for this sensitivity analysis is that pneumonia arising the day after randomization may be less likely to be influenced by study product exposure than pneumonia arising 2 or more days after initial study product exposure.

Subgroup Analyses

We will conduct 5 subgroup analyses based on baseline characteristics. These will evaluate whether these 5 baseline characteristics have an 'effect modification' when the effect of probiotics versus placebo on VAP is compared [59, 60]. Subgroup analyses will only be performed for the primary outcome.

1. We will conduct subgroup analyses among medical versus surgical versus trauma patients (the latter defined as patients cared for by a trauma service). We hypothesize that in medical patients, the treatment effect may be attenuated due to more risk factors for VAP that are non-modifiable when compared to surgical or trauma patients [1]. To perform this subgroup analysis, we will run the primary Cox regression analysis except that we will include medical versus surgical versus trauma as an independent variable instead of stratifying by it. We will also include the interaction term between medical versus surgical versus trauma and randomized treatment.

2. We will conduct subgroup analyses based on age (>75 years of age versus 65 – 75 years versus <65 years). Although little is known about the effects of probiotics in the elderly [61, 62], we hypothesize that if, overall, probiotics are associated with a lower rate of VAP than placebo, the treatment effect will be attenuated among older patients because immunosenescence renders their risk of infection less modifiable than younger patients. To perform this subgroup analysis, we will add age >75 versus 65 – 75 years versus <65 years as an independent variable as well as its interaction with randomized treatment group to the primary Cox regression model.
3. We will conduct subgroup analyses of the effect of probiotics on VAP after accounting for frailty, defined as a baseline Clinical Frailty Score of ≥5 out of 9 [63]. We hypothesize that if, overall, probiotics are associated with a lower rate of VAP than placebo, the treatment effect will be attenuated among patients who are frail, as their risk of infection may not be modifiable. To perform this subgroup analysis, we will add baseline Clinical Frailty Score of ≥5 as an independent variable as well as its interaction with randomized treatment group to the primary Cox regression model. We began measuring frailty in response to a Canadian research mandate [64], and did not start documenting frailty until 483 patients were enrolled. Thus, rather than imputing frailty status, we will restrict this subgroup analysis to patients enrolled thereafter.
4. We will conduct subgroup analyses among patients who received antibiotics for 2 days prior to randomization and the day of randomization versus patients who did not receive antibiotics for 2 days prior to, or the day of, randomization. We hypothesize that if, overall, probiotics are associated with a lower rate of VAP than placebo, the treatment effect will be attenuated in patients without recent antibiotic exposure when compared to

patients with antibiotic exposure. To perform this subgroup analysis, we will add antibiotic exposure prior to randomization defined as those receiving antibiotics for 2 days prior to randomization and the day of randomization as an independent variable as well as its interaction with randomized treatment group to the primary Cox regression model.

5. We will conduct subgroup analyses on patients with prevalent pneumonia versus no prevalent pneumonia. We hypothesize that if overall, probiotics are associated with a lower rate of VAP than placebo, the treatment effect will be attenuated among patients with pre-randomization pneumonia due to challenges interpreting whether the prevalent pneumonia has resolved prior to the development of another pneumonia event. To perform this subgroup analysis, we will add prevalent pneumonia as an independent variable as well as its interaction with randomized treatment group to the primary Cox regression model.

Steering Committee

The PROSPECT Steering Committee is responsible for overseeing the conduct of the trial, for upholding or modifying study procedures as needed, and addressing any challenges with protocol implementation. They advise as necessary on operational issues arising that are clinical, methodologic, biostatistical or ethical. The Steering Committee will review any proposed protocol amendment prior to dissemination of the revised protocol to participating centers by email correspondence, and shared on conference calls or webinars. As enrolment ensues, they share new emerging clinical, laboratory or epidemiology information that may impact on the trial. The Steering Committee has discussed and approved the interim statistical analysis plans

and final statistical analysis plan, and will assist with data interpretation, and abstract and manuscript preparation. The PROSPECT organizational chart is in Appendix 2.

Data Monitoring Committee

The PROSPECT Data Monitoring Committee (DMC) is independent from other persons involved in PROSPECT, and has the requisite expertise in randomized clinical trial design, epidemiology, biostatistics, warning guides/stopping rules, infectious diseases and critical care. The primary responsibilities of the DMC are to independently review reports prepared at the Methods Center regarding: 1) recruitment (center and patient) and screening, consent and coenrolment rates; 2) protocol procedures (randomization, stratification, protocol adherence including maintaining blinding); and 3) data tables for 2 blinded interim and final analyses. After each interim analysis, the DMC will recommend whether to continue, suspend or terminate enrolment.

