AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

BACTERIOLOGY

# *Cutibacterium acnes* Isolates from Deep Tissue Specimens Retrieved during Revision Shoulder Arthroplasty: Similar Colony Morphology Does Not Indicate Clonality

**Roger E. Bumgarner,[a] Della Harrison,[a] Jason E. Hsu[b]**

[a]Department of Microbiology, University of Washington, Seattle, Washington, USA
[b]Department of Orthopaedics and Sports Medicine, University of Washington, Seattle, Washington, USA

**ABSTRACT** *Cutibacterium acnes* is the most common bacterium associated with periprosthetic shoulder infections. Sequencing of *C. acnes* has been proposed as a potential rapid diagnostic tool and a method of determining subtypes associated with pathogenicity and antibiotic resistance patterns. When multiple deep samples from the same surgery are culture positive for the same species and the isolates show the same culture phenotype, it is typically assumed that these isolates are clonal. However, it is well-known that *C. acnes* is not clonal on the skin of most individuals. We hypothesized that the *C. acnes* bacteria recovered at the time of revision shoulder arthroplasty would often represent more than one subtype, and we tested this hypothesis in this work. For patients undergoing revision shoulder arthroplasty, multiple samples from the surgical field were taken. For those patients with multiple samples that were culture positive for *C. acnes*, isolates from each sample were subjected to full genome sequencing. Of 11 patients, 5 (45%) had different subtypes of *C. acnes* within the deep tissues even though the colony morphology was similar. One patient had four subtypes in the deep tissues, while four patients had two different subtypes. Up to four different subtypes of *C. acnes* were observed in the deep tissues of a single patient. Clonality of *C. acnes* isolates from deep specimens from a potential periprosthetic shoulder infection cannot be assumed. Sequence-based characterization of virulence and antibiotic resistance may require testing of multiple deep specimens.

**KEYWORDS** bacteriology, prosthetic infection, shoulder surgery

*Cutibacterium acnes* (formerly *Propionibacterium acnes*) exists on and under the skin surface on the upper body and has a predilection for pilosebaceous glands (1–4). It is the most common bacterium found in the deep tissues at the time of revision shoulder arthroplasty performed for pain, stiffness, and component loosening (3). Because common skin preparations and antibiotics given around the time of surgery fail to completely eradicate the bacteria (5–9), particularly those under the skin surface, transection of the dermal structures can lead to inoculation of *C. acnes* (and related *Cutibacterium* species) into the deeper tissues and implants at the time of the original shoulder arthroplasty (10, 11). Over time, these slow-growing bacteria are believed to be responsible for arthroplasty failure resulting in a need for a revision surgery. The time between index and revision arthroplasty ranges from weeks to years (12).

Multiple strains of *C. acnes* exist on the skin surface (1, 13) as well as in the pilosebaceous units. Most individuals have two or more different subtypes present on their skin simultaneously, and the distribution of various subtypes differs widely between subjects (13). Some subtypes are associated with pathogenic states rather than commensal states (13–15). Certain subtypes may be better adapted at thriving in

**Citation** Bumgarner RE, Harrison D, Hsu JE. 2020. *Cutibacterium acnes* isolates from deep tissue specimens retrieved during revision shoulder arthroplasty: similar colony morphology does not indicate clonality. J Clin Microbiol 58:e00121-19. https://doi.org/10.1128/JCM.00121-19.

**Editor** Robin Patel, Mayo Clinic



Address correspondence to Roger E. Bumgarner, rogerb@uw.edu.

For a commentary on this article, see https://doi.org/10.1128/JCM.01638-19.

**Received** 24 January 2019
**Returned for modification** 22 February 2019
**Accepted** 28 September 2019

**Accepted manuscript posted online** 23 October 2019
**Published** 28 January 2020

the deep tissues and causing clinical infection than others (13, 14, 16). Also, some subtypes may be more strongly associated with clinical markers of infection, such as hemolysis, and antibiotic resistance (16–22). Sequencing of deep tissue samples has recently become a candidate for potential rapid diagnosis (23) and for identifying virulent and antibiotic-resistant strains (14).

