

Peer Review File

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Reviewer A

Comments to the authors:

(Note: The line numbers reviewer referred to can be found in this file “TP-2020-PCC-09(TP-20-349) for review_LT comments”.)

It is mainly English editing required here for this comprehensive review. I have annotated these in the PDF attached. For even a narrative review usually we would have a brief section outlining your inclusion/exclusion criteria and search strategy. I am not sure whether the journal allows references in the abstract if it does not then ignore my comments here, but there are a number of statements within the text that just require a reference to support them, I have indicated where these are. Also as you have included adult, pediatric, lab and animal studies it would be very useful to make it clear when you start discussion the study which each is eg sometimes you say patients (and I ask adults or children?) and rather than stating this at the end of the sentence, state it at the beginning thus also saving you words, and a bit more detail about some of the studies would be helpful without adding too many words. The review is quite long, but if within the word limit that's ok. Finally I would suggest at the end be specific about the type of 'further research' that is required. ie RCTs? or just larger samples or both and perhaps it would be useful to also have a section headed up with clear bullet points indicating implications for practice or practice recommendations.

There are too many abbreviations in the paper – and it would benefit from have these all listed out at the beginning of the paper as quite difficult to read and many are not written out in full the first time used.

Reply to Reviewer A

We acknowledge the above comments and relevant English edits have been made.

We have clarified the populations involved in the studies (adults vs pediatric) we referred to throughout the manuscript. For future research directions, we intended to discuss the various interventions that can be studied. As advised, we have included more specific details for these interventions in the revised manuscript.

Specific clinical recommendations are difficult to be included as currently there are limited literature on enteral nutritional impacts on the microbiomes of the critically ill population. Existing literatures are also often contradicting. Hence the need to further research into this area.

We have included a list of abbreviations at the start of the manuscript for ease of reading.

We have also included a supplementary table of the search terms used for literature search.

Reviewer B

Comments to the authors:

(Note: The line numbers reviewer referred to can be found in this file “TP-20-349-MS for reviewer_PR SF”.)

Thank you for the opportunity to review your submission. It is obvious the authors have done extensive research in order to produce this review. I feel there are some major points still missing in addition to major grammatical and syntax errors. Please see my comments below:

Comment 1

1. Page 1-2, lines 10-41: Please cite your sources in your abstract.

Reply 1

This journal does not require references in the abstract.

Comment 2

2. Page 1, line 17: Grammatically, should be either “involved in the breaking down of macronutrients” or “involved in breaking down macronutrients”.

Reply 2

We note the above recommendation and edits have been made.

Changes in text

Page 3, line 45-47: “The gut microbiota is involved in the breaking down of macronutrients, mainly carbohydrates and proteins.”

Comment 3

3. Page 2, line 23: “potentially” instead of “potential”

Reply 3

We note the above recommendation and edits have been made.

Changes in text

Page 3, line 49-51: “Changes to selected components of macronutrient can result in alterations in gut microbiome and have potentially beneficial effects in patients in the PICU.”

Comment 4

4. Page 2, line 26: delete the comma

Reply 4

We note the above recommendation and edits have been made.

Comment 5

5. Page 2, line 31-35: keep the tense the same throughout

Reply 5

We note the above recommendation and edits have been made.

Changes in text

Page 3, line 60-63: “In ARDS, the use of immune-nutrition such as omega-3 has also increased ventilator-free days and reduced intensive care unit length of stay. However, other nutritional supplementation (e.g., oligosaccharides, inulin, glutamine and arginine) has varying results.”

Comment 6

6. Page 2, line 47: delete comma

Reply 6

We note the above recommendation and edits have been made.

Comment 7

7. Page 2, line 49: “Its” should be plural because you are referring to the trillions of bacteria. Rephrase to something like “The symbiotic relationship between bacteria and their host...”

Reply 7

We note the above recommendation and edits have been made.

Changes in text

Page 6, line 101-103: “The symbiotic relationship between bacteria and their host has been responsible for colonization resistance, immune regulation, tolerance, and gut mucosal homeostasis.”

Comment 8

8. Page 3, line 55: delete comma after “indigestible”

Reply 8

We note the above recommendation and edits have been made.

