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

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

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Abstract

Objective Research and identification of *Cutibacterium acnes* (*C. acnes*) and other microorganisms in deep tissue samples collected in clean shoulder surgeries of patients who did not undergo any previous invasive joint procedure and who had no clinical history of infection.

Methods We analyzed the results of cultures of intraoperative deep tissue samples from 84 patients submitted to primary clean shoulder surgery. Tubes containing culture medium were used for storage and transport of anaerobic agents, prolonged incubation time, and mass spectrometer for diagnosis of bacterial agents.

Results Bacteria growth was evidenced in 34 patients (40.4%) of the 84 included in the study. Of these, 23 had growth of *C. acnes* in at least one sample of deep tissue collected, corresponding to 27.3% of the total patients. The second most common agent was *Staphylococcus epidermidis*, present in 7.2% of the total individuals included. We showed a higher relationship between sample positivity and males, a lower mean age, absence of diabetes mellitus, ASA I score, and antibiotic prophylaxis in anesthetic induction with cefuroxime.

Keywords
- *Cutibacterium acnes*
- gram-positive bacterial infections
- *Staphylococcus epidermidis*
- shoulder

Multicentric study carried out in the Shoulder Surgery Group of the Department of Orthopedics and Traumatology of the Faculty of Sciences Doctors from Santa Casa of São Paulo, "Fernandinho Simonsen Pavilion" (DOT – FCMSCSP) (Director: Professor Maria Fernanda Silber Caffaro); at the German Hospital Oswaldo Cruz in São Paulo (Shoulder Surgeons Group); and at the Special Laboratory of Clinical Microbiology (LEMC) of the Discipline of Infectious Diseases, Department of Medicine, Escola Paulista de Medicina, Federal University of São Paulo (UNIFESP), São Paulo, SP, Brazil.

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Conclusions A high percentage of isolates of different bacteria was found in shoulder tissue samples of patients undergoing clean and primary surgeries, who had no history of previous infection. Identification of *C. acnes* was high (27.6%), and *Staphylococcus epidermidis* was the second most frequent agent (7.2%).

Resumo

Objetivo Pesquisa e identificação de *Cutibacterium acnes* (*C. acnes*) e de outros microrganismos em amostras de tecidos profundos coletados em cirurgias limpas de ombro em pacientes que não foram submetidos a nenhum procedimento invasivo articular prévio e que não possuíam antecedentes clínicos de infecção.

Métodos Foram analisados os resultados das culturas de amostras de tecidos profundos intraoperatórias de 84 pacientes submetidos à cirurgia limpa primária do ombro. Foram utilizados tubos contendo meio de cultivo para armazenamento e transporte de agentes anaeróbicos, tempo prolongado de incubação e espectrômetro de massa para diagnósticos de agentes bacterianos.

Resultados Foi evidenciado o crescimento de bactérias em 34 pacientes (40,4%) dos 84 incluídos no estudo. Desses, 23 apresentavam crescimento de *C. acnes* em pelo menos uma amostra de tecido profundo coletada, correspondendo a 27,3% do total de pacientes. O segundo agente mais encontrado foi o *Staphylococcus epidermidis*, presente em 7,2% do total de indivíduos incluídos. Evidenciamos maior relação da positividade de amostras com o gênero masculino, uma média de idade inferior, a ausência de diabetes mellitus, o escore ASA I e a profilaxia antibiótica na indução anestésica com cefuroxima.

Conclusões Verificou-se um elevado percentual de isolados de diferentes bactérias em amostras de tecidos de ombros de pacientes submetidos a cirurgias limpas e primárias e sem histórico de infecção anterior. A identificação de *C. acnes* foi elevada (27,6%) e o *Staphylococcus epidermidis* foi o segundo agente mais frequente (7,2%).

