Reviewer #1:

Comment: Thank you for inviting me to evaluate the Case Control Study titled " Comparison of genomic and transcriptional microbiome analysis in gastric cancer patients and healthy individuals". It is an interesting paper, the authors utilized a gastric cancer (GC) model to elaborate on the choice of modality and its effects on differences between patients with gastric cancer and control. For the comparison they not only applied the comparison to the healthy controls, but also compared the differences between tumor and adjacent tissues using 16s rRNA genome and transcript sequencing. Their study showed that bacterial 16S rRNA gene and 16S rRNA transcript sequencing results are not interchangeable. Only a small number of bacterial sequences overlapped between 16S rRNA gene and 16S rRNA transcript's sequencing. Profile of bacterial differences between case (GC) and control depended on sequencing modality. Analysis at 16S rRNA transcript level allowed us to identify rarer bacteria species and was more sensitive to reveal associations with clinical characterizations. Interestingly, the differences between tumor and adjacent tissues was of the little value in particular due to interindividual variation as compared to healthy controls. The paper is well arranged and the logic is clear, and. The cited literature is comprehensive and modern. The provided figure and tables are well composed and understandable. The quality of language of the manuscript is quite acceptable for me. So, I recommend to you that this manuscript mayd be accepted. There are a question for author: As they found the differences between tumor and adjacent tissues was of the little value in particular due to interindividual variation as compared to healthy controls.Why is the he bacterial 16S rRNA gene V1-V2 region tested instead of the commonly used V3-V4 region or V4-V5 region?Is it related to the lack of specificity of the variable region detected?

Answer: Thank You for Your review and remark. Indeed, our study did not find any significant differences according to bacterial profile, richness, or diversity alterations between tumor and tumor adjacent tissues. Also, our correlation analysis of the abundance of bacteria showed the same clusters between tumor and tumor adjacent

tissue. These results may suggest that with the onset and development of carcinogenic processes, local changes in stomach tissues lead not only to a change in the bacterial composition but are also precise uniformity between cancer-affected and still healthy tissues. We agree that the 16S rRNA region under study might influence the final results. However, currently there are no guidelines or studies, which would emphasize which region (V1-V2, V3-V4, V4-V5 or other) would be providing deeper/better look on stomach tissue microbiome. And it would be very interesting to compare the tumor and tumor adjacent tissues of the stomach using different regions of the 16S rRNA; but that would be already an object of further research. However, we want to emphasize that researchers, incl. our group, have used V1-V2 region for gastric tissue microbiome analysis previously [PMID: 34455919; PMID: 35490553; PMID: 29312210; PMID: 31175864].

Reviewer #2:

Comment: The authors characterized the microbiota of GC and compared them with that in normal controls and adjacent tissues, at both gene and transcript level. It is an interesting and hot topic. The study was excellently designed and performed perfectly. The writing, tables and figures are all present well. I suggest that this manuscript meets the requirement of publication.

Answer: Thank You for Your positive review.