

Genetics of *Cutibacterium acnes* in Shoulder Arthroplasty: A Large-Scale Bacterial Whole-Genome Sequencing Study

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INTRODUCTION:

Cutibacterium acnes is a commensal skin bacterium commonly colonizing the dermal structures of the shoulder. Frequent recovery of *C. acnes* from deep tissue cultures in cases of revision shoulder arthroplasty has generated interest in its potential role as an opportunistic pathogen contributing to arthroplasty failure. However, the clinical significance of *C. acnes* in this context has been questioned as it is traditionally considered to be a ubiquitous, non-pathogenic commensal organism, and intraarticular samples may be contaminated by extraneous sources leading to false-positive culture results. The objectives of this study were to utilize bacterial whole-genome sequencing to determine if: 1) *C. acnes* strains colonizing the skin are genetically similar to those recovered from deep tissues of the same patient; 2) genetically similar strains are commonly recovered from distinct patients, suggesting common-source contamination (i.e., environmental sources or healthcare personnel such as surgeons or laboratory technicians), and 3) specific bacterial genetic virulence factors facilitate adaptation of *C. acnes* to deep tissues and underlie the progression from colonization to chronic infection.

METHODS:

We identified *C. acnes* isolates from subjects enrolled in an institutional revision shoulder arthroplasty database. We prioritized those with the highest and lowest burdens of *C. acnes* growth from deep tissue samples for comparison with multiple, longitudinally collected samples. Isolates were collected from freezer stock, and DNA extraction and sequencing was performed. Phylogenetic analysis was performed to distinguish “related” *C. acnes* strains (those likely related by descent within the lifetime of a human host) from “unrelated” strains (unlikely to have an epidemiologic connection on a clinically relevant timescale) in this sample set. Closely-related strains from distinct patients suggestive of a common source were identified to form case clusters. We reviewed clinical, demographic, and facility data available in the electronic health record and secure web survey research registry from each case cluster. In addition to comparison of sequence type, we performed a range of genome-wide association studies (GWAS) in this data set with the goal of identifying different classes of genetic variation associated with periprosthetic joint infection (PJI).

RESULTS:

The final study cohort included samples from 89 patients (69% male) collected between 2015 and 2019. A total of 359 of 364 (99%) of isolates were successfully sequenced, 70% of which were deep tissue samples and 30% of which were superficial skin swabs.

1) **Genetic relationship between *C. acnes* from skin and deep tissue:** At the individual patient level, *C. acnes* cultured from the skin showed close genetic similarity to strains cultured from deep, intraoperative sources. Deep and superficial populations of *C. acnes* are in close continuity. This is consistent with clinical viewpoint that the skin serves as a primary reservoir for inoculation of the joint space during shoulder arthroplasty.

2) **Common source contamination:** We identified three common-source clusters of *C. acnes* involving eight patients. Two involved patients treated by the same surgeon, while the third involved patients treated by different surgeons. Demographic review revealed no evidence of shared household environments among cases. Overall, the occurrence of related strains uncommon, affecting ~5% of isolates and 9% of patients.

3) **Virulence factors:** Two complementary GWAS analyses identified loss of genes *luxS* and *TqsA* as associated with the occurrence of PJI. Most notably, *luxS* was absent in the majority (89%) of PJI cases, compared with 5% of cases without PJI. These genes control successive steps of the autoinducer-2 quorum sensing system which regulates acute infection and have been shown to influence biofilm formation.

DISCUSSION AND CONCLUSION:

Results of this study utilizing large-scale whole-genome sequencing of *C. acnes* isolates cultured from shoulder arthroplasty patients suggest:

- 1) A model of *C. acnes* PJI in which bacteria are translocated from the skin into the joint space during surgery and is not consistent with the notion of the intraarticular space acting as a primary reservoir of *C. acnes*.
- 2) That strains most commonly arise from endogenous strains from the patient skin. Strain-sharing from other sources (e.g., healthcare personnel or environment, suggestive of contamination) is infrequent.
- 3) Loss of genes (*luxS* and *TqsA*) modulating bacterial persistence and biofilm formation may facilitate establishment of chronic infection.