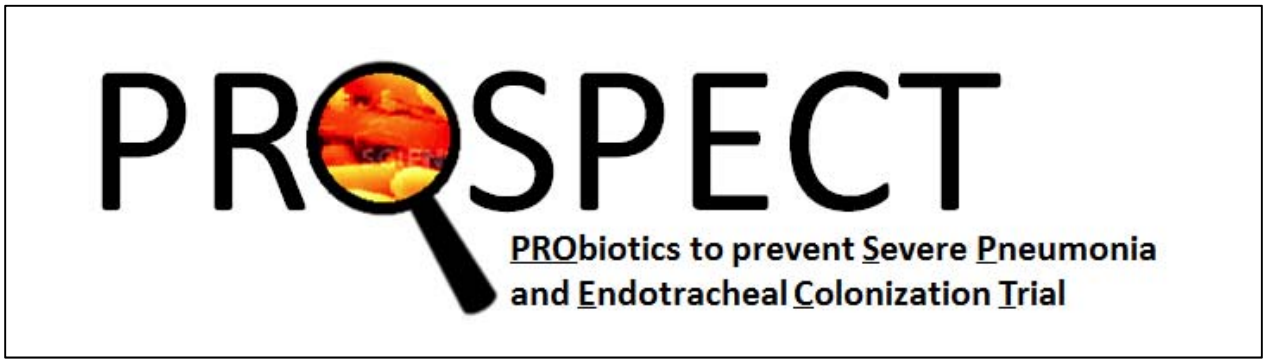

PROSPECT:
Probiotics to prevent Severe Pneumonia and Endotracheal Colonization Trial

by the PROSPECT Steering Committee, PROSPECT Investigators
and the Canadian Critical Care Trials Group

PROTOCOL # 27022015

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1.0 OVERVIEW

Probiotics are defined as live microorganisms thought to have health benefits when ingested [WHO 2002]. Many randomized controlled trials (RCTs) have shown that probiotic use impacts favourably on a range of clinical problems, including prevention of upper respiratory tract infections [Hao 2011], antibiotic-associated diarrhea [Hempel 2012, Ritchie 2012], *Clostridium difficile*-associated diarrhea [Johnson 2012, Ritchie 2012], and irritable bowel syndrome [Ritchie 2012]. Our recent meta-analysis of RCTs in the intensive care unit (ICU) suggests that the administration of probiotics to critically ill mechanically ventilated patients is associated with a 25% lower ventilator-associated pneumonia (VAP) rate [95 % CI 3% - 41%] and 18% lower infection rates overall [95% CI 1% - 31%] [Petrof 2012]. However, the estimated effect on VAP arises from 7 small, modest quality single-center RCTs yielding imprecise estimates and uncertain internal and external validity. Given the effectiveness of probiotics in the community and hospital setting, yet uncertain benefits in the ICU, a large rigorous RCT is needed. Before launching a complex costly RCT testing whether probiotics confer benefit, harm, or have no impact on infectious and non-infectious outcomes, a pilot trial was needed. We have completed such a trial. The PROSPECT (Probiotics to prevent Severe Pneumonia and Endotracheal Colonization Trial) Pilot Trial aimed to determine the feasibility of performing a large RCT in mechanically ventilated critically ill patients to test whether enteral *Lactobacillus rhamnosus* GG prevents VAP, and other infectious and non-infectious outcomes. Herein we report the results of the PROSPECT Pilot Trial and outline the protocol for the main PROSPECT Trial.

2.0 THE NEED FOR A TRIAL

2.1 What is the problem to be addressed?

2.1.1 Burden of VAP: VAP is the most common nosocomial infection in the ICU, resulting in a high burden of illness for critically ill patients. In a 2005 systematic review, the pooled cumulative incidence of VAP in critically ill patients was 23% [95% CI 18.8% - 26.9%] in RCTs and 10% [95%CI 7.0%-12.5%] in non-RCTs [Safdar 2005]. In addition, VAP conferred a 2-fold attributable risk of dying in the ICU [OR 2.02, 95% CI 1.16 - 3.56] and an attributable cost ranging from US $10,000 -$13,000 per patient [Safdar 2005]. In Canada, VAP is estimated to account for approximately 2% of all ICU days per year; the cost to the health care system is estimated at CAN $46 million (possible range, $10 to 82 million) per year [Muscedere 2008].

VAP prevention is an important patient safety goal for critically ill patients [Muscedere 2008, Muscedere CPG 2008], and a major focus of the Canada-wide quality improvement campaign *Safer Healthcare Now!* [www.saferhealthcarenow.ca]. Some uptake of effective VAP prevention strategies [Muscedere CPG 2008] has likely reduced its incidence since the 2005 systematic review described above [Safdar 2005]. VAP reporting has become mandatory for many hospitals in Canada and the United States; thus, VAP has become a prominent 'patient safety indicator' [www.jointcommission.org]. In United States where pay-for-performance is common, financially incentivized under-reporting has raised serious questions about the integrity of VAP rates as a quality of care metric. In Canada, inaccurate self-reported surveillance efforts clearly underestimate VAP rates compared to research data and clinically suspected VAP that is treated in practice. For example, in Ontario in 2010, the reported VAP incidence [health.gov.on.ca/en/pro/programs/criticalcare/ccis] was 2.80 cases/1000 ventilator days. However, our 11 center North American study found that when VAP was systematically observed and rigorously adjudicated in low risk patients, the VAP rate was 8.7 cases/1000 ventilator days (incidence of 8.2%) [Sinuff 2013]. Our recent REDOXS trial of sicker ICU patients found an adjudicated VAP rate of 14% [Heyland 2013]. VAP rates vary widely based on

different definitions and current quality of care, research metrics and financial incentives appear to result in lower VAP rates than are actually clinically suspected and treated in practice.

In summary, true rates of VAP are likely much higher than self-reported by hospital quality improvement personnel outside the context of clinical research [Morrow 2006].

2.1.2 Prevention of VAP: VAP prevention strategies have focused on methods to reduce colonization of the oropharynx with pathogenic organisms and on ways to minimize aspiration of microbes into the airway. Our recent evidence-based VAP prevention guidelines summarized existing RCT evidence in the field, and recommended using orotracheal rather than nasotracheal intubation; new ventilator circuit changes for each patient when circuits become soiled; humidifier changes every 5-7 days or as clinically indicated; a closed endotracheal suctioning system; suctioning system change for each patient; endotracheal tubes with subglottic aspiration in patients expected to be ventilated more than 72 hours; head of bed elevation at 45° or as near to 45° as possible; and suggested oral decontamination with chlorhexidine (or providone-iodine in patients with severe head injury) [Muscedere CPG 2008]. Further, selective oropharyngeal decontamination and selective digestive tract decontamination through additional oral, enteral and/or intravenous antibiotics are associated with lower VAP and lower mortality rates in the ICU [deSmet 2009, Liberati 2004]. However, prophylactic antibiotic strategies in the form of selective oropharyngeal decontamination and selective digestive tract decontamination are not recommended in national guidelines [Muscedere CPG 2008] because of concerns about increasing the risk of antimicrobial-resistant organisms. Moreover, the use of antibiotics for VAP prevention runs counter to the current universal antimicrobial stewardship movement [Laupland 2009].

