Dear Editor,

Thank you very much for your comments on our manuscript. According to the comments and suggestions, we have revised our manuscript (highlight in the revised manuscript) and responded point by point to the reviewers’ comments.

We hope that this revised manuscript will fit the World Journal of Gastroenterology. We are looking forward to your decision.

Thank you very much for your attention and consideration.

Best Wishes!

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Reviewer #1:
Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Accept (General priority)
Specific Comments to Authors:
Overall, the article is very well designed and presented. The findings potentially are of clinical impact.
Answer: Thank you very much for reviewing the manuscript!

Reviewer #2:
Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Major revision
Specific Comments to Authors:
It is an interesting study on a subject hitherto not well investigated. I have the following questions for the authors.
1. The current hypothesis as suggested by Robin and Marshall is that Helicobacter pylori is strongly causally associated with GU and DU because of its interference with normal mucosal protective mechanisms. In both DU and GU, H pylori is harboured in the gastric antrum. It is not clear, therefore why the milieu in the stomach and duodenum is different in those who are Hp positive.
Answer: This is a really intriguing finding from the current investigation. H. pylori was the most abundant bacteria in the stomach, with a relative abundance of over 90%. In contrast, the relative abundance of H. pylori in the duodenal portion varied from 0.89% to 45.53%, indicating no discernible dominance. Based on this discovery, we conjecture whether the duodenum and stomach have distinct microenvironments. In contrast to the stomach's harsh acidic environment, which poses challenges to the flora's survival, the duodenal bacterial flora exhibits greater diversity and closer interactions between the different bacterial communities, acting as a buffer to either stabilize or prevent the growth of significant amounts of H. pylori. We'll investigate and confirm this conjecture in the follow-up study.

2. What is the explanation of the bacterial flora of duodenum in those with DU being similar to those who are Hp negative.
Answer: Our earlier research similarly shown that there was no noticeable difference in flora variety between DU patients with H. pylori infection and DU patients without H. pylori infection (Ref. 21). There was no clear species dominance as the relative abundance of H. pylori in the
Duodenal division varied from 0.89% to 45.53% in this investigation. We proposed two possible explanations for this: either the duodenal mucosa's greater diversity of bacteria or the early stage of active infection. The greater diversity of the flora resulted in more intricate and close interactions amongst the flora, and the microorganisms both helped and competed with one another, which prevented the huge development of *H. pylori* and preserved the relative stability of the duodenal flora. The effect of *H. pylori* infection on the structure of the duodenal flora is still a topic that deserves to be explored.

3. What is the clinical significance of their study? How does the study contribute to understanding the relationship between Hp infection and GU and DU, namely the cause and effect controversy.
   **Answer:** It is generally recognized that most *H. pylori* are infected in early childhood and remain latent for a long time. When infected with *H. pylori*, *H. pylori* becomes dominant by continuously altering the gastric mucosal microenvironment to inhibit the growth of other flora. A very large number of studies have reported that the inflammatory response to *H. pylori* infection damages the mucosa, and ulcers may develop when the mucosal defenses and repair functions are disrupted, however the mechanism of action is still being explored. *H. pylori* infection is an indirect and continuous effect. Comparatively, it is also interesting to investigate whether the composition of the flora is altered by changes in the environment of the GI tract when a patient develops GU or DU, for example, to further promote the entry of *H. pylori* into the active phase. The notion that there may be a mutually reinforcing relationship between *H. pylori* infection and the development of ulcers deserves to be validated by further research.

4. The number of patients studied is too small for the findings to be generalised.
   **Answer:** We have conducted some relevant studies in the previous period. By comparing the structure of duodenal mucosal flora in *H. pylori* infection-positive and *H. pylori* infection-negative DU patients, it was found that *Helicobacter* spp. was not the absolute dominant genus in the duodenum, and *H. pylori* infection did not have much effect on the diversity of duodenal flora (Ref. 21). In addition, by 16S rRNA sequencing analysis of the mucosal flora of GU and DU patients with positive *H. pylori* infection, it was found that the diversity of bacterial communities in the mucosal tissues of DU patients was higher than that of GU patients (Ref. 20). On the basis of these findings, this time we recruited GU patients, DU patients, and healthy controls, and collected mucosal tissues for macrogenomic sequencing separately, and the results were consistent with the previous study. By comparing with healthy control individuals, we further explored the differential genes and differential functions of GU and DU to further explore the *H. pylori* infection associated with GU and DU and their mechanisms of occurrence.
The results of our series of studies were echoed backward and forward. In the later stage, we will further validate and explore in depth on the basis of this study.

