PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Evaluating Probiotics for the Prevention of Ventilator Associated Pneumonia: A Randomized Placebo Controlled Multicenter Trial Protocol and Statistical Analysis Plan for PROSPECT
AUTHORS	Johnstone, J; Heels-Ansdell, Diane; Thabane, Lehana; Meade, Maureen; Marshall, John; Lauzier, Francois; Duan, Erick; Zytaruk, Nicole; Lamarche, Daphnee; Surette, Michael; Cook, Deborah

VERSION 1 - REVIEW

REVIEWER	Joan Robinson University of Alberta Canada
REVIEW RETURNED	01-Aug-2018

GENERAL COMMENTS	Following a successful pilot study, the authors are part-way through an RCT of probiotics versus placebo for prevention of VAP. Major comments (page numbers are from the pdf and are one higher than the page numbers written on each page). 1. The title should provide the fact that this is a trial in adults. 2. One main comment is that the methods are often not described in sufficient detail. Methods need to be reported in sufficient detail that another research team could take over the study after reading this manuscript and would do it as the authors intended. Many definitions are missing or vague (see multiple comments below). 3. Page 7 – Line 35 – It would be helpful to refer to the footnote below the table that shows how the exclusion criteria were modified from the pilot study. 4. Page 10 – Secondary outcomes – Classification of pneumonia that occurs exactly 2 days after extubation is not clear (?is it late VAP versus post-extubation pneumonia). It looks like post-extubation pneumonia is only recorded as an outcome if the patient is still in ICU; this should specifically be mentioned. One cannot use the VAP definition for someone who is not ventilated so how is post-extubation pneumonia defined? 5. Page 11 – line 7 – It is not clear how the authors used the WHO definition and the Bristol stool chart. I suspect that they had

	to have more than 3 Bristol 6/7 stools to qualify, but why then mention the WHO criteria? 6. Page 11 – line 15 – With regard to AAD, I was unclear on "day of or within 24 hours of any antibiotic" Does that mean that the diarrhea had to start within 24 hours of starting any antibiotic or does it mean that if they were on antibiotics for weeks and got diarrhea the day after the antibiotics were stopped, that was classified as AAD? 7. Page 13 – The definition of "prevalent pneumonia" needs to be clearer as in some places the authors imply that pneumonia within 48 hours of intubation is prevalent whereas in other places they imply that the definition includes only the day of and the day following intubation. To make it more confusing, the inclusion criteria include patients already ventilated for 71 hours. In the real world, are the authors using days or hours and how is this all defined? If they are using days, if a patient is first ventilated at 23:59, how are the days counted? 8. Page 13 – line 25 – This is the first mention of stratified randomization which should be mentioned prior to "statistical analysis". 9. Page 19 – I don't think that the names of the DMSB members belong here. 10. Page 22 – The authors need to provide some detail about the trial in India. What type of patients were enrolled? The title mentions synbiotics which should be mentioned as what if it is the prebiotics that worked? 11. Antibiotic exposure needs to be defined. If a patient is on an oral antibiotics as prophylaxis for a medical condition, does that count? Do inhaled antibiotics count? Minor comments 12. Canada, the US and Saudi Arabia are not the usual three countries for a study. Is there any rationale for this (presumably just interested investigators but if there is another explanation, please provide it). 13. Page 5 – line 10 – This estimate of the incidence of VAP is from a systematic review published 13 yrs ago. Ideally the authors would quote a more recent estimate (such as the one that they quote in their sample size calculation) but if not, it would be helpful to the reader to point out that this review included only adults (who were ventilated > 48 hrs). 14. Page 8 – line 40 - ?omit "as well" 15. Page 9 – line 3 – and again on Page 11 - no need to mention who runs the laboratory 16. Page 10- line 35 – By "in hospital", I assume that the authors mean "prior to hospital discharge" – this wording seems slightly clearer to me. Diarrhea is not defined in "c".

	17. Page 10 – line 51 - ?Tell the reader where the standard definitions came from so they don't have to look up the reference if they want to know. 18. Page 11- line 33-34 – grammar needs fixed 19. Page 12 – The sentence "For example, protocol adherence regarding non-receipt of study product acknowledges sensible bedside decision making, according to metrics from our pre-specified taxonomy" needs reworded as I have no idea what that latter phrase means. 20. Page 13 – line 42 –What do the authors mean by "We will report exposures during the ICU stay such as advanced life supports and relevant cointerventions"? 21. Page 17 – line 40 – It is awkward to attribute a change to a national mandate for a study that is being conducted in three different countries. Can the authors explain this a little better? 22. Page 19 – line 5 – The term "loved one" makes many assumptions which are unfortunately not always true. I would reword. 23. Page 20 – line 47 on – I think that the details about when the DSMB will do their interim analyses belongs on the statistical analyses part of the manuscript. 24. Appendix 1 – I did not find the hypotheses to be useful but perhaps this is a recommended component of the protocol.

