Author’s response to reviews

Title: Gastric Bacterial Flora in Patients Harbouring Helicobacter pylori With or Without Chronic Dyspepsia: Analysis with Matrix-assisted Laser Desorption Ionization Time-of-Flight Mass Spectroscopy

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Reviewer comments and response

Reviewer 1

In this paper the authors aimed at characterizing the gastric microbiota of patients with H. pylori infection with or without chronic dyspepsia and absence of organic disease. The paper is globally well structured and the results show a substantial difference in the gastric microbiota composition between these two groups.

I have the following minor concerns/considerations:

- Although the study of microbiota is a topic of interest, I am not sure that these results could impact clinical decisions, since it is very difficult to understand whether microbiota differences could anticipate H. pylori infection or be the consequence of the presence of H. pylori itself. This point is described in the discussion.

Response

We agree entirely with the learned reviewer. We and other investigators accept that the field is too nascent to draw firm conclusions about cause-and-effect.

- Rome III criteria have been updated to Rome IV criteria in 2016. Although Rome III definition of “functional gastro-duodenal disorders” has been largely confirmed, the authors should refer to Rome IV criteria

Response
We agree with the learned reviewer that the Rome IV criteria are the latest updated ones for functional gastroduodenal disorders. However, at the time we conducted this study, the Rome III criteria were current and hence we used them for our definition. We have added sentences in the text to state that the criteria have been updated subsequent to our study, and mentioning the important differences between Rome III and IV. (Discussion section, lines 189-192, pages 10-11)

- Could the authors provide information about smoking and NSAIDs use in their patients?

Response

None of our patients was on long-term NSAID use; we have added a sentence in the text to that effect. Unfortunately, we do not have data on the smoking status of these patients, and have mentioned this limitation in the text. (Discussion section, lines 187-188, 266-267, pages 10, 11)

- I am a little bit puzzled by the fact that gastric biopsies were not performed in the group of patients undergoing a gastroscopy because of dyspepsia. I think that this is a limitation of the study, because we do not have information about the presence of chronic gastritis. The authors are conscious of this point, but I think that this should be further highlighted.

Response

As the learned reviewer mentions, we have acknowledged this important limitation in our manuscript. We have expanded on this in the revised manuscript. (Discussion section, line 261-266, page 14)

Reviewer 2

In this manuscript the authors seek to identify the different gut microbiota present in H. pylori infected patients with and without concurrent dyspepsia. The premise itself is not novel but the methodology/strategy is. It does however seem that the Results section is extremely brief and that the authors have missed the opportunity to further refine their analysis.

1. In the Introduction the authors describe a longitudinal study that found that incidence of GC is decreased in patients who have had an unsuccessful course of treatment to eradicate H. pylori. They propose that this might be due to successful eradication during treatment of non-H. pylori bacteria that might contribute to the development of GC. Is it also possible that treatment to eradicate H. pylori may be specifically eradicating pathogenic strains?

Response

We hope we have understood the reviewer’s query correctly. Please let us know if our understanding is incorrect.
We agree with the reviewer that there exists a theoretical possibility that eradication of only pathogenic strains of H. pylori or other organisms resulted in the decrease in the development of GC. However, the authors of the study we quoted (Ref 19) did not suggest such a possibility. Also, we are not aware of therapy targeted only against pathogenic H. pylori strains.

2. Table 1: Staphylococcus percentage. Please check calculations- 19/21 patients is not 42.8%

Response

We deeply apologize for this oversight and have corrected the value to 90.4% in the revised manuscript. This has not changed our message, but we accept our mistake.

3. Also Table 1: make sure numbers are rounded correctly. Example Micrococcus 1 patient of 21= 4.8%

Response

Yes, it is 4.8%, not 4.7%. We thank the reviewer for pointing this out. We have recalculated all our figures and have made minor changes wherever rounding off was done to the first decimal place. This, of course, has not changed our statistical calculations.

4. The Results section is very brief and the authors have missed an opportunity to perform a more detailed analysis of their findings and thus several questions remained unanswered:

   a. Are the bacterial counts different in patients with and without dyspepsia?

