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The incidence and incubation period of false-positive culture results in shoulder surgery

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Background: Postoperative shoulder infection is a significant complication requiring timely identification and treatment. Indolent infections such as those involving *Cutibacterium acnes* (formerly *Propionibacterium acnes*) provide a diagnostic dilemma as they present differently, without the acute symptoms associated with most postoperative bone and joint infections. Furthermore, *C acnes* is thought to be a common contaminant isolated from intraoperative cultures. With no consensus algorithm, long-held cultures play a major role in guiding management decisions in potential postoperative shoulder infection. Our study sought to determine the incidence of positive culture results in both open and arthroscopic procedures in noninfected patients, as well as to clarify whether an increase in the incubation time frame leads to an increased rate of culture growth.

Methods: One hundred patients were prospectively enrolled into either the open or arthroscopic procedure group. Patients with abnormal inflammatory laboratory findings, a history of shoulder surgery, or corticosteroid injection within 6 months of surgery were excluded from the study. Three cultures were obtained for each patient: superficial tissue culture, tissue culture, and ''sterile'' control swab. Cultures were held for 28 days and checked at regular intervals. All patients were followed up clinically for 6 months to ensure no signs of postoperative infection occurred.

Results: Ultimately, 95 patients were included in the final analysis. The false-positive rate was 17.0% in those who underwent open shoulder surgery and 10.4% in those who underwent arthroscopic shoulder surgery. The incidence of positive *C acnes* culture results was 6.4% in the open group, whereas *C acnes* was not isolated in the arthroscopic group. All positive bacterial culture results were reported within 7 days of collection. One culture result was positive for mold at 26 days.

Conclusion: A relatively high false-positive culture rate occurred in both open and arthroscopic shoulder surgery. *C acnes* was the most commonly identified bacterium in cultures in the open surgery group. Knowledge of one's institutional false-positive culture rate could be important in avoiding potentially inappropriate treatment. Additionally, we found that holding cultures longer than 14 days did not lead to an increased rate of false-positive culture results.

Level of evidence: Basic Science Study; Descriptive Epidemiology Study; Microbiology

Keywords: *Cutibacterium acnes* (*C acnes*); postoperative shoulder infection; false-positive rate; incubation time; open shoulder surgery; arthroscopic shoulder surgery

The Indiana University Office of Research Compliance granted institutional review board approval to this study (no. 1407463823).

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Infection following shoulder surgery, particularly in shoulder arthroplasty, is a major cause of morbidity and economic burden requiring timely identification and treatment.[1,8,11] Indolent postoperative shoulder infections (PSIs) provide a diagnostic dilemma. Persistent pain and stiffness are often the only presenting symptoms, without other local, systemic, or radiographic findings commonly associated with most postoperative bone and joint infections. The findings of inflammatory laboratory studies such as the erythrocyte sedimentation rate and C-reactive protein level are often equivocal, with reported sensitivities and specificities $< 50\%$ in *Cutibacterium acnes* (formerly *Propionibacterium acnes*[17]) shoulder infections.[11,18] Blood biomarkers have not been found to be reliable.[18] Currently, there exists no consensus algorithm to aid in the diagnosis of PSI.

C acnes is an indolent, aerotolerant, anaerobic, gram-positive bacillus considered normal flora in the sebum-containing hair follicles within the dermis around the shoulder and axilla. Once considered merely a contaminant, *C acnes* is now considered a pathogenic organism commonly isolated in both open and arthroscopic PSIs.[2,5,8,9,11,15,17] *C acnes* has been isolated in up to 70% of periprosthetic shoulder infections.[12] Previous studies suggested a commensal nature between *C acnes* and the shoulder joint; however, recent data have suggested that *C acnes* is localized to the dermis.[14] As a result, infections due to *C acnes* are thought to derive from skin contamination during surgery. Recent studies have suggested a high rate of positive culture results from intraoperative samples.[3,4,7,8,10,14] In their 2015 study, Mook et al[10] found an overall incidence of positive culture results as high as 18.3% in patients undergoing open shoulder surgery, with a greater propensity for patients with a history of ≥ 2 preoperative corticosteroid injections and male patients.

Without a reliable diagnostic test or diagnostic algorithm, intraoperative cultures play a major role in guiding management decisions in patients with potential PSI. The diagnostic strength of intraoperative cultures is further confounded by the fastidious nature of *C acnes* or other indolent bacteria. Although the majority of positive culture results for *C acnes* are typically identified at between 7 and 10 days,[3,5,6,16] some authors have advocated longer incubation times.[4,14] Theoretically, prolonged maintenance of cultures could be associated with an increased likelihood of contamination, which could result in the potential for unnecessary treatment.[3,4,7,14]

An understanding of the false-positive rate of intraoperative cultures is critical in the workup of PSI to avoid potentially inappropriate treatment. The purpose of this study was to determine the incidence of false-positive culture results in noninfected patients (clear mechanical source of pain, no suspicion of infection, negative inflammatory laboratory findings, and no corticosteroid injection within 6 months of surgery) undergoing primary open or arthroscopic shoulder surgery. Additionally, the study sought to clarify whether an increase in the incubation time frame leads to an increased rate of culture growth.