REVIEWER	Sarvin Sanaie Tuberculosis and Lung disease Research Center, Tabriz University of Medical Sciences. Tabriz, Iran.
REVIEW RETURNED	21-Oct-2018

GENERAL COMMENTS	1- In the title of the study, PROSPECT (Probiotics: Prevention of Severe Pneumonia and Endotracheal Colonization) Trial should be corrected as : PROSPECT (Probiotics: Prevention of Severe Pneumonia and Endotracheal Colonization Trial). The T stands for trial and "PROSPECT trial" has been stated in some parts of the text which should be corrected as "PROSPECT" or maybe PROSPEC trial. 2- In page 5, line 26: it has been stated that "Probitics …. through modifying the microbiome"; but probiotics have effect on microbiota, not microbiome. 3- What is the rational for choosing a single-microorganism probiotic and its dosage? 4- VAP diagnosis in this trial seems to be just clinically. Isn't any broncoscopy or microbiologic evaluation being performed to have microbiological diagnosis? CIPS criteria are not used for VAP diagnosis/risk stratification in this study. 5- Page 8, line 15: how and based on which scoring system (e.g. APACHE, SOFA, SAPS,…) the illness severity of the patients at the baseline is recorded? 6- How the matching of patients regarding illness severity, comorbidities, organ failures and… has been performed?

	7- Page 8, line 17: VAP prevention strategies should be thoroughly described. Moreover, as adherence of ICU staff to VAP bundle criteria will directly affect the VAP incidence and mortality, the adherence rate should be reported. 8- Energy intake of the patients should be reported. 9- Is GRV being measured in these patients? 10- Gastric intolerance and use of the prokinetic drugs should be defined. 11- Since VAP incidence is higher in nasogastric feeding compared to orogastric feeding, a subgroup analysis comparing the VAP Incidence in these two feeding methods will be valuable. 12- As patients during their ICU stay may need supplemental parenteral nutrition, PEG or postpyloric feeding, these statistics should be considered during subgroup analysis. 13- Is any subgroup analysis regarding MDR pathogen responsible for VAP occurrence being conducted?

REVIEWER	DR NIRANJAN BISWAL PROFESSOR (SENIOR SCALE)OF PEDIATRICS,JIPMER PONDICHERRY-605006,INDIA
REVIEW RETURNED	16-Nov-2018

GENERAL COMMENTS	This is a new study PROTOCOL to prevent infections and related co- morbidities.After its completion it will help in a bigway to save people from sufferings.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Following a successful pilot study, the authors are part-way through an RCT of probiotics

versus placebo for prevention of VAP.

1. The title should provide the fact that this is a trial in adults.

**We have modified the title by identifying the focus on adults and avoiding spelling out the entire acronym in the title, because this can be done in the text. The new title we propose is thus:

Probiotics in Critically Ill Adults:

A Trial Protocol and Statistical Analysis Plan for PROSPECT

2. One main comment is that the methods are often not described in sufficient detail. Methods need to be reported in sufficient detail that another research team could take over the study after

reading this manuscript and would do it as the authors intended. Many definitions are missing or vague (see multiple comments below).

** We have attempted to provide more fulsome detail throughout the manuscript, and believe we have addressed the comments below.

3. Page 7 – Line 35 – It would be helpful to refer to the footnote below the table that shows how the exclusion criteria were modified from the pilot study.

** We have now explicitly mentioned in the text that the full explanation can be found in the footnote of Table 1 (Page 6, Line 5). This approach retains the flow of the manuscript and we hope you agree.

4. Page 10 – Secondary outcomes – Classification of pneumonia that occurs exactly 2 days after extubation is not clear (?is it late VAP versus post-extubation pneumonia). It looks like post-extubation pneumonia is only recorded as an outcome if the patient is still in ICU; this should specifically be mentioned. One cannot use the VAP definition for someone who is not ventilated so how is postextubation pneumonia defined?

**Thank you for this question. We have clarified in the text that post-extubation pneumonia occurs 3 or more days following extubation. The text now reads (Page 9, Line 5), “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).”