Palavras-chave
- *Cutibacterium acnes*
- infecções por bactérias gram-positivas
- ombro
- *Staphylococcus epidermidis*

Introduction

Cutibacterium acnes (*C. acnes*), formerly called *Propionibacterium acnes*, is a gram-positive, lipophilic, nonsporulate, and slow-growing anaerobic bacterial agent.[1] It is a human skin commensal species present in regions with sebaceous follicles, especially in males.[2,3] Although it can be found in the hip and knee, its presence is more notorious in the shoulder.[2,3] In patients submitted for the first time to shoulder surgery, without previous clinical signs and symptoms of infection, the presence of *C. acnes* is verified in up to 41.8% of deep tissue samples.[4]

Laboratory diagnosis of *C. acnes* is challenging. Bacteriological cultures require specific care to avoid false-negative results or doubts between infection and contamination of samples.[5] Rapid transport from sample collection to laboratory should promote survival for anaerobic agents. Furthermore, the incubation period in an anaerobic environment must be prolonged for at least 14 days to allow slow bacterial growth and its isolation.[6] Faced with so many difficulties in the diagnosis of growth in culture medium, previous studies argue that there is a large number of underdiagnoses of *C. acnes* colonization.[6] In a previous study published by the same group, it was evidenced that tissue samples collected and transported in a dry vial and incubated for up to 7 days presented a 99.2% rate of negative results for the identification of any microorganism, alerting to the low diagnostic efficacy of laboratory investigation standards in our country.[7] New laboratory bacteriological investigation techniques are under development to improve diagnosis, of which we highlight genetic sequencing and mass spectrometry.[5,8,9]

The aim of this study is to identify the presence of *C. acnes* and other bacterial agents in deep shoulder tissue samples of patients who have not undergone any previous invasive procedure in this joint and who did not have a clinical history of infection (clean surgeries).

Casuistic and Methods

This is a prospective, longitudinal, sequential, observational, and multicentric cohort study in patients undergoing clean and elective shoulder surgeries from July 2020 to November 2020, from whom deep tissue samples were collected sterilely for the research of microorganisms, including *C. acnes*. The study was approved by the Research Ethics Committee of our institution.

The results of 84 shoulder cultures collected from 84 patients submitted to primary clean surgery in eight different hospitals in the city of São Paulo were analyzed. Of these patients, 45 (54%) were male and 39 (46%) were female; ages ranged from 19 to 94 years (mean of 51.5). Regarding ethnicity, 63 individuals (75%) were white, 12 (14%) were

mixed-race, 5 (6%) were Asian-American, and 4 (5%) were black. As for the surgical technique, 38 (45%) underwent arthroscopic procedure, and 46 (55%) underwent open procedure. All patients were older than 18-years-old, with no previous history of shoulder infection, and underwent primary shoulder surgery.

We correlate the comorbidities of patients with the classification of the American Society of Anesthesiologists (ASA),[10] 21 individuals (25%) were classified as ASA I, 61 (73%) as ASA II, and 2 (2.3%) as ASA III. All patients in this study received antibiotic prophylaxis, with first or second generation cephalosporins—cefazolin or cefuroxime, respectively—in anesthetic induction and for 24 hours after the surgical procedure.

In each shoulder surgery, three intraoperative samples of bone (humerus head, acromion, or distal third of the clavicle), tendon (supraspinal, tendon of the long head of the biceps, or joint tendon), and subacromial pouch of about 0.5 cm^3, were collected for laboratory analysis of bacteria proliferation in aerobic and anaerobic cultures. Of the three deep tissue samples selected, two were collected separately in sterile vials containing thioglycolate broth and one was collected in a sterile vial with 0.9% saline solution. The vials were sealed and sent to the microbiological research laboratory (Special Laboratory of Clinical Microbiology of the Universidade Federal de São Paulo) immediately after the procedure by the surgical team members themselves.

In the laboratory, the samples in the vials containing thioglycolate (►**Figure 1**) were placed in the anaerobiosis jar at 37°C for 14 days. The samples in 0.9% saline were transferred to the Trypic Soy Broth (TSB) liquid medium and incubated in aerobiosis at 37°C. After 14 days, in the tubes that presented turbidity (►**Figure 2**), 10µL of the cloudy liquid medium were inoculated in sheep blood agar plates and incubated at 37°C for another 14 days in the anaerobiosis jar. All samples in anaerobiosis and aerobiosis that showed bacterial growth were stored in TSB with 20% glycerol and frozen at −80°C (►**Figure 3**).