Comment 9

9. Page 3, line 56: Reword so “large intestine” isn’t an adjective. Maybe “anaerobic bacteria in the large intestine”.

Reply 9

We note the above recommendation and edits have been made.

Changes in text

Page 6, line 106-108: “The host, in turn, provides complex polysaccharides which are otherwise indigestible and can only be fermented by anaerobic bacteria in the large intestine.”

Comment 10

10. Page 3, line 68-69: replace comma with “and” between bacteria names

Reply 10

We note the above recommendation and edits have been made.

Changes in text

Page 6, line 117-119: “Healthy commensal genera such as *Faecalibacterium* and *Ruminococcus* are depleted while pathogenic genus such as *Enterococcus* becomes predominant.”

Comment 11

11. Page 3, line 69: Delete comma after “Although”

Reply 11

We note the above recommendation and edits have been made.

Comment 12-13

12. Page 3, line 78-79: Should be present tense: “summarize” and “describe”

13. Page 3, line 79: “animal- and lab-based”

Reply 12- 13

We note the above recommendation and edits have been made.

Changes in text

Page 7, line 136-138: “Where possible, we review pediatric studies, but also describe animal- and lab-based as well as adult studies where relevant.”

Comment 14

14. Page 4, line 87: “increase host susceptibility”

Reply 14

We note the above recommendation and edits have been made.

Changes in text

Page 7, line 145-147: “Many of these practices decrease phylogenetic diversity of the gut microbiota and increase the host susceptibility to infection by the predominant pathogenic organisms.”

Comment 15

15. Page 3, line 85-86: You mention parenteral nutrition here, but your review’s title mentions only enteral nutrition.

Reply 15

In this subsection, we highlight the various common practices in PICU that can affect the microbiome. Since PN use is common in the critically ill children, it is included here. The focus of the review would still on enteral nutrition and its impact. The role of PN in ICU would not be discussed in detail. The title of the subsection has also been edited.

Changes in text

Page 7, line 142: Gut Microbiome alterations in PICU

Page 7, line 148-149: “These common treatments can result in a state of extreme dysbiosis in the critically ill.”

Comments 16

16. Page 4, line 92: delete comma

Reply 16

We note the above recommendation and edits have been made.

Comment 17

17. Page 4, line 89-92: When mentioning this study, include which risk factors these patients were exposed to (PPIs, opioids, PN, antibiotics), as you are saying these practices are demonstrated by the study.

Reply 17

These risk factors were not specifically mentioned in the study though most of them received antibiotics. The study was included to demonstrate dysbiosis in the PICU environment in general. Hence there is no change for this

Comment 18

18. Page 4, line 93: clarify that you are referring to microbial specificity

Reply 18

We note the above recommendation and edits have been made.

Changes in text

Page 7, line 152- 154: “The same study also demonstrated a loss of microbial site specificity in patients. It is well reported that different body sites contains unique microbial community signature.”

Comment 19

19. Page 4, line 94: “among” instead of “between” because there are more than two body sites

Reply 19

We note the above recommendation and modifications are made.

Changes in text

Page 7, line 153-154: “It is well reported that different body sites contains unique microbial community signature.”

Comment 20

20. Page 4, line 98: cite

Reply 20

We note the above recommendation and references have been included.

Changes in text

Page 8, line 165-166: “The predominant presence of pathogenic microbial community can be worrying and may predispose these vulnerable individuals to nosocomial infections (14, 15).”

Comment 21

21. Page 4, line 103: Would be helpful to specify what specific duration of fasting is associated with changes in the gut microbiome

Reply 21

We note on the above suggestion. However, no human study had reported the specific duration of starvation that would cause the changes in gut microbiome. Hence no edit has been made to the line.

Comment 22

22. Page 4, line 111: add comma after “support”

Reply 22

We note the above recommendation and edits have been made.

Comment 23

23. Page 4, lines 114-117: You discuss EN vs PN in animal models here. In children admitted to the ICU who are intubated (and unable to feed enterally), we delay PN therapy for 1 week. We know EN is better than PN, and we know outcomes are better with PN if PN therapy is delayed. This information should be reflected in your review, as we would never give PN in someone able to get EN (so this is not an argument).

