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Microbiology of bone-joint infections in children at a university clinic in Medellín, Colombia

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Abstract

Objective: the objective of the study was to describe the microorganisms, antimicrobial susceptibility, and diagnostic microbiology procedures in children with osteoarticular infections.

Methods: This was a retro-prospective case series in children who consulted a university clinic in Medellín, Colombia, between 2010-2016, who had a culture-confirmed diagnosis.

Results: Out of a total of 218 registered patients, we included 39 with microbiological confirmation. There were more males (27; 69,2%), and 71% cases occurred in >5 years old. The main microorganisms were Methicillin-Susceptible *Staphylococcus aureus* (MSSA) 53,8%, Methicillin-Resistant *Staphylococcus aureus* (MRSA) 20,5%, other gram-positive cocci 10%, *Pseudomonas aeruginosa* 5,1%, *Enterobacter cloacae* complex 2,5%, *Haemophilus haemolyticus* 2,5%, *Mycobacterium tuberculosis* 2,5%, and C*andida albicans* 2,5%. The susceptibility of MSSA/MRSA to trimethoprim-sulfamethoxazole and vancomycin was 100%, and for clindamycin it was 100% and 87%, respectively. Bone tissue and bone secretion using conventional methods, bone pus or synovial fluid inoculated in a blood culture bottle, and synovial tissue were positive in 96%, 87,5% and 50%, respectively. The blood cultures were positive in 54%. In two patients only the blood cultures were positive.

Conclusion: MSSA was the most frequent microorganism followed by MRSA. However, MRSA showed high susceptibility to trimethoprim-sulfamethoxazole, clindamycin, and vancomycin. The high positivity of primary cultures suggests the importance of always obtaining them. Although the positivity of blood cultures was lower, should always be included as part of microbiological studies.

Key words: children, bone-join infection, microbiology, antibiotics, diagnostic.

Microbiología de las infecciones osteoarticulares en niños atendidos en una clínica universitaria en la ciudad de Medellín, Colombia.

Resumen

Objetivo: el objetivo del estudio fue describir los microorganismos, susceptibilidad antimicrobiana y los procedimientos diagnósticos microbiológicos en niños con infecciones osteoarticulares.

Métodos: serie de casos retro-prospectiva en niños que consultaron a una clínica universitaria en Medellín-Colombia, entre 2010-2016, con diagnóstico confirmado por cultivo.

Resultados: de 218 pacientes registrados se incluyó a 39 con confirmación microbiológica. Hubo predominio en hombres (27; 69,2%) y 71% de los casos ocurrió en >5 años. Los principales microorganismos fueron *Staphylococcus aureus* meticilino-sensible (SAMS) 53,8%, *S. aureus* meticilino-resistente (SAMR) 20,5%, otros cocos grampositivos 10%, *Pseudomonas aeruginosa* 5,1%, *Enterobacter cloacae* complex 2,5%, *Haemophilus haemolyticus* 2,5%, *Mycobacterium tuberculosis* 2,5% y *Candida albicans* 2,5%. La susceptibilidad de SAMS/SAMR a trimetroprim-sulfametoxasol y vancomicina fue del 100 % y para clindamicina 100% y 87%, respectivamente. El cultivo de hueso y secreción de hueso en medios convencionales, pus de hueso o líquido articular inoculado en botella de hemocultivo y el cultivo de tejido articular fueron positivos en el 96%, 87,5% y 50%, respectivamente. Los hemocultivos fueron positivo en el 54%. En dos pacientes los únicos cultivos positivos fueron los hemocultivos.

Conclusión: SAMS fue el microorganismo más frecuente seguido de SAMR. Sin embargo, SAMR presentó una alta susceptibilidad a trimetroprim-sulfametoxasol, clindamicina y vancomicina. La alta positividad de los cultivos primarios sugiere la importancia de siempre obtenerlos. Aunque la positividad de los hemocultivos fue menor, siempre deberán incluirse como parte de los estudios microbiológicos.

Palabras clave: niños, infecciones osteoarticulares, microbiología, antibióticos, diagnóstico.