The next step consisted of the identification of bacterial agents by the matrix-assisted laser desorption ionization-time of flight (MALDI-TOF MS, MALDI Biotyper, Bruker

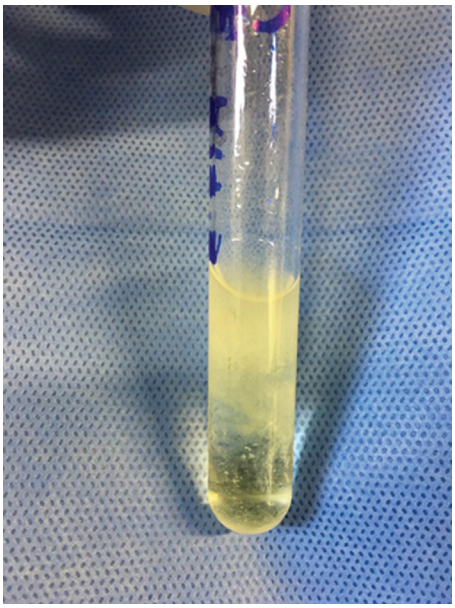

Fig. 2 Example of turbid thioglycolate broth after sample incubation, evidencing bacterial proliferation.

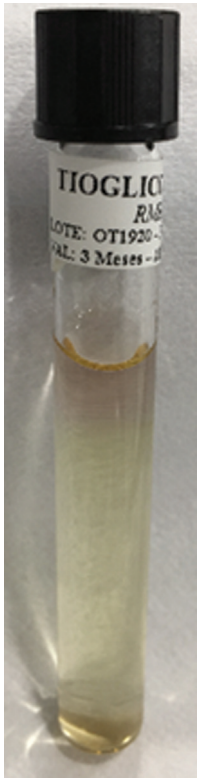

Fig. 1 Example of a vial containing thioglycolate broth, used for incubation of the collected samples.

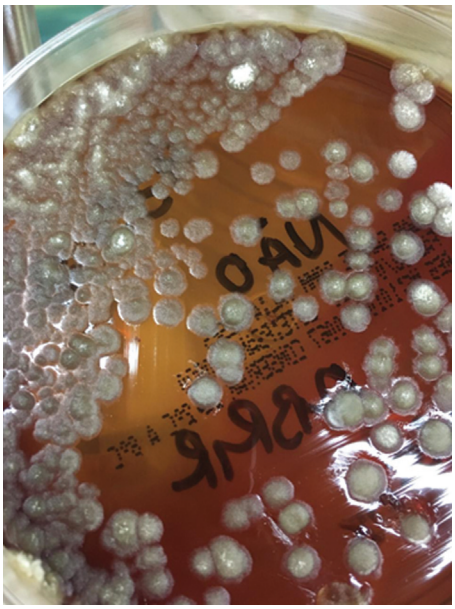

Fig. 3 Petri dish containing samples of *Cutibacterium acnes* cultures grown in sheep's blood agar.

