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COMMENTARY

The Nesting Doll of *Cutibacterium acnes* Clonality

Audrey N. Schuetz[a]

[a]Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine and Science, Rochester, Minnesota, USA

ABSTRACT In this issue of the *Journal of Clinical Microbiology*, R. E. Bumgarner, D. Harrison, and J. E. Hsu (J Clin Microbiol 58:e00121-19, 2020, https://doi.org/10.1128/JCM.00121-19) address in a retrospective analysis that clonality of *Cutibacterium acnes* isolates from deep tissue specimens obtained from patients during revision shoulder arthroplasty cannot be assumed. Given that multiple subtypes of *C. acnes* isolates are present on and around the skin pilosebaceous follicles, the finding of multiple subtypes in deep tissues around a single patient's infected joint is not entirely surprising. However, the authors also challenge laboratorians to consider whether further assessment of *C. acnes* isolates from the same joint should be performed and, if so, what testing should be undertaken.

Cutibacterium (formerly *Propionibacterium*) *acnes* is a well-known culprit of foreign-body infections of joints. A Gram-positive anaerobic bacillus which tolerates aerobic environments well, *C. acnes* comprises usual microbiota of the pilosebaceous (hair) follicles of the skin, densely populating areas of the skin surface which are rich in sebum, such as the axilla. Due to its presence in large numbers in and around the axilla, shoulder arthroplasties (or shoulder joint replacements with metal or plastic implant materials) are the most common of the joint replacements which may become infected with *C. acnes*, occurring in approximately 0.9% to 1.9% of patients undergoing such procedures (1). Despite accurate cleansing of the skin surface preoperatively, deep wound inoculation with *C. acnes* from within the sebaceous glands under the skin surface may still occur intraoperatively.

C. acnes-associated arthroplasty infections are often indolent in nature and difficult to diagnose clinically, as they usually lack clinical signs common to many infective processes, such as erythema and wound drainage. Microbiologic diagnosis is complicated by the difficulty in cultivating *C. acnes*; perioperative cultures are often held up to 3 weeks in an attempt to isolate the bacteria. Furthermore, multiple surgical specimens (i.e., at least three and optimally five or six) are collected in order to aid in determination of the clinical significance of *C. acnes*, with more than one positive culture suggesting significance, rather than likely contamination (2). As a result, clinical microbiology laboratories have been receiving an increasing number of surgical specimens from shoulder and other arthroplasty surgeries over the past several years.

In this issue of the *Journal of Clinical Microbiology*, R. E. Bumgarner, D. Harrison, and J. E. Hsu address in a retrospective analysis whether clonality of *C. acnes* isolates from deep tissue specimens can be assumed (3). They included 11 patients from whom multiple deep tissue specimens collected during revision shoulder arthroplasty were positive for *C. acnes* by culture. Not surprisingly, multiple clonal subtypes were present within single patients, as assessed by high-resolution single-locus sequence typing (SLST) (4). Five of 11 patients (45%) demonstrated the presence of multiple subtypes of *C. acnes* within deep tissue cultures from various surgical sites; one patient had four different *C. acnes* subtypes. This finding is interesting but expected, if we assume that inoculation of *C. acnes* into the deep tissues may have occurred during the initial

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Address correspondence to Schuetz.audrey@mayo.edu.

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arthroplasty surgery. Various clones may have established themselves within the tissues and as biofilm around the prosthetic material.

One of the strengths of this study is the technique of surgical deep specimen collection designed to avoid carryover from nearby skin or pilosebaceous units or between different specimen sites. Sampling of deep tissues was performed via standardized protocol, with a new sterile blade or rongeur used for each separate specimen around the shoulder joint, including explants. After initial skin incision, new instruments were used for each specimen collection. Additionally, tissue, rather than swabs, was obtained which enhances recovery of bacteria. Although not performed in this study, other author groups have compared isolates from specimens obtained over time perioperatively, such as immediately after incision of skin, and prior to wound closure, in order to assess comparability of isolates (5). Long-term prospective studies comparing subtypes of *C. acnes* present on skin surface prior to initial arthroplasty and during revision surgical procedures may further elucidate the question of clonality, keeping in mind that the skin microbiome, including *C. acnes* subtypes, likely changes over time.

Most interesting, though, is the authors' challenge to laboratorians to consider our approach to positive *C. acnes* cultures from such specimens. By testing single *C. acnes* isolates from each surgical site, the authors demonstrate that subtypes differed despite similar morphologic appearances of the colonies. Moreover, a single subtype of *C. acnes* in a particular patient in this study possessed two different colony morphologies. Clinical microbiologists expect and know that colony morphology assessment by the naked eye is not as accurate as subtyping. However, Bumgarner et al. (3) question our current approach in clinical microbiology laboratories whereby we assume that "one sample would be representative of the whole" (e.g., one *C. acnes* isolate is meant to represent all *C. acnes* recovered from the same site of infection). When multiple specimens collected from different sites around a single joint intraoperatively are positive for *C. acnes*, most laboratorians will perform definitive identification and other studies such as antimicrobial susceptibility testing (AST) on only the first isolate. Other isolates that appear morphologically similar to the original isolate may undergo limited identification by Gram stain and rapid biochemicals, rather than full identification by matrix-assisted laser desorption ionization−time of flight mass spectrometry (MALDI-TOF MS).

How far should we unpack the nesting doll of morphologically similar *C. acnes* isolates in the laboratory? One of the drawbacks of this study is that the authors saved only one isolate from each colony type per site of intraoperative culture (e.g., humeral canal tissue, periglenoid tissue). However, four different medium types were inoculated with each tissue collection. Growth patterns of isolates on the variety of agars were not recorded. Since we are aware that isolates of the same morphologic appearance may be comprised of different subtypes, it is likely that there were even greater subtype differences among the isolates growing on different agars. Other limitations of the study included the small sample size and the lack of assessment for hemolysis and AST patterns of the isolates. Despite these limitations, the small sample size would have precluded strong conclusions even if there were differences noted.

The suggestion to consider assessment of antibiotic resistance among *C. acnes* isolates from the same infected joint source is also intriguing. Although AST practices differ among laboratories, most laboratorians almost definitively would not additionally assess the AST profile of the other *C. acnes* bacteria obtained from the same intraoperative procedure but from different sampling sites around the joint. AST patterns have been shown to vary according to *C. acnes* subtypes specifically for isolates obtained from acne cases from different patients but have not yet been definitively noted for infected joints (6, 7). However, the higher resistance rates noted in such isolates to typical antiacne medications such as tetracycline, erythromycin, and clindamycin are likely linked to antibiotic exposure on the skin of patients. Further studies should be performed to assess possible links between subtypes of *C. acnes* and antimicrobial resistance.

Before we question whether to change our practice by performing additional

studies on morphologically similar isolates from the same site of infection, we need to know what we are chasing. Would these investigations make a difference for our patients? The authors of this study acknowledge that associations between hemolytic phenotypes, other virulence factors, phylotypes/subtypes, pathogenesis, and clinical outcomes of infected implants are unknown. If significant associations were to be established, perhaps laboratories will be expected in the future to perform whole-genome sequencing or other testing on such isolates routinely to assess relatedness and perhaps other factors. Is this issue of polyclonality of multiple subtypes limited to just *C. acnes* or its genus or is it a more widespread issue among other bacterial species? At what point do we stop unpacking the nesting doll?

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