Detection of *Cutibacterium acnes* in Tissue Samples from Primary Clean Shoulder Surgeries – Part I

Detecção de Cutibacterium acnes em amostras de tecidos de cirurgias limpas primárias do ombro – Parte I

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Abstract

Objective The present study aimed to identify bacterial agents in shoulder surgery specimens from patients with no history of previous shoulder infection or surgery.

Methods Tendon, bursa, and bone specimens were collected during surgery, stored in sterile dry bottles, and sent to a hospital-associated laboratory for culture growth analysis in media for aerobic and anaerobic agents. Findings from 141 samples from 47 shoulders were analyzed.

Results The cultures were negative in 46 cases (97.8%) and in 140 samples (99.2%). The culture was positive in a single patient, with growth of *Staphylococcus hominis* from one of three specimens collected.

Conclusions The rates of bacterial growth were not consistent with the international literature, indicating the low effectiveness of laboratory methods used in Brazil.

Keywords
- cutibacterium acnes
- gram-positive bacterial infections
- shoulder

Resumo

Objetivo Identificar agentes bacterianos em amostras de cirurgias do ombro de pacientes sem histórico de infecção e de cirurgias prévias no ombro.

Métodos Amostras de tendão, bursa e osso foram coletadas no intraoperatório, armazenadas em frascos estéreis a seco e enviadas para análise de crescimento de cultura em meios para agentes aeróbios e anaeróbios no laboratório credenciado ao hospital. Foram analisados os resultados de 141 amostras de 47 ombros.

Resultados Obtivemos resultados de culturas negativas em 46 casos (97,8%) e em 140 amostras (99,2%). Apenas um paciente apresentou resultado positivo, com crescimento bacteriano do *Staphylococcus hominis* em uma das três amostras coletadas.

Conclusões Não evidenciamos taxas de crescimento bacteriano condizentes com a literatura internacional, alertando para a baixa eficácia dos métodos laboratoriais utilizados no nosso país.

Palavras-chave
- cutibacterium acnes
- infecções por bactérias gram-positivas
- ombro

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Introduction

Infection is one of the most feared complications related to orthopedic surgeries, as it increases morbidity and treatment costs.[1–5] The rates of shoulder arthroplasty-associated infection are up to 9.5% in the Brazilian literature[6] and 15% in the international literature.[7] This rate can reach 3.4% in arthroscopies and up to 1.9% in open surgeries for rotator cuff repair or osteosynthesis.[7] Some authors discuss the meaning of bacterial growth and its real pathophysiological role in prosthetic components loosening, joint stiffness, pseudoarthrosis, instability, and residual pain in operated shoulders.[8–10]

Cutibacterium acnes, a gram-positive, lipophilic, nonsporulated, slow-growing anaerobic bacterial organism,[3,9,11] has a special role in this scenario. It has been isolated in up to 41.8% of primary shoulder surgeries in patients with no previous clinical signs of infection.[4,8,12–14]

This organism is part of the commensal flora of the skin, colonizing hair follicles and sebaceous glands; it is traditionally considered nonpathogenic.[10] However, *C. acnes* has been demonstrated in a series of infections, mainly related to bacterial adhesion in orthopedic implants and biofilm formation.[15] Shoulder surgery involves a great risk due to the proximity of the surgical site to the axilla, with an abundance of hair follicles and sebaceous glands.[7] This is why shoulder colonization is greater when compared with other joints, such as the knee and hip. For the same reason, the incidence of *C. acnes* infections is higher in males.[14]

The most common *C. acnes* contamination route is the migration of organisms colonizing the skin adjacent to the surgical site to deeper tissues after incision. Other potential contamination occurs due to the manipulation of the surgical site and surgical materials by the hospital team, in addition to the hematogenous spread of the organism adhered to previous implants. For these cases, the most important method for bacteriological diagnosis is culture from deep specimens.[15,16]

The present study aimed to identify bacterial growth in arthroscopies and open surgeries in shoulders not previously operated on.

Casuistry and Methods

This is a prospective, cross-sectional, sequential, observational study evaluating culture findings from specimens obtained during shoulder surgery and surveying clinical data from patients, complemented by a statistical analysis.

Culture findings of 64 shoulders from 63 patients who underwent primary shoulder surgery between April 2019 and May 2020 were analyzed. The mean age of the patients was 59 years old, ranging from 18 to 85 years old. Thirty-three (52.4%) patients were males and 30 (47.6%) were females. Shoulder arthroscopy was performed in 46 (71.9%) patients. Among those submitted to an open procedure, 6 (9.4%) underwent osteosynthesis, 6 (9.4%) underwent surgery for instability, 4 (6.2%) underwent shoulder arthroplasty, and 2 (3.1%) underwent rotator cuff repair (►**Table 1**).

