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Microbiological Profile of Periprosthetic Knee Infections in a Brazilian Unified Health System Hospital Specialized in Highly Complex Orthopedic Surgeries*

Perfil microbiológico das infecções periprotéticas do joelho em um hospital do Sistema Único de Saúde especializado em cirurgias ortopédicas de alta complexidade

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Abstract

Objective We studied the microbiological profile of periprosthetic knee infections treated in a Brazilian tertiary hospital.

Methods The study included all patients undergoing revision surgery for total knee arthroplasty (RTKA) between November 2019 and December 2021, with a diagnosis of periprosthetic infection confirmed per the 2018 International Consensus Meeting (ICM) criteria.

Results Sixty-two patients had a periprosthetic joint infection (PJI) per the 2018 ICM criteria. Cultures were monomicrobial in 79% and polymicrobial in 21% of cases. The most frequent bacterium in microbiological tissue and synovial fluid cultures was *Staphylococcus aureus*, observed in 26% of PJI patients. Periprosthetic joint infection with negative cultures occurred in 23% of patients.

Conclusion Our results show the following: i) a high prevalence of *Staphylococcus* as an etiological agent for knee PJI; ii) a high incidence of polymicrobial infections in early

Keywords
- arthroplasty, replacement, knee
- postoperative complications
- intraarticular injections
- prosthesis-related infections

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infections; iii) the occurrence of PJI with negative cultures in approximately one fourth of the subjects.

Resumo

Objetivo Identificar o perfil microbiológico das infecções periprotéticas do joelho tratadas em um hospital terciário brasileiro.

Métodos Todos os pacientes submetidos à cirurgia de revisão de artroplastia total do joelho (RATJ), no período compreendido entre novembro de 2019 e dezembro de 2021, e que tiveram o diagnóstico de infecção periprotética confirmado de acordo com critérios do *International Consensus Meeting* (ICM) 2018, foram incluídos no estudo.

Resultados Sessenta e dois pacientes foram diagnosticados com infecção periprotética (IAP) pelos critérios do *International Consensus Meeting* 2018. Culturas monomicrobianas foram identificadas em 79% e polimicrobianas em 21% dos casos. A bactéria mais frequentemente identificada nas culturas microbiológicas de tecidos e líquido sinovial foi o *Staphylococcus aureus,* presente em 26% dos pacientes com infecção periprotética. Infecções periprotéticas com culturas negativas ocorreram em 23% dos pacientes.

Conclusão Nossos resultados evidenciam: i) alta prevalência de bactérias do gênero *Staphylococcus* como causadores da IAP do joelho; ii) a alta incidência de infecções polimicrobianas nas infecções precoces e iii) IAP com culturas negativas ocorre em, aproximadamente, um quarto dos pacientes.

Palavras-chave
- artroplastia do joelho
- complicações pós-operatórias
- injeções intra-articulares
- infecções relacionadas à prótese

Introduction

Periprosthetic joint infection (PJI) represents a severe complication, with an incidence ranging from 1 to 4% after primary arthroplasties. However, this incidence can get up to 5 to 15% in high-risk patients and those undergoing revision surgery. Thus, in many series, PJI is the most significant cause for revision in modern knee arthroplasty.[1–3]

Early diagnosis and pathogen identification are critical for proper treatment and infection eradication. Nevertheless, PJI diagnosis is difficult because of its different clinical presentations and the lack of a single clinical test to confirm or rule out this complication. Thus, since 2011, several societies have proposed criteria to standardize PJI diagnosis.[4–6]

These criteria allow diagnostic confirmation of PJI, even in patients with negative microbiological cultures. However, pathogen identification remains a fundamental principle for treating these infections.[5,7] Thus, this study aims to characterize the microbiological profile of periprosthetic knee infections treated in a Brazilian tertiary hospital.

Material and methods

Study subjects

This study included all patients undergoing revision surgery for total knee arthroplasty (RTKA) from November 2019 to December 2021 with a diagnosis of periprosthetic infection confirmed per the 2018 International Consensus Meeting (ICM) criteria. The Research Ethics Committee approved this research under number 20309419.0.0000.5273. Patients confirmed their participation in the study by signing an informed consent form.

