Reviewer's report

Title: Use of 16S ribosomal RNA gene analyses to characterize the bacterial signature associated with poor oral health in West Virginia

Version: 1 Date: 9 December 2010

Reviewer: Derren Ready

Reviewer's report:

Summary:

The 13 authors describe the oral microbiota of 12 subjects as determined by culture-independent methods.

General comments:

Overall the paper is well written, interesting and easy to follow. The selection of the 12 subjects is the most puzzling aspect of the manuscript and will need clarification. Samples from 7 subjects were taken from one study group and 5 subjects from a separate study. These 7 or 5 subjects were then further subdivided into low or high disease state dependent on periodontal health or caries incidence.

Major compulsory revision:

1) The authors need to specify why it was important to study these diverse groups of subjects as currently it is unclear in the manuscript

Minor essential revisions

2) The authors must not over interpret these data on such small groups of subjects, particularly as for the sequencing data the number of clones (with data) examined ranges from 55 to 133.

3) The authors state ‘Comparisons of bacterial populations ……provided a means of assessing how bacterial populations associated with periodontal disease compared with those associates with poor caries outcome later in life.’ Are the authors really trying to determine microbial differences in high and low disease states in the young and the elderly with periodontis and caries in just 12 subjects?

4) Was statistical advice taken on the number of subjects required to be enrolled into the study? If it was then please add these details to the text of the manuscript.

5) Did the authors carryout examiner calibration to ensure that assessment of the clinical parameters in the two study groups was comparable?
6) Was plaque collected with a curette for the COHRA study group? Please state the in the text of the manuscript.

7) The COHRA group of patients had subgingival plaque collected from the first four molars and the elderly patients had plaque collected from six sites. Do the authors think that these differences may alter these data?

8) Were any additions made to the DNA extraction method to improve lysis of Gram-positive cell walls? Please provide details if carried out.

9) Please provide the strain details for the E. coli strain used for 16S cloning.

10) Page 10: The authors state ‘As expected, increased bacterial diversity was evident in plaque from individuals diagnosed with high oral disease.’ Please provide a reference to support this statement.

11) The authors describe bacterial diversity in the text of the results and discussion, but perhaps sometimes they mean species richness. The species richness is the number of different species identified whereas the diversity would include the number of individual species as well as the numbers present for each species. If the authors wish to describe diversity then they should consider using a diversity index (eg. Shannon-Weaver Index or the Evenness score) to measure this and add these details to the text. It would allow a better diversity comparison to be carried out which may be of interest to readers of this manuscript.

12) The authors highlight a potential ‘atypical bacterial phylogenetic signature’ however, are they confident that this is the case, as they did not examine non-West Virginia subjects in this study.

Discretionary revisions

13) Why was plaque placed into 100-500µl and not a standardized volume used?

14) It would be ideal to have the clinical details for all 12 of the subjects.

15) As only 5 periodontis patients were examined it may not be that surprising that only a minority of these subjects harboured ‘red-complex’ bacteria. The text could be altered to reflect this.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.