The roles and responsibilities of the DMC are as outlined and approved in the PROSPECT DMC Charter [22], modeled on the Data Monitoring Committees: Lessons, Ethics, Statistics (DAMOCLES) Study Group charter [65].

Patient and Public Involvement

In PROSPECT, we are involving patients and their families in the following ways. First, before beginning PROSPECT, we ensured that patients and families were supportive of the use of probiotics in the ICU setting. We conducted a substudy nested within the PROSPECT Pilot [16, 17] whereby patient's substitute decision makers were interviewed at the time of enrolment to explore their comfort with probiotic use during critical illness [66]. In total, 103 SDMs

participated in 8 centers. We found no difference in characteristics of substitute decision makers who consented versus declined the PROSPECT Pilot. Rather, the rationale for substitute decision maker consent was related to personal beliefs regarding possible benefits to the patient, as well as predictions of patient's wishes regarding this trial. These findings gave us confidence that patients and families were supportive of the trial. Second, in accordance with many local ICU research practices, patients who gain capacity after resolution of their critical illness are asked to agree to ongoing participation in the trial. We have no formal patient or family advisor for PROSPECT. When PROSPECT results are available following trial completion, we will ensure the results are disseminated by having the academic message of each "in press" manuscript translated into press releases for the public. Possible target hospital media include newsletters, emails and intranet bulletins. High citizen awareness and probiotic consumption predict strong public interest.

Ethical oversight

PROSPECT is approved by Health Canada [#9427-M1133-45C], the Research Ethics Boards (REBs) of all participating hospitals, and Public Health Ontario. The study is underway in accordance with Good Clinical Practices following the Tri-Council Guidelines [67] and in accordance with ethical principles of the Declaration of Helsinki [68]. Access to the database, study-related files and source documents for scientific or auditing purposes is possible during and after the trial for any PROSPECT Methods Center staff, Health Canada authorities, or REB representatives (local, provincial or central). Participants will not be identified by name, and confidentiality will be maintained unless otherwise regulated. Data will be retained for 25 years as per Health Canada.

The DMC will use conservative 'warning guides' for apparent benefit in PROSPECT; there are no stopping guides for futility.

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PROSPECT is funded by peer-reviewed grants (Canadian Institutes of Health Research, Canadian Frailty Network, Physician Services Incorporated, Hamilton Academic Health Sciences Organization, and Academic Medical Organization of Southwestern Ontario), and funds from St. Joseph's Healthcare Hamilton and McMaster University. The study products are donated by the manufacturers of *L. rhamnosus* GG (i-Health, Inc.) which have no role in the trial conception, design, conduct, oversight analysis or write up.

Knowledge Dissemination

Results of the trial and secondary manuscripts will be communicated through conventional academic channels (e.g., abstracts, posters, peer-review manuscripts), at professional healthcare fora (e.g., grand rounds, teaching sessions, in-services, quality improvement councils), and via media (e.g., newspapers, radio, television, blogs, twitter etc.).

DISCUSSION

Probiotics may be a simple, cost-effective strategy to prevent VAP [69]. However, despite encouraging findings of efficacy, trials to date have been limited by insufficient power and risk of bias [9 - 11]. Studies of probiotics in the critical care setting have been criticized as difficult to interpret due to differences in populations and heterogeneous probiotics and combination products used [70]. Indeed, experts in the field have emphasized the need for well-powered studies of probiotics in the ICU setting [70]. To address this call, PROSPECT is a large,

international, rigorous multicenter randomized trial that aims to determine whether probiotics are effective, have no benefit, or are harmful in critical illness.

Additional strengths of PROSPECT include representation of persons greater than 65 years of age to enhance the generalizability of the findings, and separate peer-review funding for this population from the Canadian Frailty Network [64]. The efficacy of probiotics may be less in the elderly, as illustrated by a recent rigorous trial that found probiotics did not prevent *C. difficile* infection in persons ≥65 years admitted to hospital and receiving at least one antibiotic [71]. We are also documenting baseline pre-hospital frailty with the Clinical Frailty Score [72] to further understand the relationship between frailty, immunosenence and critical care-associated infections.

