The Incidence and Incubation Period of False Positive Cultures in Shoulder Surgery

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This is the author's manuscript of the article published in final edited form as:

McCarroll, T. R., Jaggers, R. R., Cagle, R. A., Davis, T. E., Easton, B. L., Curless, C. T., & Misamore, G. W. (2020). The Incidence and Incubation Period of False Positive Cultures in Shoulder Surgery. Journal of Shoulder and Elbow Surgery. https://doi.org/10.1016/j.jse.2020.06.030

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Disclaimer: None

Indiana University Office of Research Compliance granted institutional review board approval (IRB# 1407463823).

Grant Support: Thomas A. Brady Trust, Methodist Sports Medicine Research and Education Foundation

Acknowledgments: The authors thank Anietra Butler, MS.

ABSTRACT

Background: Postoperative shoulder infection (PSI) is a significant complication requiring timely identification and treatment. Indolent infections such as those involving *Cutibacterium acnes* (C. *acnes*, recently reclassified from *Propionibacterium acnes*[1]) 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 hold cultures play a major role in guiding management decisions in potential PSI. Our study seeks to determine the incidence of positive cultures in both open and arthroscopic procedures in non-infected patients as well as clarify whether or not an increase in the incubation time frame leads to an increased rate of culture growth.

Methodology: One hundred patients were prospectively enrolled into either an open and arthroscopic procedure group. Patients with abnormal inflammatory labs, history of previous shoulder surgery, or corticosteroid injection within six months of surgery were excluded from the study. Three cultures were obtained for each patient (1superficial tissue culture, 2- tissue culture, and 3- “sterile” control swab). Cultures were held for 28 days and checked on regular intervals. All patients were followed clinically for 6 months to ensure no signs of postoperative infection.

Results: Ultimately ninety-five patients were included in the final analysis. The false-positive rate in open shoulder surgery was 17.02% and arthroscopic shoulder surgery was 10.4%. The incidence of positive C. *acnes* cultures was 6.4% in the open group while C. *acnes* was not isolated in the arthroscopic group. All positive bacterial cultures were reported within seven days of collection. One culture 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 bacteria in cultures in the open surgery group. Knowledge of one's own 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; Mcirobiology

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

Infection following shoulder surgery, particularly in shoulder arthroplasty, is a major cause of morbidity and economic burden requiring timely identification and treatment[1, 9, 13]. Indolent postoperative shoulder infections (PSI) 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. Inflammatory laboratory studies such as erythrocyte sedimentation rate (ESR) and C-3 reactive protein (CRP) are often equivocal with reported sensitivities and specificities below 50% in C. acnes shoulder infections[13, 21]. Blood biomarkers have not been found to be reliable[21]. Currently, there exists no consensus algorithm to aid in diagnosis of PSI.

Cutibacterium acnes (C. *acnes*, recently reclassified from *Propionibacterium acnes*[20]) is an indolent, aero-tolerant 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 postoperative shoulder infections[2, 5, 9, 11, 13, 18, 20]. C. *acnes* has been isolated in up to 70% of periprosthetic shoulder infections [14]. Previous studies suggested a commensal nature between C. *acnes* and the shoulder joint; however, recent data suggests C. *acnes* is localized to the dermis[16]. 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 cultures from intraoperative samples[3, 4, 7, 10, 12, 17]. In their 2015 study, Mook et al found an overall incidence of positive cultures as high as 18.3% in patients undergoing open shoulder surgery with a greater propensity for those patients with a history of two or more preoperative corticosteroid injection and male[12].

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. While the majority of positive cultures for C. acnes are typically identified between seven and ten days[3, 5, 6, 19] some have advocated for longer incubation times[4,17]. 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, 17].

An understanding of the false-positive rate of intraoperative cultures is critical in the workup of PSI in order to avoid potentially inappropriate treatment. The purpose of the following study was to determine the incidence of false positive cultures in non-infected patients (clear mechanical source of pain, no suspicion of infection, negative inflammatory labs, no corticosteroid injection within six months of surgery) undergoing a primary open or arthroscopic

shoulder surgery. Additionally, the study seeks 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 was approved by the local institutional review board.

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

Eligible study subjects included adult (>18 years old) patients undergoing open and 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 six months of enrollment, systemic or shoulder inflammatory disorder, and clinical or imaging findings raising suspicion of infection were excluded from the study. Additionally, preoperative erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and procalcitonin (PCT) were obtained as screening tools to further assess underlying infectious or inflammatory process.