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Introduction

Osteomyelitis, septic arthritis, and osteoarthritis (OA) are common invasive bacterial infections in children, affecting mainly those under five years of age. Osteoarticular infections (OAI) have a global incidence of 10-25 cases per 100,000 children/year, with it being lower in developed countries with 4-10 cases per 100,000 children/year[1,2].

Staphylococcus aureus is the most prevalent etiological agent involved in OAI, even though its frequency varies depending on age. Other microorganisms causing OAI in children include *Streptococcus pyogenes*, *S. pneumoniae*, *S. agalactiae*, *Haemophilus influenzae* serotype b, *Neisseria gonorrhoeae*, *Escherichia coli*, *Kingella kingae*, and *Candida* spp.[3,4]. Nevertheless, there has been a recent increase in severe invasive infection cases by Community-acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA). Consequently, OAI by CA-MRSA have increased globally[1,4-7]. CA-MRSA infections have been reported in Colombia since 2006[8]. The first report in pediatric patients back in 2009 included patients with multisystemic disseminated infection and osteomyelitis[9]. Since then, other cases have been described[10-12].

Recognizing the etiology of OAI in children is fundamental, which allows to choose the most appropriate empirical antibiotic therapy based in local epidemiology[3,4]. However, local data, and specifically in children is scarce[13-15].

The objective of this study was to describe the microorganisms, antimicrobial susceptibility, and the diagnostic microbiology procedures of children with OAI.

Methodology

This was a retro-prospective, observational case-series study. We included children with confirmed OAI who were admitted at the *Clínica Universitaria Bolivariana* (*CUB*) in the city of Medellín, Colombia, between July 2010 and July 2016. CUB is a combined terciary care adult-pediatric facility, with a 70-bed capacity in pediatric hospitalization. We reviewed the electronic medical records, between 2010 and 2014, with diagnostic codes for arthritis and osteomyelitis, according to the International Classification of Diseases (ICD), valid at that time[16]. Between 2015 and 2016, patients were recruited prospectively through daily medical rounds and the information was recorded. A total for 218 medical records were evaluated. The study was approved by the Ethics Committee of the School of Health Sciences at the *Universidad Pontificia Bolivariana* and the *CUB*. Due to the characteristics of the study, no informed consent was required or obtained.

Inclusion and exclusion criteria

We included all patients older than 30 days and up to 15 years old, hospitalized with a diagnosis of acute, subacute, or chronic osteomyelitis, arthritis or OA, confirmed by primary culture (bone culture, bone pus, synovial tissue or synovial fluid) or by positive blood cultures.

We excluded patients with no microbiology confirmation, non-infectious arthritis, those with incomplete medical information. In patients with multiple positive cultures, we accounted only for the first isolate.

Operative definitions

Acute osteomyelitis, arthritis, and OA were defined when the evolution time for symptoms was ≤14 days. They were considered subacute or chronic infections if the duration of the symptomatology was shorter than three months or longer than three months, respectively[2,4].

Microbiology

The sample for culturing were blood, bone biopsies, bone pus, synovial fluid or synovial tissue. The samples collected and the type of cultures ordered depended on the orthopedic surgeon's advice. The physicochemical study of the synovial fluid was not included in this study. The samples for the microbiological study were processed according to the established microbiology laboratory protocols and were inoculated, simultaneously, in convencional cultures and blood culture bottles. The laboratory does not have molecular techniques for the microbiological diagnosis of OAI.

Conventional cultures: The samples that arrived in dry tubes to the microbiology laboratory were inoculated in MacConkey agar plates, chocolate agar plate or Columbia colistin-nalidixic acid agar plate (bioMérieux –Marcy-l´Etoile); the synovial tissue or bone samples were inoculated in the enriched brain heart infusion medium (BHI). The media were incubated at 37°C in a 5% CO_2, and they were checked daily for five days. In the case of yeasts, solid culture media Sabouraud, chocolate and blood agar plates (bioMérieux –Marcy-l´Etoile) were used. For mycobacteria, Lowenstein-Jensen solid culture media along with susceptibility tests were used for rifampin, isoniazid, and ethambutol, and they were processed following established protocols by the National Institute of Health of Colombia *(Instituto Nacional de Salud*), and as recommended by the Pan American Health Organization[17].

