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Clinical and Microbiological Characterization of Osteoarticular Infections in Children from a clinic in Rionegro, Antioquia

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ABSTRACT

Introduction: Osteomyelitis and septic arthritis are common invasive bacterial infections in children.

Objective: To describe the microorganisms, antimicrobial susceptibility, and microbiological diagnostic procedures.

Methods: case series in children with confirmed diagnosis, between 2017 – 2022.

Results: 48/84 patients were studied. Cases of osteomyelitis, septic arthritis and osteoarthritis were 20.8%, 37.5% and 41.6%, respectively. Isolated microorganisms included Methicillin-Susceptible *Staphylococcus aureus* (MSSA) 30.7%, Methicillin-Resistant *S. aureus* (MRSA) 28.8%, *Pseudomonas aeruginosa* 5.7%, *S. epidermidis* 3.8%, *Escherichia coli* 3.8%, *Enterococcus faecalis* 3.8%, *Aeromonas hydrophila* 3.8%, *Mycobacterium tuberculosis* 1.9%, *Salmonella* spp. 1.9%, among others. In 40 infections originating from skin and soft tissues, orthopedic hardware, limb trauma, and without an identified source, MSSA/MRSA predominated (75%). MSSA/MRSA showed susceptibility to vancomycin at 100%, trimethoprim-sulfamethoxazole at 100% and 93%, and clindamycin at 100% and 86.6%, respectively. Blood cultures (n=22), synovial tissue cultures (n=7), bone cultures (n=28), and bone pus or synovial fluid in blood culture bottles (n=3) were positive in 100%.

Conclusions: *S. aureus* was the most frequent microorganism with a high proportion of MRSA. MRSA exhibited high susceptibility to trimethoprim-sulfamethoxazole and vancomycin. The high positivity of primary cultures suggests the importance of always obtaining them.

ARTÍCULO ORIGINAL

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PUBLICACIÓN ADELANTADA

Caracterización clínica y microbiológica de las infecciones osteoarticulares en niños de una clínica en Rionegro, Antioquia

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RESUMEN

Introducción: osteomielitis y artritis séptica son infecciones bacterianas invasoras comunes en niños.

Objetivo: describir los microorganismos, susceptibilidad a los antibióticos y los procedimientos diagnósticos microbiológicos.

Métodos: serie de casos en niños con diagnóstico confirmado, entre 2017 – 2022.

Resultados: se estudiaron 48/84 pacientes. Los casos de osteomielitis, artritis séptica y osteoartritis fueron de 20,8 %, 37,5 % y 41,6 %, respectivamente. Los microorganismos aislados incluyeron a *Staphylococcus aureus* susceptible a meticilina (SAMS) 30,7 %, *S. aureus* resistente a meticilina (SAMR) 28,8 %, *Enterococcus faecalis* 3,8 %, *Aeromonas hydrophila* 3,8 %, *Mycombacterium tuberculosis* 1,9 %, *Salmonella* spp. 1,9 %, entre otros. En 40 casos de infecciones originadas en piel y tejidos blandos, material de osteosíntesis, trauma sobre la extremidad y sin una fuente identificada, predominó SAMS/SAMR (75 %). La susceptibilidad de SAMS/SAMR a vancomicina fue 100 %, para trimetoprim-sulfametoxazole 100 % y 93 % y clindamicina 100 % y 86,6%, respectivamente. Los hemocultivos (n=22), cultivos de tejido sinovial (n=7), hueso (n=28) y pus de hueso o liquido sinovial en botella de hemocultivo (n= 3) fueron positivos en el 100 %. **Conclusiones**: *S. aureus* fue el microorganismo más frecuente con una elevada proporción de SAMR y presentó una alta susceptibilidad a trimetoprim-sulfametoxasol y vancomicina. La alta positividad de los cultivos primarios sugiere la importancia de siempre obtenerlos.

INTRODUCTION

Osteomyelitis, septic arthritis, and osteoarthritis (OA) are common invasive bacterial infections in children, primarily affecting those under 5 years old. The incidence of osteoarticular infections (OAI) is estimated to be 10-80 cases per 100,000 children per year, with a lower rate in developed countries at 4-10 cases per 100,000 children per year (1-3).