►**Table 1** shows exclusion criteria.

The application of exclusion criteria resulted in a final sample of 62 patients diagnosed with a periprosthetic knee infection.

Surgical procedure and biological sampling

On the day before surgery, we collected peripheral blood from all patients for routine preoperative serological tests, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and D-dimer.

All patients underwent spinal anesthesia with a peripheral nerve block. We performed all procedures under ischemia with the pneumatic cuff inflated 100 mm Hg above the systolic blood pressure.

After limb exsanguination and surgical drapes placement, we performed an arthrocentesis with a 20G needle to get

Table 1 Exclusion criteria

Exclusion criteria
- Refusal to sign the informed consent form
- Revision of an unicompartmental arthroplasty
- Reimplants in patients with spacers (second time)
- Impossibility to sample synovial fluid
- Insufficient information to confirm or rule out an infectious diagnosis
- Use of antibiotic agents within 15 days before the surgery
- Patients with other active bacterial infectious diseases
- Patients with acquired immunodeficiency syndrome

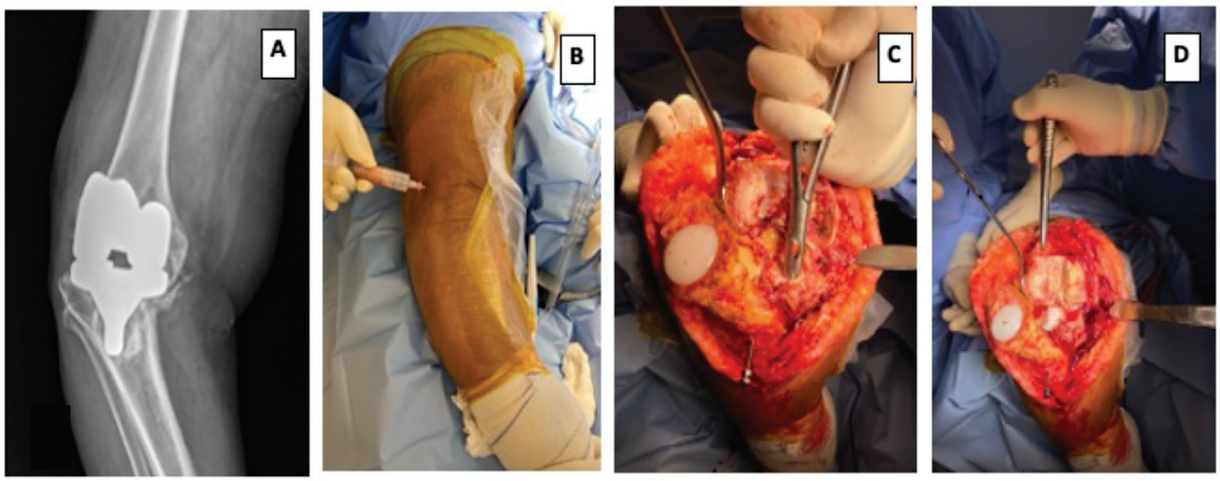

Fig. 1 Surgical procedure and biological material sampling. (A) Anterior-posterior radiograph of the right knee showing failure of the total knee arthroplasty; (B) Arthrocentesis after sterile drapes placement and before the surgical incision to avoid synovial fluid contamination with blood, (C) Periprosthetic bone tissue sampling for microbiological analysis, (D) Periprosthetic membrane sampling for histopathological analysis.

synovial fluid (SF) samples. The lack of surgical access or additional local anesthetic block minimized any chance of SF contamination by blood or other agents (►**Fig. 1**). If SF sampling was not feasible, we performed a second attempt by direct visualization after the medial parapatellar surgical approach.

We placed 1 to 2 mL aliquots of SF in vacuum blood collection tubes containing ethylenediaminetetraacetic acid (EDTA). These samples went immediately to the laboratory for a total white cell count and determination of the percentage of polymorphonuclear cells in an Abbott Cell Dyn 3700 SL equipment (Abbott Laboratories, Chicago, IL, USA).