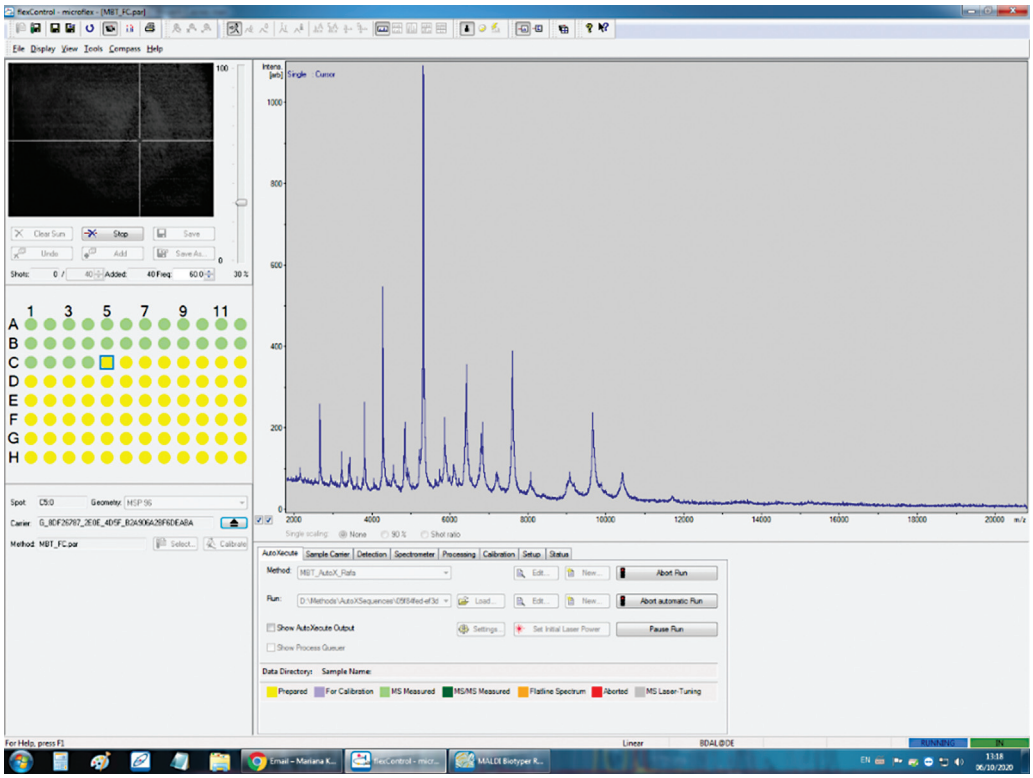

Fig. 4 Image taken from the Flexcontrol program, used to identify bacterial agents through the specific flight time graph of each microorganism. In this example, after analyzing the defined peaks, we have the identification of *Staphylococcus cohnii*.

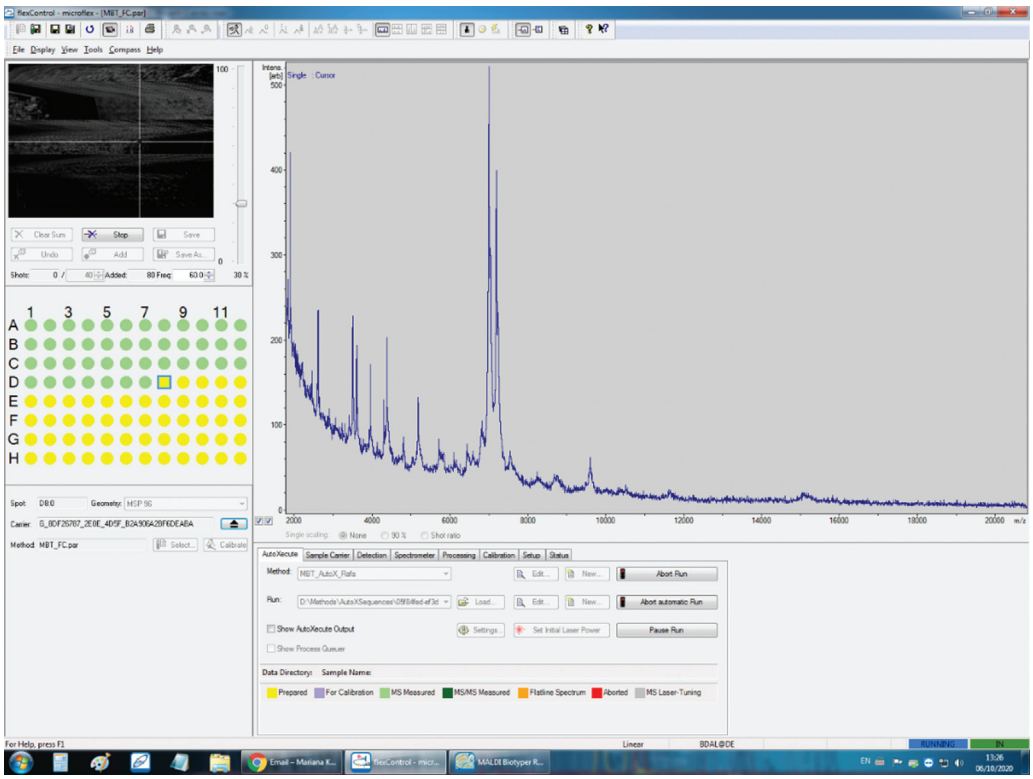

Fig. 5 Image taken from the Flexcontrol program used to identify bacterial agents through the specific flight time graph of each microorganism. In this example we have the identification of *Cutibacterium acnes*.