While *Staphylococcus aureus* has been the most common pathogen causing musculoskeletal infections, community-acquired methicillin-resistant *S. aureus* (CA-MRSA) has emerged as a significant microorganism involved in invasive infections, including OAI (1,4-6). In South America, various studies have reported CA-MRSA as a major etiological agent in cases of OAI, similar to other countries worldwide (7-12). In Colombia, the first report of multisystem invasive infection and osteomyelitis caused by CA-MRSA was documented in 2009 (13). Since then, additional cases have been described (14-16).

Other microorganisms causing OAI in children include *Streptococcus pyogenes*, *S. agalactiae*, *Neisseria gonorrhoeae*, *Escherichia coli*, *Salmonella* spp., *Kingella kingae*, and *Candida* spp. (5,11,17,18). With the introduction of conjugated vaccines into immunization schedules, microorganisms such as *S. pneumoniae* and *Haemophilus influenzae* serotype b have decreased as causative agents of invasive infections, including OAI (4,18).

Locally, the etiology of OAI in children is not well-characterized, and published data are scarce (19-23). Recognizing the microbiology involved in OAI is crucial, allowing for the selection of empirical antibiotic therapy based on epidemiology (7,9-11,24).

The objective of this study was to describe the microbiology, antibiotic susceptibility, and microbiological procedures in children with OAI treated at a high-complexity clinic.

METHODOLOGY

The study was an observational and retrospective case series. Children with a confirmed diagnosis of OAI who were hospitalized at *Clínica Somer* (CS) in the city of Rionegro, Antioquia, Colombia, between January 1, 2017, and July 30, 2022, were included. CS is a fourth-level complexity institution that provides care for both adults and children, with a capacity of 110 pediatric beds distributed across emergency services, hospitalization, and intensive care units. CS serves as a referral center for complex patients from urban and rural areas inside and outside the state of Antioquia. Medical records of patients diagnosed with septic arthritis and osteomyelitis, according to the International Classification of Diseases (ICD) valid at that time (25), were reviewed. A total of 84 medical records were evaluated. The study received approval from the Ethics and Research Committee of CS (Minutes number 54 dated July 15, 2022). Due to the nature of the study, informed consent was not required.

Inclusion and Exclusion Criteria

All patients aged over 30 days to 15 years, hospitalized with a diagnosis of osteomyelitis, septic arthritis, or acute, subacute, or chronic OA confirmed by a primary culture (bone culture, bone pus, tissue, or synovial fluid) or a positive blood culture were included.

Patients without microbiological confirmation, non-infectious arthritis, those under 1 month or over 15 years old, and those with incomplete medical information in the medical records were excluded. In patients with multiple positive cultures, only the first isolation was recorded.

Operational Definitions

Osteomyelitis, septic arthritis, and acute OA were defined when the duration of symptoms was ≤14 days. Subacute and chronic infections were considered if the symptom duration was less than 3 months or more than 3 months, respectively (25).

Microbiology

The cultured samples included blood, bone biopsy, bone pus, tissue, and synovial fluid. The samples collected and the types of cultures ordered depended on the orthopedic surgeon's criteria. The cytological study of synovial fluid was not included in the analysis. Samples for microbiological study were processed according to the laboratory's microbiology protocols. The laboratory does not have molecular techniques for the microbiological diagnosis of OAI.

Conventional Cultures: The samples were inoculated on MacConkey agar, chocolate agar, and blood agar; synovial tissue or bone tissue was inoculated in brain heart infusion (BHI) enriched medium. For mycobacteria, Ogawa Kudoh solid medium was used, following the protocols of the National Institute of Health of Colombia and recommendations by the Pan American Health Organization (26).

Blood Cultures: Bottles inoculated with blood, bone pus, or synovial fluid were incubated for 7 days in the automated Bactec™ FX system (Becton Dickinson, USA). Identification and antibiotic susceptibility testing for microorganisms isolated in conventional media or blood cultures were performed on the automated Phoenix™ M50 system (Becton Dickinson, USA), applying susceptibility breakpoints valid for the date of isolations, according to the Clinical and Laboratory Standards Institute (CLSI) (27). Antibiotics analyzed for gram-positive cocci other than *S. aureus* were oxacillin, vancomycin, penicillin, ampicillin, clindamycin, trimethoprim-sulfamethoxazole, and erythromycin; for enteric gram-negative bacilli: cefepime, imipenem, meropenem, and ciprofloxacin; for *Pseudomonas aeruginosa* and other environmental gram-negative bacilli:

