

Identification and Analysis of the Ankle Microbiome Using Next-Generation DNA Sequencing

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INTRODUCTION: Next-generation DNA sequencing (NGS) technologies have made it possible to detect microbial genome sequences within human tissues with a sensitivity that has not been reached before, allowing research to better characterize “normal” host flora. Researchers have utilized NGS to identify the shoulder microbiome, but there are no reports yet on the ankle microbiome. Using NGS, this study aims to determine whether 1) a unique microbiome exists in human ankle tissues, 2) components of the ankle microbiome impact patient outcomes, and 3) microbes found on the skin make up a normal part of the ankle microbiome within the tissue.

METHODS:

After obtaining Institutional Review Board (IRB) approval, a prospective study recruited 33 patients undergoing total ankle arthroplasty (TAA, n=24) or ankle arthrodesis via an anterior approach (n=9) between November 2020 and October 2021 with one of two fellowship-trained foot and ankle surgeons at an academic medical center. During the operation, the surgeon would swab five layers of the ankle: skin (n=33), retinaculum (n=32), tibialis anterior tendon (n=32), joint capsule (n=32), and distal tibia (n=33). These swabs (N=162) were then sent to one company for NGS. Retrospective data was also collected from patient records to include demographics, medical comorbidities, surgical indication, postoperative complications, readmission and reoperation rates, and pre- and postoperative patient-reported outcome measures. The majority of subjects underwent surgery for ankle arthritis (97.0%). Most subjects were female (51.5%) and white (93.9%), and mean age at surgery was 60.73 (range, 19-85) years. Mean follow-up duration was 0.75 (range, 0.11-1.29) years.

RESULTS:

Of the 162 swabs sent for NGS, 19 (11.7%) indicated 27 bacteria were present (positive), while the remaining 143 (88.3%) had no bacteria present (negative). The most common organisms were *Cutibacterium acnes* (40.7%) and *Staphylococcus epidermidis* (11.1%). *C. acnes* was found in the skin (n=2), capsule (n=5), and distal tibia (n=4); while *S. epidermidis* was found in the retinaculum (n=1), tibialis anterior (n=1), and tibia juxta-articular (n=1). The most bacteria were found in the retinaculum (29.6%), followed by the distal tibia (25.9%) and capsule (22.2%). No postoperative outcome was associated with the presence of bacteria, or the number of bacteria, or affected layers. Similarly, complication (positive=18.2%, negative=22.7%; $p=1.000$), nonunion (positive=0.0%, negative=28.6%; $p=1.000$), infection (positive=0.0%, negative=4.5%; $p=1.000$), 90-day readmission (positive=0.0%, negative=4.5%; $p=1.000$), and reoperation (positive=0.0%, negative=4.5%; $p=1.000$) rates did not differ if a subject’s NGS profile was positive or negative.

DISCUSSION AND CONCLUSION:

This study found that *C. acnes* and *S. epidermidis* were most commonly found in the ankle microbiome, though at a relatively low rate. While there is some evidence to suggest both *S. epidermidis* and *Staphylococcus aureus* biofilm formation are identified on explanted ankle prosthetics, no study to date has investigated whether or not these bacteria were present in the ankle joint prior to surgery. Furthermore, there is no evidence that patients presenting with these bacteria at the time of surgery are at a higher risk of failure following TAA or ankle arthrodesis. Further research should be done with a larger sample to support these findings.