Reviewer’s report

Title: Dynamics of microbiota during mechanical ventilation in aspiration pneumonia

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Reviewer: Gisli G Einarsson

Reviewer's report:

In the following submitted manuscript (PULM-D-19-00238) titled "Dynamics of microbiota during mechanical ventilation in aspiration pneumonia" by Ken Otsuji et al. (2019) the authors describe microbial community composition of the lower and upper respiratory tract in intubated patients diagnosed with aspiration pneumonia, with a further aim of developing treatment strategies against infecting microorganisms. In the study the authors applied 16S rDNA marker-gene cloning methodologies to assess the community composition of the relevant clinical specimens that were collected at predefined time-points during the intubation process. Overall I find this study interesting, especially the longitudinal design and that sampling was both carried out in a paired fashion of the upper and lower airways were possible. However, there are number of limitations to the current manuscript that in my mind would need to be addressed prior to the manuscript further progressing towards publication in BMC Pulmonary Medicine.

Major points:

1. In the current manuscript any limitations related to 16S rDNA clonal libraries are missing. For example, how does this methods compare to more recent advantages made through 16S rRNA marker-gene sequencing on the 454-FLX or Illumina MiSeq platforms? Would 16S rDNA cloning based approaches offer significantly better and more cost-effective ways of assessing microbial community composition than such more modern approaches? I guess that despite potentially allowing better phylogenetic/taxonomic assignment through Sanger sequencing of the longer 16S rRNA gene sequences from the clonal libraries (up-to near full length 16S rRNA gene), biases based on only being able to pick a limited number of clones per sample, the vector may preferentially ligate to one particular sequence type over another, high workload and cost would be a limitation in using the described methodology. This is potentially something that the authors could comment on in discussions.

2. From the current manuscript it is not entirely clear to me how the approaches employed in the current study can infer how best to treat putative bacterial infections in the lower airways of patients diagnosed with aspiration pneumonia. The data shows that there is heterogeneity in community composition, and that this appears to change over time. However, stating that opportunistic pathogens such as Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus etc. are assumed to be resistant to administrated antibiotics is probably an oversimplification. The populations of those clinically relevant taxa may be quite diverse in relations to their responses to treatment, and although many will produce beta-lactamases (incl.
ESBL) or other determinants of putative antibiotic resistance there is unlikely to be a single antibiotic treatment approach that fits all members of those taxa. Without testing this in some way, such as through susceptibility testing against a range of antibiotics, we can't speculate or propose what the best treatment option will be. If the authors are eluting towards that we should be using more personalised approaches, rather than the combination of Ampicillin/Sulbactam which is the most commonly prescribed option here, then that should be highlighted within the manuscript.

Minor Points:

1. In the manuscript "facultative anaerobes" and "facultative aerobes" are used interchangeably when talking about the same main taxa. The authors should use consistent terminology where appropriate.

2. Page 5, line 100) stating that "The tracheal aspirate (B) were cultured aerobically at 35C with 5%CO2…". This would be microaerophilic environmental conditions, not aerobic.

3. Page 12, lines 191-193, stating "Since culture-based methods cannot sufficiently analyse the causative bacteria in aspiration pneumonia…” is not strictly speaking correct as there are number of culture-based approaches that might be useful in this context. In clinical laboratories bacterial cultures attempt to capture a wide range of potentially pathogenic microorganism, including bacteria and fungi. This however can be time consuming and does not allow "rapid" detection of putative infectious agents. Using extended bacterial cultures, i.e. with a number of different bacteriological media and atmospheric conditions (anaerobic atmosphere generally not applied routinely), would allowed the capture of most of the microorganism and furthermore allow for their phenotypic analysis. In this context using 16S rDNA cloning and Sanger sequencing to assess the microbial community composition would not allow for the assessment of functional capabilities of individual members of the community, or the community as a whole, and hence cannot predict the response to antimicrobial therapy with certainty. It would be appreciated if the authors could comment on those limitations when using molecular based profiling.

4. Page 12, line 196, the use of the word "vicissitude", I would recommend that the authors would use other commonly used words such as "change", "alteration", "modification" etc.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
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