Given previous meta-analyses suggesting that probiotics may reduce all healthcare-associated infections in the ICU [9], we are evaluating all infectious outcomes in PROSPECT. Also, a recent large trial of 2556 healthy newborns conducted in rural India showed that synbiotics (*Lactobacillus plantarum* plus fructooligosaccharide) decrease the risk of sepsis and lower respiratory tract infections within 60 days [73]. It is unknown whether the benefit was from the *L. plantarum* or the addition of fructooligosaccharide; however, these results suggest that modification of microbiota can reduce infections. Given the growing interest in the dysbiosis of critical illness, this trial will advance our understanding of whether microbiota modification with probiotics has any influence on infectious and non-infectious clinically important outcomes [74 - 76].

The International Conference for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use [ICH] E9 [77] and SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) [18] guidelines endorse a separate statistical analysis

plan for clinical trials. Recommendations [18] include more technical and detailed elaboration of the principal features of the analysis described in the protocol, including procedures for executing the statistical analysis of the primary and secondary variables and other data [77]. We followed these expert recommendations [19] for 55 items in 6 sections: Title and Trial Registration (11 items/subitems); Introduction (2 items); Study Methods (9 items/subitems); Statistical Principles (8 items/subitems); Trial Population (8 items/subitems); and Analysis (17 items/subitems). Dissemination of this document aligns with calls to make statistical analysis plans publicly available [78] to aid in the transparent reporting of trial results.

TRIAL STATUS

PROSPECT is supported by a longstanding research consortium (the Canadian Critical Care Trials Group) dedicated to investigator-initiated, peer-review funded studies designed to understand and improve the outcomes of critically ill patients [21]. Recruitment is ongoing, with 73% of the target sample size accrued as of May 2018. At the first interim analysis, the DMC made no suggestions to suspend enrolment. Randomization is anticipated to continue until approximately April 2019. Final data entry, data validation and outcome adjudication will ensue for 6-9 months thereafter, with an anticipated database lock by December 2019, followed by the terminal statistical analyses. PROSPECT results will inform global practice in critical care medicine.

Author Contributions:
Concept and design: J Johnstone, D Heels-Ansdell, L Thabane, D Cook
Acquisition, analysis, or interpretation of data: J Johnstone, D Heels-Ansdell, L Thabane, M Meade, J Marshall, F Lauzier, EH Duan, N Zytaruk, D Lamarche, M Surette, D Cook
Drafting of the manuscript: J Johnstone, D Heels-Ansdell, L Thabane, D Cook
Critical revision of the manuscript for important intellectual content: everyone
Statistical analysis: D Heels-Ansdell (Trial Biostatistician), L Thabane (Senior Biostatistician), D Lamarche, M Surette
Obtained funding: everyone
Administrative, technical, or material support: D Heels-Ansdell, E Duan, N Zytaruk, L Thabane, D Lamarche, M Surette
Drs. J Johnstone and D Cook as Co-Principal Investigators take responsibility for the integrity of the data

