

January 21, 2021

World Journal of Gastroenterology

Dear Editor,

We are submitting a revised version of our manuscript, manuscript NO: 82857. The main changes are as follows:

- 1) In view of Professor Yang's great contribution to the manuscript revision, the authors wish to place Professor Yang in second place. All others have agreed with this change. We hope that this change is acceptable.
- 2) The prevalence of cholelithiasis in the abstract has been rewritten in the revised manuscript.
- 3) The revised manuscript has omitted Figure 1.
- 4) The revised manuscript lists the relevant references in Table 1.
- 5) The second paragraph in "The biliary microbiome" and the last sentence of the paragraph titled "*Helicobacter* species induce gallstone formation by precipitating calcium" have been rewritten to make them more precise.
- 6) All of the reference lists have added journal names.

Specific changes in the revised copy of the manuscript that were made in response to these comments are shown in red. Moreover, this manuscript was carefully edited by a native English speaker again. We hope that the revised manuscript will meet with the approval of the reviewers. We very much appreciate the helpful comments and suggestions made by the editorial board member and reviewers.

With best wishes,

Yours sincerely,

Zi-Kai Wang

Associate chief physician

Corresponding author

We would like to express our sincere thanks to the reviewers for their constructive and positive comments.

Responses to Reviewer 1

To Reviewer 1

Specific Comments

1. Figure 1 which appears in INTRODUCTION is difficult to understand, and it might just as well be omitted.

Answer: We are sorry for the confusing figure. This figure has been deleted in the revised version.

2. It might be inappropriate for Figure 2 to appear in the paragraph titled “The GI microbiome induces gallstone formation by regulating bile acid (BA) metabolism”.

Answer: We thank the reviewer to raise this important issue. This sentence has been moved to the last sentence of the paragraph in the revised version to address this issue.

3. How about rewriting the last sentence of the paragraph titled “Helicobacter species induce gallstone formation by precipitating calcium” as flows after changing the line? Original: Nevertheless, the mechanistic links between pathobionts and cholelithiasis formation need further exploration. ⇒Nevertheless, the mechanisms for the GI microbiome contributing to cholelithogenesis as described above (Figure 2) lack evidence to support causality, and the mechanistic links between pathobionts and cholelithiasis formation need further exploration.

Answer: Thanks for your valuable suggestion. This sentence has been rewritten in the revised version.

4. Typos should be corrected. · associationstudies⇒association studies (in “The biliary microbiome”) · defectin⇒defect in (in “The GI microbiome induces gallstone formation by regulating bile acid (BA) metabolism”).

Answer: Correction has been made in the revised version.

5. In REFERENCES, some journal names are missing.

Answer: Thanks for your careful review. The missing journal names have been added in the revised version.

Responses to Reviewer 2

To Reviewer 2

Specific Comments

1. The prevalence of cholelithiasis in the abstract should be based on world wide epidemiology.

Answer: Thanks for your comments. The prevalence of cholelithiasis in the abstract has been rewritten to “Cholelithiasis is a common digestive disease affecting 10% to 15% of the adult population” to address this issue in the revised version.

2. The microbial composition, taxonomic profile, and metabolites are mixed in same paragraphs. If it is corrected, it might be better.

Answer: We are sorry for the confusable concepts with “ microbial composition”, “taxonomic profile”, and “metabolites” in our previous manuscript. The paragraph has been rewritten as follows to make it more precise:

There are some resemblances and dissimilarities between the biliary and GI microbiomes. The comparative metagenomic analysis demonstrated no significant differences between the GI tract and bile in the predominant phylum *Firmicutes* and the rare phylum *Fusobacteria*. However, the microbial diversity in the biliary tract is more diverse than in the GI tract^[9]. The bile duct and duodenum share the core microbiota with the genus *Escherichia-Shigella*, *Fusobacterium*, and *Enterococcus*^[10]. Given that the bile duct is anatomically connected to the GI tract via the duodenal papilla, it was hypothesized that the biliary microbiota originates from intestinal bacteria and migrates retrograde into the biliary tract. Consistent with the hypothesis, studies demonstrated that the biliary microbiota shared a compositional similarity to duodenal microbiota, and all bacteria in bile were detected in the upper GI using 16S sequencing^[11, 12]. It is noteworthy that the intestinal microbiome contributes to the heterogeneity of the biliary microbiome in cholelithiasis patients, despite the high prevalence of oral cavity and respiratory tract inhabitants to intestinal

inhabitants^[13].

Responses to Reviewer 3

To Reviewer 3

Specific Comments

1. Please list the references you used in a table to make it easier for the reader.

Answer: Thanks for your valuable suggestion. The references have been added in Table 1 to make it more clear in the revised version.