Blood culture bottles: The bottles inoculated with blood, bone pus or synovial fluid were incubated for seven days in the automated equipment BacT/Alert3D™ (bioMérieux –Marcy-l´Etoile). Bottles which flagged positive were processed for Gram staining, and according to the findings, subcultures were carried out in chocolate, Columbia CNA, MacConkey, blood or Sabouraud agar plates.

Identification and susceptibility to antimicrobials for the isolated microorganisms in conventional media or blood cultures, was performed in the automated equipment Vitek2™ *compact* (bioMérieux –Marcy-l´Etoile), and *Clinical and Laboratory Standards Institute* (CLSI) susceptibility breakpoints, valid for the date of isolations, were applied at the time. For the analysis, the following antibiotics were included for gram-positive cocci other than *S. aureus*: oxacillin, vancomycin, penicillin, clindamycin, and erythromycin; for

enteric gram-negative bacilli: cefepime, imipenem, meropenem, and ciprofloxacin; for *Pseudomonas aeruginosa*: ceftazidime, cefepime, piperacillin-tazobactam, imipenem, meropenem, and ciprofloxacin; for other gram-negative bacilli: cefotaxime; for yeasts, fluconazole; and for *Mycobacterium tuberculosis*: rifampin, isoniazid, and ethambutol. For *S. aureus* vancomycin, oxacillin, clindamycin, erythromycin, and trimethoprim-sulfamethoxazole were analyzed; susceptibility to vancomycin was discriminated by the different minimal inhibitory concentrations (MIC) reported (MIC ≤0.5 µg/ml, 1 µg/ml, and >1 µg/ml)[18].

Data collection and statistical analysis

From the medical records, we collected demographic, clinical, and microbiological data of the variables of interest. We recorded age, gender, joint, bone, or bone and joint compromise, acute, subacute or chronic infection, medical history, such as previous trauma to the affected limb, skin or soft tissue infection (furuncles, cellulitis or abscesses), infected trauma wound, orthopedic hardware, fractures or no identifiable factor. The microbiological information consisted of the name of the microorganism, susceptibility to the tested antibiotics, obtained cultures, type of specimens, and the use of antibiotics prior to taking cultures. The microbiological information was complemented, if necessary, directly from the microbiology laboratory. The collection of the information was done exclusively by one of the researchers and it was recorded using an Excel™ database. We performed descriptive statistics including calculating absolute frequencies and proportions for the qualitative variables, using SPPS®.

Results

We reviewed 218 medical histories with a recorded diagnosis of arthritis and osteomyelitis according to the ICD. From the initial 218, 179 were non-eligible or excluded (because there were no isolates, microbiological cultures were not taken, age or incomplete information), for a total of 39 clinical histories corresponding to the same number of patients. The number of negative cultures was not included in the analysis. Figure 1 shows the flowchart for the selection of the patients.

Of 39 patients, most were male (66,7%) for all types of OAI. Additionally, 71 % were older than five, and in 71% some importance background events in relation to the OAI was identified. For OAI by *S. aureus*, skin and soft tissue infection (furuncles, cellulitis or abscesses), and previous infected post-traumatic wounds were found as background events in 2/8 cases (25%) for MRSA, and 6/21 cases (28,5%) for MSSA. Nevertheless, for the studied group, trauma to the affected limb (33,3%) was the most frequent event (Table 1).

Regarding microbiology the main microorganisms were MSSA and MRSA, with 21 (53,8%) and 8 (20,5%), respectively. They were followed in order of frequency by other gram-positive cocci (10,2%) and gram-negative bacilli (10,2%). *Candida albicans* (2,5%) and *M. tuberculosis* (2,5%) were isolated from patients with subacute and chronic infectious processes, while patients with *P. aeruginosa* isolates (5,1%) had orthopedic hardware (Table 2). The antimicrobial susceptibility profile for all microorganisms showed high susceptibility to the analyzed antibiotics (Table 3). Regarding MSSA and MRSA, susceptibility to trimethoprim-sulfamethoxazole and vancomycin was 100%, and for clindamycin 100% and 87%, respectively. All analyzed *S. aureus* isolated presented a MIC ≤1 µg/ml for vancomycin (Table 4).

