RESEARCH ARTICLE

Cutibacterium acnes is Isolated from Air Swabs: Time to Doubt the Value of Traditional Cultures in Shoulder Surgery?

Surena Namdari, MD, MSc[1]; Thema Nicholson, MSc[1]; Javad Parvizi, MD[1]

Research performed at the Rothman Institute, Thomas Jefferson University Hospitals, Philadelphia, Pennsylvania, USA

Received: 07 November 2019 *Accepted: 13 June 2020*

Abstract

Background: Given high rates of positive *Cutibacterium acnes* (*C. acnes*) cultures in cases of both primary and revision shoulder surgery, the ramifications of positive *C. acnes* cultures remain uncertain. Next generation sequencing (NGS) is a molecular tool that sequences the whole bacterial genome and is capable of identifying pathogens and the relative percent abundance in which they appear within a sample. The purpose of this study was to report the false positive culture rate in negative control specimens and to determine whether NGS has potential value in reducing the rate of false positive results.

Methods: Between April 2017 and May 2017 swabs were taken during primary shoulder arthroplasty. After surgical time out, using sterile gloves, a sterile swab was opened and exposed to the air for 5 seconds, returned to its contained, and sealed. One swab was sent to our institution's microbiology laboratory for aerobic and anaerobic culture and held for 13 days. The other sample was sent for NGS (MicroGen Dx, Lubbock, TX), where samples were amplified for pyrosequencing using a forward and reverse fusion primer and matched against a DNA library for species identification.

Results: For 40 consecutive cases, swabs were sent for culture and NGS. *C. acnes* was identified by culture in 6/40 (15%) swabs and coagulase negative staphylococcus (CNS) was identified in 3/40 (7.5%). Both cases with positive NGS sequencing reported polymicrobial results with one sample (2.5%), including a relative abundance of 3% *C. acnes*. At 90 days after surgery, there were no cases of clinical infection in any of the 40 cases.

Conclusion: We demonstrate that the two most commonly cultured organisms (*C. acnes* and CNS) during revision shoulder arthroplasty are also the two most commonly cultured organisms from negative control specimens. Contamination can come from air in the operating room or laboratory contamination.

Level of evidence: III

Keywords: Air swabs, Cultures, Cutibacterium acnes, Infection, Shoulder surgery

Introduction

Cutibacterium acnes (*C. acnes*) is an anaerobic aerotolerant bacteria commonly isolated during revision shoulder surgery (1). It is increasingly recognized as a pathogen, mainly in implant-related infections. As an anaerobe, it usually needs a prolonged culture incubation time of up to 14 days for growth and the association between implant surgery and *C. acnes* infection is not always obvious (2). Unfortunately,

Corresponding Author: Surena Namdari, Rothman Institute, Thomas Jefferson University, Department of Orthopaedic Surgery, Philadelphia, PA, USA
Email: surena.namdari@rothmaninstitute.com

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Arch Bone Jt Surg. 2020; 8(4): 506-510. Doi: 10.22038/abjs.2020.40642.2095 http://abjs.mums.ac.ir

prolonged incubation also increases the risk of false positive cultures in isolating organisms that may exist as a result of contamination. The optimal time for incubation of culture samples, based on a recent study, appears to be 10 days (3).

Given high rates of positive *C. acnes* cultures in cases of both primary and revision shoulder surgery, the ramifications of positive *C. acnes* cultures for clinical decision making remains uncertain (4, 5). Based on one study by Mook et al., 13% of negative control specimens were culture positive for *C. acnes* (6). We have similarly demonstrated, in a prior investigation, that 20% of our negative control specimens from the operating room were culture positive for *C. acnes* (7).