5. Page 11 – line 7 – It is not clear how the authors used the WHO definition and the Bristol stool chart. I suspect that they had to have more than 3 Bristol 6/7 stools to qualify, but why then mention the WHO criteria?

**We are documenting each bowel movement in enrolled patients. There is no generally accepted metric for diarrhea in critically ill patients, so we will report diarrhea incorporating 2 definitions: the WHO definition and the Bristol Stool Chart. This is now clarified in the text as follows (Page 10, Line 3), “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].”

6. Page 11 – line 15 – With regard to AAD, I was unclear on “day of or within 24 hours of any antibiotic” Does that mean that the diarrhea had to start within 24 hours of starting any antibiotic or does it mean that if they were on antibiotics for weeks and got diarrhea the day after the antibiotics were stopped, that was classified as AAD?

**We agree this was not clear. The diarrhea would need to follow the antibiotic exposure obviously. The definition in the text now reads (Page 10, Line 7), “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].”

7. Page 13 – The definition of “prevalent pneumonia” needs to be clearer as in some places the authors imply that pneumonia within 48 hours of intubation is prevalent whereas in other places they imply that the definition includes only the day of and the day following intubation. To make it more confusing, the inclusion criteria include patients already ventilated for 71 hours. In the real world, are the authors using days or hours and how is this all defined? If they are using days, if a patient is first ventilated at 23:59, how are the days counted?

** Thank you for this comment. We have taken the opportunity to clarify how we use the term ‘prevalent pneumonia’ throughout the manuscript. We used the term ‘prevalent pneumonia’ to capture any pneumonia present the day of randomization or the day following, as we believe that these prevalent pneumonias could not plausibly be influenced by probiotics. Prevalent pneumonia could be community-acquired pneumonia, healthcare-associated pneumonia or ventilator-associated pneumonia, and thus, the term ‘prevalent’ is independent of the intubation timing but is used in reference to the randomization. In other words, we label the type of pneumonia as per clinical practice, but the word 'prevalent' is in reference to the day of the randomization. We have attempted to make this clearer in the text, and we have now defined prevalent infections (Page 12, Line 7) as, “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.”

**In this trial, we use days rather than hours to inform the classification. The onset of pneumonia is not as clear as the onset of say, a surgical procedure, so we avoid false precision this way. With respect to timing of different types of pneumonia, we have tried to standardize all mention of timing to days, not hours. However, we have retained our original use of the word hours for the trial inclusion criteria since this was used precisely to ascertain eligibility, as per all of our scientific, regulatory and ethics documents. If a patient is first ventilated at 23:30, that would still be counted as day 1 of mechanical ventilation. We used this pragmatic approach as per many other ICU trials, which allows more consistency in application.

8. Page 13 – line 25 – This is the first mention of stratified randomization which should be mentioned prior to “statistical analysis”.

** We agree this should be mentioned prior to the statistical analysis section, and it is captured in the “Consent and Randomization” subheading of the methods as follows (Page 6, Line 13), “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.”

9. Page 19 – I don't think that the names of the DMSB members belong here.

**These have been removed.

10. Page 22 – The authors need to provide some detail about the trial in India. What type of patients were enrolled? The title mentions synbiotics which should be mentioned as what if it is the prebiotics that worked?

**Thank you. We have expanded this section in the Discussion to read as follows (Page 22, Line 2), "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. "

11. Antibiotic exposure needs to be defined. If a patient is on an oral antibiotics as prophylaxis for a medical condition, does that count? Do inhaled antibiotics count?

**Thank you for this point. Whether antibiotics are intended as prophylaxis or treatment, they will count as exposure. We have clarified the definition of antimicrobial exposure in the updated manuscript as follows (Page 10, Line 11): "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."

Minor comments

12. Canada, the US and Saudi Arabia are not the usual three countries for a study. Is there any rationale for this (presumably just interested investigators but if there is another explanation, please provide it).

**Interested sites were welcome to participate in PROSPECT if the investigators and their staff had the requisite expertise. We have a long-standing collaboration with a very academic ICU in Riyadh led by an internationally accomplished trialist, Dr. Y. Arabi, and his ICU is active in the trial.

13. Page 5 – line 10 – This estimate of the incidence of VAP is from a systematic review published 13 yrs ago. Ideally the authors would quote a more recent estimate (such as the one that they quote in their sample size calculation) but if not, it would be helpful to the reader to point out that this review included only adults (who were ventilated > 48 hrs).