Regarding sample collection, all patients had primary cultures obtained (bone, bone pus, synovial fluid or tissue). Almost half patients (48,7%) received at least one dose of empirical antibiotic therapy before cultures were obtained. In 25 patients, bone cultures were done, and 96% (24/25) were posi-

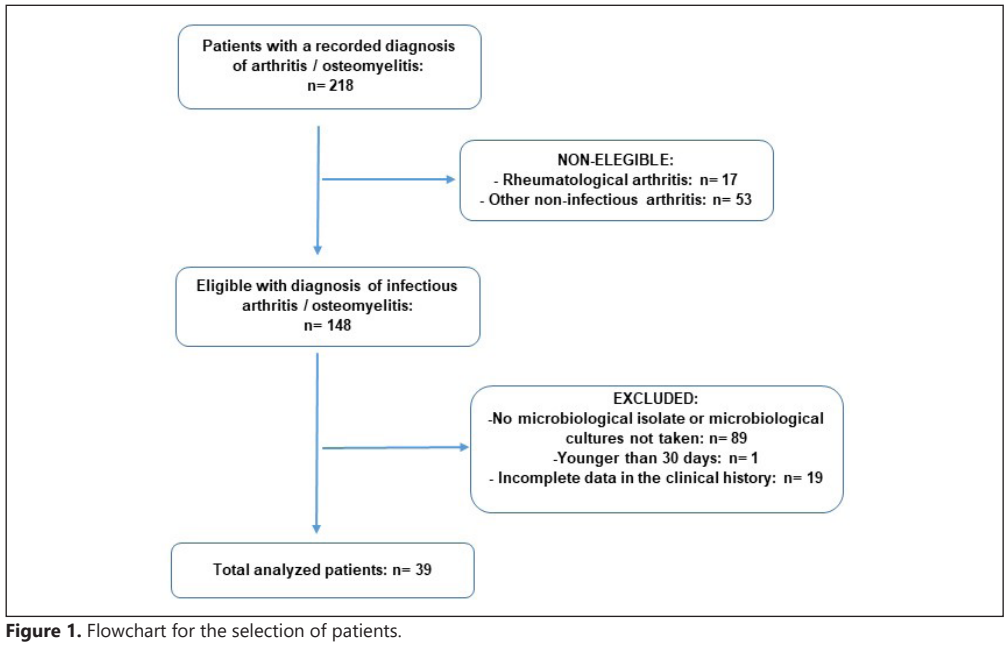

Figure 1. Flowchart for the selection of patients.

Table 1. General clinical characteristics.

Characteristics		n= 39 (%)
Male gender		**27 (69,2)**
Age		
	1-11 months	1 (2,6)
	12-23 months	3 (7,7)
	24-35 months	3 (7,7)
	36-47 months	1 (2,6)
	48-59 months	3 (7,7)
	5-10 years	14 (35,9)
	11-15 years	14 (35,9)
Type of infection		
	ASA	11 (28,2)
	SASA	1 (2,5)
	CSA	1 (2,5)
	AOM	3 (7,6)
	SAOM	5 (12,8)
	COM	4 (10,2)
	AOA	12 (30,7)
	SAOA	2 (5,1)
	COA	0
Clinical history		
	Trauma	13 (33,3)
	Skin and soft tissue infection[1]	6 (15,3)
	Infected trauma wound	5 (12,8)
	Orthopedic hardware	4 (10,2)
	Fracture	1 (2,6)
	Unidentified or no information	10 (25,6)
Bone and/or joint involved [2]		
	Knee	16 (41)
	Femur	11 (28,2)
	Tibia	9 (23)
	Ankle	4 (12,5)
	Hip	3 (7,6)
	Sacroiliac	2 (5,1)
	Humerus	2 (5,1)
	Elbow	2 (5,1)
	Shoulder	1 (2,5)
	Others [3]	5 (12,8)