Materials and methods

Study design and cohort

This single-surgeon prospective cohort study sought to enroll 100 consecutive patients: 50 each in the arthroscopic and open repair groups. Patients were enrolled from May 2015 to November 2017 from a private-practice clinic prior to surgery. Informed consent was obtained from those patients eligible for the study.

Eligible study subjects included adult patients (aged > 18 years) undergoing open or arthroscopic shoulder surgery for a clearly identified mechanical source of primary shoulder symptoms, including rotator cuff tear, labral tear, instability, tendinosis, and osteoarthritis. Patients with prior shoulder surgery, prior glenohumeral injection within 6 months of enrollment, systemic or shoulder inflammatory disorder, and clinical or imaging findings raising the suspicion of infection were excluded from the study. Additionally, the preoperative erythrocyte sedimentation rate, C-reactive protein level, and procalcitonin level were obtained as screening tools to further assess underlying infectious or inflammatory processes.

Procedure and follow-up

All study participants prewashed the surgical area with chlorhexidine soap the night before surgery. For all open surgical procedures, patients were placed in a semi-sitting position. For all arthroscopic surgical procedures, patients were placed in the lateral decubitus position. Hair was shaved from the shoulder with electric clippers immediately prior to preparation and draping if it interfered with incisions, draping, or postoperative dressings. Presurgical skin cleansing was performed over the entire shoulder and down the operative extremity to the wrist. The skin was painted with 100% isopropyl alcohol, which was allowed to completely dry. It was then painted with povidone-iodine 10%, which was allowed to completely dry prior to draping. Adhesive-edged drapes were applied circumferentially about the shoulder, and a sterile stockinet was placed over the upper extremity, near the axilla. For open surgical procedures, all exposed skin about the shoulder was covered and sealed with an occlusive adhesive dressing. Administration of prophylactic antibiotics was delayed until all cultures were obtained. All cultures were taken as quickly as possible after initiation of surgery so that antibiotics were not unduly delayed.

At the time of surgery, 3 cultures (superficial, deep, and ''sterile'' control) were obtained. For open surgical procedures, a superficial specimen was a tissue biopsy specimen composed of a small piece of fat and/or connective tissue (approximately $1 \times 1 \times 0.5$ cm) harvested immediately below the dermal layer. For arthroscopic surgical procedures, a superficial specimen was obtained by using an arthroscopic punch to harvest 5-6 bites of subacromial bursal tissue. A deep specimen was a tissue biopsy of the glenohumeral synovial and/or capsular tissue. The sterile control specimen was handled using all the same

procedures, except that the swab never made contact with the patient. The surgical assistant passed the swab to the surgeon, who moved the swab through the air around the patient's shoulder without contacting any surface for 3 seconds and then returned it to the culture vial in the standard fashion.

All samples were tested with gram stain, and cultures were performed in chocolate agar, blood agar, MacConkey agar, CNA agar, and thioglycolate broth. Cultures were monitored daily for 28 days for colony growth; the plates were opened only if growth was observed. A culture result was considered positive if any microbial growth occurred.

Because all included study patients had a clear mechanical source of primary shoulder pain, no previous shoulder surgery, negative inflammatory laboratory findings, no recent injections, and appropriate imaging findings, all positive culture results were considered, for the purposes of this study, to be false-positive results due to contamination during sampling, transport, and/or laboratory handling errors.

Patients were followed up clinically for 6 months postoperatively to ensure that preoperative pain resolved after surgery, consistent with an effectively treated mechanical disorder, and there was no clinical suspicion of infection (shoulder or wound redness, swelling, warmth, tenderness, or drainage; fever; or unexplained malaise).

Results

In total, 128 patients were screened for the study. Of these, 22 (17.1%) were excluded because of elevated inflammatory laboratory findings and 6 did not receive the planned surgical procedure. Of the 100 participants enrolled in the study, 5 were removed postoperatively because of laboratory error in which either cultures were not held for the entire 28-day protocol or culture specimens were lost. Ultimately, 95 patients were included in the final analysis: 47 in the open group and 48 in the arthroscopic group. In the open group, surgical procedures included 22 replacement arthroplasties, 22 rotator cuff repairs, and 3 instability repairs. In the arthroscopic group, surgical procedures included 24 acromioplasties, 17 rotator cuff repairs, 4 distal clavicular resections, and 3 instability repairs. At 6 months postoperatively, all patients had progressed through the postoperative rehabilitation protocol as expected, with no signs of infection. However, 1 patient, treated for multidirectional instability, did have some residual pain without clinical signs or concerns of infection.