The high rate of false positive cultures from shoulder samples, including negative controls has led some investigators to seek an alternative method for isolation of pathogens from the shoulder. Next generation sequencing (NGS) is a molecular tool that sequences the whole bacterial genome University of Malaya, Kuala Lumpur, Malaysia.
School of Pharmacy, Faculty of Science, University of Nottingham Malaysia Campus, Jalan Broga, Selangor, Malaysia.
Sengenics, High Impact Research (HIR. This technology is capable of identifying pathogens and the relative percent abundance of microbial DNA in each sample (8). The clinical value of NGS has been proven in identification of pathogens in patients with neurological infections and systemic sepsis (9, 10).

The purpose of this study was to evaluate the role of NGS, compared to traditional cultures, in isolating organisms, particularly C. acne, from air swabs taken during shoulder arthroplasty. The hypothesis of the study was that NGS would be less prone to contamination and would carry a lower rate of false positive samples.

Materials and Methods

This prospective, single-institution investigation was approved by our institutional review board and was undertaken between April 2017 and May 2017. In forty consecutive cases, swabs of the air were taken during primary shoulder arthroplasty cases in operating rooms that utilized laminar air flow. We excluded any cases with a prior history of shoulder infection. Once the patient was in the operating room, the operative shoulder was prepped and draped per usual protocol and perioperative IV antibiotics were administered. After surgical timeout and before the skin incision, and while wearing sterile gloves, a trained research coordinator opened two separate sterile swabs (COPAN eSwab™ and Puritan™ Sterile Flock Swab) and exposed them to the air, by waving it around for 5 seconds. Each swab was then sealed in its container and transported appropriately. Patients were followed for 90 days after surgery for any clinical signs of early infection.

Culture protocol

The COPAN eSwab was sent to the hospital's microbiology laboratory. Swabs were inoculated in aerobic blood agar plate, chocolate agar plate, MacConkey agar plates, anaerobic blood agar plates and thioglycollate broth. One drop of the broth was placed onto a glass and processed for gram staining. The plates were incubated for 48 hours before removing them from the chamber and examining them for growth. If the thioglycollate broth was cloudy and there were organisms observed on the original gram stain which did not grow on plated media, it was subcultured on the agar plates described above. Each plate was examined every other day for the first week and then on days 7 and 13 or until positive. Semi-quantitative scoring of cultures was determined by the four-quadrant method and classified as follows: no growth, very light growth, light growth, moderate growth, heavy growth (11).

Next Generation Sequencing

The Sterile Flock Swab was sent overnight for NGS analysis (MicroGenDx, Lubbock, TX). Upon arrival at the lab, the first step was DNA extraction and performance of a quantitative PCR to determine the bacterial burden present in the sample. This process is described in Appendix A. The second step was the NGS assay. Initially, the DNA was amplified via a PCR reaction using forward and reverse primers flanking the region of interest. For the detection of bacterial and fungal species, the two regions of interest were the 16S and internal transcribed spacer (ITS), which were highly conserved regions of the rRNA gene in bacteria and fungi, respectively (12, 13). Following the amplification process, the amplified DNA was then pooled based on amplification strength. Sample DNA was then loaded onto beads for the emulsion PCR. Emulsion PCR was then carried out to generate high levels of the sample DNA for NGS. The sample was then sequenced on the Ion Torrent PGM sequencing platform (ThermoFisher Scientific, Waltham, MA). The Ion Torrent sequencer relied on the principle that a hydrogen ion was released each time a nucleotide was incorporated into the DNA, thus generating a change in pH. This change in pH corresponded to the number of nucleotides incorporated into the growing sequence, which was then detected by the sequencer. The final step before data analysis consisted of denoising, to remove short sequences that could interfere with the interpretation of the data generated (14). The sequence reads generated were then compared against a curated NIH/Genbank database. The comparison against the database was performed using USearch and an agreement of at least 90% between the sequence reads and the database was necessary (15, 16).

Statistical Analysis

Comparative statistics between groups were performed using Fisher's exact test for non-continuous variables.

