Dear Editor and Reviewers,

Thank you for the constructive comments! We have carefully considered the comments and revised the manuscript accordingly. Below are our responses to the reviewer's comments. Revisions are in Purple in the text. Thank you for the opportunity to improve our manuscript!

Reviewer(s)' Comments to Author:
Reviewer: 1
The major point is how and when the saliva samples are taken especially in healthy controls. The author mention that samples are taken 30 minutes before operation but are these taken in fasting condition all the same time of the day? More importantly are healthy control normalized in term of timing? How variable are the results if samples are taken in different time of the day (i.e. morning vs afternoon or evening)? Are 30 minutes without eating or drinking sufficient? More details are required in the methods section.
Thank you for your positive feedback!
In order to ensure the consistency of saliva collection time, enrolled patients are arranged to collect at about 4:00 p.m. on the day of admission and the collection time of healthy population is also arranged in the afternoon.
The preoperative saliva sample refers to the saliva sample collected in the afternoon on the day of admission, and the patient will have surgery in our department 1-2 days later.
Please see the revised manuscript at page 8.
Page 8: To ensure all sample collection at the similar time period in a day, we collected patient samples around 4:00 pm on the day of admission prior to biopsy or surgery for cancer diagnosis. Healthy subjects' saliva samples were also collected around 4:00 pm in the afternoon.
Other points: 1. In the introduction section, the first paragraph, the authors briefly mention a lack of early detection. I was wondering if it would be a good idea to talk about the existing biomarker (CA19) commonly used in the clinic.
Page 5: Biomarker Ca19-9 has been commonly used for diagnosis and prognosis of pancreatic cancer with diagnostic sensitivity of 0.78 and specificity of 0.77, but this biomarker test has limited sensitivity among patients with jaundice, pancreatitis, enteritis and elevated blood glucose since such patients usually have elevated Ca19-9 concentrations[9-11]. In addition, 7%-10% Lewis (a-/b-) populations could not express Ca19-9[12].

2. The study excludes non-cancers PDAC lesions from sequencing (last line in methods section under research design and participants). The supplementary data comparing the Vellionella specie decline from healthy to resectable to non-resectable PDAC is very well presented. Although the authors do attempt to characterise the changes in the microbiota as PDAC advances, since IPMN can give rise to PDAC, it would be interesting to know how the microbiome changes between IPMN, resectable and non-resectable PDAC vs healthy.

We also collected the saliva sample from intraductal papillary mucinous neoplasm (IPMN) patients, pancreatic serous cystadenoma patients, solid pseudopapillary neoplasm patients and neuroendocrine tumors patient, but the sample size was not enough for testing. The projects is continuing and we will show our results in the future.

3. How many patients had resectable and non-resectable PDAC is not clear from the manuscript?
Among the 41 patients with PDAC, 31 (76%) had head pancreatic cancer. 20 (49%) patients were resectable pancreatic cancer.

4. I was wondering how many times the symptom data collection was done from PDAC patients in this study. What were the symptomatic differences found in resectable and non resectable PDAC? What was their effect on microbiome from these different cohorts?
We collected symptom data one time on the day of admission. We did not show the results of symptomatic features of resectable PDAC and non-resectable PDAC because symptomatic features data were not the fucos of this study. We showed the microbiome of resectable PDAC and non-resectable PDAC in figure 2B and the symptomatic feature data will be showed in another paper.

5. The symptoms used in the assessment are not mentioned in the methods section under subheading phenotype measures. Although the study mentions the list of symptoms under abundance of bacteria and symptom (subheading under statistical analysis), it would be clearer to list to symptoms here as well.
Page 8: Since there is no measure or checklist for the symptoms specific to pancreatic cancer, we developed a checklist based on literature review to assess symptoms specific to pancreatic cancer, such as bloating, jaundice, nausea, vomiting, dark brown urine, diarrhea, constipation, pale stools, pruritus, lack of appetite, pain, fatigue, disturbed sleeping. Patients report the presence and absence of symptoms by checking "Yes" or "No."

We showed at page 10 of data analysis part: ***Abundance of bacteria and symptom.*** We used Wilcoxon rank-sum test to compare the abundance of bacteria (top 10 positively expressed flora) in PDAC patients with and without typical symptoms of PDAC, including pain, fatigue, disturbed sleeping, nausea, vomiting, lack of appetite, diarrhea, constipation, bloating, jaundice, dark brown urine and pale stool.

6. From the manuscript, it is not clear how many patients form the symptomatic and asymptomatic cohort. A table on the same can help add more clarity.
Each symptom of PDAC patients was divided in to symptomatic group and asymptomatic group according to the presence and absence of each symptom. We analyzed differences of the median abundance of bacteria between symptomatic group and asymptomatic group.