Effective VAP prevention strategies have been variably used in practice [Heyland 2002]. Our recent multicenter study encouraging VAP guideline uptake incorporating education, reminders, and opinion leaders showed a high awareness of best practices [Sinuff 2013]. However, guideline concordance for the 14 recommendations in 11 sites was 51% at baseline, 54% at 6 months, and reached a plateau of 59% at 24 months. The subset of VAP prevention strategies had uptake rates from 2%-100%, based on complexity and availability of the strategy.

In summary, the ongoing morbidity, mortality and cost of VAP remain high [Safdar 2005, Muscedere 2008]. Effective VAP prevention strategies are poorly applied in practice and [Sinuff 2013] underscore the need for simple, cost-effective, strategies to reduce VAP. Probiotics represent one such novel intervention. Safely and widely used outside the ICU, probiotics have an appealing but uncertain impact in critical illness.

2.1.3 Probiotics as a potential strategy to prevent VAP: Probiotics have emerged as a plausible strategy to prevent VAP by enhancing gut barrier function and reducing pathogenic bacterial load (Section 2.1.4). In 2010, a systematic review and meta-analysis of RCTs evaluating the efficacy of probiotics on VAP prevention in the ICU included 5 RCTs, showing that probiotics compared with control (placebo or other) reduced the incidence of VAP in mechanically ventilated patients [60/316 (19%) versus 102/373 (27%), OR 0.55, 95% CI 0.31 – 0.98, I^2 39%] [Siempos 2010]. However, despite the benefit suggested by this meta-analysis, the precise effect of probiotics on VAP remains unclear. Of 5 trials in the meta-analysis, 3 had unique features which makes interpretation challenging. One also used prebiotics (non-digestible ingredients that stimulate the growth and activity of bacteria in the gut [Knight 2009, Spindler-Vesel 2007, Kotzampassi 2006]). One used probiotics as a mouthwash rather than oral ingestion [Klarin 2008]. One trial used a single strain of organism [Forestier 2008] and found no difference in VAP: 24/102 (24%) cases in the group that ingested *L. casei rhamnosus* strain 35 versus 24/106 (23%) in the placebo arm, RR 0.94, 95% CI 0.54 – 1.64 [Forestier 2008].

Since this meta-analysis was published, a high quality RCT compared a combination of oropharyngeal plus gastric *L. rhamnosus* GG to corresponding placebos in 146 patients

expected to remain intubated for at least 72 hours (a group at relatively high risk of VAP) [Morrow 2010]. Those patients treated with *L. rhamnosus* GG had lower rates of VAP [17/73 (23%) vs. 33/73 (45%), RR 0.46, 95% CI 0.26-0.82]. This trial suggests that *L. rhamnosus* GG, specifically, is a promising probiotic to prevent VAP in a selected high-risk ICU population. However, it is not certain whether the benefit was derived from the ingested probiotic, the mouthwash component or the combination. Using probiotic mouthwash is problematic because oral decontamination with chlorhexidine is common in many ICUs, supported by RCTs in our recent meta-analyses [Chan 2007] and VAP prevention guidelines [Muscedere CPG 2008]. Further, chlorhexidine co-administration may substantially alter or even neutralize the effect of probiotics in critical illness.

We completed a systematic review and meta-analysis on probiotic use in the ICU for the prevention of a wider range of clinically important outcomes [Petrof 2012]. We included 23 RCTs enrolling critically ill adults that evaluated probiotics compared to a placebo and reported infections, mortality, or length of stay. Among 11 RCTs, probiotics were associated with reduced infectious complications overall (RR 0.82, 95% CI 0.69 - 0.99, p = 0.03; I^2 44%). Probiotics were associated with a trend towards reduced ICU mortality (RR 0.80, 95% CI 0.59 -1.09, p = 0.16; I^2 0%) but did not influence hospital mortality. Probiotics had no effect on ICU or hospital length of stay. Our updated estimate pooling results from 7 RCTs showed that probiotics significantly reduced VAP rates (RR 0.75, 95 % CI 0.59, 0.97, p = 0.03; I^2 35%). However, in an *a priori* subgroup analysis evaluating the impact of study quality on the treatment effect, the signal of benefit was in RCTs at high risk of bias; there was no overall effect in rigorously performed trials. A succinct critique of all RCTs in these 2 meta-analyses is found in Appendix A; the VAP meta-analysis results are found in Appendix B.

In summary, although RCT data indicate that probiotics *may* prevent VAP, these estimates arise from small (n=50 to 300), mostly low quality single-center RCTs yielding imprecise estimates of effect, and findings of uncertain internal and external validity. Based on this RCT evidence, forthcoming VAP prevention guidelines in 2015 will not 'recommend' probiotics, but 'suggest' that probiotics be considered [Dr. J Muscedere, personal communication]. It is possible that their use may increase, as apparent salutary effects of probiotics become propagated. However, to inform practice, a large rigorous RCT is needed.

2.1.4. Proposed Mechanism for Probiotics to Prevent VAP: Despite the signal from RCTs showing that probiotics may decrease infections in general, and VAP in particular [Petrof 2012], the potential mechanisms of this effect during critical illness remain incompletely understood. One possible mechanism for the clinical effect of probiotics is maintenance of the endogenous gastrointestinal (GI) tract microbiota, which inhibit gut colonization with exogenous pathogenic microorganisms by competition for epithelial binding sites and luminal nutrients, support of intraepithelial immune defenses, and production and release of specific antibacterial factors [Marshall 1999]. Intact endogenous microflora is necessary for normal development of small bowel epithelium microvasculature [Stappenback 2002] and for maturation of the gut-associated lymphoid tissues [Tanoue 2012]. Enhanced physical and immunological barrier function and a reduced pathogenic bacterial load may reduce bacterial translocation from the gut lumen into regional lymphatic tissues or portal vein, further reducing nosocomial infection risk [Brenchley 2012]. Finally, the GI tract harbours up to 25 grams of bacterial lipopolysaccharide or endotoxin; augmented gut barrier function with probiotics may reduce GI absorption of endotoxin [van Deventer 1988].

One group of trialists have partnered with translational biologists to examine how the effect of probiotics might be mediated [Tan 2011]. In this RCT of 52 severely brain-injured patients, those receiving an ingested probiotic mixture containing $0.5x10^8$ *Bifidobacterium longum*, $0.5x10^7$ *Lactobacillus bulgaricus* and $0.5x10^7$ *Streptococcus thermophilus* (Golden Bifid) had increased serum cytokine levels associated with pro-inflammatory Th1 response (IL-12p70

and IFNγ) and decreased serum cytokine levels associated with the anti-inflammatory Th2 response (IL-4 and IL-10) compared to the placebo group [Tan 2011]. A suppressed Th1 response is associated with higher risk of infectious complications in critically ill brain injured patients; authors hypothesized that probiotics may prevent Th1 suppression [Meisel 2005].