Results

The study consists of air swabs taken during 40 consecutive shoulder arthroplasty cases, where one swab was processed in the hospital microbiology lab and another analyzed using the NGS technology. The swabs processed in the microbiology lab isolated an organism in 6/40 (15%) cases whereas NGS isolated organisms in only 2/40 (5%) cases. *C. acnes* was identified by culture in all 6 (15%) of the positive swabs and Coagulase Negative Staphylococcus (CNS) was also identified in 3 (7.5%)

Table 1. Positive Next Generation Sequencing Results

Case Number	Species Identified (Relative %)
Case #6	P. aeruginosa (26%) P. melaninogenica (19%) C. tuscaniense (12%) S. mitis (9%) B. japonicum (8%) A. wolffii (7%) C. appendicis (4%) B. antiquum (4%) A. schindleri (2%)
Case #20	S. hominis (31%) S. epidermidis (24%) M. subterranean (11%) C. kroppenstedtii (8%) E. biforme (8%) P. submarinus (4%) N. oleivorans (3%) P. acnes (3%)

of the 6 positive samples. Mean time for these cultures to become positive was 7.2 days (range, 5 to 9 days). When considering the semi-quantitative assessment of bacterial growth, all six samples with isolated *C. acnes* were rated as very light growth and all three CNS positive samples were rated as moderate growth. Both cases with positive NGS sequencing reported polymicrobial results with one sample (2.5%), showing a very low abundance of 3% *C. acnes* [Table 1]. As none of the cases with positive culture results also had positive NGS results, there was no concordance between the two testing strategies. At 90 days after surgery, there were no cases of clinical infection in any of the 40 cases.

Discussion

C. acnes is abundant in the dermal layers of the skin limiting its eradication with topical skin preparations, and making interpretation of cultures from shoulder surgery difficult to interpret (1, 2, 17-20). Given that clinical and intraoperative signs of infection are commonly absent in *C. acnes* infections, cultures are currently considered as the "gold standard" for diagnosis of shoulder infections (21). This study detected a high rate (15%) of false positive cultures from samples taken from the room air during shoulder surgery. Interestingly, all samples isolating an organism by culture were positive for C. acne. NGS, on the other hand, showed a single sample with a very low abundance of *C. acnes*.

A very high rate of positive *C. acnes* cultures in primary shoulder surgery exists, ranging from 9% to73% (4, 7, 22-24). *C. acne* is also isolated from a relatively high percentage of negative control samples (6, 7). The fact that *C. acne* exists in abundance in many samples taken from patients undergoing shoulder surgery generates a dilemma for clinicians on deciding which patients to treat and what cultures to consider as false positive. The reason for a relatively high rate of false positive cultures in shoulder surgery relates to numerous factors including inoculation of the deep tissues by bacteria that colonize the skin layers, use of dirty instruments for specimen acquisition, specimen mishandling prior to transfer to sterile containers, contamination of the samples by the air, or contamination in the microbiology laboratory.

One possible explanation for false positive samples, in general, and the air swabs from this study, is the fact that some organisms do exist in the air in the operating rooms. The longer incubation period that is usually deemed to be necessary for isolation of *C. acnes* allows for some of these organisms that exist in low quantities in the room air to be isolated. The fact that bacteria in the operating room exist has been demonstrated by numerous studies. In a study by Smith et al cultures from sterile basins that were left exposed to air during lower extremity arthroplasty under laminar flow isolated Bacillus species in 13% of cases (25). Our data demonstrates that 15% of samples exposed to the air for as little as 5 seconds can be culture positive for *C. acnes*. Interestingly, half of the *C. acnes* positive samples were also positive for CNS (7.5%). In a study of 221 revision shoulder arthroplasties, Lucas et al reported a 25% rate of positive CNS cultures and considered it the second most commonly identified organism in revision shoulder arthroplasty. The findings of this study are sobering in demonstrating that the two most commonly identified organisms during revision shoulder arthroplasty (*C. acnes* and CNS) are also the most likely bacterial contaminants from the air in the operating room. Another explanation for the high false positive cultures in shoulder surgery may relate to bacterial contamination of specimens during collection, transport, or laboratory processing. In this study, collection was performed in a very controlled and reproducible manner and specimens were sealed and transported to the laboratory immediately after collection. Because of this, if there was contamination, it was more likely to occur in the laboratory. Though protocols were present within the institution's microbiology laboratory to prevent specimen mishandling, factors present during the processing of clinical specimens could contribute to contamination include faulty exhaust systems, breached decontamination solutions, or dirty microscopy slides (15, 26). Because of the risk of single specimen contamination, it is generally recommended that multiple tissue samples be sent when attempting to diagnose a *C. acnes* infection (27). Despite this, simply increasing the number of specimens does not eliminate the concern for false-positive diagnoses.