Daltonics, Germany) according to the technical description of Dingle et al.[11] (►**Figures 4** and **5**).

The patients were followed up in the postoperative period with face-to-face physical examinations and serial radiographs during a period of up to 10 months from the first surgery performed in a patient included in the study until treatment conclusion. In patients who presented identification of bacteria in the collected samples, blood count and blood inflammatory tests were collected serially. The evaluations of patients in the postoperative period, as well as their radiographs and laboratory tests were documented in medical records, aiming at the formation of a database for a future study of the group with longer clinical follow-up time.

For statistical analysis, we used the Fisher exact test, chi-square test, Person correlation, two-sample t-test, confidence interval, and *p*-value, when applicable. The level of significance adopted was 0.05 (5%).

Results

After laboratory analysis of the collected samples, the growth of bacterial agents was evidenced in 40.5% of the patients (34/84). Of these, 23 had *C. acnes* in at least one of the samples collected, corresponding to 27.3% of the total patients, and 67.6% of the patients presented positive samples for growth of any microorganism. There were 4 patients (4.8%) who presented more than one positive sample for *C. acnes*. The second most identified microbial agent was *Staphylococcus epidermidis*, present in 6 individuals (7.2% of the total and 18.2% of the positive ones), while the third agent was *Escherichia coli*, identified in 3 individuals (3.5% of the total and 8.8% of the positive). (►**Table 1**)

Among the 23 individuals who had growth of *C. acnes* in the samples, 18 (78.3%) were male. There was a statistically significant difference for males both in those positive for *C. acnes* ($p = 0.05$) and in the total number of positives ($p = 0.01$). There was no statistically significant difference for ethnicity or for the surgical technique applied (open or arthroscopic surgeries).

Of the 34 individuals positive for any microorganism, 11 (32.4%) were classified as ASA I, while in individuals with no growth of microorganisms, this group corresponded to only 14% (7 among 52 individuals). Statistical significance was evidenced for this group of patients ($p = 0.04$). When the patients positive for *C. acnes* were evaluated, it was found that 8 (34.7%) were classified as ASA I, and we found no statistical significance, but a trend ($p = 0.08$). It was also observed that individuals with diabetes mellitus had lower isolation of bacteria, corresponding to only 3 (13%) individuals of the 23 positives for *C. acnes* ($p = 0.04$), and 6 (17.6%) of the 34 positives for any bacterium ($p = 0.04$).

In 8 (35%) of the individuals with samples positive for *C. acnes*, cefazolin was administered as a prophylactic antibiotic in anesthetic induction and during the 24 hours postoperatively. In 15 (65%) individuals, cefuroxime was administered in the same period, 5 of which presented two or more positive samples for *C. acnes* (83% of individuals with two or more positive samples). We showed a statistically significant difference in antibiotic therapy prophylaxis for the detection of a positive sample for *C. acnes* ($p = 0.03$) and there was no statistical significance for two or more positive samples for *C. acnes*, but there was a trend ($p = 0.09$).

Among the 252 samples collected, 53 (21%) were positive for microorganism identification. Of the positive samples, 30.5% were collected in vials with saline solution and 69.8% were collected in vials containing thioglycolate. Tendon samples had 35% bacterial identification, followed by subacromial pouch (34%), and bone (31%) samples. Additionally, 22 patients (64.7% of the positive) presented monomicrobial results—that is, only one of the samples positive for bacterial identification—while 12 (35.3%) presented polymicrobial results—that is, two or more samples positive for the same or different agents. *C. acnes* was the most identified agent in patients with monomicrobial outcome (81%) and polymicrobial result (100%), and 43% of patients positive for *C. acnes* presented polymicrobial samples.

Discussion

The identification of potential pathogenic bacterial agents has gained emphasis in the orthopedic literature. In the study of shoulder disorders, *C. acnes* is highlighted as a possibly pathogenic agent during the postoperative period of open and arthroscopic surgeries. More recently, its presence in samples of primary shoulder surgeries has been evidenced.[4,12,13] In the present study, we tried to evaluate the presence of *C. acnes* in deep tissue samples from the shoulders of patients undergoing primary clean surgeries, with no history of previous infection.