2.1.5 *L. rhamnosus* GG as an intervention: The term 'probiotic' is non-specific and includes a large and variable number of species of microorganisms. A recent consensus report on probiotic science outlined the importance of clearly defining not only the genus and species of probiotic to be studied but also the strain, as some effects of probiotics are strain-specific [Forsythe 2011]. *L. rhamnosus* GG, a variant to *L. casei rhamnosus* is the most widely studied in adults and children [Gorbach 2000]. *L. rhamnosus* GG is approved in Canada for managing and preventing antibiotic-associated diarrhea (Health Canada Natural Product # 80011341). *L. rhamnosus* GG has been tested in critically ill patients in 2 trials [Morrow 2010, Ferrrie 2011]. One trial (described in Section 2.1.3) found *L. rhamnosus* GG (a dose of $2x10^9$ of *L. rhamnosus* GG twice daily; $1x10^9$ was ingested and $1x10^9$ was used as a mouthwash) reduced the incidence of VAP in 146 patients at high risk of VAP compared to placebo [Morrow 2010]. Another small trial compared *L. rhamnosus* GG ($1x10^{10}$ ingested twice daily) to placebo as a therapy in 36 critically ill patients with diarrhea and found no difference in diarrhea duration [Ferrie 2011]. Neither trial observed adverse events related to *L. rhamnosus* GG [Morrow 2010, Ferrie 2011]. Indeed, *L. rhamnosus* GG has an excellent safety profile, as discussed further in Section 2.6.
For the PROSPECT Pilot Trial, we used $1x10^{10}$ *L. rhamnosus* GG twice daily. Recent *in vivo* studies in mice showed that a dose of *L. rhamnosus* GG of $\geq 1 \times 10^9$ was optimal to reduce bacteremia and mortality from peritonitis and pneumonia. This dose could reduce intestinal epithelial apoptosis and restore colonic epithelial cell proliferation, and attenuate the local and systemic inflammatory response, potentially by down-regulation of TLR-2/TLR-4 signaling pathway in the colon. [Wischmeyer 2012]. The dose we will use is higher than the dose showing prevention of VAP in the Morrow RCT (total of 4 billion *L. rhamnosus* GG) [Morrow 2010] and the same dose as the other RCT using *L. rhamnosus* GG in ICU patients (total of 20 billion *L. rhamnosus* GG) [Ferrie 2011]. For the PROSPECT Trial we will use $1x10^{10}$ *L. rhamnosus* GG twice daily.

2.2 Research Questions

Research Question for PROSPECT Pilot Trial: Is it feasible to perform a large RCT in mechanically ventilated critically ill patients to investigate whether enteral *L. rhamnosus* GG prevents VAP, based on successful and timely pilot trial recruitment; high adherence to protocol; minimal contamination; and an acceptable VAP rate? We originally hypothesized that a full scale adequately powered RCT would be feasible, and confirmed this in the PROSPECT Pilot Trial, the results of which are reported below.

Research Question for Main PROSPECT Trial: What is the effect of enteral *L. rhamnosus* GG on VAP, other ICU-acquired infections, diarrhea (total, antibiotic associated and *Clostridium difficile*-associated diarrhea), antibiotic use, duration of mechanical ventilation, ICU and hospital length of stay, ICU and hospital mortality compared to placebo among mechanically ventilated critically ill patients? We now hypothesize that *L. rhamnosus* GG will decrease rates of VAP and other ICU-acquired infections, and decrease all-cause, antibiotic-associated diarrhea, and *Clostridium difficile*-associated diarrhea.

2.3 Why is a trial needed now? First, the clinical and economic burden of VAP remains high [Safdar 2005] (Section 2.1.1). Second, use of existing VAP prevention strategies are variable but disappointing in Canada [Sinuff 2013] (Section 2.1.2); thus, a simple inexpensive VAP prevention strategy could further lower VAP rates. Third, VAP continues to be a key

'quality indicator' for most hospitals in Canada [www.saferhealthcarenow.ca]) which motivates identification of inexpensive VAP prevention strategies that can be successfully implemented and which do not involve antibiotics, given the worldwide resistance problem [Kollef 2011]. Fourth, meta-analyses of small trials often yield results discordant with results of large trials; larger trials tend to show more modest treatment effects [Nuesche 2010], and single-center trials tend to show larger treatment effects than multicenter trials, even after adjusting for sample size [Deschartres 2011]. As such, a large multicenter RCT is required to better understand the treatment effect of probiotics in the prevention of VAP in critically ill patients. Fifth, probiotics are a promising method to prevent VAP, with biologic plausibility and clinical promise, but existing data do not support their widespread use and more robust evidence is needed before 'indication creep' occurs.

2.4 Systematic reviews and this trial in the light of these reviews: As detailed in Section 2.1.3, 2 systematic reviews and meta-analyses have summarized the evidence of probiotic use for prevention of VAP; one in 2010 [Siempos 2010] and ours [Petrof 2012]. Both meta-analyses concluded that although promising for VAP prevention, further research is needed before probiotics can be recommended [Siempos 2010, Petrof 2012]. There are other additional recent systematic reviews on this topic, offering the same message [Wang 2014, Bo 2014].

A recent Cochrane Review [Bo 2014] including 8 RCTs enrolling 1083 patients compared a form of probiotic (*Lactobacillus casei rhamnosus*; *Lactobacillus plantarum*; Synbiotic 2000FORTE; Ergyphilus; combination *Bifidobacterium longum* + *Lactobacillus bulgaricus* + *Streptococcus thermophilus*) versus a control group (placebo; glutamine; fermentable fibre; peptide; chlorhexidine). Probiotics significantly decreased the incidence of VAP (odds ratio (OR) 0.70, 95%CI 0.52 to 0.95, low quality evidence). Although estimates do not suggest harm and trends suggest benefit, the aggregated results were uncertain for ICU mortality (OR 0.84, 95%CI 0.58 to 1.22 very low quality evidence), hospital mortality (OR 0.78, 95% CI 0.54 to 1.14, very low quality evidence), length of ICU stay (mean difference (MD) -1.60, 95% CI -6.53 to 3.33, very low quality evidence), duration of mechanical ventilation (MD -6.15, 95% CI -18.77 to 6.47, very low quality evidence). Antibiotics for VAP were used for a shorter duration when patients received probiotics in one small trial (Mean Difference -3.00 days, 95% CI -6.04 to 0.04). Only 3 RCTs reported diarrhea and found a trend toward a lower incidence (OR 0.72, 95% CI 0.47 to 1.09, very low quality evidence). There were no reports of nosocomial probiotic infections. The overall quality of these RCTs, based on a risk of bias assessment, was moderate; half of the trials had low risk of bias while the other half were at high risk of bias across one or more domain. An intention-to-treat analysis yielded similar results to the per-protocol analysis. This Cochrane review concluded that trials to date do not provide sufficient evidence to draw conclusions on the efficacy and safety of probiotics for the prevention of VAP in the ICU.

We searched www.clinicaltrials.gov and found no new RCTs using enteral probiotics for VAP prevention that are planned, underway or completed.