ceftazidime, cefepime, piperacillin-tazobactam, imipenem, meropenem, and ciprofloxacin; for *Mycobacterium tuberculosis*: rifampicin, isoniazid, and ethambutol. For *S. aureus*, vancomycin, oxacillin, clindamycin, erythromycin, and trimethoprim-sulfamethoxazole were analyzed. Vancomycin susceptibility was discriminated for different reported minimum inhibitory concentrations (MIC) [MIC ≤0.5 µg/mL, 1 µg/mL, and >1 µg/mL] (27).

Data Collection and Statistical Analysis

Demographic, clinical, and microbiological data of interest people were collected. Age, gender, origin (Metropolitan area of Medellín, east of Antioquia, and other), type of health insurance affiliation, affected joint, bone, or both joint and bone, acute, subacute, or chronic infection, and medical history such as trauma to the affected limb, skin or soft tissue infection (furuncle, cellulitis, or abscess), infected trauma wound, orthopedic hardware, fractures, or no identifiable factor were recorded.

Microbiological information included the microorganism's name, susceptibility to the evaluated antibiotics, obtained cultures, sample types, and prior antibiotic use before culturing. The information was collected exclusively by one of the researchers and recorded using an Excel™ database. The open-source statistical package PSPP was used, applying descriptive statistics that included absolute frequencies and proportions for qualitative variables.

RESULTS

A total of 84 medical records with a diagnosis of septic arthritis or osteomyelitis according to the ICD were reviewed. Out of the 84 records, 21 patients were excluded due to diagnoses of non-infectious arthritis, 2 with less than 1 month, 12 with negative cultures for OAI (which were treated as culture-negative infections), and 1 case due to incomplete information, resulting in a total of 48 medical records corresponding to an equal number of patients. The number of negative cultures was not included in the analysis.

Among the 48 patients, the majority were males (68.7%), and 50% had health insurance under the contributory regime. 58% of the patients were over 6 years old. 89.5% of the patients came from the eastern region of Antioquia or outside the state. Regarding the type of infection, acute OA predominated (37.5%), followed by acute septic arthritis (20.8%), acute osteomyelitis (16.7%), and chronic osteomyelitis (14.6%). The femur was the primary bone involved (29.2%), followed by the tibia (20.8%), and the most affected joints were the knee and the hip (14.6% each) (Table 1).

Table 1. Demographic and general clinical characteristics

Characteristics		**n= 48 (%)**
Gender	Male	33 (68.7)
	Female	15 (31.2)
Health affiliation	Contributory	24 (50.0)
	Subsidised	23 (47.9)
	Other	1 (2.0)
Age		
	1-12 months	9 (18.7)
	1-5 years old	11 (22.9)
	6-10 years old	13 (27.0)
	11-15 years old	15 (31.2)
Origin		
	Medellín Metropolitan Area	5 (10.4)
	East of Antioquia	25 (52.0)
	Other	18 (37.5)
Type of infection		
	SA	10 (20.8)
	OM	11 (22.9%)
	COM	7 (14.6)
	OA	20 (41.6%)
Bone and/or joint involved *		
	Femur	14 (29.2)
	Tibia	10 (20.8)
	Knee	7 (14.6)
	Hip	7 (14.6)
	Humerus	6 (12.5)
	Elbow	6 (12.5)
	Ankle	5 (10.4)
	Fibula	2 (4.2)
	Shoulder	2 (4.2)
	Ulna	2 (4.2)
	Others †	7 (14.6)

* Some patients presented more than one compromised anatomical site. † Hallux, phalanx, radius, metatarsal. OA: osteoarthritis; OM: osteomyelitis; SA: septic arthritis; COM: chronic osteomyelitis

Source: own elaboration

In 37 (77.0%) patients, some history related to OAI was identified. Skin and soft tissue infections were the main antecedent (20/48; 41.5%), followed by open fractures (6/48; 12.5%) and the use of orthopedic hardware (6/48; 12.5%). Methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) were the predominant microorganisms in OAI related to skin and soft tissue infections, osteosynthesis material, limb trauma, and infections without an identifiable antecedent, accounting for 85% (17/20), 50% (3/6), 100% (3/3), and 63.3% (7/11) of cases, respectively. Environmental and enteric gram-negative bacilli predominated in cases of open fractures (Figure 1).