Procedure and Follow-up

All study participants pre-washed the surgical area with chlorhexidine soap the night before surgery. For all open surgeries, patient's were placed into a semi-sitting position. For all arthroscopic surgeries, patients were placed into a lateral decubitus position. Hair was shaved

from the shoulder with electric clippers immediately prior to prepping and draping if the hair interfered with incisions, draping, or postoperative dressings. Pre-surgical skin cleansing was performed over the entire shoulder and down the operative extremity to the wrist. Skin was painted with 100% isopropyl alcohol which was allowed to completely dry. The skin was then painted with provodine iodine 10% which was allowed to completely dry prior to draping. Adhesive edged drapes were applied circumferentially about the shoulder and a sterile stockinette was placed over the upper extremity to near the axilla. For open surgeries, all exposed skin about the shoulder was covered and sealed with an occlusive adhesive dressing. Prophylactic antibiotics were 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, three cultures (superficial, deep, and "sterile" control) were obtained. A superficial specimen for an open surgery was a tissue biopsy comprised of a small piece of fat/connective tissue (approximately 1 x 1 x 0.5 cm) harvested immediately below the dermal layer. A superficial specimen for an arthroscopic surgery was obtained by using an arthroscopic punch to harvest 5-6 bites of subacromial bursal tissue. A deep specimen was a tissue biopsy of glenohumeral synovial/capsular tissue. The "sterile" control specimen was handled using all of 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 three seconds, and then returned it to the culture vial in the standard fashion.

All samples were tested with gram stain and cultures were done in chocolate agar, blood agar, Maconkey agar, CNA agar, and thioglycolate broth. Cultures were monitored daily for

twenty-eight days for colony growth, opening the plates only if growth was observed. A culture was considered to be positive if any microbial growth occurred.

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

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

Results

In total, 128 patients were screened for the study. Twenty-two (17.1%) were excluded due to elevated inflammatory labs and six patients did not receive the planned surgery. Of the 100 participants enrolled in the study, five patients were removed postop due to laboratory error in which cultures were either not held for the entire twenty-eight-day protocol or culture specimens were lost. Ultimately, ninety-five patients were included in the final analysis, forty-seven patients in the open group and forty-eight in the arthroscopic group. In the open group, surgeries included 22 replacement arthroplasties, 22 rotator cuff repairs, and 3 instability repairs. In the arthroscopy group, surgeries included 24 acromioplasties, 17 rotator cuff repairs, 4 distal clavicle resections, and 3 instability repairs. At six months postoperative, all patients had progressed through the postoperative rehabilitation protocol as expected with no signs of

infection. However one patient, treated for multidirectional instability, did have some residual pain without clinical signs or concerns for infection.

Open Surgery Group

In the open group, positive culture results were reported for eight of the forty-seven patients (17.02%) with one patient yielding positive superficial and deep culture specimens, each growing a different organism. Nine of the 141 cultures (6.3%) obtained were positive for bacterial growth. Five positive cultures were from superficial tissue, two positive cultures were isolated from deep tissue, and two positive cultures were isolated from the control "sterile" swab group. All positive bacterial cultures were reported within seven days.

C. *acnes* was isolated in three of the forty-seven patients (6.4%), twice from a superficial tissue specimen and once from a deep tissue specimen. C. *acnes* was the most common isolated organism (33% of positive cultures) in the open group followed by Coagulase-negative *Staphylococcus aureus* (CoNS, 22% of positive cultures). All three patients yielding positive C. *acnes* growth were men.

Arthroscopic Shoulder Group

In the arthroscopic group, positive culture results were reported five of forty-eight patients (10.4%). Eight of the 144 cultures (5.5%) were positive for bacterial growth and one of the 144 cultures (0.7%) was positive for "mold". Of the nine positive cultures, three were obtained from superficial tissue, three were obtained from deep tissue, and three were obtained from the "control" swab. Two patients had more than one positive culture with one of the patients growing Methicillin Resistant *Staphylococcus aureus* (MRSA) from the superficial, deep, and "sterile" control cultures. The patient showed no signs of infection postoperatively. The patient was not treated and progressed through the postoperative protocol as expected with

no sequelae. All positive bacterial culture results were reported within seven days. Only one specimen was isolated beyond one week, which was positive with "mold" at twenty-six days.

No specimens from the arthroscopic group were positive for C. *acnes*. The most common isolated organism was MRSA (50% of positive cultures) followed by CoNS (25% of positive cultures). (Table I)

Statistical Analysis

We determined, using 1-way analysis of variance (ANOVA), there was no statistically significant difference between control and tissue sample positive culture ($P = 0.35$) and positive C. *acnes* ($P = 0.78$) rates in the open surgery group. Similarly, in the arthroscopic group, there was no difference between control and tissue sample positive culture ($P = 1$) and positive C. *acnes* ($P = 1$) rates. Statistical significance was considered for P-values < .05.

Discussion

Previous studies have shown high rates of positive cultures in operative shoulders when no infection was suspected preoperatively. However, those studies included patients who had had previous shoulder surgery or who had received a recent preoperative cortical steroid injection, either of which could have been a possible source of contamination resulting in an indolent, undiagnosed infection[5, 10, 12, 20].