Response

Unfortunately, we have not checked bacterial counts. Our aim was to explore if there indeed is a qualitative difference between the two groups, because this has not been shown before in literature. Now that we did find a difference, we will, as suggested by the learned reviewer, extend the study with quantitative analysis.

   b. It appears the authors did not analyze strains of H. pylori? Why not?

Response

We agree with the learned reviewer that the strain of H. pylori contributes to its pathogenicity and, so, symptoms. We admit that this could be a confounding factor deciding the symptoms in our patients. There is also the possibility that the concomitant gastric microbiota may be influenced by the H. pylori strain, although we are not aware of any literature to support this. Although we do not expect to find a difference in the concomitant gastric microbiota between strains, we have mentioned this as a limitation of our study in the revised manuscript (Discussion section, lines 263-266, page 14)
c. Were there differences in the types of bacteria found between men and women?

Response

Although we have not included this information in the manuscript because the numbers are small for subgroup analysis, there were no obvious qualitative differences observed between the genders.

d. It appears that the authors did not separately analyze the bacterial contribution in the antrum and body specimens. This decision was based on a previous publication by Bik et al. In the same publication these Bik et al found no difference in H. pylori in bacterial diversity. Given that the current authors hypothesised that there IS a difference it would have made more sense to analyse these independently. Can the authors screen a small number of additional patients to either prove or refute this?

Response

We have probably been misunderstood. We mentioned in the manuscript that we combined samples from the antrum and body because Bik et al. had already shown that there is no difference in bacterial diversity between the two regions. We admit that we have not studied the two regions separately, and will certainly follow up on the reviewer’s suggestion in a future study to support or refute the findings mentioned by Bik et al.

e. What is the distribution of the microflora amongst individuals? Did some patients have much more representation than others?

Response

We did not find wide variation between individuals in the two groups in the range of microbial distribution. All individuals had only one or two isolates; the median (range) number of species identified in the two groups was similar. We have provided this information in the revised manuscript. If the editor and reviewer advise, we can provide a table listing the species in each individual; this may be included as supplementary material in the publication. (Results section, lines 165-167, page 9)

f. Findings in the non-dyspeptic groups analyzed in terms of their clinical indications? Why not?

Response

We have mentioned in the manuscript the spectrum of indications for endoscopy in the non-dyspeptic group. Unfortunately, the number in each indication is small to make meaningful distinctions between them. (Methods section, line 120-122, page 7)
5. Did the authors explore potential pathogenicity of the bacterial types? This should also be mentioned in the discussion?

Response

No, we did not explore the potential pathogenicity of the bacterial types we isolated. Although some of the organisms we isolated are known to be pathogens in other sites and clinical situations, we do not know yet about their pathogenic ability in the gastric environs. We have mentioned this in the revised manuscript. We are grateful to the reviewer for this suggestion for further exploration. (Discussion section, lines 267-269, page 14)

6. Can the authors clarify what they mean by colony character study Pg 9 line 4.

Response

We meant the physical characteristics of the bacterial colony (size, shape, colour, margin, opacity, elevation). This line has been added in the revised manuscript. (Methods section, line 133, page 8)

7. Why is table 2 not represented in terms of percentages?

Response

Since the numbers (except the number 11) in Table 2 were in single digits, we had not converted them to percentages. But, we have done this now in the revised manuscript.

8. Page 12 lines 28-40 appear to be duplicate sentences with the same information

Response

We are sorry we could not identify which sentences the reviewer is referring to. We will be happy to revise any sentence that the reviewer finds inappropriate. We have empirically deleted from one place a sentence that featured in two places: “…a clinical scenario that is much more common in this infection than the development of organic disease.”

9. Can the authors comment on the future clinical ramifications of this work?

Response

We had made a brief and modest mention about the possible clinical ramifications of our findings. On the advice of the learned reviewer, we have expanded a little on this in the revised manuscript. (Conclusion section, lines 289-291, page 15)