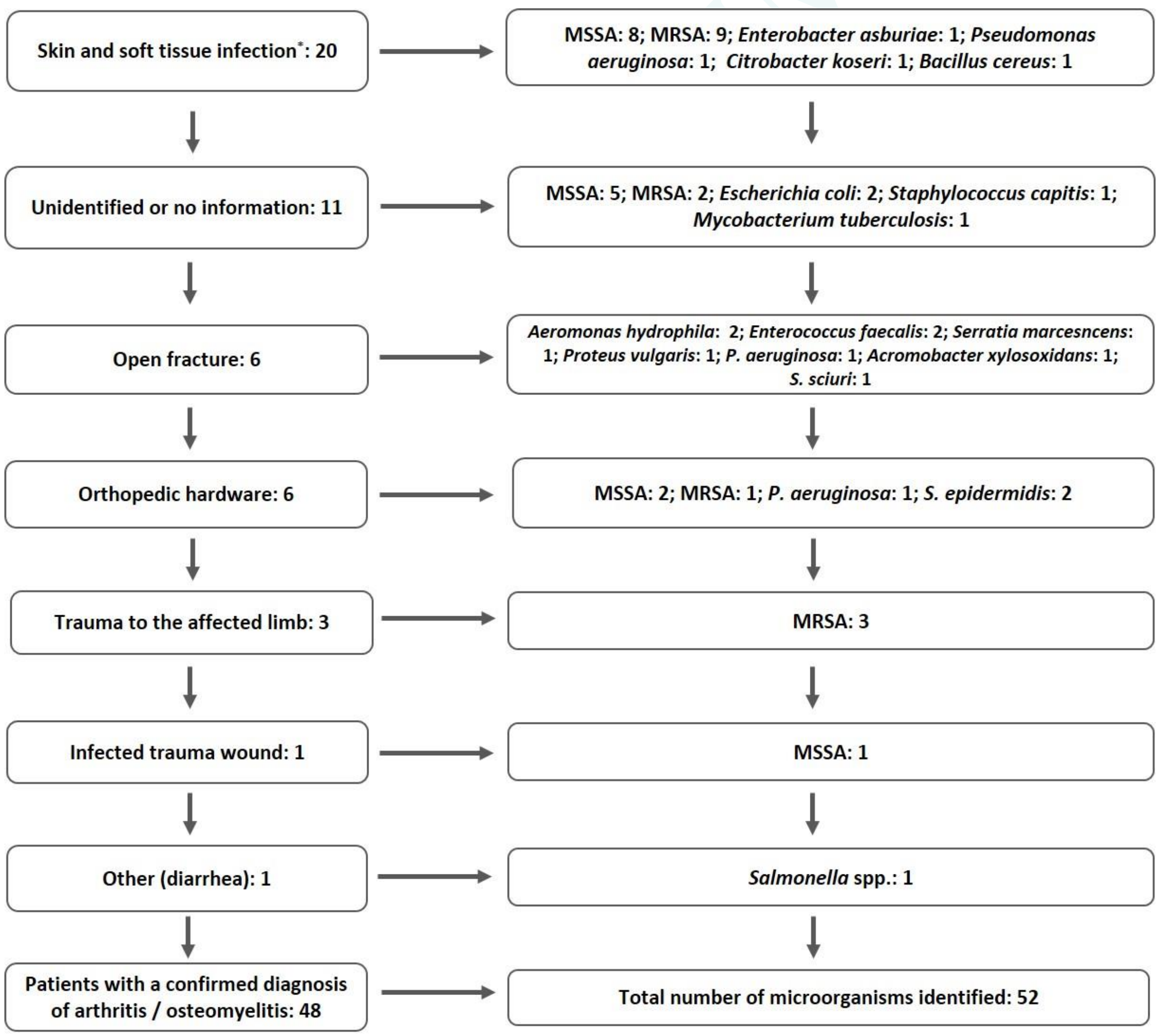

Figure 1. Clinical history and identified microorganisms

* Furuncles, cellulitis or abscess. MSSA: methicillin susceptibility *Staphylococcus aureus*; MRSA: methicillin resistant *Staphylococcus aureus*
Source: own elaboration

When evaluating age and its relationship with etiology, *S. aureus* infections predominated in all age ranges: 6/31 (19.3%) under 12 month, 6/31 (19.3%) between 1-5 years, and 19/31 (61.2%) over 6 years. Infections with environmental gram-negative bacilli (*P. aeruginosa*, *Aeromonas hydrophila*, *Acromobacter xylosoxidans*) mostly occurred in those over 6 years old. In cases of enteric gram-negative bacilli, the ages with the most cases were under 12 months and over 6 years old (Table 2).