Reply 23

The animal studies were included to describe the biochemical changes in the intestinal lining between mice who are enterally fed, and those who are not (calories provided via PN). This review focuses on the effect of enteral nutrition on gut microbiome changes. We note the suggestion to include timing of initiation of PN and findings of the PEPanic trial by T Fizez et al in 2016. These are included in the revised manuscript.

Changes in text

Page 8, line 182- 187: Added the following text

“When EN cannot be established, PN would often be initiated to ensure adequate delivery of calories. However, there are growing concerns regard to early PN use. In a multi-center RCT involving 1440 critically ill children, delaying PN for 1 week was shown to have more superior outcome than early PN. This was specific for lower new infection rate (adjusted odds ratio 0.48; [95% CI 0.35- 0.66]) and shorter mean duration of ICU (6.5 ± 1.4 days in late PN group vs 9.2 ± 0.8 days in early PN group).”

Comment 24

24. Page 5, line 125: delete comma (I will now stop commenting on incorrect comma placement; please review and correct)

Reply 24

We note the above recommendation and edits have been made.

Comment 25

25. Page 5, line 125-126: The structure of this sentence right now is such that “influence” is your subject, and so when you say “revert”, you are saying “influence revert”. This is incorrect. Please rephrase your sentence.

Reply 25

We note the above recommendation and edits have been made.

Changes in text

Page 9, line 213-214: “Such changes in microbiome diversity can also revert to its original state in two days.”

Comment 26

26. Page 5, line 131-132: delete first sentence; you don’t go into the studies here (if you keep this sentence, you must cite them here).

Reply 26

We note the above recommendation and the sentence is deleted.

Comment 27

27. Page 5, 133: “between” implies only two carbs. Change to “among”.

Reply 27

We note the above recommendation and modifications have been made.

Changes in text

Page 9, line 221-222: “This difference is also observed among various digestible carbohydrates (i.e. glucose, fructose and lactose).”

Comment 28

28. Page 5, line 135: delete comma

Reply 28

We note the above recommendation and modifications have been made.

Comment 29

29. Page 5, line 140: delete “with either diet in” and insert “a”

Reply 29

We note the above recommendation and modifications have been made.

Changes in text

Page 10, line 237-238: “The result of dysbiosis was shown in an experiment involving 4 groups of mice fed with a fructose, glucose, fat or normal diet.”

Comment 30-31

30. Page 5, line 149: “to a cohort”

31. Page 5, line 150: “reintroduction of lactose.”

Reply 30-31

We note the above recommendation and modifications have been made.

Changes in text

Page 10, line 245-247: “In a matched case-control study, lactose-free extensively-hydrolysed formula was given for two months to a cohort of infants with cow’s milk protein allergy, followed by reintroduction of lactose again.”

Comment 32

32. Page 5, line 151: “lactose-containing”

Reply 32

We note the above recommendation and modifications have been made.

Changes in text

Page 10, line 247-248: “Population of *Bifidobacterium* significantly increased with lactose-containing milk feeds, and that of *Bacteroides* decreased.”

Comment 33

33. Page 6, line 155: There is no need to discuss the division of carbohydrates. Start with talking about the fermentable carbs. Could start the paragraph with “Non-digestible fermentable carbohydrates...”

Reply 33

We note the above recommendation and the first sentence of the paragraph has been deleted.

Comment 34

34. Page 6, line 166: “has been seen even when delivered as PN and has...” because you are reviewing the literature broadly and want to avoid wording your information like you are discussing your own studies’ results

Reply 34

We note the above recommendation and modifications have been made.

Changes in text

Page 11, line 268-269: “This effect of butyrate has been seen even when delivered as PN and has a dose-response relationship. ”

Comment 35

35. Page 6, line 171: delete “certain”

Reply 35

We note the above suggestions and edits have been made.

Changes in text

Page 11, line 271- 273: “Given the beneficial effects of SCFAs, it is important to understand the production of these metabolites by microbiota in the large intestine, as they are currently being explored as possible therapeutic options for diseases such as inflammatory bowel disease (IBD). ”

Comment 36

36. Page 6-7: line 185-204: You start talking about infants here, which is straying from the patient population mentioned in your title. I would either rename your review or discard your paragraph on neonatal formula. One or two sentences is probably okay. Line 185: If you keep this sentence, rephrase for grammar.