[1] Furuncles, cellulitis or abscess. [2] Some patients presented more than one compromised anatomical site. [3] Ulna, radius, clavicle, phalanx and iliac. AOA: acute osteoarthritis; AOM: acute osteomyelitis; ASA: acute septic arthritis; COA: chronic osteoarthritis; COM: chronic osteomylitis; CSA: chronic septic arthritis; SAAS: subacute septic arthritis; SAOA: subacute osteoarthritis; SAOM: subacute osteomylitis.

tive. For synovial tissue cultures, in 4/6 the result was positive (66,6%). In 16 patients, bone pus or synovial fluid were obtained and cultured in blood culture bottles, with 14 positive cases (87,5%). Synovial fluid in conventional cultures was positive in 7/14 processes samples (50%) (Figure 2). Twenty-four (61,5%) patients had blood cultures available. Of those, 13/24 (54,1%) were positive. In patients with positive primary site cultures and positive blood cultures (bacteremia), there were no discrepancies among the isolated microorganisms. Two patients had positive blood cultures (MRSA and MSSA) but negative primary cultures.

Discussion

This study allowed for an approximation to the microbiology in a group of patients younger than 15 years with OAI hospitalized in a university clinic in the city of Medellín, Colombia. We highlight the following results: most cases occurred in males older than five years of age; the main isolated microorganism was *S. aureus*, with a 27.5% proportion for MRSA; *S. aureus* presented high susceptibility to clindamycin, trimethoprim-sulfamethoxazole, and vancomycin; the types of specimens that presented more positive results in the cultures were bone biopsy, and bone pus or synovial fluid in blood culture bottles. In several patients, microbiological isolation was achieved even with the use of antibiotics prior to the cultures; blood cultures were not ordered routinely in all patients.

OAI occur mostly in males younger than five years[2-4,19]. In the studied group, most patients were older than five. This could be related to a higher tendency to trauma as consequence of starting physical activity and contact sports in school children or teenagers[20,21]. However, there was a high number of patients from different ages who were not included in the study due to the absence of a microbial isolate, in whom we do not know the clinical background prior to the onset of the infection, which does not make it possible to generalize this result.

Between 70-90% of cases of OAI with positive cultures the most frequent infectious agent identified is *S. aureus* in variable proportions, depending on the geographical area, MSSA and CA-MRSA[3-6,22,23]. Other important reported microorganisms are *S. pyogenes*, *S. agalactiae*, *Escherichia coli*, *K. kingae*, and *Candida* spp.[3,4]. *Streptococcus pneumoniae* and *H. influenzae* serotype b were very prevalent agents before the introduction of conjugate vaccines against these microorganisms[3,4,6]. Our results coincide with what has been reported in the literature in relation to the prevalence of MSSA and MRSA; no OAI cases by *S. pneumoniae* or *H. influenzae* serotype b were identified, which might be explained by the introduction of both vaccines in the amplified national immunization program.

At a local and national level, there are few studies that describe microbiology in OAI. Sierra M *et al.* in a study about non-gonococcal septic arthritis in a group of patients older than 12 years (n: 54), in a university hospital in the city of Me-

Table 2. Etiology and distribution by age.

Age / Micro organisms	1-11 months	12-23 months	24-35 months	36-47 months	48-59 months	5-10 years	11-15 years	Total
SAMS		3	1	1	1	5	10	21
SAMR					1	6	1	8
Staphylococcus hominis			1					1
S. epidermidis							1	1
Streptococcus pyogenes			1					1
S. agalactiae							1	1
Pseudomonas aeruginosa					1	1		2
Enterobacter cloacae							1	1
Haemophilus haemolyticus						1		1
Mycobacterium tuberculosis							1	1
Candida albicans	1							1
Total	1	3	3	1	3	13	15	**39**

MSSA: methicillin-susceptible *Staphylococcus aureus*; MRSA: methicillin resistant *Staphylococcus aureus.*

Table 3. Identified microorganisms and their susceptibility to the analyzed antibiotics.