5. In both groups of patients with either DU or GU, biopsies should have been taken from both the stomach and the duodenum to compare the flora. In this study duodenal biopsies have been done only in patients with DU and not GU and vice versa.

**Answer:** I understand what you are trying to express is that both GU and DU patients with positive HP infection should have both gastric and duodenal biopsies, and then the following comparisons should be made: gastric mucosa of HP+/GU vs. gastric mucosa of HP+/DU, duodenal mucosa of HP+/GU vs. duodenal mucosa of HP+/DU. There could be a lot of confounding influences in this comparison. In the comparison between gastric mucosa of HP+/GU and gastric mucosa of HP+/DU, does duodenal *H. pylori* infection as well as DU have any effect on the gastric mucosal flora, and is it consistent with normal gastric mucosal flora? Similarly, whether gastric *H. pylori* infection as well as GU has an effect on duodenal mucosal flora in the duodenal mucosal comparison between HP+/GU and HP+/DU needs to be explored. These influences are some interesting lines of research and need to be further confirmed by additional studies. The influence of these factors was excluded in this study by using healthy individuals as a control group in order to respond more clearly to the effect of *H. pylori* infection on GU and DU. In addition, duodenal mucosa sampling from healthy individuals was missing in this paper. Our previous study found that *H. pylori* infection had little effect on the diversity of duodenal flora (Ref. 21), so we only performed duodenal mucosal sampling of GU patients with positive *H. pylori* infection.

6. The effect of diet and ingestion of other probiotics is not studied.

**Answer:** We appreciate your proposal, as we did not take the patients' nutrition into account when doing the study in before. Referring back to the data collected during the patient's initial subject visit, we discovered that the study participants had no notable dietary deviations in the month before the sample was taken, ruling out the possibility of ulcers resulting from short-term gastrointestinal tract damage from bad lifestyle choices. Furthermore, none of the patients included in this research were probiotic users. As indicated, further details on these topics are now included in the article's "Results-Participants" section. Furthermore, as the study did not originally impose a follow-up requirement, it was challenging to determine whether dietary disparities occurred across participants. Variations in dietary patterns may also lead to variations in microbial populations. The variations in eating patterns were minimal, nevertheless, given that all of the participants were from the same area. However, we think this is a very worthy line of inquiry, and we appreciate your advice. We will consider this in our follow-up study.
7. There are a number of technical issues. The introduction section is too large. There is a mixup of information of methodology and results with the two not being separated in the manuscript. Results section has methodology and vice versa.

Answer: Thank you very much for your suggestion. We have streamlined the introductory section of the article and adjusted the mixed description of the methods and results sections, as described in the highlighted changes to the article ("Methods and materials-Bioinformatics and statistical analyses" at the end of paragraph 4, as well as in the "Results" section).

Reviewer #3:
Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Accept (General priority)
Specific Comments to Authors:
Helicobacter pylori (H. pylori) plays a significant role in chronic gastritis, peptic ulcers, and the formation and progression of gastric cancer. Whether the pathogenesis of gastric ulcer (GU) and duodenal ulcer (DU) is consistently related to H. pylori remains inconclusive. This study performed metagenomic sequencing on the gastrointestinal microbiota of patients with H. pylori-infected GU and H. pylori-infected DU. We found that the microbial diversity in H. pylori-infected DU patients showed no significant difference. However, the gastric mucosal microbiome was found to be dominated by H. pylori and reduced in biodiversity in patients with H. pylori-infected GU. H. pylori derived the intergroup differential functions, particularly concerning tRNA Q-modification and demethylmenaquinones and menaquinones synthesis. uibE gene was detected to be an enrichment in the synthesis pathway. Neither the entire paper nor any part of its content has been published or has been accepted elsewhere.

Answer: Thank you very much for reviewing the manuscript!