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REFERENCES

1. Safdar N, Dezfulian C, Collard H, Saint S. Clinical and economic consequences of ventilator-associated pneumonia: A systematic review. Crit Care Med 2005; 33: 2184.
2. Muscedere J, Dodek P, Keenan S, et al. Comprehensive evidence-based clinical practice guidelines for ventilator-associated pneumonia: Prevention. J Crit Care 2008; 23: 126.
3. http://www.jointcommission.org. Accessed January 11, 2018.
4. Sinuff T, Muscedere J, Cook D, et al. The active observational study of the adoption and transfer of clinical practice guidelines through education, for ventilator-associated pneumonia study (ABATE VAP). Crit Care Med 2013; 41: 15.
5. Marshall J. The gastrointestinal flora and its alterations in critical illness. Curr Opin Crit Care 1999; 5: 119.
6. Stappenback T, Hooper L, Gordon J. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proc Natl Acad Sci USA 2002; 99: 15451.
7. Tanoue T, Honda K. Induction of Treg cells in the mouse colonic mucosa: a central mechanism to maintain host-microbiota homeostasis. Semin Immunol 2012; 24: 50.
8. Brenchley J, Douek D. Microbial translocation across the gastrointestinal tract. Annu Rev Immunol 2012; 30: 149.
9. Petrof E, Dhaliwal R, Manzanares W, et al. Probiotics in the critically ill. Crit Care Med 2012; 40: 3290.
10. Bo L, Li J, Tao T, et al. Probiotics for preventing ventilator-associated pneumonia. Cochrane Database of Syst Rev 2014; 10: CD009066.
11. Manzanares W, Lemieux M, Langlois PL, Wischmeyer PE. Probiotic and synbiotic therapy in critical illness: a systematic review and meta-analysis. Crit Care. 2016; 19: 262.
12. Nuesch E, Trelle S, Reichenbach S, et al. Small study effects in meta-analyses of osteoarthritis trials: meta-epidemiological study. BMJ 2010; 341: c3515.
13. Dechartres A, Boutron I, Trinquart L, et al. Single-center trials show larger treatment effects than multicenter trials. Ann Intern Med 2011; 155: 39.
14. Weng H, Li JG, Mao Z, et al. Probiotics for preventing ventilator-associated pneumonia in mechanically ventilated patients: A meta-analysis with trial sequential analysis. Front Pharmacol 2017; 8: 717.
15. Morrow L, Kollef M, Casale T. Probiotic prophylaxis of ventilator-associated pneumonia. Am J Respir Crit Care Med 2010; 182: 1058.
16. Johnstone J, Meade M, Marshall J, et al. PROSPECT: Protocol for a feasibility randomized pilot trial. Pilot and Feasibility Studies 2015; 1: 19.
17. Cook D, Johnstone J, Marshall J, et al. Probiotics: Prevention of Severe Pneumonia and Endotracheal Colonization Trial - PROSPECT: A Pilot Trial. Trials 2016; 17: 377.
18. Chan A, Tetzlaff J, Altman D, et al. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med 2013; 158: 200 - 207.
19. Gamble C, Krishan A, Stocken D, et al. Guidelines for the content of statistical analysis plans in clinical trials. JAMA 2017; 318: 2337 – 2343.
20. Duan EH, Johnstone J, Meade MO, et al. Refining exclusion criteria following pilot trials: Lessons from PROSPECT. Canadian Critical Care Forum 2016, Toronto, ON, October 2016 Can Crit Care Forum Abstract Book 2016.

21. Marshall JC, Cook DJ for the Canadian Critical Care Trials Group. Investigator-led clinical research consortia: The Canadian Critical Care Trials Group. Crit Care Med 2009; 36:S165-S172.
22. PROSPECT. www.prospecttrial.com Accessed May 14 2018.
23. Smith O, McDonald E, Zytaruk N, et al. Enhancing the informed consent process for critical care research: strategies from a thromboprophylaxis Trial. Intensive and Critical Care Nursing 2013; doi.org/10.1016/j.iccn.2013.04.006.
24. Lamarche D, Rossi L, Shah M, et al. Quality control in the conduct of a probiotic randomized trial. Can Crit Care Forum, Toronto, ON, October 28, 2014.
25. Grossman R, Fein A. Evidence-based assessment of diagnostic tests for ventilator-associated pneumonia. Chest 2000; 117(4 Suppl 2): 177S – 181S.
26. Muscedere J, Sinuff T, Heyland D, et al. The clinical impact and preventability of ventilator-associated conditions in critically ill patients who are mechanically ventilated. Chest 2013; 144: 1453.
27. Canadian Critical Care Trials Group. A randomized trial of diagnostic techniques and empiric broad-spectrum antibiotics for suspected ventilator-associated pneumonia. N Engl J Med 2006; 355(25):2619-2630
28. Heyland D, Muscedere J, Wischmeyer P, et al. A randomized trial of glutamine and antioxidants in critically ill patients. N Engl J Med 2013; 368: 1489.
29. Calandra T, Cohen J for the International Sepsis Forum Definition of Infection in the ICU Consensus Conference. Crit Care Med 2005; 7: 1538.
30. Pugin J, Auckenthaler R, Mill N, et al. Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic blind bronchoalveolar lavage fluid. Am Rev Respir Dis 1991; 143: 1121.
31. Horan T, Andrus M, Dudeck M. CDC/NHSN surveillance definition of healthcare associated infections and criteria for specific types of infections in the acute care setting. Am J Infect Control 2008;36:309.
32. Chastre J, Wolff M, Fagon J, et al. Comparison of 8 vs 15 days of antibiotic therapy for ventilator-associated pneumonia in adults: a randomized trial. JAMA 2003; 290: 2588 – 2598.
33. Chastre J and Fagon J. Ventilator-associated pneumonia. Am J Respir Crit Care Med 2002; 165: 867–903
34. Cohen S, Gerding D, Johnson S, et al. Clinical practice guidelines for C. difficile infection in adults: 2010. Infect Control Hosp Epidemiol 2010; 31: 431.
35. World Health Organization. http://www.who.int/topics/diarrhoea/en/ Accessed May 14 2018.
36. Heaton K, Lewis S. Stool form scale as a useful guide to intestinal transit time. Scan J Gastroenterol 1997; 32: 920.
37. Thibault R, Graf S Clerc A, et al. Diarrhea in the intensive care unit. Crit Care 2013; 17: R153.
38. Polk R, Fox C, Mahoney A et al. Measurement of adult antibacterial drug use in 130 US hospitals: comparison of defined daily dose and days of therapy. Clin Infect Dis 2007; 44: 664-70.
39. Kuster S, Ruef C, Ledergerber B et al. Quantitative antibiotic use in hospitals: comparison of measurements, literature review, and recommendations for a standard of reporting. Infection 2008; 36:549-59.