Isolation of *C. acnes* is difficult. In a previous study of our group, it was evidenced that the culture medium used for anaerobic microorganisms in the collection and transport containers, as well as in the prolonged incubation time of samples, are determining factors for the identification of this bacterium in primary shoulder surgeries without history of infection.[7] Using sterile vials for dry transport, with transport carried out by outsourced laboratories to the hospital and with incubation time of up to 7 days, we obtained only one positive sample for coagulase-negative *Staphylococci* among the 47 shoulders studied,[7] constituting a result below that found in the literature.[4,12,13] We understand, therefore, that the use of a methodology involving: the storage and transport of samples in saline and thioglycolate (means of transport and culture for anaerobic agents), the rapid transport from the operating room to the laboratory, the incubation time of more than 14 days, and the identification of the agents with greater sensitivity and specificity by MALDI-TOF MS, were essential factors for the correct analysis of the shoulder's microbiota in the present study.

Following this methodology, we obtained 40.3% of positive samples for different microorganisms, of which 67.3% were positive for *C. acnes* (27.6% of the sample). When comparing these values with other studies, we noted that Sethi et al.[12] found a rate of 21.8% of positive samples for *C. acnes* in primary arthroscopies of the shoulder, Hudek et al.[4] found a rate of 36.4% in open shoulder surgeries, and Levy

Table 1 Total number of individuals who presented bacterial identification of at least one sample, the bacterial agents identified, and the respective risk factors evidenced: age, gender, ASA, antibiotic prophylaxis and diabetes mellitus

Individual	Bacteria identified	Age (years)	Gender	ASA	Antibiotic prophylaxis	Diabetes mellitus
2	*Pmi, Bl*	91	F	III	1	(+)
4	*Ca*	61	M	II	1	(-)
6	*Ca, Ec*	57	M	II	2	(-)
7	*Ca, Ml, Se*	33	M	I	2	(-)
8	*Ca, Eh*	19	M	I	2	(-)
9	*Cs*	56	F	II	1	(-)
14	*Sp*	28	M	I	2	(-)
15	*Ml*	67	F	II	1	(+)
17	*Ca, Ab*	42	M	II	2	(-)
18	*Ca, Sca*	32	M	I	1	(-)
22	*Ca, Sco*	25	M	I	2	(-)
26	*Ca, Ef*	30	M	II	2	(-)
29	*Ca*	52	M	II	2	(-)
30	*Ca*	66	M	II	1	(-)
33	*Ca, Ec*	56	F	II	2	(+)
34	*Ca, Se*	58	M	II	1	(+)
37	*Ca*	49	F	II	2	(-)
41	*Pmo*	53	F	II	1	(-)
42	*Ca*	20	F	I	2	(-)
44	*Ec*	24	M	I	1	(-)
48	*Ca*	56	M	II	2	(-)
49	*Ca*	51	M	II	2	(-)
53	*Pa*	30	M	II	1	(-)
55	*Ca*	21	M	I	1	(-)
57	*Ca*	57	F	I	2	(-)
58	*Se*	69	F	II	2	(+)
59	*Se*	58	M	I	1	(-)
64	*Ca*	71	F	II	2	(-)
72	*Se, Sl*	27	M	II	1	(-)
74	*Ca*	54	M	II	2	(-)
76	*Ca, Cg*	30	M	I	1	(-)
77	*Sco*	42	M	II	1	(-)
81	*Ca*	36	M	II	1	(-)
83	*Ca*	52	M	II	1	(-)

Abbreviations: ASA, American Society of Anesthesiologists; Ab, *Acinobacter baumannii*; Bl, *Bacillus licheniformes*; Ca, *Cutibacterium acnes*; Cg, *Cutibacterium granulosum*; Cs, *Corynebacterium simulans*; Ec, *Escherichia coli*; Eh, *Escherichia hermannii*; Ef, *Enterococcus faecalis*; Ml, *Micrococcus luteus*; Pa, *Propionibacterium acidifaciens*; Pmi, *Proteus mirabilis*; Pmo, *Pseudomonas moteillii*; Sca, *Staphylococcus capitis*; Sco, *Staphylococcus cohnii*; Se, *Staphylococcus epidermidis*; Sl, *Staphylococcus lugdunensis*; Sp, *Staphylococcus pasteuri*; F, female, M, male. **Notes:** 1, antibiotic prophylaxis with cefazolin; 2, antibiotic prophylaxis with cefuroxime; (+) has diabetes mellitus; (-) does not have diabetes mellitus.

