Reviewer’s report

Title: Apical Periodontitis: preliminary assessment of microbiota by 16S rRNA high throughput amplicon target sequencing.

Version: 0  Date: 18 Dec 2017

Reviewer: Yasuo Takeuchi

Reviewer's report:

This is a preliminary study examined the microbiota of periapical granulomas (PGs) and radicular cysts (RCs). The samples of periapical lesions were collected from 5 PGs and 5 RCs, and the bacterial presence was examined by pyrosequencing of the 16S rRNA genes. In the present study, facultative anaerobes were mainly found in both PGs and RCs, while PCoA plots with a weighted UniFrac distance matrix showed the difference of microbiota between PGs and RCs. In addition, abundance of several minor OTUs differed among these two groups. They concluded that characteristics of microbiota differed between PGs and RCs.

Main criticism of this study is very small sample size. it is fact that there are limited studies performed comprehensive analysis of microbiota associated with apical periodontitis. This reviewer also understands that studies of this nature using the sequencing approach are usually plagued by a small sample size. However, each five sample is not enough for an evidence based evaluation / comparison of microbiota among two groups.

Specific comments are as follows;

- English style should be refine throughout the paper. It should get some English proofreading from native speakers. Correct the spelling of bacterial names (ex; Staphilococcus warneri → Staphylococcus warneri).

- P3, Abstract / P5, Paragraph 2: What is the mean of the abbreviation "OPT"?

- P3, Abstract / P5, Paragraph 2: State more clearly where and how clinical samples were collected in the study. The authors mentioned that "The main cause of apical periodontitis is the bacterial colonization of the root canal space (in Abstract)". If so, it seems reasonable to collect the sample from the root canal space.
- Materials and Methods: All of the statistical methods used in the study should be written in this section, not in the Results.

- Results: Although the authors showed the relative abundance of bacterial species, it is difficult to understand the whole picture of microbiota in PGs and RCs. This reviewer recommends to show the data of microbiota at phylum and/or genus level.

- P7, Paragraph 1: What is the mean of the abbreviation, "G and C samples."?

- P8, Paragraph 1: The richness of the samples varied from 22 to 202 OTUs, didn't they?

- P8, Paragraph 2: Alpha-diversity indices were showed in Table 2, weren't they?

- P8, Paragraph 3: The words "ADONIS" and "ANOSIM" are recommended to write in all capital letters.

- Discussion: The authors mentioned that some facultative anaerobes such as Lactococcus lactis, Propionibacterium acnes, Staphylococcus warneri, Acinetobacter johnsonii and Gemellales dominated in periapical lesions. I'm not sure but these are unfamiliar as usual bacteria in oral cavity, and it is something of a surprise for me. The authors cited Ref No. 24 and mentioned that "these species are all normal commensals of the human oral cavity and were isolated in radicular cyst (Page 10, Paragraph 2)". However, it was the case report and was also not mentioned the characteristics and/or prevalence of these bacteria. I cannot get over the possibility that the samples were contaminated. The authors should collect more periapical samples and confirm the prevalence of these bacteria using PCR or other bacterial identificational methods. Otherwise, if you collected the saliva from the same subjects, you can examine the presence of these bacteria for checking the contamination of saliva. How do you think that these bacteria are associated with the pathogenicity of periapical lesions? Add the discussion about this topic.

- P10, Paragraph 3: Although the authors mentioned the anaerobic taxa were abundant in RCs samples, there were no information about it in Results. Again, the sentence "Aerobe and facultative anaerobic bacteria growth was seen in 10.8% of the cases." should be written in Results.

- P11, Paragraph 2: Show the proportions of aerobic and anaerobic bacteria to whole microbiota in Results.

- Figure 1: What is the mean of descriptions (Numbers) at "Region" and "Canal Root therapy" part?
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.
No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.
No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.
Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.
I recommend additional statistical review

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Please indicate the quality of language in the manuscript:
Not suitable for publication unless extensively edited

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