40. The Guidance Document for Industry – Reporting Adverse Reactions to Marketed Health Products: www.hc-sc.gc.ca/dhp-mps/pubs/medeff/_guide/2011-guidance-directrice_reporting-notification/index-eng.php
41. Cook D, Lauzier F, Rocha M, et al. Serious adverse events in academic critical care research. CMAJ 2008; 178: 1181.
42. Bhatt A. Protocol deviation and violation. Perspect Clin Res. 2012; 3: 117.
43. Lauzier F, Adhikari N, Seely A, et al. Protocol adherence for continuously titrated interventions in randomized trials: An overview of the current methodology and case study. BMC Medical Research Methodology 2017; 17: 106.
44. Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. Trials 2010, 11: 32.
45. National Healthcare Safety Network. https://www.cdc.gov/nhsn/pdfs/pscmanual/6pscvapcurrent.pdf Accessed May 14 2018.
46. Little R, Rubin D: Statistical analysis with missing data. 2nd edition. Hoboken, NJ: Wiley; 2002; Rubin DB: Multiple imputation for nonresponse in surveys. New York: J Wiley & Sons; 1987.
47. Zhang Y, Alyass A, Vanniyasingam T, et al. A systematic survey of the methods literature on the reporting quality and optimal methods of handling participants with missing outcome data for continuous outcomes in randomized controlled trials. J Clin Epidemiol 2017; 88: 67-80.
48. Schoenfeld D: Partial residuals for the proportional hazards regression model. Biometrika 1982, 69:239–241.
49. Wei LJ: Testing goodness of fit for proportional hazards model with censored observations. J Am Stat Assoc 1984, 79:649–652.
50. Harrell FE, Lee KL: Verifying Assumptions of the Cox Proportional Hazards Model. Proceedings of the Eleventh Annual. SAS User's Group International Conference. Cary, N.C.: SAS Institute, Inc.; 1986:823–828.
51. Haybittle, JL. Repeated assessment of results in clinical trials of cancer treatment. British J Radiology 1971; 44: 793.
52. Peto R, Pike M, Armitage P, et al. Design and analysis of randomized control trials requiring prolonged observations of each patient. British J Cancer 1976; 34: 585.
53. Li G, Taljaard M, Van den Heuvel E, et al. An introduction to multiplicity issues in clinical trials: the what, why, when and how. International Journal of Epidemiology, 2017, 746–755.
54. Thabane L, Mbuagbaw L, Zhang S, et al. A tutorial on sensitivity analyses in clinical trials: the what, why, when and how. BMC Med Res Methodol 2013, 13(1):92.
55. de Souza RJ, Eisen R, Perera S, et al. Best (but oft-forgotten) practices: sensitivity analyses in randomized controlled trials. Am J Clin Nutr 2016; 103: 5-17.
56. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. Journal of the American Statistical Association 1999;94(446):496-509.
57. Bakoyannis G, Touloumi G. Practical methods for competing risks data: A review. Statistical Methods in Medical Research 2012;21(3):257-272.
58. Cramer JA, Roy A, Burrell A, et al. Medication compliance and persistence: terminology and definitions. Value Health. 2008; 11: 44–7.
59. Rothwell P. Subgroup analysis in randomised controlled trials: importance, indications, and interpretation. Lancet 2005; 365: 176–186.