2.5 The PROSPECT Pilot Trial Results: The PROSPECT Pilot Trial was designed to be as rigorous as possible, according to published guidelines [Arnold 2009, Thabane 2010]. It was conducted in 14 ICUs: 8 in Ontario [St. Joseph Healthcare, Hamilton (1 unit), Hamilton Health Sciences, Hamilton (2 units), St. Michael's Hospital, Toronto (1 unit), Mount Sinai Hospital (1 unit), Ottawa Health Research Institute (2 units) and the University Health Network – Toronto General Hospital (1 unit)]; 1 in Québec [CHU de Québec-Hôpital de l'Enfant-Jésus (3 units)]; 3 in British Columbia [St Paul's Hospital (1 unit), Vancouver General (1 unit) and the Royal Jubilee Hospital, Vancouver Island (1 unit)]; and 2 in the United States of America: Mayo Clinic, Rochester Minnesota (1 unit) and the St. John's Mercy Hospital, St. Louis, Missouri (1 unit).

The PROSPECT Pilot Trial feasibility objectives were: 1) Timely recruitment of 150 patients over the period of 1 year; 2) Maximal protocol adherence, defined as successful if ≥90%

of prescribed doses are actually administered; 3) Minimal contamination, defined as successful if ≤5% of patients receive a single dose of open-label probiotics; and 4) Minimal VAP rates, defined as successful if ~10% patients develop VAP overall. No significance testing between groups was needed to analyze the PROSPECT Pilot Trial feasibility objectives; patients were analyzed as a single cohort. 1) 150 patients were enrolled over 11 months. Recruitment was 1.8 patients per month among actively recruiting centers. 2) Adherence of study product was 96.3% of doses prescribed were doses actually received. 2) Contamination did not occur; no patients received a dose of open-label probiotic at any time. 4) The clinically suspected VAP rate was 15%. Other diagnostic criteria for VAP led to different pneumonia rates, as predicted.

Successful completion of the PROSPECT Pilot Trial led to extension to the PROSPECT vanguard phase enrolling 250 patients. The protocol described in this document is for the PROSPECT Main Trial.

2.6 Describe any risks to the safety of participants involved in this trial: In healthy persons, the pathogenic potential of *L. rhamnosus* GG is extremely low. A 4 year population-based study analyzing the prevalence of *Lactobacillus* spp. in Finland where *L. rhamnosus* GG is heavily consumed (>3 million kg/year), revealed 8/3,317 (0.24%) positive blood cultures positive for *Lactobacillus* spp. isolates; none were the *L. rhamnosus* GG strain [Saxelin 1996]

L. rhamnosus GG has been tested in 2 trials enrolling 174 critically ill patients [Morrow 2010, Ferrie 2011] and no adverse events were reported. However, reports of infection related to the ingestion of *L. rhamnosus* GG are published. In a review evaluating the safety of probiotics in patients receiving nutritional support, 5 cases of *L. rhamnosus* GG bacteremia were found [Whelan 2010]. All arose in children, all with co-morbidities including congenital heart disease, neurological disorder and short gut syndrome [Whelan 2010]. To our knowledge, there has only been one published adult case report of *L. rhamnosus* GG infection [Rautio 1999]. A 74-year old woman was admitted to hospital with acute onset fever and mild abdominal pain, and found to have a liver abscess; culture of the liver abscess grew *Lactobacillus* spp. and the strain was confirmed as *L. rhamnosus* GG. When questioned, the patient reported consuming one-half litre of dairy drinks containing *L. rhamnosus* GG daily [Rautio 1999]. We will exclude patients with structural heart disease, gastroesophageal or intestinal injury, and those at increased risk of an endovascular infection (e.g., long-term indwelling dialysis catheters). *Lactobacillus* spp. infection has been documented in immunosuppressed patients including those with advanced HIV and post-transplantation, likely related to disruption of endogenous microbiota [Oggioni 1998, Kalima 1996, Ledoux 2006]. Thus, patients with HIV (CD4 count <200cells/μL) and those on chronic immunosuppressive medications will also be excluded from our trial.

The safety of probiotics in critically ill patients was further evaluated in an Agency for Healthcare Research and Quality systematic review [Hempel 2011]. Of 17 RCTs reporting on adverse events (almost all of which included *Lactobacillus* strains), ICU patients taking probiotics were not more likely to have adverse events than control patients with similar health status (RR 0.79; 95% CI 0.51 - 1.22). No differences in GI adverse events were observed (RR 0.91; 95% CI 0.56-1.50), or infections (RR 1.15; 95% CI 0.70-1.88), or other adverse events (RR 0.88; 95% CI 0.72-1.08). One of 17 RCTs showed higher mortality [24/152 (16%) versus 9/144 (6%), RR 2.53, 95% CI 1.22 - 5.25] [Besselink 2008]. This trial used the probiotic Ecologic 641, containing 6 different strains of bacteria in patients with severe pancreatitis. Suspected reasons for the higher risk of death include virulence of the probiotic, use of in insoluble fiber, and nasojejunal administration to patients with severe pancreatitis and locally impaired GI integrity [Besselink 2008]. Patients with severe pancreatitis will also be excluded from our trial.

In summary, although we consider it very unlikely, probiotics may confer an increased risk of acquired infections in some critically ill patients. We have designed appropriate exclusion

criteria with safety in mind, without unduly affecting the generalizability of the results. Please see section 3.8 for further information on serious adverse events (SAEs).

3.0 THE PROPOSED TRIAL

3.1 What is the proposed trial design? A stratified parallel group blinded RCT in which patients will be randomized to placebo or probiotic in a fixed allocation ratio of 1:1 (*L. rhamnosus* GG).

3.2 What are the planned interventions?

3.2.1 Probiotic: Patients allocated to the intervention group will receive $1x10^{10}$ colony forming units (CFU) of *L. rhamnosus* GG (Culturelle, Locin Industries Ltd) in 1 capsule suspended in tap water, administered through a nasogastric (or orogastric) or nasoduodenal (or oroduodenal) tube twice daily while patients are in the ICU. The first dose will be within 72 hours of intubation. Patients in the ICU who await discharge and can swallow pills will take the capsules *orally*.

The intervention is packaged in sheets of 10. To ensure consistency and quality of the probiotic administered, one capsule from every 10 sheets of both probiotic and placebo will be cultured and quantified in the Surette Laboratory at McMaster University.

3.2.2 Placebo: Patients allocated to the placebo group will receive a capsule identical in appearance to the *L. rhamnosus* GG capsule, but containing microcrystalline cellulose. The placebo will also be suspended in tap water and similarly administered twice a day. When suspended in water, the placebo has identical appearance and consistency as the probiotic. The placebo will be prepared by the manufacturer of *L. rhamnosus* GG, Culturelle, and has been used successfully in a recent RCT in the ICU population [Morrow 2010]. This has also been used successfully in the PROSPECT Pilot Trial.

3.3 What are the proposed practical arrangements for allocating participants?
Allocation will be according to a computer based random number generator produced on the RANDOMIZE.NET website. Randomization will be stratified by center and by status of medical, surgical (directly out of operating room or post-operative recovery room) and trauma (cared for by trauma service), given the possible different VAP rates, and differential compliance between surgical and trauma patients due to *nil per os* (NPO) status. Patients will be randomized in variable unspecified block sizes. Research Coordinators will screen all ventilated patients during weekdays. A de-identified log of screened patients will be kept, recording each inclusion/exclusion criterion. Reason(s) for being eligible non-randomized will be recorded. Once the Research Coordinator determines that a patient is truly eligible, s/he will obtain written informed *a priori* consent either from the patient or substitute decision maker. Then s/he will notify the local Study Pharmacist who will obtain the allocation from the PROSPECT website. All other members of the local research team and the ICU clinical team, patients and families will be blinded to allocation.