For this study, we excluded any patient with a history of any prior shoulder surgery or who had received a corticosteroid injection within 6 months of surgery. We also excluded any patient with elevated serum inflammatory markers. Still, those measures do not absolutely rule out the possibility of an indolent, undiagnosed infection in our patients. Therefore, we also performed a "sterile" control culture on each patient by doing cultures from swabs that never had

contact with the patient. Thus, any positive control culture would clearly have to be considered to be a false-positive.

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

Overall, the rate of positive cultures was similar for both open and arthroscopic surgery groups. There were 9 positive cultures from 141 specimens in the open surgery group (6.4%) and 9 positive cultures from 144 specimens in the arthroscopic surgery group (6.3%). Our study's overall incidence of positive cultures 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% open group and 0% arthroscopic group) was lower than in previous reports.

C. *acnes* was the most commonly isolated organism in the open surgical 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 one deep tissue sample positive for C. *acnes* could be to sampling error or contamination from the dermis during surgery[7, 10, 11, 12, 16]. While 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's results. Further higher-powered studies with controls would be needed to determine whether previous seeding truly increases the rate of false-positive cultures.

Despite a low overall incidence of PSI in arthroscopic surgery of ~0.27%, the false-positive rate of cultures in our study was surprisingly high (approximately 19% of all patients had a positive culture)[5]. The high false-positive rate highlights the need to minimize contamination secondary to sampling, transport, or handling errors. Additionally, clinical judgment should not be based solely off culture results, as exemplified by our patient with MRSA-positive cultures from the superficial, deep, and control samples. Given no clinical symptomology 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 positive C. *acnes* rate reported by Chuang et al [Deep tissue rate 19.6% and Sethi et al (21.8% at 14 days, 25.1% positive rate 28 days][5, 20]. An absence of positive C. *acnes* cultures was not expected given superficial tissue samples and prolonged culture incubation times. Patient pre-surgical 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 consensus algorithm and/or more specific diagnostic test.

Holding cultures beyond fourteen days has been suggested in the literature but has been questioned by some authors[7, 12]. Recent studies suggest incubation times from seven to thirteen days decrease the false-positive rate of C. *acnes*. Our results demonstrate that all positive bacterial cultures were identified within seven days (one culture positive to “mold” at twenty six days) and that holding cultures longer than 14 days did not lead to increase rate of false positive culture results.

Our study does have limitations. We are not able to prove the positive cultures are truly false positives. Our study does include patients with history of remote glenohumeral injection, which has been associated with an increased rate of positive cultures[12]. We tried to reduce the potential impact of that factor by excluding all patients who had received an injection within six months of surgery. Still, we did not definitively eliminate the possibility of preoperative seeding by injection since one could appropriately argue that any injection at 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 six month follow-up period may not allow for enough time to reliably determine whether positive cultures were inconsequential, given PSI secondary to C. *acnes* can first present as late as two years post operatively[1]. However, all but one patient experienced complete resolution of preoperative pain and none of the patients exhibited clinical symptomology 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 eventually would 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 labs, 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 based on an inaccurate diagnosis for infection, when no infection was actually present, due to a false postitive culture could be quite significant.

There are disturbing findings when reviewing the results of our study. One concern, was the high rate of lab errors. We had to exclude five patients due to blatant errors, including lost samples and removing cultures prior to 28 days. Additionally, five of the eighteen positive

cultures were from the “sterile” control swabs, which suggest 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 for implementing institutional regulations to limit culture mishandling in the future.

Our study supports previous findings of high positive culture rates associated with shoulder surgery. While our results may not be generalizable outside of our health system, it highlights the importance of determining one’s own institutional false-positive rate. While the utility of prolonged incubation times beyond fourteen days is debatable, our study did not find an increase in false positive cultures beyond fourteen days.

Conclusion

Our study’s primary objective was to determine the incidence of positive cultures 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 in open shoulder surgery was 17.02% and arthroscopic shoulder surgery was 10.4%. The incidence of positive C. *acnes* cultures was 6.4% in the open group while C. *acnes* was not isolated in the arthroscopic group.

Our study’s second objective was to determine if there was an increased rate of positive cultures with prolonged incubation time of cultures in the microbiology laboratory. All positive bacterial cultures were reported within seven days of collection. One culture positive for “mold” was isolated at twenty-six days. Thus, this study did not find an increasing rate of false positive cultures by prolonging incubation times to 28 days.

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Table Legend

Table I. Results

TABLE I

Open surgery

Total	**47 subjects**		**141 specimens**
+ cultures	**9 (19.1%)**		**9 (6.4%)**
	Deep	**Superficial**	**Control**
+ culture	**2**	**5**	**2**
+ C acnes	**1**	**2**	**0**

9 positive cultures

Arthroscopic surgery

9 positive cultures

Total	**48 subjects**		**144 specimens**
+ cultures	**9 (18.7%)**		**9 (6.2%)**
	Deep	**Superficial**	**Control**
+ cultures	**3**	**3**	**3**
+ C acnes	**0**	**0**	**0**