Open surgery group

In the open group, positive culture results were reported for 8 of the 47 patients (17.0%), with 1 patient yielding positive superficial and deep culture specimens, each growing a different organism. Of the 141 culture results obtained, 9 (6.3%) were positive for bacterial growth. Five positive culture results were from superficial tissue, 2 positive culture results were isolated from deep tissue, and 2 positive culture results were isolated from sterile control swabs. All positive bacterial culture results were reported within 7 days.

C acnes was isolated in 3 of the 47 patients (6.4%), twice from a superficial tissue specimen and once from a deep tissue specimen. *C acnes* was the most commonly isolated organism (33% of positive culture results) in the open group, followed by coagulase-negative *Staphylococcus aureus* (22% of positive culture results). All 3 patients yielding *C acnes*–positive growth were men.

Arthroscopic shoulder group

In the arthroscopic group, positive culture results were reported in 5 of 48 patients (10.4%). Of the 144 culture results, 8 (5.5%) were positive for bacterial growth and 1 (0.7%) was positive for "mold." Of the 9 positive culture results, 3 were obtained from superficial tissue, 3 were obtained from deep tissue, and 3 were obtained from control swabs. Two patients had >1 positive culture result. One of these patients showed growth of methicillin-resistant *Staphylococcus aureus* (MRSA) from the superficial, deep, and sterile control cultures. This patient showed no signs of infection postoperatively, was not treated, and progressed through the postoperative protocol as expected, with no sequelae. All positive bacterial culture results were reported within 7 days. Only 1 specimen was isolated beyond 1 week, which was positive for mold at 26 days.

No specimens from the arthroscopic group showed positive findings for *C acnes*. The most commonly isolated organism was MRSA (50% of positive culture results), followed by coagulase-negative *S aureus* (25% of positive culture results) (Table I).

Statistical analysis

We determined, using 1-way analysis of variance, that there was no statistically significant difference between control and tissue sample positive culture ($P = .35$) and *C acnes*–positive ($P = .78$) rates in the open surgery group. Similarly, in the arthroscopic group, there was no difference between control and tissue sample positive culture ($P > .999$) and *C acnes*–positive ($P > .999$) rates. Statistical significance was considered for $P < .05$.

Discussion

Previous studies have shown high rates of positive culture results in operative shoulders when no infection was suspected preoperatively.[5,8,10,17] However, these studies included patients who had undergone previous shoulder surgery or who had received a recent preoperative cortical steroid injection, either of which could have been a possible

Table I Results of open surgery (9 positive cultures) and arthroscopic surgery (9 positive cultures)

	Subjects	Specimens
Open surgery		
Total	47	141
Positive culture results	9 (19.1%)	9 (6.4%)
Deep	2	
Superficial	5	
Control	2	
Results positive for *C acnes*		
Deep	1	
Superficial	2	
Control	0	
Arthroscopic surgery		
Total	48	144
Positive culture results	9 (18.7%)	9 (6.2%)
Deep	3	
Superficial	3	
Control	3	
Results positive for *C acnes*		
Deep	0	
Superficial	0	
Control	0	

source of contamination resulting in an indolent, undiagnosed infection.

In our study, we excluded any patient who had a history of any shoulder surgery or who received a corticosteroid injection within 6 months of surgery. Moreover, we excluded any patient with elevated serum inflammatory markers. Still, these measures do not absolutely rule out the possibility of an indolent, undiagnosed infection in our patients. Therefore, we also performed a sterile control culture in each patient by obtaining cultures from swabs that never had contact with the patient. Thus, any positive control culture result would clearly have to be considered a false-positive result.

We identified 9 positive culture results in each of the open and arthroscopic groups. Two of the 9 positive culture results for the open surgery group and 3 of the 9 positive culture results for the arthroscopic surgery group occurred in control specimens. Because the rate of positive culture results for control specimens did not statistically differ from the rate of positive culture results that were actually taken from patients, we surmise that all positive culture results were false-positive results for the purpose of this study.

Overall, the rate of positive culture results was similar for both the open and arthroscopic surgery groups. There were 9 positive culture results from 141 specimens (6.4%) in the open surgery group and 9 positive culture results from 144 specimens (6.3%) in the arthroscopic surgery group. Our study's overall incidence of positive culture results for open surgery (17%, 8 of 47 patients) and arthroscopic surgery (10%, 5 of 48 patients) is consistent with the high false-positive rates quoted in previous studies, but the positive culture rate of *C acnes* (6% in the open group and 0% in the arthroscopic group) was lower than in previous reports.