7. The manuscript does mention ethnic differences between different Chinese provinces and non-Chinese populations. This study also estimates the lifestyle difference as it mentions 61% of PDAC population has a high-fat diet in comparison to controls. Does this cohort percentage have an increase in specific microbial diversity?
We defined the high-fat diet as eating fat meat, hot pot and other greasy food（people in Sichuan Province preferred）for more than 5 days a week, but we could not determine the specific amount of these fat intake per day. We only put the high-fat diet as a high-risk factor into the logistic regression to analyze the risk of predicting pancreatic cancer. The result showed "having high-fat diet or not" was not one risk factor. In future studies, we will find the appropriate way to quantize specific components in diet.

8. Was there any of the microbial differences in the PDAC resectable and non-resectable affected by symptoms?
The microbiome differences of resectable PDAC and non-resectable PDAC were shown in figure 2B. All of PDAC patients had at least one symptom in this study, so each symptom of PDAC patients was divided in to symptomatic group and asymptomatic group according to the presence and absence of each symptom. We analyzed differences of the median abundance of bacteria between symptomatic group and asymptomatic group.

9. It will be interesting to see whether this microbial diversity is specific for symptomatic PDAC population by looking at previous literature or is it generalizable for the symptom.
All of PDAC patients had at least one symptom in this study, so we could not divided the PDAC population into asymptomatic group and symptomatic group. Each symptom of PDAC patients was divided in to symptomatic group and asymptomatic group according to the presence and absence of each symptom. We analyzed differences of the median abundance of bacteria between symptomatic group and asymptomatic group.

10. How to the differences in asymptomatic and symptomatic PDAC population correlate with a healthy cohort? A table summarising the results of the main microbial population will put things in perspective for the reader and make results clearer.
All of PDAC patients had at least one symptom in this study, so we could not divided the PDAC population into asymptomatic group and symptomatic group. Each symptom of PDAC patients was divided in to symptomatic group and asymptomatic group according to the presence and absence of each symptom. We analyzed differences of the median abundance of bacteria between symptomatic group and asymptomatic group.

Reviewer: 2

Comments to the Author

Concerning the quality and importance of this manuscript, only saliva sample was collected might be a limitation, and it might be important to compare the microbiome of the cancer patient saliva and the tissue samples. For the data analysis, more information could be further explored from the 16s Seq data.
Thank you for your positive feedback! You make an important point that it might be important to compare the microbiome of the cancer patients' saliva and the tissue samples. The data published in this article is part of our center's project to explore new diagnostic markers for pancreatic cancer. Due to resource constraints, we did not include the detection of tumor tissue flora. However, from the current research results, there are differences of oral mircobiome distribution between pancreatic cancer patietns and the normal population. In the future, we will explore the association of bacterial profile and cancer detection by testing the saliva, feces, and tumor tissue samples. I explain this in the discussion part. Please see the revised manuscript.
Page 16: Currently, no studies focus on comparing advantages and disadvantages of using different sample collection techniques, future study should compare the effectiveness of using different sample collection techniques, such as saliva, tongue coating, and oral wash on sample quality for microbiota profiles and preference of patients.

From the methodology, Saliva sample collection was only mentioned that all the subjects were instructed to not eat and drink for 0.5 hour prior to saliva sample collection. Did all patients wash their mouth or brush their teeth before sample collection? Did all sample collection at the same time period in a day?

We did not let the participants brush their teeth before collecting the saliva, because brushing may affect the distribution of oral flora. But we ask to rinse out the residue in the oral cavity before collection. We collected patient samples at 4 pm on the day of admission. Please see the revised manuscript.

Concerning of the parameters (for instance diet) or the symptoms, it will be better to include standard score or relatively clinical chemistry parameters for a more subjective evaluation.

Page 8：Participants were also instructed not to brush their teeth at least 8 hours prior to saliva sample collection since brushing teeth may remove part of the oral flora. Participants were asked to rinse their mouths to remove debris from the oral cavity before saliva collection. To ensure all sample collection at the same time period in a day, we collected patient samples at 4 pm on the day of admission.

Thirdly, Since there is no measure or checklist for the symptoms specific to pancreatic cancer, we developed a checklist based on literature review to assess symptoms specific to pancreatic cancer, such as bloating, jaundice, nausea, vomiting, dark brown urine, diarrhea, constipation, pale stools, pruritus, lack of appetite, pain, fatigue, disturbed sleeping. Patients report the presence and absence of symptoms by checking “Yes” or “No.”

Please see the revised manuscript at page 8.

Besides, there are some limitations could be paid more attention: In discussion, it was mentioned that Four known Main periodontal disease contributors: aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia and prevotella intermedia were more prevalent in PDAC patients in Fan et al.’s study.thus by collecting patients should they also consider to exclude the patients with periodontal disease? The study did not clarify this issue yet and might be important to check.