**We have now qualified this statement in the Introduction as follows (Page 4, Line 4), “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].”

14. Page 8 – line 40 - ?omit “as well”

**This has been deleted.

15. Page 9 – line 3 – and again on Page 11 - no need to mention who runs the

Laboratory

** Dr. Surette’s name has been removed in the second location in the manuscript. The Surette Laboratory is the official name of this specific laboratory at McMaster, so we retained it in the first spot and hope that this is acceptable.

16. Page 10- line 35 – By “in hospital”, I assume that the authors mean “prior to hospital discharge” – this wording seems slightly clearer to me. Diarrhea is not defined in “c”.

** Thank you for identifying this typographical error. We have clarified the wording as follows (Page 9, Line 11), “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].”

17. Page 10 – line 51 - ?Tell the reader where the standard definitions came from so they don’t have to look up the reference if they want to know.

**We have clarified that the definitions came from the International Sepsis Forum Consensus Conference on Definitions of Infection in the Intensive Care Unit as follows (Page 9, Line 18), “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].”

18. Page 11- line 33-34 – grammar needs fixed

**Thank you. The grammar has been corrected.

19. Page 12 – The sentence “For example, protocol adherence regarding non-receipt of study product acknowledges sensible bedside decision making, according to metrics from our prespecified taxonomy” needs reworded as I have no idea what that latter phrase means.

**We have moved this sentence to help explain our approach to protocol deviations, introduced by the word 'thus' which hopefully helps with clarity. The text now reads, following a more detailed sentence (Page 11, Line 20), “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].”

20. Page 13 – line 42 –What do the authors mean by “We will report exposures during the ICU stay such as advanced life supports and relevant cointerventions”?

**Thank you for the chance to clarify. We will be reporting classical cointerventions in ICU trials (e.g., life supports) which will be expected in a critical care trial report, so we retained this sentence. We will also report other relevant cointerventions specific to this particular trial (e.g., other pneumonia prevention strategies). We have clarified the sentence to read (Page 13, Line 4), “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.”

21. Page 17 – line 40 – It is awkward to attribute a change to a national mandate for a study that is being conducted in three different countries. Can the authors explain this a little better?

**We have clarified this sentence, as we agree it was confusing. We received a grant for PROSPECT from the Canadian National Frailty Network, which encourages all funded investigators to document baseline frailty as a condition of acceptance. The sentence now reads as follows (Page 17, Line 9): “We began measuring frailty in response to a Canadian research mandate [64], and did not start documenting frailty until 483 patients were enrolled.”

22. Page 19 – line 5 – The term “loved one” makes many assumptions which are unfortunately not always true. I would reword.

**We agree - yet do not see the words 'loved one' in this document actually. Please let us know if we have missed it and we will delete this.

23. Page 20 – line 47 on – I think that the details about when the DSMB will do their interim analyses belongs on the statistical analyses part of the manuscript.

**Thank you. We have moved some of the detail into the statistical analysis section.

24. Appendix 1 – I did not find the hypotheses to be useful but perhaps this is a recommended component of the protocol.

**Indeed, this is a recommended component of the protocol and aids in interpreting subgroup results as per Sun et al (Reference 60 in the revised manuscript). We have retained Appendix 1.

Reviewer: 2

1. In the title of the study, PROSPECT (Probiotics: Prevention of Severe Pneumonia and Endotracheal Colonization) Trial should be corrected as: PROSPECT (Probiotics: Prevention of Severe Pneumonia and Endotracheal Colonization Trial). The T stands for trial and "PROSPECT trial" has been stated in some parts of the text which should be corrected as "PROSPECT" or maybe PROSPEC trial.

**Thank you. This change has been made throughout.

2. In page 5, line 26: it has been stated that "Probiotics …. through modifying the microbiome"; but probiotics have effect on microbiota, not microbiome.

**This is a great point and has been corrected.

3. What is the rationale for choosing a single-microorganism probiotic and its dosage?

**We agree our rationale should be included. We have added the following to our Introduction (Page 4, Line 17), "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]." This choice is also reflected in trials evaluating the effect of probiotics on antibiotic associated diarrhea in children. Among 22 randomized trials testing the effect of probiotics on the outcome of antibiotic associated diarrhea, one of the 2 commonest probiotics tested was Lactobacillus rhamnosus used in 4 trials, 3 of which were Lactobacillus rhamnosus GG [Goldenberg JZ, et al. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. Cochrane Database of Systematic Reviews 2015, Issue 12. Art. No.: CD004827]. The pooled results from these 4 trials (n = 611 patients) showed a statistically significant protective effect (relative risk 0.35). However, we did not modify our manuscript with regard to this additional pediatric literature.