Reply 36

We note the suggestion regarding studies on infant formula milk. These studies were included to demonstrate the changes in stool microbiome with the addition of prebiotics such as FOS or GOS to formula milk. These changes can be relevant even in older paediatric population who are formula milk in PICU. However, we do note your suggestion and have limited the discussion on these infant formulas and adult studies. This sentence has also been rephrased.

Changes in text

Page 11, line 284-288: “In two randomised control trials (RCTs) involving healthy infants, those who were fed with formula milk supplemented with GOS had an increased abundance of *Bifidobacterium* and *Lactobacillus* than those who were on regular formula milk feeding. The counts of *Clostridium* species were also lower counts in those were supplemented with GOS.”

Comment 37

37. Page 7, lines 200-205: Avoid talking about adults except maybe for a brief summary statement describing what we know in adults and how we don't have info in kids; I wouldn't discuss this adult study in a pediatric review.

Reply to comment 37

We acknowledge the suggestions and have limited the discussions of adult studies in the revised manuscript.

Changes to text

Page 12, line 303: Deleted the description of adult studies after this line.

Comment 38

38. Page 7, line 209: Rephrase

Reply 38

We note the above suggestions and edits have been made.

Changes in text

Page 12, line 308- 310: “In the stomach and duodenum, protein is broken down into amino acids via the action of digestive enzymes pepsin, trypsin and chymotrypsin. In the small and large intestine, several gut bacterial are involved in further catabolism, assimilation and utilisation of amino acids.”

Comment 39

39. Page 7, line 214: Rephrase (“alongside with” is not grammatically correct) – could say instead, “and to a lesser extent”

Reply 39

We note the above suggestions and edits have been made.

Changes in text

Page 12, line 312- 313: “Bacteria of the *Clostridium* genus are noted to be the key drivers of amino acid fermentation, and to a lesser extent *Bacteroides*, *Fusobacterium*, and *Veillonella* genera.”

Comment 40

40. Page 7, line 215: Rephrase

Reply 40

We note the above suggestions and edits have been made.

Changes in text

Page 12, line 313-314: “The most abundant end products from the fermentation process are SCFAs. ”

Comment 41

41. Page 7, line 219: Delete “till date”

Reply 41

We note the above suggestions and edits have been made.

Comment 42

42. Page 8, lines 223-252: I feel it may be more useful to talk about why lipids are used (calories, decr carbohydrate needs, etc) vs discuss adult literature. Try to refrain from talking about adults, as this data is not applicable to peds.

Reply 42

We acknowledge the above comment and have added the paragraph on the use of enteral lipid supplements in PICU. Since the review focus on enteral nutrition and subsequent paragraphs discuss dietary fat, we have focused on the use of enteral lipid supplement. We have also limited discussion on adult literature as much as possible unless these have potential application in children.

Changes in text

Page 13, line 348- 353: Added the following text

“Critically ill children often receive enteral lipid supplementation, such as medium chain triglyceride oil, in addition to their standard milk feeds. This is because the volume of fluid they can receive in a day is often restricted, which could limit the amount of calories received. Additional of enteral lipid supplementation allows optimisation of calories without proportionate increment in the milk feed volume. Hence it is important to review the effects of lipid on microbiome and clinical outcomes.”

Page 14, line 377-379: Deleted description of adult studies and summarized its findings. “The use of omega-3 PUFA supplement had been shown to reduce overall mortality in critically ill adult with sepsis and sepsis induced ARDS (68).”

Page 14, line 386: Deleted description of adult studies.

Comment 43

43. Page 10, line 296: don’t abbreviate to “omega”

Reply 43

We note the above suggestions and edits have been made.