Finally, Another limitation of our study was that we were not able to exclude participants with dental disease since our participants were not able to provide accurate history of dental disease and there were no medical record regarding dental disease for us to verify participants’ dental disease status. In future study, it may beneficial to have a professional dentist to examine participant’s oral health status so as to ascertain the potential impact of oral health status on microbiome flora profile among patients with pancreatic cancer. Pleasse see the limitation part at page 19.

Reviewer: 3

Comments to the Author

Thanks! We revised the manuscript to make it clear as below.

Pg #5/Par #1/Line 8: "remains a great challenging" Please change to "remains a great challenge".

Page 5: Early detection of pancreatic ductal adenocarcinoma (PDAC) remains a great challenge.

pg #5/Par #2/Line 6-8: "Dysbacteriosis...lung cancer" It is mandatory that you briefly explain the general concept of oral or gut microbiota dysbiosis along with mentioning its implication and importance in malignant disease.

Page 5: Oral or fetal microbiota profile of gastrointestinal and colorectal cancer, oropharyngeal cancer, liver cancer and lung cancer may be novel and potential diagnostic biomarker.

Pg #5/Par #2/Line 8-10: "Accumulated studies...healthy individuals" How does the abundance of microbiota shift in these patients? Furthermore bacterial diversity is a crucial factor of microbiota dysbiosis. How does diversity change in these patients?

We described the microbiota shift in these patents in detail after this sentence " Accumulated studies have revealed that oral and gastrointestinal microbiome differ in abundance in patients with pancreatic cancer compared with healthy individuals".

Pg #6/Par #1/Line 4-6: "Tongue coating...oral microbiota" There are some points to be made here regarding the choice of the sampling method.

Among all the oral mucosal surfaces the tongue the is the most populated niche and has a significant impact on other regions in the oral cavity, being a reservoir from which oral bacteria travel around the oral cavity to colonize other regions, facilitated by saliva [1]. Compared with the other parts of the oral cavity, the distinct surface characteristics (fissures, crypts, papillae, saliva) of the tongue coating are prone to the colonization, growth, and proliferation of microbiota [2]. Recent studies have reported that tongue coating microbiome could serve as a stable non-invasive biomarker in gastrointestinal cancer [3], significantly distinguishing patients with pancreatic head cancer from healthy individuals [4]. Furthermore, the microbial communities and intra-personal diversity of the tongue and salivary microbiota have shown high levels of similarity [5]. Hence, according to these data and the fact that swabbing of tongue dorsum is less complicated, more specific, and more cost-effective than saliva collection, why did you choose saliva as the most appropriate sample for the evaluation of oral microbiota? Additionally, it is well-known that cancer patients, especially these with pancreatic neoplasms, present systemic immune dysfunction [6]. Since the human throat is rich with lymphoid tissue (Waldeyer's ring) where immune interactions occur and the oral microbiota greatly interferes with local mucosal or systemic immunity [7], would the swabbing from the oropharyngeal rear serve

as a more representative biomarker for distinguishing several features between pancreatic cancer patients and healthy controls?

Thanks for telling us in detail the differential distribution of the bacterial flora in the tongue coating of different cancer patients (including pancreatic cancer) and tongue coating collection can be used as a sample collection technique for oral flora research. Currently, no studies focus on comparing advantages and disadvantages of using different sample collection techniques, future study should compare the effectiveness of using different sample collection techniques, such as saliva, tongue coating, and oral wash on sample quality for microbiota profiles and preference of patients.

Please see the revised manuscript in the discussion part at page16.

Pg #7/Par #1/Line 5-9: "Participants...enrollment" According to the NIH Human Microbiome Project - Core Microbiome Sampling Protocol A [4] the following exclusion criteria should also be taken into consideration regarding microbiota studies: - Use of any of the following drugs within the last 6 months: oral, intravenous, intramuscular, nasal or inhaled corticosteroids; presence of oral disease However, they are not mentioned in the Study Design. Moreover, no specific tool is mentioned regarding the validation (or estimation) of oral health between subjects, which is an important factor when evaluating the oral microbiota.

We mentioned that subjects who 4) use of antibiotics (including oral, intravenous or intramuscular) and probiotics within 4 weeks prior to enrollment; and 5) use of corticosteroids (nasal or inhaled) or other immunosuppressants.

Pg #7/Par #3/Line 3-4: "All the...sterile tube". It should be clarified whether the collection of the saliva was stimulated or unstimulated.

About 3 mL saliva was collected after it unstimulated accumulated on the mouth floor and collected in a sterile tube.

Pg #11/Par #2/Line 11-14: "Patients with...unresectable PDAC" It would be nice to add another Table presenting the alterations in diversity between resectable and unresectable PDAC patients.