When multiple deep tissue samples from the same revision patient are culture positive for *C. acnes* and the isolates appear phenotypically identical by culture, it is often assumed that the bacteria are clonal across the samples. Thus, to reduce the expense to the patient in such cases, if additional characterization is done (typically measurement of antibiotic susceptibility or hemolytic activity), only one isolate per patient is analyzed. It is also often the case that only a single isolate per patient is subjected to multilocus sequence type (MLST) analysis in research studies. However, to our knowledge, there have not been any studies investigating the clonality, or lack thereof, in deep tissue specimens from a potential periprosthetic shoulder infection. In this study, we retrospectively analyzed a subset of revision patients in which multiple deep tissue cultures were positive for *C. acnes* utilizing whole-genome sequencing to identify whether deep tissue isolates were clonal. Given that *C. acnes* is not clonal on the skin of a given individual, we hypothesized that multiple deep tissue cultures from an individual revision patient may also not be clonal even from different cultures that yielded morphologically similar colonies of the same species.

## MATERIALS AND METHODS

From August 2014 to February 2016, we identified a subset of 11 patients in which multiple deep tissue specimens were positive for *Cutibacterium acnes* growth. As was our standard practice at the time, an isolate from each culture-positive deep tissue specimen was grown in brain heart infusion (BHI) and banked as a glycerol stock. When multiple colony morphologies were observed, an isolate from each colony type was banked. DNA from the isolate(s) from each specimen was extracted for whole-genome sequencing, and the single locus sequence typing (SLST) locus in each genome sequence (24) was used to rapidly determine whether clonality existed between isolates from the patient's shoulder.

**Specimen handling and culturing protocol.** A standardized protocol for revision shoulder arthroplasty was followed. Multiple deep tissue specimens were taken from each patient's shoulder, including capsule, collar membrane, humeral canal tissue, humeral explant(s), glenoid explant(s), and periglenoid tissue. A new sterile rongeur or sterile knife blade with sterile forceps was used for each collected specimen. The instruments were individually peel-packed and opened just prior to specimen sampling, and contact with the dermal structures was avoided.

Specimens were cultured according to methods previously described (25, 26). Specimens were processed by the laboratory within 1 h after the surgical procedure in a class 2 laminar flow biological safety cabinet. Fluid and homogenized tissue specimens were inoculated onto the following microbiological media: blood agar (Trypticase soy agar with 5% sheep blood), chocolate agar, brucella agar (with blood, hemin, and vitamin K), and brain heart infusion broth. All media, with the exception of the brucella agar, were incubated at 37°C with 5% carbon dioxide for 21 days. Brucella agar plates were incubated anaerobically at 37°C for 21 days. Media were examined daily for growth visually but were opened only if growth was noted. Species identification was performed using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Billerica, MA). When colonies from multiple samples taken from the same surgery appeared biochemically and morphologically similar, only a single colony from a single sample was subjected to mass spectrometric identification. Gram stain, catalase, and indole test spot results were used to refer an identification of *C. acnes* among morphologically identical colonies isolated from additional media. For each culture-positive sample on solid media, a single isolate for each colony morphotype (typically one) was subcultured in BHI broth and stocked as a glycerol stock. For samples that were culture positive in liquid media, the liquid medium was plated on blood agar, and a single isolate was subcultured in BHI broth and then maintained as a glycerol stock.

Culture results were recorded in a semiquantitative manner, categorized in terms of the specimen *Cutibacterium* value (SpCuV) (27): 0 as no growth, 0.1 as one colony, 0.2 as growth in broth only, and 1, 2, 3, 4 for culture reports of 1+, 2+, 3+, and 4+ growth, respectively, as previously described (27). The individual SpCuVs of each of the deep specimens (capsule, humeral head, humeral stem, etc.) were summed and reported as the total shoulder *Cutibacterium* score (total ShCuS). We selected patients with a threshold total ShCuS above 1.1 as an indication of substantial bacterial burden. This threshold level typically signifies that the patient had multiple positive cultures at a level higher than what would be considered a laboratory contaminant (25, 28, 29).

**Isolate sequencing and SLST classification.** For subjects from whom multiple deep tissues cultured positive, the banked isolates from positive culture were grown anaerobically in BHI medium for 5 days. Bacteria were pelleted, and DNA was isolated following the protocols in the MasterPure Gram Positive DNA purification kit (Lucigen, Middleton, WI). For each isolate, the DNA was purified and then subjected to full genome shotgun sequencing. In brief, the DNA was fragmented using a Nextera DNA library

preparation kit and then sequenced on an Illumina miSeq sequencer using the 2 × 300 paired-end read protocol (Illumina Inc., San Diego, CA). Reads were imported into the software Geneious (Biomatters Ltd., Auckland, New Zealand) and trimmed at both ends to remove sequences with estimated error rates of greater than 2%. Reads shorter than 150 bp after trimming were filtered out. Genome assembly was performed on the filtered and trimmed reads using the Geneious *de novo* assembler. On average, the assembled genomes had approximately 50-fold read coverage across the contigs.