We inoculated 3 to 5 mL aliquots of SF into aerobic blood culture tubes and, if possible, the same amount into anaerobic blood culture tubes. Blood culture tubes were sent immediately for microbiological culture. All samples were cultured for 14 days.

After removing the prosthetic components, we collected the following samples for microbiological analysis: three samples of femoral bone tissue, three samples of tibial bone tissue, and a fragment of the periprosthetic membrane. If the obtention of a periprosthetic membrane fragment was not feasible, we collected a peri-implant soft-tissue sample. For histopathological analysis, we sampled the periprosthetic membranes from the femur and tibia. Antibiotic therapy started only after collecting all biological samples.

We placed the bone fragments in sterile tubes with 1 mL of 0.9% saline solution. The samples went immediately to the laboratory for microbiological cultures. All samples were cultured for 14 days.

For the histopathological examination, we collected one or two periprosthetic membrane fragments and stored them in flasks containing 10% formalin solution. Membrane classification followed the parameters proposed by Morawietz et al.[8]

Diagnostic definition and group allotment

The following 2018 ICM criteria confirmed PJI diagnosis: i) growth of the same pathogen in two or more periprosthetic tissue cultures, or ii) presence of a fistula. These major criteria are enough for diagnostic confirmation. In addition, a score equal to or greater than 6 confirmed infections per the proposed algorithm (►**Table 2**).

Statistical analysis

Quantitative data, depicted as mean, standard deviation (SD), median, minimum, and maximum values, underwent descriptive analysis. The analysis of categorical variables, expressed as frequencies and percentages, used the chi-squared or Fisher exact test when necessary. We performed all analyses with the Med Calc and GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA) software. Significance was set at a $p > 0.05$ level.

Results

Study population

Based on clinical data and laboratory tests, we evaluated 84 patients who underwent RTKA for PJI diagnosis per the 2018 ICM criteria. In total, we included 62 patients with PJI in the study. ►**Table 3** summarizes the demographic data from these patients.

►**Fig. 2** shows the temporal evaluation (30 days) from infected patients.

Pathogen identification

The microbiological cultures showed positive results, allowing pathogen identification in 77% (48 patients) of the cases. Cultures were monomicrobial in 79% and polymicrobial in 21% of the subjects. Periprosthetic joint infection with negative cultures occurred in 23% of patients.

We identified gram-negative agents in 24% of the cultures. Considering only monomicrobial infections, 86% of the

Table 2 Criteria to diagnose a knee periprosthetic infection

Major criteria (at least one positive)				**Decision**
Two cultures positive for the same organism				Infected
Fistulae				
Preoperative diagnosis	**Minor criteria**		**Score**	**Decision**
	Serum	Elevated CRP or D-dimer	2	≥ 6 infected
		Elevated ESR	1	
	Synovial fluid	Elevated leukocyte count or positive leukocyte esterase	3	2–5 Potentially infected*
		Positive alpha-defensin	3	0–1 not infected
		Elevated neutrophil %	2	
		Elevated CRP	1	
Intraoperative diagnosis	**Inconclusive preoperative criteria or lack of synovial fluid**		Score	Decision
	Preoperative score		–	≥ 6 infected
	Positive histopathological analysis		3	4-5 potentially infected
	Presence of pus		3	
	One positive culture		2	≤ 3 not infected

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

patients presented gram-positive agents alone and 14% had exclusively gram-negative agents. The most frequent agent in cultures of periprosthetic tissues and SF samples was *Staphylococcus aureus*, present in 26% of patients with PJI. ►**Fig. 3** reveals the bacteria identified at cultures from PJI patients and their rates.

When evaluating only patients with positive microbiological cultures, we found out that polymicrobial infections were significantly more frequent in early infections, that is, occurring up to 3 months after surgery, compared with intermediate or late infections ($p = 0.02$) (►**Fig. 4**). The distribution of PJI patients with negative cultures was similar in acute, intermediate, and chronic infections.