Table 1 Study participants according to length of clinical follow-up, surgical technique, diagnosis, gender, age, and bacterial growth in cultures

#	Follow-up (months)	Technique	Diagnosis	Gender	Age	Culture
1	19	A	3	F	79	(-)
2	19	A	3	F	67	(-)
3	18	B	1	M	54	(-)
4	18	B	1	F	69	(-)
5	18	B	1	M	66	(-)
6	18	A	3	M	18	(+)
7	18	B	1	F	68	(-)
8	18	B	1	F	60	(-)
9	17	B	3	F	43	(-)
10	17	B	1	M	36	(-)
11	17	B	3	M	30	(-)
12	17	A	2	F	85	(-)
13	17	B	1	F	71	(-)
14	16	A	3	M	77	(-)
15	16	B	1	M	51	(-)
16	16	B	1	M	71	(-)
17	16	B	1	F	41	(-)
18	16	A	3	M	37	(-)
19	16	A	1	M	53	(-)
20	15	A	1	M	54	(-)
21	15	A	1	M	72	(-)
22	15	A	1	F	68	(-)
23	14	A	1	M	75	(-)
24	14	B	3	M	40	(-)
25	14	A	1	M	75	(-)
26	14	A	1	M	58	(-)
27	13	A	1	M	68	(-)
28	13	A	1	M	70	(-)
29	13	A	1	F	65	(-)
30	13	A	1	F	66	(-)
31	12	A	1	F	75	(-)
32	12	A	1	F	74	(-)
33	12	A	1	F	77	(-)
34	11	B	3	M	28	(-)
35	11	A	1	M	55	(-)
36	11	A	1	M	56	(-)
37	11	B	3	M	63	(-)
38	10	A	1	F	55	(-)
39	10	A	1	M	60	(-)
40	10	A	1	M	31	(-)
41	9	A	3	M	21	(-)

(*Continued*)

Table 1 (*Continued*)

#	Follow-up (months)	Technique	Diagnosis	Gender	Age	Culture
42	9	B	1	F	80	(-)
43	9	B	2	F	77	(-)
44	9	A	3	M	34	(-)
45	9	A	1	F	46	(-)
46	8	A	1	F	58	(-)
47	8	A	1	F	84	(-)

Abbreviations: A, arthroscopic technique; B, open technique; 1, rotator cuff injury; 2, arthrosis; 3, trauma; (+), positive bacterial growth in culture; (-), negative bacterial growth in culture; 0, unavailable culture result.

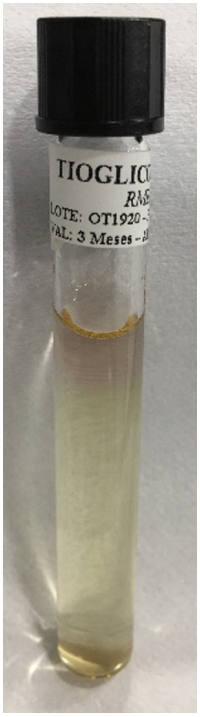

Fig. 1 Tube with thioglycolate medium used for clinical samples culture.

The inclusion criteria were patients > 18 years old, with no previous history of shoulder infection and who underwent primary shoulder surgery. Patients with a history of previous shoulder surgery and/or infection were excluded.

For open procedures, skin preparation followed the pattern used in patients undergoing osteosynthesis or arthroplasty: careful degermation of the shoulder, arm, forearm, hand, and axilla with 10% povidone-iodine (PVP-I) solution, preparation of the surgical site with a 10% PVP-I alcoholic solution in 2 layers, and surgical site enveloping with an incisional antimicrobial field of hypoallergenic acrylic adhesive impregnated with iodine (Ioban).

For patients undergoing arthroscopic surgery, the preparation followed another protocol, consisting of careful degermation of the shoulder, arm, forearm, hand, and axilla with a 4% chlorhexidine gluconate solution and preparation of the surgical site with a 2% alcoholic chlorhexidine solution in 2 layers, with no enveloping.

All patients receive an infusion of cefuroxime, 1.5 g, during anesthetic induction and for 24 hours after the procedure.

Intraoperatively, 0.5-cm^3 specimens of bone (humerus, acromion, or clavicle), tendon (long head of the biceps tendon or joint tendon), and subacromial pouch were sent for laboratory analysis of bacterial proliferation in culture media. These specimens were collected in dry sterile bottles, with no addition of another substrate or contact with the surgical field, gloves, aprons, or any other equipment. In < 1 hour, the bottles were taken by the hospital support team to a hospital-associated laboratory.