4. VAP diagnosis in this trial seems to be just clinically. Isn't any bronchoscopy or microbiologic evaluation being performed to have microbiological diagnosis? CIPS criteria are not used for VAP diagnosis/risk stratification in this study.

** Microbiologic confirmation is indeed one of many different diagnostic criteria we are using for the outcome of pneumonia. In addition to our primary outcome of adjudicated VAP, we have recorded VAP using 4 other common definitions of VAP including the Clinical Pulmonary Infection Score (Reference 30 of our revised manuscript), Calandra criteria from the International Sepsis Forum Consensus Conference on Definitions of Infection in the Intensive Care Unit (Reference 29 of our revised manuscript), Centers for Disease Control and Prevention VAP definition (Reference 31 of our revised manuscript) and the VAP definition from Heyland et al's large Canadian trial REDOXS (Reference 28 of our revised manuscript). Some of these definitions mandate a microbial isolate, some require interpretation that the isolate is pathogenic, and some of which require invasive techniques to identify a potential pathogen. However, we are not mandating bronchoscopy in patients with suspected pneumonia in PROSPECT. This is based on a trial by Heyland et al (Heyland et al. 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, Reference 27 of our revised manuscript), which we now cite, that showed no difference in outcomes when diagnostic testing was invasive versus noninvasive for non-immunocompromised critically ill patients. We have added the following sentence to the manuscript (Page 8, Line 15), "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 allowing VAP reporting according to several other definitions [28 - 31]."

5. Page 8, line 15: how and based on which scoring system (e.g. APACHE, SOFA, SAPS,…) the illness severity of the patients at the baseline is recorded?

**We have clarified that we are using the APACHE II score (Page 7, Line 18). Thanks.

6. How the matching of patients regarding illness severity, comorbidities, organ failures and… has been performed?

**As this is a randomized controlled trial, we are not matching patients per se; ideally in a large trial, patient characteristics will be comparable between the 2 groups as a result of the randomization process. We will present all baseline characteristics in a Table and we and the readers will interpret how similar the patients are in the 2 arms of the trial.

7. Page 8, line 17: VAP prevention strategies should be thoroughly described. Moreover, as adherence of ICU staff to VAP bundle criteria will directly affect the VAP incidence and mortality, the adherence rate should be reported.

**We are collecting unit-based VAP prevention strategies in each center on a site information form (e.g., whether selective digestive decontamination (SDD) is used or not, whether there is a semirecumbancy target policy, etc.). This type of information will be available upon request (e.g., we may not include the statement that 'no participating centers used SDD during this trial'). We are recording other VAP strategies used for each patient each day on the daily data collection form (e.g., subglottic secretion drainage endotracheal tubes, toothbrushing). In a large blinded pragmatic trial such as this, whatever the utilization is, it is expected to be comparable between groups for these latter cointerventions.

8. Energy intake of the patients should be reported.

**Thank you for this comment. Each day, we are recording route of nutrition, as well as type and volume of enteral nutrition received. A recent large RCT did not show any difference in outcomes with high caloric enteral nutrition (The TARGET Investigators. Energy-Dense versus Routine Enteral Nutrition in the Critically Ill. N Engl J Med 2018; 379:1823-1834). While we could calculate caloric intake each day during the trial, we are not planning to report this in the main manuscript as this additional level of detail is not a focus of PROSPECT. We agree that this will be an interesting substudy and secondary publication though.

9. Is GRV being measured in these patients?

**Gastric residual volume is not a reliable predictor of aspiration risk and pneumonia as per the large French trial by Reignier et al (Effect of not monitoring residual gastric volume of risk of ventilator-associated pneumonia in adults receiving mechanical ventilation and early enteral feeding: a randomized controlled trial. JAMA 2013; 309: 249-56). Therefore, we are not recording this each day.

10. Gastric intolerance and use of the prokinetic drugs should be defined.

** Please see our response above regarding gastric residual volume. We do not define or collect gastric intolerance in PROSPECT (e.g., regurgitation or vomiting) as this is not a trial focused on feeding. Each day we are collecting any prokinetic or aperient (laxative) administered, which we agree, qualifies as being a cointervention for this trial for the outcome of diarrhea. We agree that this will be an interesting substudy and secondary publication about risk factors for diarrhea though.