Table 3 Demographics distribution

Variable	Infection
N	62
Gender, *n (%)*	
Female	23 (37%)
Male	39 (63%)
Age (years), *mean (±standard deviation)*	68.9 (±8.7)
BMI (kg/m^2), *mean (±standard deviation)*	27.4 (±9.9)
Diabetes, *n (%)*	12 (19%)
Inflammatory disease, *n (%)*	11 (18%)
Previous implant, *n (%)*	
Primary prosthesis	38 (61%)
Revision	18 (29%)
Rate of events characterizing infection, *n (%)*	
Fistulae	16 (25%)
≥ 2 positive cultures	46 (74%)
Time between prosthesis implant and revision, *n (%)*	
≤ 3 months	23 (37%)
3–12 months	9 (15%)
> 12 months	30 (48%)

Abbreviations: n, Number of patients; BMI, body mass index.

Discussion

Although the risk of periprosthetic infection after knee arthroplasty is low, the exponential boost in the amount of

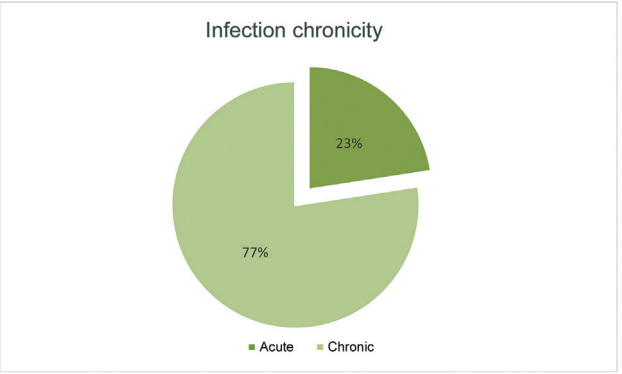

Fig. 2 Distribution of patients with acute or chronic periprosthetic infection.
Cronicidade da infecção = Infection chronicity
Aguda = Acute
Crônica = Chronic

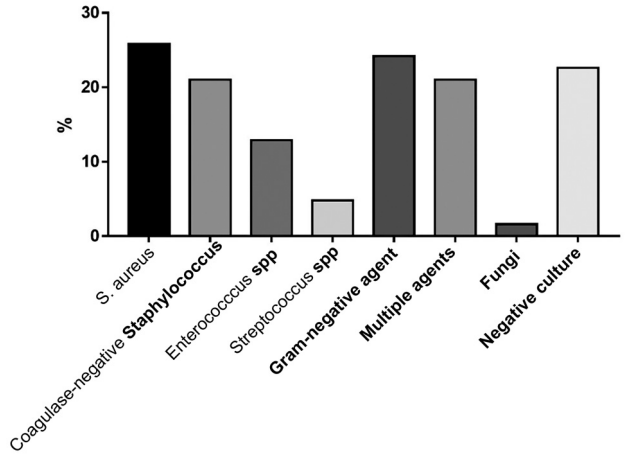

Fig. 3 Distribution of culture-identified pathogens.
S. aureus = *S. aureus*
Staphylococcus coagulase negative = Coagulase-negative *Staphylococcus*
Enterococccus spp = *Enterococccus* spp
Streptococcus spp = *Streptococcus* spp
Gram negativas = Gram-negative agent
Polimicrobianas = Multiple agents
Fungos = Fungi
Cultura negativa = Negative culture

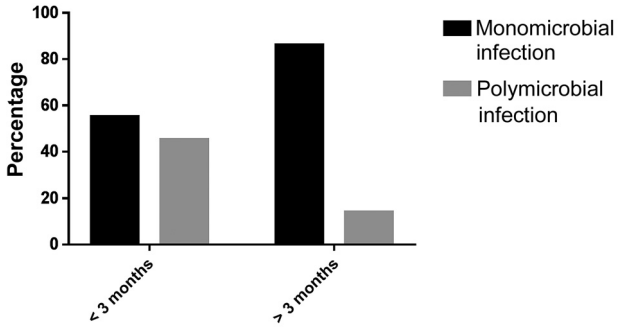

Fig. 4 Frequency of monomicrobial or polymicrobial infections regarding the time between the previous surgery and the revision surgery for total knee replacement.
Percentual = Percentage
< 3 meses = < 3 months
> 3 meses = > 3 months
Monomicrobiana = Monomicrobial infection
Polimicrobiana = Polymicrobial infection

TKAs performed annually makes this complication a significant and increasingly frequent problem.