Since there were no significant differences of alpha diversity between resectable and unresectable PDAC patients, we showed the p value in text respectively. However, Shannon (p= 0.273), Simpson (p= 0.715), Chao1 (p= 0.159), ACE (p=0.137) were not able to distinguish resectable PDAC and unresectable PDAC.

Pg #12/Par #2/Line 5-9: "In addition...without diarrhea" These results regarding the bacterial abundances do not match with the respective ones in Table 3. For example, Prevotella presents greater abundance in patients without jaundice (669.4±384.3)

compared to those with jaundice (403.2±310.8) as the mean values suggest, thus they should be revised accordingly.

Page14: Table 3 presented flora abundance differences between the PDAC patients with symptoms and without symptoms. Patient reporting bloating has greater abundance of *Porphyromonas* (660.4±461.0, p=0.039), *Fusobacteria* (490.0±186.6, p = 0.024) and *Alloprevotella* (155.4±124.1, p = 0.041) compared to those without bloating (412.0±394.3, 361.8±184.4 and 99.3±81.9, respectively). *Prevotella* presents greater abundance in patients without jaundice (669.4±384.3, p = 0.008) compared to those with jaundice (403.2±310.8). *Veillonella* presents greater abundance in patients without dark brown urine (1863.8±1449.2, p = p=0.035) compared to those with dark brown urine (1018.6±766.7). *Alloprevotella* presents greater abundance in patients without vomiting (130.3±100.9, p = 0.036) compared to those with vomiting (91.8±134.4) while *Neisseria* presents greater abundance in patients with vomiting (3343.3±1829.9, p = 0.024) compared to those without vomiting (1360.3±1256.6). *Campylobacter* presents greater abundance in patients with diarrhea (130.5±59.7, p = 0.034) compared to those without diarrhea (74.9±87.2).

Pg #13/Par #2/Line 1-2: "This prospective...adenocarcinoma" Please clarify how the results reflect and oral dysbiosis.

This sentence is a general summary of the results of the entire study. The later part of the discussion elaborates on the flora distribution of our PDAC patients' saliva sample.

Pg #13/Par #2/Line 9-11: "This provides...collect samples" Could this result also reflect the possible translocation of oral bacteria in the gut microenvironment, as it is already evident in colorectal cancer [8]?

I don't think the current research results reflect the possible translocation of oral bacteria in the gut microenvironment because *Enterobacter* were not the common type of bacteria in the oral cavity.

Pg #15/Par #3/Line 1: "Our study had limitations" The sampling method of the oral microbiota is also a limitation and it should be mentioned here.

Page 16: Currently, no studies focus on comparing advantages and disadvantages of using different sample collection techniques, future study should compare the effectiveness of using different sample collection techniques, such as saliva, tongue coating, and oral wash on sample quality for microbiota profiles and preference of patients.

Pg #26/Table 4: Please fix the headings of the columns so that the results can be easily compared without confusion.

Thanks, we will.

Science editor:

1 Scientific quality: The manuscript describes an observational study of the saliva microbiota for cancer detection. The topic is within the scope of the WJG. (1) Classification: Grade B and Grade C; (2) Summary of the Peer-Review Report: Overall it is a sound study with impressive microbiome analysis for potential application of early detection of pancreatic cancer. However, there are still some pointes need to be further improved. From the innovation of the study, the research team also compared the microbiota profiles difference between the patients with different symptoms and without symptoms, which may help in early detection of pancreatic cancer. The questions raised by the reviewers should be answered; and (3) Format: There are 4 tables and 2 figures. A total of 42 references are cited, including 16 references published in the last 3 years. There are no self-citations. 2 Language evaluation: Classification: Grade B and Grade B. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the Institutional Review Board Approval Form, and written informed consent. The authors need to provide the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement, and fill out the STROBE checklist with page numbers. No academic misconduct was found in the CrossCheck detection and Bing search. 4 Supplementary comments: This is an unsolicited manuscript. The study was supported by expert funding of National Natural Science Foundation of China; the 1·3·5 project for disciplines of excellence-Clinical Research Incubation and Innovation Project, West China Hospital, Sichuan University; Clinical Research Incubation and Innovation Project of West China Hospital; and Science and technology project of Sichuan Province. The topic has not previously been published in the WJG. The corresponding author has not published articles in the BPG. 5 Issues raised: (1) I found the authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s); (2) I found the authors did not provide the original figures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor; (3) I found the authors did not write the "article highlight" section. Please write the "article highlights" section at the end of the main text; and (4) the author should number the references in Arabic numerals according to the citation order in the text. The reference numbers will be superscripted in square brackets at the end of the sentence with the

citation content or after the cited author's name, with no spaces. 6 Re-Review: Required. 7 Recommendation: Conditionally accepted.

We will upload the grant application form(s), original figures. We will add the "article highlights" section at the end of the main text. All the revisions are in Purple in the text.