Table 2. Etiology and distribution by age

Age / Micro-organisms	1-12 months	1-5 years old	6-10 years old	11-15 years old	Total *
MSSA	3	2	6	5	16
MRSA	3	4	3	5	15
Staphylococcus capitis	-	1	-	-	1
S. epidermidis	-	-	-	2	2
Escherichia coli	1	1	-	-	2
Enterobacter asburiae	-	-	-	1	1
Citrobacter koseri	1	-	-	-	1
Mycobacterium tuberculosis	-	-	1	-	1
***Salmonella* spp.**	1	-	-	-	1
Bacillus cereus	-	1	-	-	1
Pseudomonas aeruginosa	-	1	2	-	3
Aeromonas hydrophila	-	-	-	2	2
Enterobacter cloacae	-	-	-	1	1
Proteus vulgaris	-	-	-	1	1
S. sciuri	-	-	-	1	1
Serratia marcescens	-	-	1	-	1
Enterococcus faecalis	-	1	1	-	2
Acromobacter xylosoxidans	-	-	1	-	1

* A total of 52 microorganisms were identified in 48 patients. In 4/6 open fractures two microorganisms were isolated. MSSA: methicillin susceptibility *Staphylococcus aureus*; MRSA: methicillin resistant *Staphylococcus aureus*
Source: own elaboration

The analysis of the evaluated antibiotics revealed that MSSA was more susceptible than MRSA (Table 3). For vancomycin, all *S. aureus* isolates were susceptible, with an MIC

≤ 1 μg/mL. *Enterococcus faecalis* isolates were susceptible to ampicillin (100%), as were 100% of enteric and environmental gram-negative bacilli to carbapenems (Figure 2). Antibiotic susceptibility testing was not performed for *A. xylosoxidans* due to a lack of reference cut-offs.

Table 3. Antimicrobial susceptibility in *Staphylococcus aureus*

					VAN / MIC (μg/mL)		
	No.	**CLI**	**ERY**	**SXT**	**≤ 0.5**	**1**	**> 1**
MSSA	16	16 (100%)	14 (87%)	16 (100%)	1 (7%)	15 (94%)	0
MRSA*	15	13 (86.6%)	11 (73%)	14 (93%)	1 (7%)	14 (93%)	0

* Positive D-test in one isolate. CLI: clindamycin; ERY: erythromycin; SXT: trimethoprim-sulfamethoxazole; VAN: vancomycin. MIC: minimal inhibitory concentration; MSSA: methicillin susceptibility *Staphylococcus aureus*; MRSA: methicillin resistant *Staphylococcus aureus*
Source: own elaboration