60. Sun X, Briel M, Walter SD, Guyatt GH. Is a subgroup effect believable? Updating criteria to evaluate the credibility of subgroup analyses. BMJ 2010; 340: 85-854.
61. Patel PJ, Singh SK, Panaich S, Cardozo L. The aging gut and the role of prebiotics, probiotics, and synbiotics: a review. J Clin Gerontol Geriatr 2014;5:3e6.
62. Wachholz PA, P Boas. Evidence on the role of prebiotics, probiotics, and synbiotics in gut health and disease prevention in the elderly. J Clin Gerontol Geriatr 2014; 5: 1-2.
63. Bagshaw S, Stelfox T, McDermid R, et al. Association between frailty and short- and long-term outcomes among critically ill patients: a multicentre prospective cohort study. CMAJ 2013; DOI:10.1503/cmaj.130639.
64. Canadian Frailty Network. http://www.cfn-nce.ca/ Accessed May 14, 2018.
65. DAMOCLES Study Group. A proposed charter for clinical trials data monitoring committees: helping them to do their job well. Lancet 2005; 365: 711-722.
66. Shears M, Clarke F, Mehta S, et al. Inter-Prob:INTERviewing substitute decision makers about PROBiotics. Can Crit Care Forum, Toronto, ON, October 25-28, 2015.
67. Grimes D, Hubacher D, Nanda K, et al. The Good Clinical Practice guideline: a bronze standard for clinical research. Lancet 2005; 366: 172–74
68. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013; 310(20):2191-4.
69. Branch-Elliman W, Wright S, Howell M. Determining the ideal strategy for ventilator-associated pneumonia prevention. Am J Respir Crit Care Med 2015; 192: 57.
70. Crooks NH, Snaith C, Webster D, et al. Clinical review: Probiotics in critical care. Critical Care 2012, 16: 237.
71. Allen S, Wareham K, Wang D, et al. Lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhoea and Clostridium difficile diarrhoea in older inpatients (PLACIDE): a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 2013; 382: 1249–1257.
72. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly people. CMAJ 2005; 173: 489–495.
73. Panigrahi P, Parida S, Nanda N, et al. A randomized synbiotic trial to prevent sepsis among infants in rural India. Nature 2017; 548: 407–412.
74. McDonald D, Ackermann G, Khailova L et al. Extreme dysbiosis of the microbiome in critical illness. mSphere 2016; 1: e00199-16.
75. Relman D. The human microbiome and the future practice of medicine. JAMA 2015; 314: 1127-8.
76. Lamarche D, Johnstone J, Zytaruk N, et al. Microbial dysbiosis and mortality during mechanical ventilation: a prospective observational study. Respir Res 2018; 19:245.
77. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline: Statistical Principles for Clinical Trials E9. London, England: European Medicines Agency; 1998.
78. Finfer S, Bellomo R. Why publish statistical analysis plans? Crit Care Resusc 2009; 11: 5-6.

Table 1: Inclusion and Exclusion Criteria

Inclusion criteria
1. Adults ≥18 years of age admitted to a medical, surgical or trauma ICU 2. Receiving invasive mechanical ventilation, estimated to be required for ≥72 hours
Exclusion criteria
1. Invasively mechanically ventilated >72 hours at the time of screening 2. Potential increased risk of iatrogenic probiotic infection including specific immunocompromised groups: HIV <200 CD4 cells/μL, chronic immunosuppressive medications, previous transplantation at any time, chemotherapy in the last 3 months, absolute neutrophil count <500. Previous or current corticosteroids use is not exclusionary 3. Risk for endovascular infection: rheumatic heart disease, congenital valve disease, surgically repaired congenital heart disease, unrepaired cyanotic congenital heart disease, valvular replacement (mechanical or bio-prosthetic), previous or current endocarditis, permanent endovascular devices (e.g., endovascular grafts, inferior vena cava filters, dialysis vascular grafts), tunneled hemodialysis catheters, pacemakers or defibrillators. These are not exclusions: coronary artery stents or bypass grafts, mitral valve prolapse, bicuspid aortic valve, temporary catheters (central venous, peripherally inserted, extra-corporeal life support-related) or neurovascular coils 4. Primary diagnosis of severe acute pancreatitis 5. Percutaneously inserted feeding tubes in situ, as per Health Canada 6. Strict contraindications or inability to receive enteral medications 7. Intent to withdraw advanced life support 8. Previous enrolment in this trial, or current enrolment in a potentially confounding trial

*Changes from the PROSPECT pilot are as follows: 1. Omitted radiation therapy as an exclusion criterion; 2. Omitted steroid exposure as an exclusion criterion; 3. Better defined transplant to explicitly exclude all transplant patients (autologous stem cell patients are now excluded); 4. Better defined the cardiac valvular diseases at risk; 5. Removed surgery of esophagus/stomach/small bowel as exclusion criteria and replaced with any strict contraindication or inability to receive enteral medications; 6. Replaced severe acute pancreatitis with organ dysfunction with primary diagnosis of severe acute pancreatitis; 7. Omitted pregnancy as exclusion criterion [20].

