

Answering Reviewers

Manuscript ID: 80143

Title: Alterations of the gut microbiota in coronavirus disease 2019 and its therapeutic potential

We greatly appreciate the reviewer and editor for reviewing our manuscript entitled “**Alterations of the gut microbiota in coronavirus disease 2019 and its therapeutic potential**”. We also thank the reviewer and editor for the positive comments on the improvement of our manuscript. We have made careful modifications to the original article according to these thoughtful comments and suggestions. **In order to highlight the changes, the main modifications in the manuscript are marked in red.** In the revised manuscript, we corrected all inappropriate descriptions and fully addressed all concerns. Attached please find our point-by-point responses. We hope the revised paper will satisfy the reviewer and editor.

Point-by-Point Response to Reviewer

Response to Reviewer 1’ s Comments

1. I request that the researchers change the title of the paper to reflect the purpose of the current study.

Response: We thank the reviewer for the excellent suggestion and we sincerely apologize for the inappropriate title of the paper, so we have changed the title as below.

“Alterations of the gut microbiota in coronavirus disease 2019 and its therapeutic potential”

2. Reread the study abstract and shorten it even more, ensuring that the most important findings of the current study are presented in the results section of the study abstract.

Response: Thanks to the reviewers' suggestion, we have rewritten the abstract in the revised manuscript as follow.

Abstract. The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses a serious threat to global health. SARS-CoV-2 infects host cells primarily by binding to angiotensin-converting enzyme 2, which is coexpressed in alveolar type 2 cells and gut epithelial cells. It is known that COVID-19 often presents with gastrointestinal symptoms and gut dysbiosis, mainly characterized by an increase in opportunistic pathogens and a decrease in beneficial commensal bacteria. In recent years, multiple studies have comprehensively explored gut microbiota alterations in COVID-19 and highlighted the clinical correlation between dysbiosis and COVID-19. SARS-CoV-2 causes gastrointestinal infections and dysbiosis mainly through fecal-oral transmission and the circulatory and immune pathways. Studies have shown that the gut microbiota and its metabolites can regulate the immune response and

modulate antiviral effects. In addition, the gut microbiota is closely related to gastrointestinal symptoms, such as diarrhea, a common gastrointestinal symptom among COVID-19. Therefore, the contribution of the gut microbiota in COVID-19 should not be overlooked. Strategies targeting the gut microbiota via probiotics, prebiotics and fecal microbiota transplantation should be considered to treat this patient population in the future. However, the specific alterations and mechanisms as well as the contributions of gut microbiota in COVID-19 should be urgently further explored.

3. The final paragraph of the introduction to the study should emphasize the purpose of writing the paper within the context of the research problem.

Response: We thank the reviewer for the valuable suggestion, and we sincerely apologize for the unsuitable content of the introduction, so we have rewritten the part of the introduction and the final paragraph of the introduction in the revised paper as below.

...Gut dysbiosis is closely associated with gastrointestinal symptoms and disease severity[11, 12]. Therefore, the crosstalk between the gut microbiota and COVID-19 is gaining attention.

The gut microbiota has become a hot research topic in recent years. The resident microbial composition of the human gut mainly includes bacteria, archaea, viruses and fungi[13]. The human gut microbiota consists of more than 10¹⁴ bacteria and comprises approximately 500 to 1000 species. Gut bacteria in healthy individuals are mainly Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes[13]. The complex gut microbiota communities have important genomic and enzymatic properties and perform a critical role in the immune system, which protects against pathogens and helps maintain gut microbiota homeostasis. Gut microbiota homeostasis is essential for maintaining human health. Conversely, dysbiosis can lead to metabolic disturbance, immune dysfunction and systemic inflammation and has been linked to various diseases[14].

Therefore, this review mainly summarizes the gut microbiota alterations and the possible mechanisms of dysbiosis in COVID-19. Furthermore, we highlight the theoretical basis that the gut microbiota can be considered a promising therapeutic target in COVID-19, potentially interfering with immune and inflammatory responses and antiviral effects. Finally, we also reviewed multiple interventions targeting the gut microbiota, such as prebiotics, probiotics and fecal microbiota transplantation (FMT), which could optimize COVID-19 treatment.

11 Villapol S. Gastrointestinal symptoms associated with COVID-19: impact on the gut microbiome. *Transl Res* 2020; 226: 57-69 [PMID: 32827705 DOI: 10.1016/j.trsl.2020.08.004]

12 Yeoh YK, Zuo T, Lui GC, Zhang F, Liu Q, Li AY, Chung AC, Cheung CP, Tso EY, Fung KS, Chan V, Ling L, Joynt G, Hui DS, Chow KM, Ng SSS, Li TC,

Ng RW, Yip TC, Wong GL, Chan FK, Wong CK, Chan PK, Ng SC. Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. *Gut* 2021; 70(4): 698-706 [PMID: 33431578 DOI: 10.1136/gutjnl-2020-323020]

13 Wade WG. The oral microbiome in health and disease. *Pharmacol Res* 2013; 69(1): 137-143 [PMID: 23201354 DOI: 10.1016/j.phrs.2012.11.006]

14 Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol* 2021; 19(1): 55-71 [PMID: 32887946 DOI: 10.1038/s41579-020-0433-9]

4. Examine the current study's results for any errors that could derail the study's progress.

Response: Thanks to the reviewers' suggestion, we have re-examined the current study's results carefully to ensure that there are no errors that could derail the study's progress.