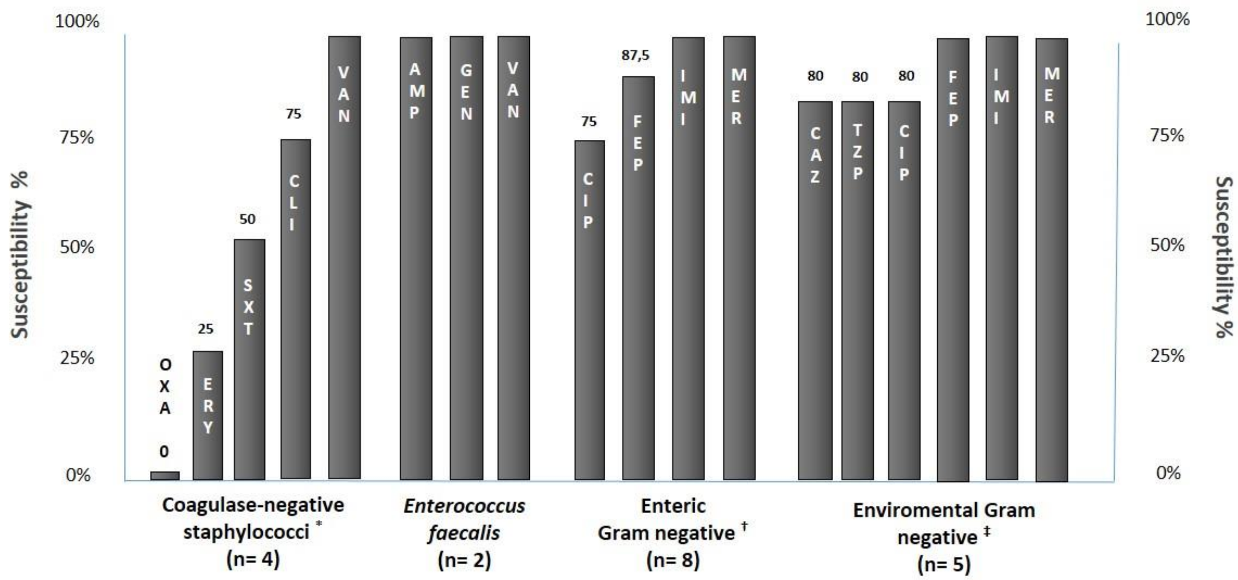

Figure 2. Identified microorganisms and their susceptibility to the analyzed antibiotics
* *Staphylococcus epidermidis:* 2*; S. capitis:* 1; *S. sciuri:* 1. † *Escherichia coli*: 2*; Proteus vulgaris*: 1*; Citrobacter koseri:* 1*; Serratia marcescens:* 1; *Enterobacter cloacae:* 1; *E. asburiae:* 1; *Salmonella* spp.: 1. ‡ *Pseudomonas aeruginosa*: 3*; Aeromonas hydrophila*: 2. OXA: oxacillin; VAN: vancomycin; CLI: clindamycin; ERY: erythromycin; SXT: trimethoprim-sulfamethoxazole; AMP: ampicillin; GEN: gentamacin; CIP: ciprofloxacin; CAZ: ceftazidime; FEP: cefepime; TZP: piperacillin-tazobactam; IMI: imipenem; MER: meropenem
Source: own elaboration

Regarding the collected samples, primary cultures (bone, bone pus, synovial fluid, or tissue) were taken in all patients. Hemocultures were taken from 22 patients, with 100%

positivity (Figure 3). Empirical antibiotics were initiated before the collection of primary cultures in 33 patients (68.7%).