C acnes was the most commonly isolated organism in the open surgery group. Consistent with the literature, all of our *C acnes* cultures were isolated from male patients and the majority were taken from superficial tissues. The 1 deep tissue sample positive for *C acnes* could be due to sampling error or contamination from the dermis during surgery.[7,8,9,10,14] Although patient preoperative preparation, sampling, and laboratory handling play a more obvious role, previous seeding via previous surgery and/or recent (<6 months) corticosteroid injections might play a small role in the differences in our study results. Further higher-powered studies with controls would be needed to determine whether previous seeding truly increases the rate of false-positive culture results.

Despite a low overall incidence of PSI in arthroscopic surgery of approximately 0.27%, the false-positive rate of cultures in our study was surprisingly high (approximately 19% of all patients had a positive culture result).[5] The high false-positive rate highlights the need to minimize contamination due to sampling, transport, or handling errors. Additionally, clinical judgment should not be based solely on culture results, as exemplified by our patient with MRSA-positive culture results from the superficial, deep, and control samples. Given no clinical symptomatology combined with resolution of pain postoperatively, the patient avoided inappropriate antibiotic therapy.

C acnes was not isolated in the arthroscopic group in our study, which differed from the *C acnes*–positive rate reported by Chuang et al[5] (deep tissue rate, 19.6%) and Sethi et al[18] (21.8% positive rate at 14 days and 25.1% at 28 days). An absence of positive *C acnes* culture results was not expected given superficial tissue samples and prolonged culture incubation times. Patient presurgical preparation, sampling, and laboratory handling differences are thought to account for the majority of our differing results. Perhaps more important than the reason for the differences is the discrepancy in results present in the literature, which further supports the need for a consensus algorithm and/or more specific diagnostic test.

Holding cultures beyond 14 days has been suggested in the literature but has been questioned by some authors.[7,11] Recent studies have suggested that incubation times from 7 to 13 days decrease the false-positive rate of *C acnes*. Our results demonstrate that all positive bacterial culture results were identified within 7 days (1 culture result was positive for mold at 26 days) and that holding cultures longer than 14 days did not lead to an increased rate of false-positive culture results.

Our study does have limitations. We were not able to prove the positive culture results were truly false-positive results. Our study did include patients with a history of remote glenohumeral injection, which has been associated

with an increased rate of positive culture results.[11] We tried to reduce the potential impact of this factor by excluding all patients who had received an injection within 6 months of surgery. Still, we did not definitively eliminate the possibility of preoperative seeding by injection because one could appropriately argue that any injection in any time frame prior to surgery could cause contamination of the joint.

Another limitation of the study was the short clinical follow-up period. A 6-month follow-up period may not allow enough time to reliably determine whether positive culture results were inconsequential, given that PSI due to *C acnes* can first present as late as 2 years postoperatively.[1] However, all but 1 patient experienced complete resolution of preoperative pain, and none of the patients exhibited clinical symptomatology consistent with infection. Still, one could reasonably argue that multi-year follow-up would be necessary to be absolutely sure that none of our patients would eventually present with a shoulder infection.

Another important limitation of this study is the fact that the results reflect an experience in a single health system. Our results may not be generalizable to other microbiology laboratories, surgeons, or surgery centers. It would certainly be important for all clinicians to understand the false-positive risk for their health systems. The clinical and economic implications of treating a patient on the basis of an inaccurate diagnosis of infection when no infection is actually present, owing to a false-positive culture result, could be quite significant.

There are disturbing findings when reviewing the results of our study. One concern is the high rate of laboratory errors. We had to exclude 5 patients because of blatant errors, including lost samples and removal of cultures prior to 28 days. Additionally, 5 of the 18 positive culture results were from the sterile control swabs, which suggests a high rate of contamination during sampling, transport, or laboratory handling. The high rate of errors was considered a significant finding in our study and highlights the need to implement institutional regulations to limit culture mishandling in the future.

Our study supports previous findings of high positive culture rates associated with shoulder surgery. Although our results may not be generalizable outside our health system, they highlight the importance of determining one's institutional false-positive rate. Although the utility of prolonged incubation times beyond 14 days is debatable, our study did not find an increase in false-positive culture results beyond 14 days.

Conclusion

Our study's primary objective was to determine the incidence of positive culture results in open and arthroscopic shoulder surgery in patients who were deemed (based on the inclusion and exclusion criteria of this study) to have no preoperative infection. The false-positive rate was 17.0% in the open shoulder surgery group and 10.4% in the arthroscopic shoulder surgery group. The incidence of positive *C acnes* culture results was 6.4% in the open group, whereas *C acnes* was not isolated in the arthroscopic group.

Our study's second objective was to determine whether there was an increased rate of positive culture results with prolonged incubation times of cultures in the microbiology laboratory. All positive bacterial culture results were reported within 7 days of collection. One culture positive for mold was isolated at 26 days. Thus, this study did not find an increased rate of false-positive culture results by prolonging incubation times to 28 days.

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