5. Rewrite the study conclusion to clarify whether or not the current study met its objectives, thereby answering the question of whether or not the research problem was solved.

Response : We thank the reviewer for raising this important point. Accordingly, we have rewritten the conclusion section in our revised paper as below.

COVID-19 was previously identified as a respiratory infectious disease. However, accumulating clinical studies have subsequently found that a large proportion of patients have gastrointestinal symptoms, such as abdominal pain, diarrhea, vomiting, and acid reflux, as well as significant gut microbiota alterations^[7, 24]. The gut microbiota not only significantly affects the COVID-19 development and disease severity but also reflects the susceptibility of COVID-19 patients to long-term complications^[101]. Studies have confirmed that dysbiosis increases the poor prognosis of COVID-19^[101]. However, controversial results exist regarding gut microbiota alterations in COVID-19 patients. A variety of factors, such as sex, age, basic health status, medication use, genetics, ethnicity, and geographic location, can affect the composition of the gut microbiota and lead to individual differences and varying responses to SARS-CoV-2 infection. Therefore, further exploration of the specific gut microbiota alterations in COVID-19 patients and the clinical correlation between the gut microbiota and COVID-19 will be a very challenging and valuable research direction in the future.

Unfortunately, direct evidence for the contribution of the gut microbiota in COVID-19 remains lacking. For example, the exact mechanism of dysbiosis remains unclear. Moreover, the regulation of host immune and inflammatory responses and antiviral effects by the gut microbiota during SARS-CoV-2 infection relies largely on inferences or conjectures from previous studies. Given that specific drugs for COVID-19 remain enigmatic, vaccines represent

the most effective prevention and control strategy; however, the continuous mutation of the virus has exacerbated this conundrum. Therefore, gut microbiota intervention through probiotics, prebiotics and FMT is undoubtedly one of the promising cosupplementation strategies for the future treatment of COVID-19, but large-scale studies are lacking and there are no corresponding treatment guidelines. The therapeutic prospects of the gut microbiota in COVID-19 are promising, but there is still a long way to go to realize its potential.

7 Cheung KS, Hung IFN, Chan PPY, Lung KC, Tso E, Liu R, Ng YY, Chu MY, Chung TWH, Tam AR, Yip CCY, Leung KH, Fung AY, Zhang RR, Lin Y, Cheng HM, Zhang AJX, To KKW, Chan KH, Yuen KY, Leung WK. Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples From a Hong Kong Cohort: Systematic Review and Meta-analysis. *Gastroenterology* 2020; 159(1): 81-95 [PMID: 32251668 DOI: 10.1053/j.gastro.2020.03.065]

24 Weiser JN, Ferreira DM, Paton JC. *Streptococcus pneumoniae*: transmission, colonization and invasion. *Nat Rev Microbiol* 2018; 16(6): 355-367 [PMID: 29599457 DOI: 10.1038/s41579-018-0001-8]

101 El-Salhy M, Hatlebakk JG, Gilja OH, Bråthen Kristoffersen A, Hausken T. Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study. *Gut* 2020; 69(5): 859-867 [PMID: 31852769 DOI: 10.1136/gutjnl-2019-319630]

6. The references used are recent, which is great, but some of them could be combined.

Response: Thanks to the reviewer's valuable comments, we have combined some references in the manuscript. (page 4, line 12, 16; page 7, line 7; page 8, line 13, 17; page 9, line 17, 28; page 10, line 2, 6; page 11, line 25-26; page 12, line 15; page 13, line 18, 21; page 13, line 24.)

7. Examine the percentage of plagiarism and self-citation to ensure that it is within the journal's acceptable limit.

Response: Thanks to the reviewers' comments, we have revisited and revised our manuscript carefully (the revised parts: page 5, line 17-20; page 6, line 14-17; page 6, line 23-27; page 10, line 9-11; page 10, line 15-17; page 11, line 8-16; page 12, line 17-20; page 13, line 7-10; page 14, line 1-2) and checked the plagiarism percentage using "iThenticate/CrossCheck" to ensure that the percentage of plagiarism is within the journal's acceptable limits. In addition, we sent the revised manuscript to "Nature Publishing Group Language Editing: <http://languageediting.nature.com>", a professional English editing company recommended by the journal, for further polishing. We ensure that the percentage of self-citation is within the journal's acceptable limit.

8. Taking into account the approval of a third party in using images 1 and 2 in part for the results due to intellectual property rights laws if the two images were not designed by the authors.

Response: Figures 1 and 2 were designed by the authors and redrawn them according to the journal image guidelines, and ensure that each part of the image is decomposable in PowerPoint, there will be no property rights disputes.

Response to Reviewer 2' s Comments

Authors discuss the role of reprogramming gut microbiota in treatment of Covid- 19 infections. While large scale studies are lacking and there are no treatment guidelines for this modality, it raises interesting discussion points for further studies.

Response: We thank the reviewer for the highly praise, which is the greatest affirmation and support for our work. The recognition is our motivation to continue our further study in the field in the future, and we sincerely hope to continue our cooperation. Sincerely thanks and congratulations again.