et al.[13] found a rate of 41.8% in primary arthroplasties. Our study did not identify a statistically significant difference between the results of patients undergoing open or arthroscopic surgeries. The second most common agent found in our study was *Staphylococcus epidermidis*, present in 7.2% of the total individuals included and in 18.2% of those with bacterial growth identified in the samples. This data is in accordance with the literature.[5] We evidenced from our study data that *C. acnes* plays an important role in shoulder surgeries' postsurgical care, since it corresponds to the bacterial agent most found in primary surgeries of this joint.

Epidemiologically, we were able to establish correlations between the positivity of samples, including *C. acnes,* and age, gender, and presence of previous comorbidities of

patients. We showed that male patients presented higher rates of positive samples for any microorganism and, more specifically, for *C. acnes*.

The mean age in patients with positive samples was 46.2 years; and, in those with *C. acnes* isolation, it was 44.7 years. Both values were lower than the means of patients without isolation.

The predominance of males in the findings of *C. acnes* has been previously reported by several studies.[2,3,14,15] Kaveeshwar et al.[15] found a correlation between isolation of this agent and age lower than 40 years. The higher prevalence stake in males and young people are related to the more active production of the sebaceous glands in these individuals.[15] Finally, Nagaya et al.[16] showed that patients with an anesthetic risk score higher than ASA III had a higher risk of developing infection after shoulder arthroplasty. Interestingly, in our study we found a higher correlation of microorganism isolation in ASA I patients, with a tendency to isolate *C. acnes*. Another important relationship we found was the lower isolation of *C. acnes* and other bacterial agents in patients with diabetes mellitus, which is in accordance with Kaveeshwar et al.,[15] who also did not evidence this comorbidity as a risk factor for the isolation of *C. acnes*.

We found that prophylactic antibiotic therapy in anesthetic induction was related to the isolation of *C. acnes*. Proportionally, there was greater isolation in patients receiving cefuroxime when compared to those receiving cefazolin. The international literature demonstrates rates of reduction of more than 50% of superficial and deep infections after the administration of cephalosporins in anesthetic induction of orthopedic surgeries. Cefazolin and cefuroxime are the antibiotics of choice by the American Academy of Orthopaedic Surgeons (AAOS) for elective orthopedic surgeries, with no clear predilection for one or the other.[17] Cephalosporins, in addition to having coverage for most *Staphylococcus aureus* and some gram-negative organisms, have a good safety profile, a long half-life time, and an efficient penetration into the bones.[17] To our knowledge, there are no previous studies demonstrating the comparative relationship between cefazolin or cefuroxime prophylaxis in the laboratory growth rate of *C. acnes*.

During the follow-up of the individuals, only one patient who had the isolation of *Proteus mirabilis* and two others with isolation of *C. acnes* presented nonspecific inflammatory signs 2 weeks after surgery. All patients underwent oral antibiotic therapy and were asymptomatic, with no radiographic or laboratory alterations after follow-up of up to 10 months.

It is noteworthy that the present study is the first of its kind in Brazilian orthopedics. As a positive point, it exhibits the importance of an effective methodology for collecting and transporting intraoperative samples, as well as for identifying microorganisms by new and different microbiological techniques as a substantial factor for diagnostic success, and for improving care in combating orthopedic infections. We understand that our study shows a different perspective of bacterial research in our country, which allows the identification of possible risk factors for bacterial growth. We highlight as limiting factors of the study the infeasibility of performing a multivariate analysis of the risk factors found due to the sample size, as well as the absence of a control group.

The observation and clinical correlation of inflammatory signs and symptoms over longer periods in individuals who obtained bacterial isolation in this study is a topic of interest of our group for a new ongoing study, which aims to contemplate the importance of the qualification and quantification of the positive samples found.

Conclusion

There was a high percentage of isolates of different bacteria in shoulder tissue samples of patients submitted to clean and primary surgeries and with no history of previous infection. Identification of *C. acnes* was high (27.6%), and *Staphylococcus epidermidis* was the second most frequent agent (7.2%).

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

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