At the laboratory, the samples were manipulated in a microbiological safety cabinet. Bone fragments were inoculated only in thioglycolate medium (►**Figure 1**). The medium was incubated for up to 72 hours at 36°C ± 1°C. In case of turbidity, the medium was subcultured in 5% sheep blood agar (►**Figure 2**) and incubated for up to 72 hours in a 5% CO_2 atmosphere at 36°C ± 1°C. Non-bone tissues were homogenized aseptically, using a sterile disposable scalpel, and ~ 0.5 mL of sterile 0.9% saline solution. For aerobic culture, aliquots of ~ 0.05 mL of the homogenate were placed in thioglycolate medium and supplemented chocolate agar (►**Figure 2**). The thioglycolate medium was incubated for up to 72 hours at 36°C ± 1°C. The plates were incubated for up to 72 hours in a 5% CO_2 atmosphere at 36°C ± 1°C and inspected daily for the presence of colonies. For anaerobic culture, the homogenate was placed on Brucella agar with equine blood and supplemented with hemin and NAD, in addition to the thioglycolate medium. The plates and the tubes were incubated for up to 7 days in anaerobiosis.

Colonies present in solid media were identified by mass spectrometry. In cases of absence of colonies on the plates and turbidity of the thioglycolate medium in aerobic

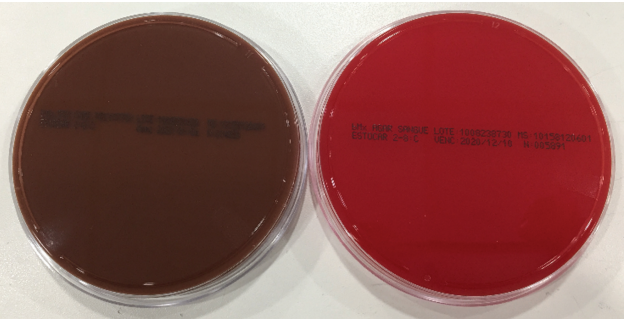

Fig. 2 Chocolate agar and blood agar plates used for clinical samples culture.

Table 2 Results of intraoperative samples cultures. The lines indicate the results of the samples collected from each patient (positive or negative); the columns indicate the surgeries performed (open or closed procedures)

	Open surgery	Closed surgery	Total
Positive culture	1	0	1
Negative culture	9	37	46
Total	10	37	47

culture, the samples were subcultured on sheep blood agar and incubated for up to 72 hours in a 5% CO_2 atmosphere at 36°C ± 1°C; the plates were inspected daily for the presence of colonies. For the anaerobic routine, in the absence of colonies on the plates and thioglycolate medium turbidity, the broth was subcultured in supplemented blood agar and incubated in anaerobiosis for 72 hours. Colonies obtained on a solid medium were identified by mass spectrometry.

In total, 189 tissue fragments from the 63 operated shoulders were collected and sent to culture. Sixteen individuals were excluded: 15 had no culture results, as samples were lost, and 1 patient did not meet the inclusion criteria (he had previously undergone a shoulder arthroplasty). The final sample consisted of 47 shoulders (141 specimens).

The present work was approved by the Research Ethics Committee (CAAE: 34265620.5.0000.0070). All patients signed an informed consent form to participate in the study; there are no conflict of interests.

Results

Of the 47 shoulders included in the study, totaling 141 tissue fragments for analysis, negative cultures were found in 46 cases (97.8%) and in 140 samples (99.2%).

Only one patient had a positive result, with bacterial growth of *Staphylococcus hominis* in one of the three samples collected. This patient, a male, underwent open surgery for anterior shoulder instability. Clinical data and supplementary tests (serial radiographs, complete blood count, C-reactive protein, and erythrocyte sedimentation rate) revealed a diagnosis of an infectious clinical condition of the shoulder. At the end of the present study, the patient presented with 12 months of postoperative follow-up with no loosening of the synthesis material (cannulated screws with washers), increased inflammatory parameters or complaints of residual pain, functional limitation, or instability recurrence.

During follow-up, no other patient presented any clinical complaint suggestive of infection (pain, hyperemia, edema, heat, or secretion drainage).

►**Table 1** shows the total study population. ►**Table 2** shows the findings of the study.