3.4 What are the proposed methods for protection against other sources of bias?
Patients will be randomized to probiotics or placebo, stratified by ICU and medical, surgical or trauma status, given possible difference in compliance and co-interventions. Randomization will be concealed to avoid selection bias. Post randomization, identical placebo will ensure blinding of all possible parties (patient, family, bedside clinicians, clinical and laboratory research team laboratory personnel, and biostatistician) to avoid unequal co-interventions, ascertainment bias, outcome modification, and analytic bias. The Data Base Manager and Site Study Pharmacists will necessarily be aware of allocation; these persons have no clinical role in the ICU or

interpretive role in the results. Protocol compliance will be documented and reasons for non-compliance will be recorded daily, along with any contamination by non-study probiotic. Co-interventions will likely be comparable between groups, given the blinded design, but will be recorded daily, including other VAP prevention strategies and antibiotics (type, dose, frequency and duration). We do not anticipate any loss to follow-up in this ICU trial; we are tracking patients only to hospital death or hospital discharge. We will register and publish the trial protocol to avoid publication bias.

3.5 What are the planned inclusion/exclusion criteria? Balancing the foundations of maximum benefit and minimum harm, enrolment criteria address generalizability and safety:

Inclusion criteria:

1) Adults ≥ 18 years of age
2) Admitted to any ICU and receiving invasive mechanical ventilation
3) Anticipated ventilation of ≥72 hours at the time of screening, as per the ICU physician.

Exclusion criteria:

1) Invasively mechanically ventilated ≥72 hours at the time of screening;
2) Patients at potential increased risk of iatrogenic probiotic infection (see Section 2.6 for detailed explanation) including specific immunocompromised populations (HIV <200 CD4 cells/μL, those receiving chronic immunosuppressive medications (e.g., azathioprine, cyclosporine, cyclophosphamide, tacrolimus, methotrexate, mycofenolate, Anti-IL2), previous transplantation (including stem cell) at any time, malignancy requiring chemotherapy in the last 3 months, neutropenia [absolute neutrophil count < 500]). However, patients receiving corticosteroids previously or presently or projected to receive corticosteroids are not excluded;
3) Patients at risk for endovascular infection (previously documented rheumatic heart disease, congenital valve disease, surgically repaired congenital heart disease, unrepaired cyanotic congenital heart disease, any intracardiac repair with prosthetic material [mechanical or bio-prosthetic cardiac valves], previous or current endocarditis, permanent endovascular devices (e.g., endovascular grafts [e.g., aortic aneurysm repair, stents involving large arteries such as aorta, femorals and carotids], inferior vena cava filters, dialysis vascular grafts), tunnelled (not short-term) hemodialysis catheters, pacemakers or defibrillators. Patients with temporary central venous catheters, central venous dialysis catheters or peripherally inserted central catheters (PICCs) are not excluded and patients with coronary artery stents, coronary artery bypass grafts (CABG) or neurovascular coils are not excluded; patients with mitral valve prolapse or bicuspid aortic valve are not excluded providing they have no other exclusion criteria;
4) Patients with a primary diagnosis of severe acute pancreatitis, without reference to a Ranson score [Ranson 1974]). However, patients with mild or moderate pancreatitis are not excluded;
5) Patients with percutaneous gastric or jejunal feeding tubes already in situ as per Health Canada guidance;
6) Strict contraindication or inability to receive enteral medications;
7) Intent to withdraw advanced life support as per the ICU physician;
8) Previous enrolment in this or current enrolment in a potentially confounding trial.

3.6 What is the proposed duration of treatment period? Patients will receive study product from the time of first administration until: 1) death or discharge from ICU; or 2) isolation of *Lactobacillus* spp. in a culture from a sterile site or if reported as the sole or predominant organism in a culture from a non-sterile site; or 3) censored at 60 days from randomization if patient remains in the ICU.

3.7 What is the proposed frequency and duration of follow-up? Patients will be reviewed daily by the Research Coordinator in the ICU, where most information will be collected. This will involve baseline data (e.g., demographics, illness severity, advanced life support), and daily data (e.g., study intervention administration and reasons why not administered, relevant medications including antibiotics and prokinetics, VAP prevention co-interventions, culture results, clinical diagnoses, diarrhea episodes, antibiotic use, length of mechanical ventilation and ICU and hospital stay, ICU and hospital mortality), and Methods Center data (e.g., infection adjudication forms). Vital status at hospital discharge will be documented.

3.8 Serious Adverse Events: The rationale for, and operational details of, our approach to serious adverse events (SAEs) accord with our published guidelines for academic drug trials in critical care [Cook 2008]. SAEs are already incorporated as trial outcomes, defined *a priori.*

Any culture obtained by the ICU clinical team and processed by the clinical microbiology laboratory positive for *Lactobacillus* spp. will be recorded. Also, when possible, the bacterial sample will be sent to the Surette Laboratory at McMaster University for sequencing to determine whether it is consistent with the administered *L. rhamnosus* GG strain. Isolation of *Lactobacillus* spp. in a culture from a sterile site or if reported as the sole or predominant organism in a culture from a non-sterile site will be a criterion for discontinuing the trial intervention. We will be vigilant about monitoring for infection caused by *L. rhamnosus* GG particularly in patients who develop ischemic bowel and who may be at higher translocation risk.

In the Pilot Trial, one patient after 4 days in the trial had *Lactobacillus* identified in a blood culture drawn from an arterial catheter but not the central venous catheter drawn at the same time; his catheters were removed and he was treated with ciprofloxacin for 10 days and recovered. It is unclear whether this *Lactobacillus* isolate represents translocation from the gut, or contamination from the hands of the person breaking the capsule then attending to the arterial catheter. This was classified as an adverse event according to the St. Joseph's Healthcare REB definitions. In the Pilot Trial, no SAEs were documented. An SAE definition is any adverse occurrence or event, or response to a drug/intervention, whether expected or not; that requires in-patient hospitalization or prolongation of existing hospitalization; that results in persistent or significant disability/ incapacity; or is a congenital anomaly/birth defect; that is life threatening; that results in death.

We will record all SAEs, anticipating they will be captured as one of the trial outcomes, as per our definitions [Cook 2008]. Any events that ICU physicians or Site Investigators label as unexpected will be described fully. These will be collated and submitted to the independent Data Safety Monitoring Board.