Comment 44

44. Page 10, lines 300-314: There are many meta analyses and studies of probiotic use in critically ill children. Please include this literature in your paper, as their clinical benefits are associated with microbiome alterations. You mention there is inconsistent evidence regarding clinical outcomes, but you do not speak to the outcomes. This article has a chart with the meta analyses you can use to find them and the associated studies: Singhi SC, Kumar S. Probiotics in critically ill children. F1000Res. 2016;5:F1000 Faculty Rev-407. Published 2016 Mar 29. doi:10.12688/f1000research.7630.1.

Reply 44

We acknowledge the above comment and have included studies of probiotics use in the septic critically ill children in the revised manuscript. As this section talks about pediatric sepsis, we focused our literature search on its use in this specific disease population.

Changes in text

Page 16, line 442-451: Added the following texts

“In a RCT involving 100 critically ill children with severe sepsis, 50 of them were randomized to receive multi-strain probiotics consisting of Lactobacillus, Bifidobacterium, and Streptococcus. At the end of 7 days, those who received probiotics had lower proinflammatory cytokine (IL-6, IL-12p70, IL-17 and TNF- α) compared to placebo group ($p \leq 0.01$) (91). The use of the same probiotics strains had also been reported by Banupriya B et al in an open label RCT involving 150 critically ill children who were expected to be mechanically ventilated for more than 48 hour. Children who received probiotic containing Lactobacillus, Bifidobacterium, and Streptococcus stains had lower incidence of ventilator associated pneumonia than those in the control group (adjusted relative risk of 0.227; $p = 0.016$) (92).

Despite these benefits, routine use of probiotics in PICU is not recommended and should be done with caution (93). This is because its use had been associated with development of Lactobacillus bacteremia in the critically ill population (94, 95). Large scale pediatric RCTs are still lacking to determine the true efficacy and safety profile of the use of probiotics in this special group of patients.”

Comment 45

45. Page 10, lines 300-314: Please discuss the trophic effects of probiotics on intestinal mucosa.

Reply 45

We note the potential trophic effect of probiotics on the intestinal mucosa. However, limited studies are found during our search. We have included the relevant findings from animal- and lab-based experiments.

Changes in text

Page 16, line 453-457: Added the following texts

“The mechanism through which probiotics helps to maintain a healthy gut ecosystem and prevent colonization of pathogens include competitive exclusion and production of bioactive compounds such as bacteriocins and hydrogen peroxide that have antipathogenic properties. (93, 94). In various animal- and lab-based experiments, its

use has also been shown to tighten intestinal barrier, increase cell proliferation and re-epithelialization (95-97).”

Comment 46

46. Page 10, line 316: Is PARDS a medically common acronym? I have never seen it. I have only seen ARDS. Perhaps pediatric ARDS is more appropriate; please verify.

Reply 46

PARDS is used in the Paediatrics ARDS definition paper in 2015: Pediatric Acute Respiratory Distress Syndrome: Consensus Recommendations From the Pediatric Acute Lung Injury Consensus Conference. *Pediatr Crit Care Med*. 2015 June ; 16(5): 428–439. doi:10.1097/PCC.0000000000000350. Hence, in our opinion, this acronym should be kept.

Comment 47

47. Page 14, line 434: There are many more interventions that tilt the balance of pathogens than those listed. Consider adding additional interventions, e.g. steroids, immunosuppressive agents, H2RAs and antacids (or combine with PPIs), mechanical ventilation, endotracheal intubation, and invasive central lines to your table.

Reply 47

We note the suggestions to include other interventions which could affect the microbiome of the critically ill children. The selected practices focus on the alterations to the gut microbiome of these patients. Table 1 summarizes the changes caused by some common practices and are not meant to be exhaustive as the focus of the review is on enteral nutrition and gut microbiome of the critically ill children.

We have explained this in the footnote of the table.

Comment 48

48. Critically ill children are often on PN and not EN, and there are many considerations for initiating EN, e.g. respiratory support. I would recommend discussing these points somewhere in your paper.

Reply 48

We acknowledge the above comment and have included limitation and published barriers to initiations of EN in the revised manuscript.

Changes in text

Page 8, line 153-158: “In a survey involving PICUs in 57 countries, fasting for procedures or surgeries, lack of dietician support and prioritizing other aspects of care in a stable resuscitated patients over nutrition were amongst the top perceived barriers to enteral feeding (19)”