11. Since VAP incidence is higher in nasogastric feeding compared to orogastric feeding, a subgroup analysis comparing the VAP Incidence in these two feeding methods will be valuable.

** Yes, we are recording orogastric or nasogastric feeding tubes in place each day. While we may consider this exposure as a relevant cointervention for the outcome of pneumonia, we will not include a detailed analysis about enteral nutritional delivery route in the main report of this trial unless this is

requested. This would make for somewhat tedious reporting since the type of tube can change with accidental or planned removal over time in the ICU. However, this detail could be useful for an interesting substudy and secondary publication.

12. As patients during their ICU stay may need supplemental parenteral nutrition, PEG or postpyloric feeding, these statistics should be considered during subgroup analysis.

** Patients with PEG at the time of randomization were excluded as per Health Canada as indicated in our original manuscript (Exclusion criteria #5 in Table 1 of the manuscript). We have documented that for any patient who needed a PEG during the ICU stay, enteral nutrition was held for approximately 72 hours after a PEG is placed; however, we will not likely have room to report this level of detail in the main publication. Yes, we did record parenteral nutrition exposure each day. Postpyloric feeding is challenging to be sure of on a daily basis due to migration of the tip of the feeding tube, so we will not be reporting this in detail in the main report, as it is not a main focus of this trial. However, this detail could be useful for an interesting substudy and secondary publication.

13. Is any subgroup analysis regarding MDR pathogen responsible for VAP occurrence being conducted?

**We agree this analysis would be of interest. We have recorded each microbial cause of VAP when present, and do have susceptibilities for each microorganism. However, at this time, they have not been classified as multi-drug resistant or susceptible. As our primary analysis is the clinical diagnosis of VAP, we will not be able to report the presence or absence of multidrug resistant (MDR) pathogens for all patients in the main publication. We are currently funded to conduct a nested substudy within 3 ICUs in Hamilton, Ontario to examine MDR pathogens. However, we hope to secure further funding to do the work to classify all organisms according to their drug resistance profile, and ultimately, examine the effect of probiotics on MDR pathogens as a tertiary outcome.

Reviewer: 3

1. This is a new study PROTOCOL to prevent infections and related co- morbidities. After its completion it will help in a big way to save people from sufferings.

**Thank you.

VERSION 2 – REVIEW

REVIEWER	JOAN ROBINSON University of Alberta, Edmonton, Alberta, Canada
REVIEW RETURNED	07-Feb-2019

GENERAL COMMENTS	The authors did a masterful job of addressing all comments. I have just four very minor residual comments:

	1. “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” – Using this definition, diarrhea that started the day before antibiotics were started would count. 2. The explanation that you used dates rather than hours for most parameters (Day 2 starts at 00:01 even if the patient started probiotics just before midnight) is pragmatic and logical but should this be mentioned in the methods? Methods should be sufficiently clear that if you retire to a tropical country tomorrow, someone else could carry out your study exactly as you intended. 3. The phrase “loved one” still appears under “Patient and Public Involvement”. Try CTRL F to find it – my kids would laugh that I am providing advice to anyone on how to do anything on a computer! 4. Is it not Clostridioides difficile now?

REVIEWER	Sarvin Sanaie Tabriz University of Medical Sciences, Tabriz, Iran
REVIEW RETURNED	31-Jan-2019

GENERAL COMMENTS	Changes are fully done and are acceptable.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

The authors did a masterful job of addressing all comments. I have just four very minor residual comments:

1. “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” – Using this definition, diarrhea that started the day before antibiotics were started would count.

**Thank you for catching this. We have clarified as follows, “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”

2. The explanation that you used dates rather than hours for most parameters (Day 2 starts at 00:01 even if the patient started probiotics just before midnight) is pragmatic and logical but should this be mentioned in the methods? Methods should be sufficiently clear that if you retire to a tropical country tomorrow, someone else could carry out your study exactly as you intended.

**We have now added the following sentence to the Methods section (Page 11, Line 14), “For the timing of all pneumonia outcomes, we use days rather than hours to inform the classification.”

3. The phrase “loved one” still appears under “Patient and Public Involvement”. Try CTRL F to find it – my kids would laugh that I am providing advice to anyone on how to do anything on a computer!

**Thank you! We have now removed this statement.

4. Is it not Clostridioides difficile now?

**“Clostridium difficile” has now been changed to “Clostridioides difficile” throughout the manuscript.

Reviewer: 2

Changes are fully done and are acceptable.

**Thank you once again for your time and suggestions.