3.9 Outcomes for the PROSPECT Main Trial: The primary outcome for the main PROSPECT RCT is VAP. Secondary outcomes will include other ICU-acquired infections, diarrhea (total, antibiotic-associated and *Clostridium-difficile* associated), antibiotic use, duration of mechanical ventilation, ICU and hospital stay, and ICU and hospital mortality.

a) The primary outcome of the main trial will be VAP. VAP will be diagnosed clinically at each site in patients who are receiving invasive mechanical ventilation for at least 48 hours, when there is a new, progressive or persistent radiographic infiltrate with no other obvious cause and the presence of any 2 of the following symptoms or signs: 1) fever (temperature >38°C) or hypothermia (temperature <36°C as measured by core body temperature); 2) relative neutropenia (<3.0 x 10^6/L) or leukocytosis (>10 x 10^6/L) and 3) purulent sputum [Grossman 2000]. Presence of a pathogen is not a criterion for the diagnosis of VAP, given the prevalence of culture negative pneumonia. In addition to rates according to the foregoing definition, we will report clinically suspected and treated VAP rates, the Clinical Pulmonary Infection Score [Pugin 1991], incidence rates (cases/1000pt-days), and overall respiratory infection rates (comprised of

any pneumonia following randomization occurring in the ICU, tracheobronchitis, empyema and/or lung abscess).

b) Any infection acquired during the ICU stay, defined as respiratory or other infections including bloodstream infections, intravascular catheter-related sepsis, intra-abdominal infections, urosepsis and surgical wound infections. These individual infections will be defined using an adaptation of the International Sepsis Forum Consensus Conference on Definitions of Infections in the ICU [Calandra 2005], as adapted for REDOXS [Heyland 2013] and ABATE [Sinuff 2013].

c) *Clostridium difficile*-associated diarrhea: 3 or more episodes of unformed stools in ≤24 hours and *C. difficile* toxin positive stool or colonoscopic or histopathologic findings demonstrating pseudomembranous colitis [Cohen 2010];

d) Antibiotic-associated diarrhea [Bartlett 2002]; and defined as more than 2 liquid stools a day for 3 or more days in quantities in excess of normal for each patient [Hickson 2007];

e) Diarrhea: defined as 3 or more loose or watery bowel movements [WHO 2013], according to the Bristol Stool Chart (type 6 or 7) and use of a fecal management device;

f) Antibiotic use (defined daily dose (DDD); daily doses of therapy (DOT), and antibiotic-free days in ICU)

g) Duration of mechanical ventilation by endotracheal tube or tracheostomy, length of ICU stay and length of hospital stay: recorded as number of days;

h) ICU mortality and in-hospital mortality: recorded at ICU discharge and hospital discharge.

3. 10 Will health service research issues be addressed? As cost surrogates, we will collect length of ICU and hospital stay in the PROSPECT Pilot and main trial. Relative to other VAP prevention strategies, probiotics are very inexpensive. We plan to write an independent grant for a cost-effectiveness study (E-PROSPECT).

3.11 What is the proposed sample size for the Main PROSPECT Trial?

For the main PROSPECT Trial, the primary objective is to determine the effect of enteral *L. rhamnosus* GG on VAP compared to placebo among mechanically ventilated critically ill patients. Widely cited data from quality of care and patient safety initiatives are seriously biased by under-reporting. By contrast, adjudicated VAP rates from a recent Canadian study of unselected ICU patients ventilated >48 hours was 8% [Sinuff 2013]. The adjudicated VAP rate in patients with 2 organ dysfunctions enrolled in our recent REDOXS trial was 14% [Heyland 2013]. In the PROSPECT Pilot Trial, the clinically suspected and treated VAP rate was 15%.

The sample size is 2650 patients. Based on an estimated 15% VAP rate, 2650 patients (1325/group) will be required to detect a 25% relative risk reduction with 80% power (alpha 0.05, beta 0.80). The 25% RRR was the observed RRR in our meta-analysis of probiotics versus placebo [Petrof 2012]. The sample size calculation was based on the Chi square test of the null hypothesis that rates of VAP in the 2 arms (enteral *L. rhamnosus* GG and placebo) are equal. The criterion for significance (alpha) has been set at 0.05, and the allocation ratio was set at 1:1.

We will enrol 1325 patients/arm or 2650 patients. We project enrolling in 38 centers an average of 1.8 patients/month over 12 months = 821 patients/year over 3 years = 2462 (in addition to the 250 patients previously enrolled during the Pilot Trial and Vanguard Phase) = 2712. This projection allows any for randomization errors, consent withdrawals and non-enrolment weeks (e.g., Research Coordinator illness, vacation, any termination and rehiring).

The Canadian Critical Care Trials Group has studied the many reasons why eligible patients are not enrolled in an ICU trial. A recent Canadian multicenter study documented that 57% of opportunities to recruit eligible patients are either missed or not realized for many reasons [Burns 2013]. Potentially modifiable reasons include research team workload and availability, narrow time windows for inclusion, co-enrolment prohibition and physician refusals. We endorse co-enrolment with scientific, ethical and logistic provisos [Cook 2008]. During the

PROSPECT Pilot we observed a 30% co-enrolment rate. Because probiotics are widely marketed in the lay and medical press, we predict high clinician and citizen interest. We project a consent rate of ≥80% (it was 82% for PROTECT, testing a familiar intervention (blood thinners) and known outcome (leg clots) [PROTECT 2011]), and 82% for the PROSPECT Pilot Trial.

3.12 Are there likely to be any problems with compliance? On what evidence are the compliance figures based? We were sufficiently concerned about compliance that protocol adherence was one of our PROSPECT Pilot Trial feasibility objectives. Based on pilot compliance of over 95%, we are satisfied that satisfactory compliance will continue. High compliance was achieved in other blinded drug RCTs such as PROTECT [PROTECT 2011] and other blinded supplement trials such as REDOXS [Heyland 2013]. Estimates of compliance are further informed by knowledge of the excellent ICU research infrastructure of participating institutions.

3.13 What is the likely rate of loss to follow-up? On what evidence is the loss to follow-up rate based? We do not anticipate any patients lost to follow-up given the hospital time horizon for vital status ascertainment, and the ICU stay for key data collection. Indeed, our group has followed 100% of ICU survivors successfully to hospital discharge, and we have not 'lost' one patient in hospital over 24 years of ICU research.

3.14 Planned analyses: Patients will be analyzed according to the intention to treat principle. Interim analyses are planned at one third and two-thirds of enrolment using the Haybittle-Peto method. We plan to evaluate the primary endpoint using the Haybitte-Peto Method using a fixed simple conservative p=0.001 for each of the 2 interim analyses at one third and two thirds of projected total enrollment [Haybitte 1971, Peto 1976]. We will compare the proportion of patients in the 2 groups with the primary and secondary outcomes using the Mantel-Haenszel Chi square test or the Fisher exact test. We will calculate the relative risk reduction, absolute risk reduction with 95% confidence intervals. If appropriate, we will calculate metrics such as the number needed to treat or number needed to harm. These may be expressed as the number needed to treat with probiotics during the ICU stay to prevent 1 case of VAP or 1 case of *Clostridium difficile*. For durations of mechanical ventilation, ICU and hospital stay, we will compare the 2 arms using a non-parametric approach, presenting medians and interquartile range because these data are usually skewed. We plan subgroup analysis for medical, surgical and trauma patients using a Mantel-Haenszel Chi square test. We will perform sensitivity analyses to adjust for potential centre effects [Thabane 2013].

Patients will be analyzed according to the intention to treat principle. All tests of significance will be at the 5% significance level, and 2 sided.