Because of the inherent concerns with culture, we also examined the *C. acnes* identification rate with NGS. Because NGS identified *C. acnes* in only 1 case, the false positive rate appears to be lower compared to culture. In our opinion, the goal of a diagnostic test for infection should be to identify clinically important organisms that are contributing to or causing the patient's disease process. Because of this, we consider the organisms positively identified by either culture or NGS from air swabs of the operating room to be false positives.

Certainly this data raises concerns regarding the accuracy of our current methods of identifying bacteria and defining infection in shoulder surgery. Further investigation on the ability of NGS to identify *C. acnes* is necessary to prove it useful as a standard diagnostic tool. Future study should be aimed at determining the false negative rate of NGS as compared to culture in cases of true shoulder infection.

This study has a number of weaknesses. Due to budgetary limitations and the cost of NGS, our sample size was admittedly small and only included one sample for culture and NGS from each case. Given that this was a hypothesis generating study, we believe that the results remain valuable despite the lack of statistical power. Assuming that a 15% reduction in the false-positive culture rate would have been clinically relevant, 75 patients would have been necessary (alpha 0.5, power 0.8). The current study was powered at 0.5. Because there was no agreement between the positive samples identified by culture or by NGS, comparative analysis of groups would have been misleading, even if adequately powered. Additionally, we did not include any tissue cultures or skin swabs from cases and cannot determine the positive culture rate from the surgical field. Finally, this study evaluated the false positive rate for *C. acnes* in negative controls, and we cannot comment on the false negative rate of cultures or NGS in cases of true *C. acnes* infection.

We demonstrate that the two most commonly cultured organisms (*C. acnes* and CNS) during revision shoulder arthroplasty are also the two most commonly cultured organisms from negative control specimens. Contamination can come from air in the operating room or laboratory contamination. Given the high rate of false positive cultures and the lack of concordance with NGS findings, diagnosis of *C. acnes* infections using culture data may be subject to inaccuracy.

Disclosure: This study was funded by the Orthopaedic Research and Education Foundation (OREF) / Goldberg Arthritis Research Grant: #16-005

Surena Namdari MD MSc[1]
Thema Nicholson MSc[1]
Javad Parvizi MD[1]
1 Rothman Institute, Thomas Jefferson University, Department of Orthopaedic Surgery, Philadelphia, PA, USA

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Appendix A

DNA extraction and real-time PCR:

DNA extraction was performed using the Roche High Pure PCR Template Preparation Kit (Roche Diagnostics, Basel, Switzerland). The extraction process was modified by the inclusion of a beading step for tissue and cell disruption using 5mm steel beads, 5mm Zirconium Oxide beads and the use of the Qiagen tissuelyser II instrument (Qiagen, Hilden, Germany). The lysate generated from this step was then prepared using the Roche High Pure PCR Template Preparation Kit. The initial step in the performance of the assay was the performance of real-time polymerase chain reaction (real-time-PCR), using the lifecycler 480 (Roche Diagnositics, Basel, Swtizerland). This assay provides a quantitative assessment of the bacterial burden of the assay, and covers a range of organisms and antibiotic resistance genes including the following: Enterococcus faecalis, Klebsiella pneumoniae, Streptococcus agalactiae, Streptococcus pyogenes, Candida albicans, Enterococcus faecium, Pseudomonas aeruginosa, Staphylococcus aureus, Serratia marcescens, methicillin resistance, vancomycin resistance.