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Response to Long et al regarding: "*Cutibacterium acnes* and the shoulder microbiome"

In reply:

We thank Long et al for their letter highlighting the limitations of using V4 16S ribosomal RNA (rRNA) gene primers for detecting *Cutibacterium* (formerly *Propionibacterium*) *acnes* in microbiome analyses. All of the analyses reported in our study were performed in the first quarter of 2016,[3] prior to the publication by Meisel et al[2] detailing the limitations of this primer set for detecting *Cutibacterium*. We were previously unaware of this problem with the V4 primer set, and as we were successful in detecting *C acnes* in skin samples using these primers, we had no reason to suspect that our approach was biased against polymerase chain reaction (PCR) amplification of *Cutibacterium* 16S rRNA gene sequences. Having assessed the references Long et al have provided, we agree that the use of V4 primers in our study may have resulted in an underestimation of the true frequency of *C acnes* in the tissue samples we assessed in our study.[3]

We would like to clarify the impact of this issue on the conclusions drawn in our study.[3] The primary aims of this project were twofold: (1) to determine whether there were microbial DNA within the human shoulder and (2) to determine whether *C acnes* was a component of the bacterial sequences recovered. On the basis of our findings, we discovered the presence of genomic DNA from various *Acinetobacter* species and *Oxalobacteraceae* family members. These genomic sequences were found in approximately three-quarters of the rotator cuff tendon tissue samples and therefore were consistent with the presence of low-abundance microbiota in the rotator cuff. As this V4 primer set is still widely used to efficiently amplify this informative region of the 16S rRNA gene in a wide variety of bacterial genera (the exception being *Cutibacterium*), we have no reason to believe that our findings indicating the presence of a microbiome in the human shoulder are compromised.

Regarding the use of PCR assays to detect *C acnes* DNA in shoulder tissue lysates,[1] we agree that this approach is critically dependent on obtaining shoulder tissue samples that have not been contaminated by exposure to the skin during collection. Despite the technical challenges involved, in our experience such informative shoulder tissue samples can be collected by a careful surgeon who is well aware of the skin contamination issue. Nonetheless, we will continue to work toward designing assays that retain the sensitivity of PCR for detecting *C acnes* while incorporating additional features that will allow us to reliably distinguish between an active periprosthetic joint infection with *C acnes* and skin contamination of the sample.

Disclaimer

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