4.0 TRIAL MANAGEMENT

4.1 What are the arrangements for day to day management of the trial? The PROSPECT Project Coordinator is a Registered Nurse with extensive experience running ICU trials, including a recently completed international thromboprophylaxis trial (NZ) [PROTECT 2011]. The Clinical Advances through Research and Information Translation (CLARITY) Research Group at McMaster University is responsible for overall management. Three co-applicants are CLARITY faculty (DC, LT, MM) who consult to clinical investigators around the world. Our staff has a wealth of experience in implementation of pilot studies and large international, national, and provincial randomized trials and observational studies. We have the requisite breadth and depth of knowledge in study design, implementation, and biostatistics. CLARITY infrastructure affords a unique, cost-effective opportunity to generate new clinical knowledge about probiotics. We have a wealth of experience and publications on calibration, adjudication and efficiencies therein

for VAP [Cook 1998], bleeding [Cook 2009, Arnold 2013], lung cancer [Walter 1997] and thrombosis [Saunders 2011, Zytaruk 2009, Lamontagne 2009].

As PROSPECT is ongoing, recruitment will be reviewed monthly through screening logs; eligible patients who are missed will be examined. If applicable, barriers to enrolment will be discussed and strategies to improve recruitment devised base on prior trials. Thus, recruitment will be maximized as necessary. Research Coordinators will review the medication profile daily to determine doses actually received and reasons for non-administration using a taxonomy. Protocol deviations (e.g., late administration) will be distinguished from protocol violations (e.g., missed doses) in characterizing non-adherence. Research Coordinators will submit relevant clinical, radiologic, and microbiologic data to the Methods Center from patients with suspected VAP. We will use our website as a communication tool as well [www.prospecttrial.com].

4.2 Steering Committee & Advisors: The PROSPECT Steering Committee includes Drs. Cook (Chair), Johnstone, Meade, Lauzier, Thabane and Marshall**.** Our experienced Steering Committee will be responsible for the conduct of this trial, for upholding or modifying study procedures as needed, addressing challenges with protocol implementation, refining the protocol as needed, reviewing and interpreting the data, and preparing the abstracts and manuscripts. Steering Committee meetings will be held in-person or by conference call quarterly.

The PI is internationally recognized for her ICU trials and VAP knowledge; she has led several large multinational CIHR funded studies. She will meet with the PROSPECT Project Coordinator weekly. In terms of the Steering Committee, Dr. Johnstone is an Infectious Diseases physician who completed her PhD in the McMaster Health Research Methodology Program, and whose Independent Study protocol stimulated this grant (supervised by Drs. Meade and Cook). As an expert in respiratory infections and their prevention, she recently led a multi-center CIHR funded study on influenza in 4 Canadian centers as part of her PhD. Dr. Meade is an international expert in ICU trials who will provide valuable insights into RCT methodology and overcoming operational challenges. Dr. Lauzier is a clinician-scientist with a strong academic track record of peer-reviewed projects who will offer scientific and practical advice. Dr. Thabane will provide biostatistical advice and trial methods support throughout the pilot. Dr. Marshall is an international trialist with expertise in infection in critical care, holding many grants for both basic and clinical sciences. Drs. Cook, Meade, Lauzier, and Marshall will also be Site Investigators overseeing patient enrolment in their center. Drs. Dawn Bowdish and Michael Surette will be PROSPECT Advisors, and provide critical immunological knowledge and infrastructure for performing culture-independent techniques and microbiome analysis in a PROSPECT mechanistic substudy (funded separately).

PROSPECT assimilates experts with a strong track record in clinical (MM, FL, DC) and basic science (JJ, MS, DB, JM), biostatistics (LT), critical care (MM, FL, JM, DC), infectious disease (JJ, DB, MS), immunology (JJ, JM, DB, MS) and microbiome research (MS). We are clinical epidemiologists (MM, FL, JJ, DC), and a biostatistician (LT). Our mentorship of new investigators is reflected in a senior (MM, JM, MS, DC) and junior (JJ, FL, DB) team; 3 of us have held CIHR Mentoring Awards (MM, DC, LT). We are CIHR-funded (JJ, MM, LT, FL, JM, MS, DB, DC), and NIH-funded (DB) scientists, with a CIHR Fellowship Award (JJ), a provincial career award (FL), and 2 Canada Research Chairs in Interdisciplinary Microbiome Research (MS) and Critical Care (DC).

4.3 Clinical Site Investigators: PROSPECT will be conducted in collaboration with many key Clinical Site Investigators. These leads have the expertise, scholarship and scientific track record to conduct randomized trials in today's complex research environment.

4.4 The Canadian Critical Care Trials Group: Dedication to this trial through the CCCTG is reflected in many Investigators being members of the CCCTG. The mission of the CCCTG is to

conduct clinically relevant, rigorous studies to understand and address diagnostic, preventive, therapeutic or palliative issues in critical illness [www.ccctg.ca]. The CCCTG is dedicated to improving the process of care and outcomes for critically ill patients [Cook 2002, Marshall 2009].

5.0 ETHICAL ISSUES:

When carefully evaluated in the ICU setting, probiotics may have salutary effects decreasing nosocomial infections such as VAP; alternatively, probiotics may have no demonstrable effect, or actually cause iatrogenic infections in ICU patients with impaired immune function. As responsible investigators, we believe that we have an ethical imperative to understand the impact of probiotics before they become encoded in practice.

The PROSPECT Trial protocol and informed consent forms will be approved by each hospital's Research Ethics Boards. The trial will be conducted in accordance with Good Clinical Practice following the Tricouncil Guidelines. Substitute decision makers and patients, when they are able, will provide written informed consent before randomization (*a priori* consent). Consenting persons will be informed that their care will not be affected in any way should they decide to refuse participation or withdraw from the trial. Confidentiality will be maintained by coded identification, password protected files and websites, locked filing cabinets and offices.

The PROSPECT Trial will have a 3-member independent Data Safety and Monitoring Committee. The primary roles of the DSMC will be ongoing independent review of reports received directly from the Methods Center regarding: 1) regular study progress; 2) procedures such as randomization, crossovers, and protocol adherence; 3) indicators of trial management (e.g. enrolment, consent); 4) efficacy and safety reports including serious adverse events; and 5) 2 interim and final analyses. The DSMC will advise the Steering Committee on these issues. It will guard the safety of the participants at all times. The DSMC will monitor performance reports to detect deficiencies in data collection processes, and recommend corrective action as needed. The DSMC will receive all reports from the PROSPECT Statistician, they will evaluate these, and disseminate their reports to the Steering Committee, PROSPECT Investigators and local REBs as per their terms of reference. We have adopted the DSMB charter from the DAMOCLES Study Group outlining DSMC roles, responsibilities, and reporting relationships [Damocles 2015]. The DSMC charter will be reviewed and modified by the DSMC as necessary.

6.0 KNOWLEDGE TRANSLATION:

Ongoing PROSPECT Trial knowledge translation will be as per Methods Center trial management, and will be facilitated by our website [www.prospectpilottrial.com]. Centers will be provided with timely, detailed site-specific feedback. We will update our peers in the CCCTG on progress, any problems and their solutions, at the thrice yearly meetings until the trial is complete.

