Reviewer’s report

Title: The microbiome of chronic rhinosinusitis: culture, molecular diagnostics and biofilm detection

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Reviewer: Michael Surette

Reviewer’s report:

The manuscript by Boase et al compares the microbiology of chronic rhinosinusitis using culture and molecular techniques. Mucosal tissue was obtained from 28 CRS patients and 6 controls. Limited standard culture was carried out. DNA was extracted and microbial species (bacterial and fungal) identified using IBIS technology. Finally samples were also examined by FISH using specific probes for Staphylococcus aureus and as well as with fungal specific probes. The authors detected bacteria in 100% of sample and found multiple organisms with Ibis although the average was between 2 and 4 species using the molecular approach in CRS patients and 1-3 in controls. Only 73% of patients had an organisms cultured. The data suggests that the molecular method is more sensitive in detecting organisms. The findings are not surprising but the methodology is very limiting. Based on many current published studies the microbiology of CRS is much more complex than revealed by Ibis and the limited culture approaches used do not really provide a suitable comparison. The comparison here is made with very limited methodologies.

Major revisions.

Page 5 2nd paragraph. This discussion about culturing is somewhat superficial. Most significantly the referenced paper refers to broth enrichment not culture on solid media. The challenge in culturing environmental organisms is also more severe than human microbiome associated organisms given that many nutrient rich media are toxic to extremely slow growing organisms. This is certainly not the case for microbes associated with the animal hosts. And what’s more striking all of the organisms identified in this study are very easy to culture. Even a little bit of effort would be able to recover all of these organisms. Anaerobic conditions would have helped The Nocardia may have been a little bit harder and required longer incubation times. I also suspect that the organisms actually cultured were under estimated.

A second major consideration that is not dealt with is that the molecular methods employed (Ibis, although this would be true of other molecular methods such as 16S metagenomic profiling) is that a positive result indicates that the DNA for an organism was present in the sample, not a viable organism. I think it is a fair question whether the fact that culture of S aureus for example is a better measure of viable organisms. Mucosal surfaces are graveyards of bacterial (and host DNA) which can persist for some time. The molecular data should always be
discussed in this context.

The number of organisms detected is surprisingly low and no doubt reflects a limitation of the ibis instrument to accurately resolve only the most abundant PCR products. Other molecular methods, such as 16S rRNA profiling with next generation sequencing, reveal much more complex microbiota associated with healthy sinuses (or at least nasopharyngeal swabs) and CRS.

Minor considerations.

The FISH results are in quite good agreement with culture: as this method stains ribosomes it is usually a good measure of viable organisms. However, it is usually common practice to also include a universal eubacterial probe and a negative control (scrambled probe). This would be helpful as would be a figure with representative images.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare I have no competing interests.