To type the isolates rapidly, the 484-bp sequence for SLST subtyping and the SLST subtypes as described by Scholz et al. (24) was extracted from each genome sequence. In brief, the consensus sequence of all known SLST subtypes was created and used to find the SLST locus in each sequenced isolate within the assembled contigs. For each isolate, the SLST locus was extracted and compared to a local SLST database of all SLST sequences (downloaded from http://medbac.dk/slst/pacnes).

## RESULTS

An average number of specimens taken from each patient was 6.0 ± 2.4, and the average number of positive isolates recovered was 3.7 ± 1.3. The average total ShCuS was 3.1 ± 1.8.

Of the 11 patients, 5 (45%) had different subtypes of *Cutibacterium acnes* within the deep tissues (Table 1) even when isolates from different samples appeared morphologically similar and grew at similar rates in culture. One patient had four different subtypes, while four had two different subtypes. Within a single subject, when isolates with the same subtype were recovered, they were sequence identical within the error of the sequence assembly, as was the case of one sample from which two visually distinct colony types were observed (Table 1, patient 7). SLST subtypes C, G, K, and L were more common in the polyclonal isolates, while types D and H were more common in the monoclonal isolates (Table 2).

## DISCUSSION

The clinical relevance of cutibacteria in the deep tissue has been debated, and the presence of virulence factors, such as those related to hemolysis, has been suggested as a method of determining the need for more aggressive surgical or medical management or for differentiating true infections from contaminants of the surgical field (22, 30). However, the correlation between the hemolytic phenotype of *Cutibacterium acnes* and clinical outcome remains unclear (31, 32). Similarly, several authors have attempted to correlate various subtypes of *C. acnes* with increased pathogenicity (13, 16, 33–38). While the correlation of certain subtypes with acnes vulgaris is clear (13, 34), the correlation of subtypes with pathogenicity of infected implants is less so (16). A major difference between such studies is that for acnes vulgaris, studies typically look at subtype distributions on the skin of many individuals, while for implant infections, most studies make use of banked isolates, and typically only a single isolate per subject is banked.

The presence or absence of multiple subtypes of *C. acnes* in the deep tissues at the time of revision shoulder arthroplasty has never been tested. This study demonstrates polyclonality of *C. acnes* species in a proportion of the deep tissues obtained at the time of surgery even when isolates appear to have the same colony morphology. Our study demonstrates that testing a single deep sample may be insufficient to properly characterize the presence of *C. acnes* in deep tissues. If multiple subtypes are present within the deep tissues as this study suggests, then testing of virulence factors, subtypes, and antibiotic resistance may need to be performed on all, or at least multiple positive samples, rather than assuming that one sample would be representative of the whole.

This study has a few limitations. First, given the infrequent nature of revision shoulder arthroplasties with a substantial bacterial load (in this case, a total ShCuS above 1.1), our sample size was limited to 11. This sample size was sufficient to test our hypothesis but insufficient to make any conclusions regarding the pathogenicity of specific SLST subtypes found in the deep tissues. Second, we are not able to correlate bacterial load and polyclonality with each patient's clinical presentation and outcome. Third, we were not able to test virulence factors such as hemolysis or antibiotic resistance patterns. Such information may have been helpful in determining the