More traditional end-of-grant KT activities of the PROSPECT Pilot Trial will include provision of feasibility results for circulation to multidisciplinary colleagues via email and in-person. Our guideline research in the ICU underscores the increasing preference, particularly of ICU nurses, for web-based educational tools; we will provide these through PowerPoint presentation slide decks and structured abstracts of our results. Structured abstracts and executive summary reports will be offered to pharmacists. PROSPECT investigators will be encouraged to share final feasibility results with a broad range of local stakeholders including local quality improvement teams and colleagues at grand rounds, research symposia, and similar hospital events. In selected fora, this information will help to garner awareness and interest in the future trial. We also plan to share PROSPECT Trial results via the Canadian Critical Care Society (CCCS) periodic publication called Critical Care Rounds. The KT Sub-Committee of the CCCTG and the CCCS will assist us further in dissemination to our ICU community.

PROSPECT Investigators will coauthor several abstracts and 2 manuscripts arising from the Pilot Trial (the protocol paper and the results manuscript), as well as other abstracts and manuscripts from the PROSPECT Main Trial. All participating centers will have an opportunity to contribute to the manuscripts prior to peer review journal submission. We will present results at local, national, and international professional society meetings for ICU and infectious disease clinicians. Concurrently, each 'in press' abstract or manuscript will be sent to a local institutional media specialist for revising the academic message into press releases to suit the lay public or other target audiences such as hospital employees. Possible target hospital media include newsletters, emails and intranet bulletins. Possible target public media include newspapers, radio and television. High citizen awareness and consumption of probiotics suggest that media will be interested. With each of the foregoing initiatives, our funders and iHealth will be acknowledged.

7.0 SUMMARY:

Before launching the PROSPECT Trial testing whether probiotics confer benefit, harm, or have no impact on infectious and non-infectious outcomes, we successfully completed the PROSPECT Pilot Trial. It met all 4 feasibility objectives of timely recruitment; high adherence to protocol; minimal contamination; and an acceptable VAP rate. The PROSPECT Pilot Trial also helped to refine pharmacy randomization and product preparation, finesse consent documents, develop, and pretest CRFs. Results have helped to estimate realistic timelines for successful large trial completion.

With the help of coinvestigators, supporters, funders and colleagues, we look forward to launching the PROSPECT Trial in collaboration with the Canadian Critical Care Trials Group.

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Appendix A. Summary Table of all RCTs Evaluating Probiotics for VAP prevention in Critically Ill Patients

Lead Author Journal Year (Country)	Inclusion Criteria	Intervention	Control	Dose	Proportion with suspected VAP (Intervention vs. Control)	Risk of Bias (low, high or unclear)	Definition of VAP
Tan [2011] Crit Care 2011 (China)	Adults with severe closed head injury requiring ICU	Golden Bifid*	-	7 sachets NG TID x 21d	7/16 (44%) vs. 13/19 (68%)	*Randomization:* Low *Concealment:* Low *Blinding:* High	CDC criteria
Morrow [2010] Am J Respir Crit Care Med 2010 (USA)	Adults, expected ventilation ≥72h	*L. rhamnosus* GG	Placebo	$2x10^9$ CFU BID – 1 to mouth, 1 to NG until extubation	17/68 (25%) vs. 33/70 (47%)	*Randomization*: Unclear *Concealment*: Unclear *Blinding*: Low	ACCP + microbially confirmed
Barraud [2010] Intensive Care Med 2010 (France)	Adults, expected ventilation ≥48h	*Ergyphilus* (mainly *L. rhamnosus* GG)	Placebo	5 capsules OD ($2x10^{10}$ CFU/capsule) while ventilated	23/87 (26%) vs. 15/80 (19%)	*Randomization*: Low *Concealment*: Low *Blinding*: Low	X-ray and one of purulent secretions/fever/ increased WBC and positive culture from BAL
Knight [2009] Intensive Care Med 2009 (UK)	Adults, expected ventilation ≥48h	Synbiotic 2000 Forte**	Placebo	10^{10} bacteria/ sachet BID NG from <24h until day 28, death or discharge from ICU	12/130 (9%) vs. 17/129 (13%)	*Randomization*: Low *Concealment*: Low *Blinding*: Low	ACCP + radiologist and microbiologist had to agree on diagnosis
Besselink [2008] Lancet 2008 (Netherlands)	Adults with first episode of severe pancreatitis	Ecologic 641***	Placebo	10^{10} bacteria/ sachet BID NJ <72h from onset of pancreatitis until day 28, resolution of pancreatitis, death or infection of pancreatic necrosis	24/152 (16%) vs. 16/144 (11%)	*Randomization*: Low *Concealment*: Unclear *Blinding*: Low	Cough, dyspnea, X-ray, lowered blood gas with positive sputum culture
Forestier [2008] Crit Care 2008 (France)	Adults, expected ICU stay≥48h, NG in place	*L. rhamnosus* Lcr35	Placebo	10^9 CFU BID NG from day 3 until ICU discharge or death	24/102 (24%) vs. 24/106 (23%)	*Randomization*: Low *Concealment*: Unclear *Blinding*: Low	CDC/NHSN criteria
Klarin [2008] Crit Care 2008 (Sweden)	Adults, expected ICU stay ≥24h	*L. plantarum* 299 (DSM 6595)	Chlorhex-idine wash	Oral decontam-ination with 10ml of 10^{10} CFU from randomization to extubation	1/23 (4%) vs. 3/21 (14%)	*Randomization*: Unclear *Concealment*: Unclear *Blinding*: High	X-ray +3/4 criteria: purulent, positive culture, temperature, increased WBC

Spindler-Vesel [2007] J Parenter Enteral Nutr 2007 (Slovenia)	Adult trauma patients, expected ICU stay ≥4 days	Synbiotic 2000**	No prebiotic	10^{10} CFU within 24hrs of injury until day 7	4/26 (15%) vs. 34/87 (39%)	*Randomization*: Unclear *Concealment*: Low *Blinding*: High	CDC criteria
Kotzampassi [2006] World J Surg 2006 (Greece)	Adult trauma patients, expected 'long' ICU stay	Synbiotic 2000 Forte**	Placebo	10^{11} CFU (1 sachet)/day x 15d	19/35 (54%) vs. 24/30 (80%)	*Randomization*: High *Concealment*: High *Blinding*: Low	ACCP

VAP - ventilator associated pneumonia; ICU - intensive care unit; h – hours; d – day(s); NG – nasogastric; CDC – Center for Disease Control; CFU – colony forming unit; ACCP – American College of Chest Physicians; WBC – white blood cell count; BAL – bronchoalveolar lavage; NJ – nasojejunal; NHSN – National Health and Safety Network

*Golden Bifid – Combination probiotic including *Bifidobacterium longum*, *Lactobacillus bulgaricus*, *Streptococcus thermophiles*

**Synbiotic 2000 Forte – A combination of probiotics and prebiotics including *Pediococcus pentoseceus*, *Leuconostoc mesenteroides*, *Lactobacillus paracasei* ssp 19 and *Lactobacillus plantarum* 2362 as well as inulin, oat bran, pectin and resistant starch

***Ecologic 641 – *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus salivarius*, *Lactococcus lactis*, *Bifidobacterium bifidum* and *Bifidobacterium lactis*

Appendix B.

Updated Meta-analysis of RCTs Testing Probiotics Compared to Placebo for VAP Prevention in Critically Ill Patients
[Petrof 2012].