Microorganisms	No.	OXA	VAN	CLI	ERY	PEN	CAZ	FEP	TZP	IMI	MER	CIP	CTX	FLU	HRE
Staphylococcus hominis	1	S	S	R	R										
S. epidermidis	1	S	S	S	R										
Streptococcus pyogenes[1]	1					S									
S. agalactiae[1]	1					S									
Pseudomonas aeruginosa	2						S	S	S	S	S	S			
Enterobacter cloacae	1							S		S	S	S			
Haemophilus haemolyticus	1												S		
Mycobacterium tuberculosis	1														S
Candida albicans	1													S	

1 Susceptibility to clindamycin or erythromycin was not performed. CIP: ciprofloxacin; CTX: cefotaxime; CAZ: ceftazidime; FEP: cefepime; TZP: piperacillin-tazobactam; FLU: fluconazole; IMI: imipenem; MER: meropenem; PEN: penicillin; OXA: oxacillin; VAN: vancomycin; CLI: clindamycin; ERI: erythromycin; HRE: isoniazid, rifampin, etambutol; S: susceptible; R: resistant.

dellín, found that the main microorganism in synovial fluid cultures was *S. aureus* in 75,5% of cases, followed by other gram-negative bacilli like *Klebsiella* spp. (5,7%), *E. coli* (3,8%), *Salmonella* spp. (1,9%), and *Enterobacter* spp. (1,9%). In this work they did not discriminate between MSSA and MRSA isolates[14]. Recently, Atehortua S *et al.* in a study in the same hospital, described the etiology in 60 patients younger than 12 with a diagnosis of septic arthritis, in whom the culture was positive (27/60; 45%). The distribution of the isolated microorganisms was as follows: MSSA (21,6%), MRSA (8.3%), *S. pyogenes* (3,3%), methicillin-resistant *S. epidermidis* (3,3%), *S. pneumoniae* (1,6%), and *N. meningitidis* (1,6%), among others[13]. Even though in the studies by Sierra M and Atehortua S *et al.* the OAI was septic arthritis, the microbiology is similar to that reported in our paper. It can also be noted that no isolates of *K. kingae* were present.

In regard to MRSA isolates, the antibiotic susceptibility profile suggests a CA-MRSA phenotype, keeping a high susceptibility to trimethoprim-sulfamethoxazole, clindamycin, and vancomycin. Similar findings have been reported by other researchers in cases of OAI by CA-MRSA in South America[6,5,13]. Finding a proportion of MRSA of 27,5% (8/28) as the cause of OAI in the studied group, could suggest that empirical management should consider an effective antibiotic against MRSA as initial therapy[3,4,24,25].

When comparing among the various types of specimens cultured, we found that in general, primary cultures (bone, bone pus, synovial tissue) presented a higher proportion of positive results than blood cultures. Only 61,5% of our patients had blood cultures done, and in two patients the only positive result was the blood culture, which indicates that they should always be included them as part of the microbiological studies in OAI. Atehortua *et al.* reported that in confirmed cases of septic arthritis, 49 % of blood cultures were positive, and in 36,9% synovial cultures were negative, indicating the importance of ordering blood cultures routinely[13]. Zuluaga AF *et al.* evidenced, in chronic osteomyelitis cases, that bone biopsies had high microbiological diagnostic performance (94/100; 94%), and were superior to cultures from non-bone biopsies[15]. It has been mentioned that the use of antibiotics prior to sampling for cultures during surgery could decrease diagnostic performance. In our case, in 19 patients (48,7%)

Table 4. Antimicrobial susceptibility in *Staphylococcus aureus*.