**TABLE 1** *Cutibacterium acnes* load, subtype, and clonality

| Patient | Specimen type | SpCuV[a] | SLST[b] subtype | Colony morphology[c] | Clonal |
|---|---|---|---|---|---|
| 1 | Capsule, sample 1 | 1 | K8 | 1 colony type | No |
| | Capsule, sample 2 | 0.2 | C1 | 1 colony type | No |
| | Capsule, sample 3 | Neg | | 1 colony type | No |
| | Humeral stem explant | 0.1 | D1 | 1 colony type | No |
| | Humeral head explant | 1 | K8 | 1 colony type | No |
| | Glenoid explant | 1 | K8 | 1 colony type | No |
| | Glenoid cement | 0.1 | G1 | 1 colony type | No |
| | Synovium | Neg | | NA | No |
| | Glenoid membrane | Neg | | NA | No |
| | Joint fluid | Neg | | NA | No |
| 2 | Humeral stem explant | 1 | K8 | 1 colony type | No |
| | Humeral canal tissue, sample 1 | 0.1 | C1 | 1 colony type | No |
| | Humeral canal tissue, sample 2 | 0.1 | K8 | 1 colony type | No |
| | Humeral canal tissue, sample 3 | 0.1 | K8 | 1 colony type | No |
| | Joint fluid | Neg | | NA | No |
| | Head explant | Neg | | NA | No |
| 3 | Humeral stem explant | 2 | C1 | 1 colony type | No |
| | Humeral head explant | 1 | C1 | 1 colony type | No |
| | Humeral canal tissue, sample 1 | 1 | G1 | 1 colony type | No |
| 4 | Humeral head explant | 1 | G1 | 1 colony type | No |
| | Humeral stem explant | 1 | C1 | 1 colony type | No |
| | Periglenoid tissue | 1 | G1 | 1 colony type | No |
| 5 | Collar membrane | 0.1 | K1 | 1 colony type | No |
| | Humeral stem explant | 0.1 | K1 | 1 colony type | No |
| | Humeral head explant | 1 | K1 | 1 colony type | No |
| | Humeral canal tissue | 1 | L1 | 1 colony type | No |
| 6 | Humeral head explant | 1 | H1 | 1 colony type | Yes |
| | Humeral canal tissue, sample 1 | 2 | H1 | 1 colony type | Yes |
| | Humeral canal tissue, sample 2 | 2 | H1 | 1 colony type | Yes |
| | Humeral canal tissue, sample 3 | 1 | H1 | 1 colony type | Yes |
| | Periglenoid tissue | 1 | H1 | 1 colony type | Yes |
| 7 | Humeral stem explant | 2 | D1 | 2 different colony types | Yes[d] |
| | Glenoid explant | 2 | D1 | 1 colony type | Yes[d] |
| 8 | Collar membrane | 1 | D1 | 1 colony type | Yes |
| | Periglenoid tissue | 0.1 | D1 | 1 colony type | Yes |
| 9 | Humeral bone | 1 | H1 | 1 colony type | Yes |
| | Humeral canal tissue | 1 | H1 | 1 colony type | Yes |
| | Glenoid explant | 0.1 | H1 | 1 colony type | Yes |
| 10 | Collar membrane | 1 | D1 | 1 colony type | Yes |
| | Periglenoid tissue | 0.1 | D1 | 1 colony type | Yes |
| 11 | Capsule | 1 | K1 | 1 colony type | Yes |
| | Humeral membrane, sample 1 | 1 | K1 | 1 colony type | Yes |
| | Humeral membrane, sample 2 | 1 | K1 | 1 colony type | Yes |
| | Periglenoid tissue | 1 | K1 | 1 colony type | Yes |

[a]SpCuV, specimen *Cutibacterium* value; Neg, negative.
[b]SLST, single locus sequence typing.
[c]NA, not available.
[d]Both colony morphologies were identical within sequencing and assembly error.

importance of testing multiple samples from a single patient rather than assuming that a single specimen is representative of the whole. Finally, while this study demonstrates that *C. acnes* isolates from deep tissues are often polyclonal, it is not at all clear whether this is unique to this genus or whether other species of bacteria isolated from patient

**TABLE 2** *Cutibacterium acnes* subtype distribution

| SLST[a] subtype | % (no.) of subtypes found in: | |
|---|---|---|
| | Monoclonal cultures | Polyclonal cultures |
| C | 0 (0) | 29 (5) |
| D | 33 (6) | 6 (1) |
| G | 0 (0) | 24 (4) |
| H | 44 (8) | 0 (0) |
| K | 22 (4) | 35 (6) |
| L | 0 (0) | 6 (1) |

[a]SLST, single locus sequence type.

tissues may also be polyclonal. Given that clonality of morphologically similar colonies is often assumed in clinical laboratories, this question merits more research.

**Conclusion.** In this study, up to four different subtypes of *Cutibacterium* were observed in the deep tissues of a single patient. *C. acnes* clonality of deep specimens at the time of revision shoulder arthroplasty cannot be assumed. Thus, sequence-based characterization of virulence and antibiotic resistance may require testing of multiple deep specimens.

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