Dear Editor and Reviewers,

Thank you for your insightful comments and suggestions regarding our manuscript "Relationship Among Parkinson's Disease, Constipation, Microbes, and Microbiological Therapy (ID:88123)". We greatly appreciate the time and effort you have dedicated to reviewing our paper and providing feedback to strengthen it.

In response to reviewers' comments and suggestions, we have made revisions accordingly. The major changes are highlighted in yellow color in the revised manuscript. Below we provide a point-by-point response detailing how we have addressed each comment:

Reviewer #1:

Specific Comments to Authors: 1. Title can include that the manuscript is a descriptive review

Response: Thank you for the suggestion, we have revised the title to "Relationship Among Parkinson's Disease, Constipation, Microbes, and Microbiological Therapy (Review)"

2. What about the use of enteric α-synuclein as a possible biomarker in the early identification of Parkinson's?

Response: Response: Your comment is valuable and has been very helpful for improving our manuscript. We have added a paragraph in the Introduction section discussing the potential of enteric α-synuclein as a biomarker for early identification of Parkinson's disease (Page 6-7).

These findings demonstrate that a-syn detection in the ENS could provide an opportunity to identify early PD neuropathology before the disease spreads to other regions and motor symptoms become evident. Shannon et al[29] reported a-syn detection in the neurites of the colonic submucosa in colonic biopsies collected 2-5 years before motor symptom onset in patients with PD[29]. This

evidence suggests that a-syn detection in colonic mucosal biopsy samples could serve as a presymptomatic biomarker for PD. Additional evidence revealing a-syn accumulation in colonic biopsies for up to 8 years before motor symptom manifestation further supports the potential of enteric a-syn as a diagnostic biomarker for PD[30]. Pouclet et al[31] performed a comparative analysis of a-syn deposition using biopsy samples collected from the rectum, descending colon, and ascending colon of 26 patients with PD and 9 control subjects. The authors discovered that 23%, 42%, and 65% of patients with PD had a-syn deposition in the rectum, descending colon, and ascending colon, respectively, while control subjects had no a-syn deposition. These findings indicate that enteric α-syn detection has the potential to be used as a sensitive, PD-specific, and clinically useful biomarker for early PD detection.

3. The role of gut dysfunction in the response of medications used in Parkinson's can be included

Response: Thank you for your suggestions. We have added a statement in the Gut Dysfunction and PD Medications section about the role of gut dysfunction in response to PD medications (Page 8).

Evidence suggests an association between gastrointestinal dysfunction and PD medication[47]. Compared to patients with PD who have a normal colonic transit, those with a slow colonic transit require a considerably higher levodopa equivalent daily dose[48]. This indicates that slow colonic transit may delay peak plasma concentration and cause a reduction in the clinical efficacy of levodopa. Long-term PD-related constipation can lead to an abnormal overgrowth of bacterial decarboxylases in the gut [49]. Du et al[11] reported a significant increase in the abundance of the order Lactobacillales in the intestines of patients with PD-related constipation. Levodopa plasma availability has a negative association with Lactobacillus abundance[50], particularly as several bacterial species of the genus Lactobacillus contain genes

encoding tyrosine decarboxylase[51].

4. Discussion can cite the role of the enteric nervous system in the pathogenesis of Parkinson's

Response: We sincerely appreciate the reviewer's suggestion, we have incorporated discussion of the enteric nervous system's role in PD pathogenesis in the revised Conclusions section (Page 16-17).

In prodromal PD, abnormalities related to α-syn can be detected in the colon. Subsequently, α-syn spreads from the gut to the brain through the vagus nerve, which may lead to the development of PD. Constipation is considered one of the precursor symptoms of PD, potentially stemming from α-syn pathology in the ENS.

5. What about the role of medications used in Parkinson's which can aggravate constipation?

Response: Thank you for your suggestion. We have added content in the " Constipation in PD" section noting that some medications for Parkinson's disease can exacerbate constipation(Page8-9).

Complex interactions occur between anti-PD medications and gastrointestinal symptoms[52]. Healthy rats treated with PD medication for 14 days exhibited significantly reduced gut motility and altered microbiota composition, including increased abundance of Bifidobacterium and Lactobacillus and decreased abundance of the families Prevotellaceae and Lachnospiraceae[50]. Alterations in microbiota composition may lead to microbial metabolite changes, leading to constipation. A comprehensive meta-analysis demonstrated that pramipexole administration increased constipation risk relative to placebo[53]. Evidence suggests that constipation marginally increased after 1 year in patients with PD on dopaminergic medication,

particularly levodopa[54]. Another randomized, double-blind trial showed that pramipexole extended release led to a higher constipation likelihood versus placebo in patients with early PD[55]. A high levodopa equivalent dose increases constipation risk, which nearly doubles with the combination of levodopa and a dopamine agonist[56].

6. Study limitations can be listed

Response: We appreciate the Reviewer's comment. We have included a Study Limitations paragraph at the end of the Discussion section(Page 17) .

This review covered longitudinal studies on gut dysbiosis in PD-related constipation. However, it has a few weaknesses. The limited number of studies may not have accurately captured the full longitudinal changes in the microbiota associated with PD-related constipation. Furthermore, there is a scarcity of clinical studies examining intestinal flora specifically in PD-related constipation, making it difficult to infer the particular microbial taxa linked to this condition. In addition, as most studies have been conducted at the phylum and genus levels, further research at the species and strain levels could provide greater mechanistic insights. Therefore, future studies should focus on identifying specific bacterial species that promote PD-related constipation development. Finally, pinpointing the causative microbes could enable targeted microbial therapies for PD-related constipation in the future. However, more rigorous clinical studies are needed to elucidate the precise microbiota compositional and functional changes underlying PD-related constipation before such therapeutic approaches can be applied.

Reviewer #2:

Specific Comments to Authors: The article is deserved to be published in WJG.

Response: Thank you for your efforts in the revision of our manuscript and for your positive comments on our work.

Thank you again to both reviewers for your thoughtful suggestions. We believe the manuscript is now much improved. We hope these revisions sufficiently address all of the comments, but we would be happy to provide any clarification or make additional changes as needed. We look forward to your decision on our revised submission.

Sincerely,

Zhou Liu