The 2018 ICM criteria for periprosthetic infection diagnosis allow its confirmation even in the absence of positive cultures. However, pathogen identification remains a fundamental principle for the proper diagnosis and treatment of bacterial diseases, including determining the most appropriate antibiotic agent. A significant issue with microbiological assays is their sensitivity since cultures fail to identify the etiological agent in 5 to 45% of periprosthetic infections.[9–11] Negative cultures pose a critical challenge for treating periprosthetic infections since the lack of pathogen identification leads to the empirical use of antimicrobial agents, which may not be active against the actual infectious organism. In addition, they are associated with a 4.5-fold higher risk of reinfection when compared with cases with positive cultures.[12–14] Our findings show the microbiological profile of periprosthetic knee infections treated in a Brazilian tertiary hospital specialized in highly complex orthopedic surgery.

In our series, the most frequently identified pathogen was *S. aureus*, followed by coagulase-negative *Staphylococcus*. This finding is consistent with other series, in which these pathogens accounted for 50 to 60% of cases.[15,16] Polymicrobial infections totaled 21% of the cases, especially early infections. Cobo et al.[17] had similar results, with an incidence of 32% of polymicrobial infections in subjects with early PJI. Other studies corroborate this finding, suggesting that this higher frequency of polymicrobial infections in early infections may reflect the inoculation of multiple organisms during surgery or contiguous dissemination from the surgical incision.[15,18,19]

For Tan et al.,[10] the incidence of suspected culture-negative periprosthetic infection was 22%. However, according to the Musculoskeletal Infection Society (MSIS) diagnostic criteria, the incidence of infections with negative cultures was 6.4%. Per the 2018 ICM criteria, the actual rate of periprosthetic infection with negative cultures ranges from 7 to 15%.[11] These findings reinforce the importance of differentiating whether periprosthetic infections with negative cultures are actually sterile or a false-negative result, that is, a failure to identify the organism infecting an implant.[11]

The main factors contributing to a negative culture are the following: (1) administration of antibiotic therapy before culture sampling, (2) using a culture medium inadequate for atypical or biofilm-encapsulated agents, (3) improper sample handling and transportation, (4) inadequate incubation time (especially for rare and indolent agents), (5) a limited number of samples or inadequate tissue collection, (6) delay in transportation to the laboratory, (7) infection by a low virulence organism.[9–11] It is important to emphasize that, unlike in other areas of microbiological diagnosis, there are no standardized culture methods for PJI diagnosis. The 2018 ICM recommends the collection of at least three and ideally five or more peri-implant tissue samples during revision surgeries, in addition to the synovial fluid sample. However, there is no consensus on the type of solid tissue most suitable for conventional cultures. Thus, further studies standardizing culture methods are required to optimize the efficiency of this test.[18]

Approximately one fourth of our patients had a negative biological culture. In these subjects, infection diagnosis was based on other tests from the ICM diagnostic criteria. Studies indicate that surgeons often minimize PJI and perform incomplete assessments for diagnosis confirmation.[20] In addition, we know that many of these tests are unavailable in Brazilian public hospitals. However, given this scenario, we emphasize the importance of adopting a careful routine to evaluate patients with a persistent exudative wound or a hot, swollen, or painful joint to rule out or confirm PJI

diagnosis. This routine would allow for an early diagnosis and more effective treatment.

Limitations of this study include the lack of evaluation of the antibiotic resistance profile of the identified bacteria and the fact that it occurred during the pandemic, which may have impacted the profile of patients treated at our institute.

Conclusion

Our results show i) a high prevalence of *Staphylococcus* spp. as causes of knee PJI, ii) a high incidence of polymicrobial infections in early cases, and iii) the occurrence of PJI with negative culture in approximately one fourth of the patients.

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

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