	No.	CLI	ERI	SXT	VAN / MIC (µg/mL)		
					≤ 0.5	1	> 1
MSSA[1,2]	21	21 (100%)	14 (66%)	21 (100%)	4 (19%)	15 (71%)	0
MRSA	8	7 (87%)	7 (87%)	8 (100%)	1 (12%)	7 (87%)	0

CLI: clindamycin; ERI: erythromycin; MIC: minimal inhibitory concentrarion; MSSA: methicillin-susceptibile *Staphylococcus aureus*; MRSA: methicillin resistant *Staphylococcus aureus*; SXT: trimethoprim-sulfamethoxazole; VAN: vancomycin. [1] In two MSSA isolates susceptibility to vancomycin was not reported. [2] Positive D-Test in one isolate.

a microbiological isolation was achieved in primary cultures after initiating antibiotic therapy. This result diverges from what was reported by Burnett RS *et al*. who did not find any effect on the performance of intraoperative cultures due to the use of prophylactic antibiotics, in the revision cases of total knee arthroplasty[26]. Ratnayake K *et al*., in a study about acute osteomyelitis in children, found that in 42/50 (84%) of cases a positive primary culture was obtained after starting antibiotics[27]. These results indicate that antibiotics in OAI may be started before surgical procedures, making sure that blood cultures are previously obtained and adequately sampling during surgery, including using the most appropriate culture media. The use of blood culture bottle to inoculate with pus or synovial fluid should be considered to increase microbiological recovery[13]. The use of prophylactic antibiotics before surgery is indicated and directed to the most common microorganisms including *S. aureus*. However, the impact on recovery of microorganisms could be less for *S. aureus* than for other bacteria.

The limitations of this study are diverse, including a reduced number of patients, and using data from patients from only one institution; when including retrospectively recruited patients, it is possible that some individuals with osteomyelitis or septic arthritis registered with different diagnoses were not identified; information about the characteristics of the cytochemical study of the synovial fluid, in the cases of septic arthritis, was not included and microbiological cultures were not done homogeneously in all patients; the number of isolates and susceptibility to different antibiotics was low for microorganisms other than *S. aureus*, which does not allow generating any recommendation for empirical treatment. Nevertheless, this did not significantly influenced the main objectives or results of the research.

In our cohort, OAI predominated in school children and teenagers. MSSA was the most frequent etiological agent, with an important proportion of MRSA. However, MRSA presented high susceptibility to trimethoprim-sulfamethoxazole, clindamycin and vancomycin. The high positivity of primary cultures suggests the importance of always obtaining them. Although the positivity of blood cultures was lower, should always be included as part of microbiological studies.

Ethical considerations

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Protection of human and animal subjects. The study was approved by the Ethics Committee of the School of Health Sciences at the *Universidad Pontificia Bolivariana* and the *Clínica Universitaria Bolivariana*. No experiments were performed on humans or animals in this research.

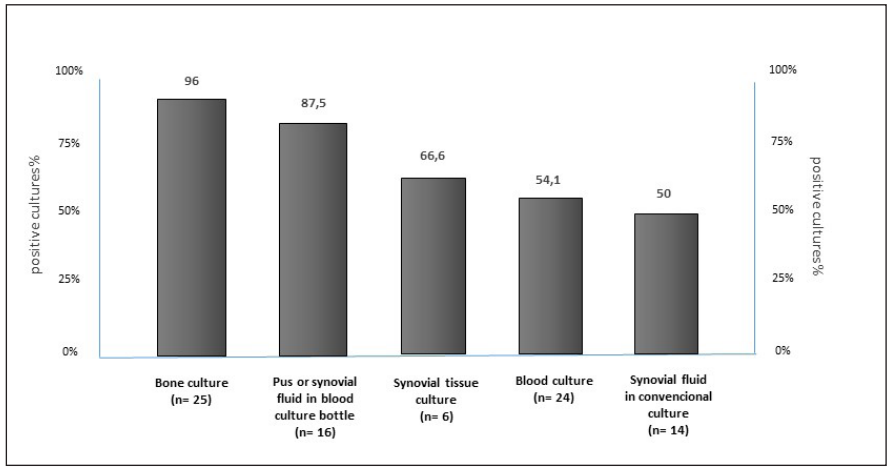

Figure 2. Comparison among the different types of specimens cultured.

Confidentiality and privacy. The confidentiality and anonymization of the information was guaranteed.

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Author contributions. Álvaro Hoyos: conceptualization, investigation, formal analysis, validation, writing, review & editing. Luisa Mantilla: investigation, formal analysis, validation, writing, review.

Conflict of interest statement. None declared.

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