Table 2: Determination of the sample size: based on an estimated 15% VAP rate, 2650 patients (n=1325 in each arm) will be required to detect a 25% relative risk reduction with 80% power.

***Per Group* Sample Size for 80% power and alpha=0.05, using continuity correction.**

	RRR				
Baseline Risk	**10%**	**15%**	**20%**	**25%**	**30%**
8%	17473	7635	4221	2653	1809
9%	15374	6720	3716	2337	1594
10%	13695	5988	3313	2084	1422
12%	11176	4891	2707	1704	1164
14%	9377	4107	2275	1433	979
15%	8657	3793	2102	**1325**	906
16%	8028	3519	1951	1230	841
18%	6978	3061	1699	1072	734
20%	6139	2695	1497	945	647
22%	5452	2396	1332	842	577
24%	4879	2147	1194	756	518
25%	4627	2037	1134	718	493
30%	3620	1598	892	566	389
35%	2900	1284	719	458	316
40%	2361	1049	589	376	260
50%	1605	719	408	262	183

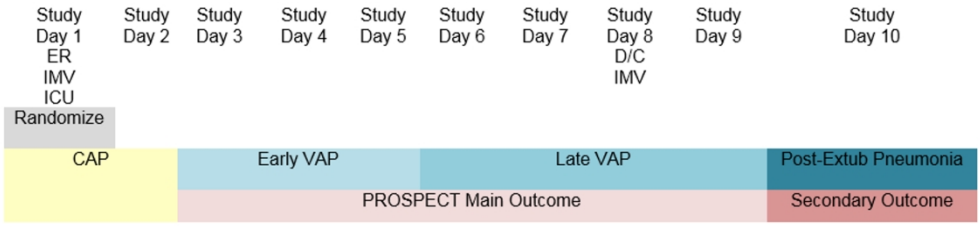
Figure 1 and 2: Pneumonia Classification

Legend for Figure 1 and 2: These figures illustrate the pneumonia classification that we are using, according to when the lung infection develops in a patient's hospital trajectory. The different classifications over time in each example relate to the day of hospital admission, day of ICU admission, day of initiation of mechanical ventilation (via endotracheal intubation or tracheostomy), day of randomization in the trial, and day of discontinuation of mechanical ventilation. Note that the pneumonia classifications over time do not reflect persistent or progressive lung infections, but rather the pneumonia classification that would be ascribed if a new infection develops on each day shown.

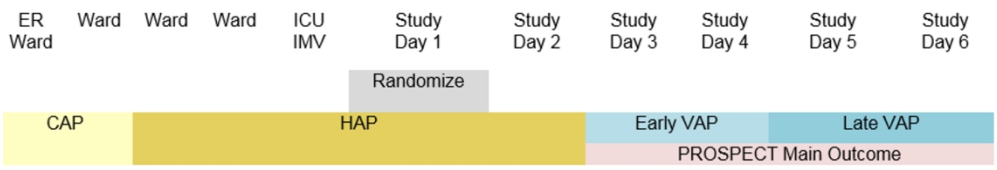
Abbreviations: ER – emergency room; Extub – Extubation; IMV – invasive mechanical ventilation; ICU – intensive care unit; ICUAP – intensive care unit associated pneumonia; Rand – randomization; CAP – community-acquired pneumonia; VAP – ventilator-associated pneumonia; HAP – hospital-acquired pneumonia;

Figure 1: Pneumonia classifications that could arise in patients who require ICU admission and invasive mechanical ventilation at the time of presentation to the emergency room, and are randomized into PROSPECT that day. The primary outcome is adjudicated VAP (any, including early or late), arising on study day 3 or later. Secondary outcomes illustrated include early VAP, late VAP and post-extubation pneumonia.

