Minimal Bacterial Load for Detection of C. acnes in Clinical Isolates: Comparison of Next-Generation Sequencing to Bacterial Culture

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INTRODUCTION: *Cutibacterium acnes* has been described as the most commonly isolated causative microorganism in prosthetic shoulder infections. It is also commonly isolated from deep tissue cultures obtained during primary shoulder arthroplasty, making interpretation of positive cultures in the revision setting challenging. *C. acnes* is less commonly identified in cases of primary shoulder arthroplasty by Next Generation Sequencing (NGS), appearing to demonstrate a lower false positive rate. Recent work suggests *C. acnes* potentially exists within the normal microbiota of the shoulder, and there is controversy with regards to whether culture may be too sensitive, identifying what may be low-load and lacking clinical impact. In this study we aimed to explore and compare the minimum bacterial load required for the identification of *C. acnes* by both conventional anaerobic culture as well as NGS analysis.

METHODS: Five *C. acnes* strains were tested in duplicate for this study. We included the ATCC 6919 and four strains isolated from heart, knee, shoulder, and cerebrospinal fluid (CSF) procedures. A standard bacterial suspension was prepared at 1.5×10^8 CFU/mL. Serial dilutions were prepared from 1.5×10^6 CFU/mL to 1.5×10^1 CFU/mL. Regarding the culture methodology, 100 uL were inoculated into CDC anaerobic blood agar plates and incubated for 7 days at 37°C in an anaerobic chamber. The remaining volume from each bacterial suspension was sent for NGS analysis.

RESULTS: There was a clear difference in the appearance and the speed of growth between strains. Three strains (ATCC 6919, CSF, and heart) appeared white on the agar culture, with heart and CSF being the fastest to grow (~3 days). The knee and shoulder isolates appeared light gray, and shoulder strain grew the slowest (~5 days). All the strains depicted gamma hemolysis (non-hemolytic strains). No antibiotic resistance genes were identified in any of the strains tested. The plates with the highest bioburden (1.5×10^6 and 1.5×10^5 CFU/mL) showed growth within 72h. Plates with lower bioburden required at least 96h of incubation. All plates were positive by the 7^{th} day. Culture results improved with incubation time. At 3 days, plates were positive for bacterial suspensions as low as 1.5×10^4 CFU/mL and by the 7^{th} day as low as 1.5×10^1 CFU/mL [Table 1]. NGS analysis was able to identify *C. acnes* in bacterial suspensions as low as 1.5×10^2 CFU/mL [Table 2].

DISCUSSION AND CONCLUSION: This study evaluated five morphologically different strains of *C. acnes* bacteria that demonstrated similar genetic backgrounds with regard to their antimicrobial susceptibility profile. The shoulder and knee isolates had the slowest growth of all samples. Culture results varied with time, with 3 days of incubation proving to be less sensitive for *C. acnes* detection compared to 7 days. Yet, even at the lowest bacterial loads, culture incubation greater than 7 days was not necessary for *C. acnes* identification. This study corroborates our prior findings in that NGS and culture vary markedly in their ability to detect *C. acnes*, particularly at lower bacterial loads. Traditional culture may detect *C. acnes* that are part of the natural microbiome of skin or joint.