Discussion

We emphasize the importance of our study in initiating the scientific approach to *C. acnes* in the Brazilian literature. This organism is a bacterial agent attributed to the high incidence of postsurgical infection of the shoulder. The samples were sent from surgery to a specialized, hospital-associated laboratory, which uses a protocol to investigate aerobic and anaerobic bacteria; this is one of the largest, most important, reputable laboratory centers in our country.

Hudek et al.[12] demonstrated a 36.4% incidence of *C. acnes*-positive postoperative samples from patients with no previous history of infection who underwent primary open surgery for rotator cuff repair, subacromial decompression, arthroplasty, and anterior shoulder instability correction. These authors found an even higher rate for primary shoulder arthroplasties, at 41.8%.[14] Our findings were not consistent with their study. Methodologically, these studies followed similar criteria for patient selection. Antisepsis and asepsis were also similar, except for the use of an iodinated solution for degermation instead of chlorhexidine in our study. Regarding specimen selection,[12] the study base was expanded, collecting specimens from superficial sites, while other authors[14] included samples from deep tissues alone. Another difference was the use of vials containing thioglycolate solution (a medium suitable for anaerobic agent culture) for immediate specimen storage by Hudek et al.,[12] while other authors[14] immediately placed the specimens in dry vials. Both the storage and the material collection methodology of our study followed the model used by Levy et al.[14]

An important factor to consider for *C. acnes* detection is the incubation time. The literature shows an average of 6 days for growth, ranging from 2 to 15 days.[16] At the laboratory from our study, the standard culture time for anaerobic agents is up to 5 days. This period, lower than the one required by *C. acnes*,[1,17] allows only the growth of a smaller series of organisms. The unique finding of *S. hominis* demonstrates the inefficiency of the 7-day incubation period for extensive research and suggests the underdiagnosis of deep tissue contamination by *C. acnes*, since coagulase-negative staphylococci are the most associated agents.[16] We believe that the research protocol is inefficient for the correct analysis of bacterial agents, mainly *C. acnes*.

The storage medium used for the specimens from collection to laboratory processing also deserves attention, mainly for the research of anaerobic agents. Immediate inoculation in a nutrient medium and the prevention of contact with ambient air, with vial sealing, can preserve survival and favor anaerobic bacterial growth.

The literature shows lower rates of shoulder infection after arthroscopies compared with open procedures;[7] this occurs because arthroscopies have more limited incisions and less tissue manipulation, in addition to the intense rising process of wounds with saline solution

virtually during the whole surgery. In our study, most surgical procedures were arthroscopies (71.9%), but this fact, alone, does not justify the lack of bacterial growth. For open surgeries, the most used surgical route in our study was the deltopectoral approach, which is associated with a two-fold lower rate of *C. acnes* growth compared with the anterolateral approach to the shoulder, since it is assumed that colonization is greater next to the acromion.[12] We emphasize that, although these considerations are important for the analysis of the lower incidence of *C. acnes* in comparison with that reported in the literature, there was no statistical significance between open and closed surgery for culture positivity ($p = 0.213$).

The typically insidious, frustrating clinical picture of *C. acnes* infections does not indicate a potential long-term postoperative complication, although it is known that its significant housing adjacent to the surgical site increases the risk in shoulder surgeries.[16] In a review of 75 patients who underwent revision arthroplasty of the shoulder due to material failure, Topolski et al.[18] showed 60% growth of *C. acnes*. This highlights the importance in identifying and studying this organism to improve antiseptic and aseptic techniques for the therapeutic success of shoulder surgery.

Regarding the only case with bacterial growth, we emphasize that it was not consistent with a shoulder infection since no clinical, laboratory or radiographic criteria were met; as such, the diagnosis of specimen contamination is evident.[19]

The greatest importance of the present study is to alert for the low effectiveness of the methods currently used in Brazil for bacterial identification, even in the largest laboratories.

Further studies, following well-defined antisepsis and asepsis standards, but with a new laboratory methodology, standardizing the selection of storage medium and proper culture for anaerobes, with longer incubation time than that conventionally employed for anaerobic agents, in addition to evaluation and comparison with most recent microbiological research methodologies (mass spectrometry and genetic sequencing) are essential for a more reliable analysis of the real activity and importance of *C. acnes* in our environment. To contemplate all these factors, our group has a new study in progress, using tubes with thioglycolate broth for immediate specimen storage after surgical collection, a minimum incubation time of 14 days, and routine mass spectrometry for positive cultures.

Conclusion

We did not evidence bacterial growth rates consistent with the international literature in tissue samples from shoulders undergoing primary surgery, which were collected and cultured in a laboratory considered a reference in our country, following the usual methods.

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Conflict of Interests
The authors have no conflict of interests to declare.

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