Figure 2: Pneumonia classifications that could arise in patients who require ICU admission and invasive mechanical ventilation after an initial hospital stay, and are randomized into PROSPECT the day following ICU admission. The primary outcome is adjudicated VAP (any, including early or late), arising on study day 3 or later.



362x90mm (300 x 300 DPI)



165x28mm (300 x 300 DPI)

Appendix 1: Objectives, Outcomes, Hypothesis and Methods of Analysis

Objectives	Outcomes	Hypothesis	Methods of Analysis
1) **Primary:** To compare the effects of *Lactobacillus rhamnosus* GG versus placebo on:	Ventilator-associated pneumonia (VAP)	*Lactobacillus rhamnosus* GG will reduce the risk of the primary outcome	Cox proportional hazards
2) **Secondary:** To compare the effects of *Lactobacillus rhamnosus* GG versus placebo on:	a) Early VAP, late VAP, post-ventilation ICU-acquired pneumonia, and a composite of all three.	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	b) Clostridium difficile	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	c) Any infection acquired during the ICU stay	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	d) Diarrhea in the ICU	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	e) Antibiotic-associated diarrhea	*Lactobacillus rhamnosus* GG will reduce the risk	Cox proportional hazards
	f) Antimicrobial use	*Lactobacillus rhamnosus* GG will reduce the risk	Independent samples paired t-test or Wilcoxon rank sum test
	g) Duration of mechanical ventilation, ICU stay and hospital stay	*Lactobacillus rhamnosus* GG will have no effect	Independent samples paired t-test or Wilcoxon rank sum test
	h) ICU mortality and hospital mortality	*Lactobacillus rhamnosus* GG will have no effect	Cox proportional hazards
	i) Serious adverse events	*Lactobacillus rhamnosus* GG will have no effect	Cox proportional hazards
3) **Sensitivity Analyses:**			
i. Compare proportion of patients with VAP in the two groups.	VAP	Results remain robust	i. Mantel-Haenszel Chi square test ii. Competing risk

ii. Check for competing risk of death				analysis
iii. Efficacy analysis				ii. Cox proportional hazards
iv. Include all VAP events that occur after the day of randomization				iii. Cox proportional hazards
4) **Subgroup Analyses:**				Cox proportional hazards with interaction test between each subgroup variable and treatment group evaluating the credibility of subgroup findings using our 11 previously published criteria [6]
i. Medical vs surgical vs trauma patients	VAP	Treatment effects may be attenuated in one group compared to another group (see text for details)		
ii. >75 years vs 65 – 75 years vs <65 years old				
iii. Frail patients versus not frail patients				
iv. Antibiotics prior to randomization vs no antibiotics prior to randomization				
v. Pneumonia at baseline vs no pneumonia				

Legend for Appendix 1: In all analyses, results will be expressed as estimate of effect, corresponding 95% and associated p-values. All tests will be two-sided using alpha = 0.05 level of significance in accordance with a superiority hypothesis.

Appendix 2: PROSPECT Organizational Chart

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym p.1.
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry p.3 (Abstract), p5. (Intro).
	2b	All items from the World Health Organization Trial Registration Data Set N/A (it is on the Registry website)
Protocol version	3	Date and version identifier p.5 (Intro).
Funding	4	Sources and types of financial, material, and other support p20. (Methods
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors p1, 24
	5b	Name and contact information for the trial sponsor p.1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities p20 (Method
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) p18+19 (Me
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention p4+5
	6b	Explanation for choice of comparators p4+5
Objectives	7	Specific objectives or hypotheses p4+5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) p5+6

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained p5+6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) p5+6 + Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered p6+7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) p.7.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) p.11.
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial p. 13.
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended p. 8 - 10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) Table 1, Figure 1+2
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations p. 11.
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size p.5.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions p. 6.

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned p. 6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions p. 6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how p. 6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial p. 6
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol p. 7+8, 11+12
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols p. 14 (very little missing da
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol p. 7, 8, 11, 12
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol p12 - 15
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) p. 15 - 18
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) p. 12, 14
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed p. 18

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial p. 14., 19
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct p10+11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor p11+12
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval p20.
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) p. 18
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) p.6.
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable N IA.
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial p.20., 7+8
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site p24
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators p14+15.
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation N/A.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions p21.
	31b	Authorship eligibility guidelines and any intended use of professional writers p24 (no use of a medical writer).
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code N/A.

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates p 6.
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable N/A.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.