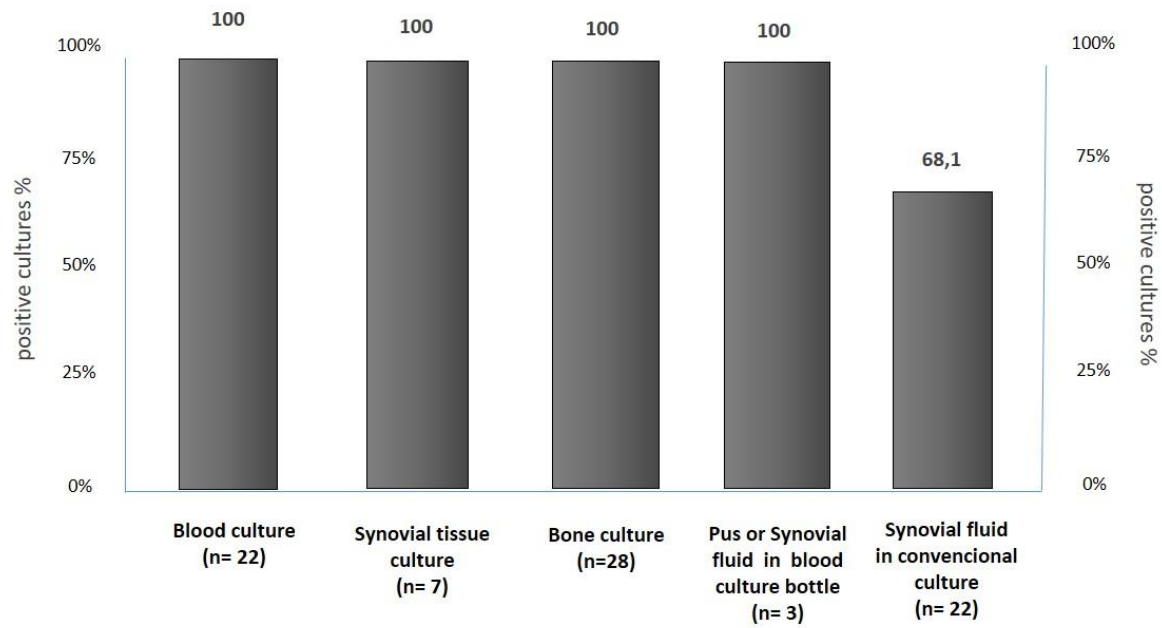

Figure 3. Comparison among the different types of specimens cultured
Source: own elaboration

DISCUSSION

This study provided insights into the microbiology of OAI in a group of patients under 15 years old hospitalized at a high-complexity clinic in Rionegro, Antioquia.

Most OAI cases typically occur in male patients under 5 years old (2,5,17,28). However, in the studied group, the majority of OAI cases occurred in males over 5 years old, consistent with recent publications by various authors (7,22,23). OAI in males over 5 years old may be related to a greater tendency for trauma as a consequence of the initiation of physical activity and contact sports in school-age children and adolescents (29,30). In our series, due to the low number of patients, OAI with negative cultures (not included in the analysis), and the fact that not all cases recorded the clinical history preceding the infection, this finding cannot be generalized.

At the local level, there are few studies describing the microbiology of OAI in children. Recently, in the city of Medellín, a descriptive retrospective study reported the microbiology of OAI in children from a university clinic (23). Of the 39 characterized cases, the main identified microorganisms were MSSA (53.8%), MRSA (20.5%), other gram-positive cocci (10%), *P. aeruginosa* (5.1%); *Enterobacter cloacae*, *Haemophilus haemolyticus*, *S. agalactiae*, *S. pyogenes*, *M. tuberculosis*, and *C. albicans*, each with one case (2.5%). In the same city, at San Vicente Foundation University Hospital, several researchers have reported the etiology of OAI at different times. Urrego JD *et al*. conducted a study to describe the epidemiological profile of patients aged 0 to 15 years with OAI. Of the 72 patients, the main identified microorganisms were MSSA (38.9%), MRSA (26.4%), *S. pyogenes* (2.5%), and *M. tuberculosis* (1.5%), among others (22). Atehortúa S *et al*. described the etiology in 60 patients under 12 years diagnosed with septic arthritis, with a positive culture (27/60; 45%). The distribution of isolates 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%) (19). Sierra M *et al*. in a study on non-gonococcal septic arthritis in a group of patients over 12 years old (n=54) found that the main microorganism in synovial fluid culture was *S. aureus* in 75.5% of cases, followed by other gram-negative bacilli such as *Klebsiella* spp. (5.7%), *E. coli* (3.8%), *Salmonella* spp. (1.9%), and *Enterobacter* spp. (1.9%). In this latter study, a distinction between MSSA and MRSA was not made (20). Our results are similar to those reported locally, and it is noteworthy that no cases of *S. pneumoniae* or *H. influenzae* serotype b were presented, which could be explained by the introduction of both conjugate vaccines into the expanded immunization program (8,9,11). It is also emphasized that no isolates of *K. kingae* were present, similar to the studies mentioned locally. Various hypotheses have been proposed to explain the difficulty in isolating *K. kingae* in conventional culture media, such as a low bacterial inoculum at the infection site, demanding nutritional requirements of the microorganism, and some growth-inhibiting factors present in traditional culture media (19). This last result contrasts with the recently published study by Cañete I *et al*., who, in a study describing the characteristics of pediatric OAI in a hospital in Santiago, Chile, South America, found that in patients under 5 years old, the most frequent microorganism was *K. kingae* (n=12), followed by *S. aureus* (n=9). The researchers used different diagnostic techniques such as polymerase chain reaction (PCR), which could explain better performance in etiological diagnosis (8,19).

Regarding etiology, *S. aureus* was the most frequently identified microorganism. MRSA showed high susceptibility to trimethoprim-sulfamethoxazole, clindamycin, and vancomycin, suggesting a phenotype of community-acquired MRSA (CA-MRSA). The high proportion of positive isolates for MRSA (48.3%) is striking, representing almost half of the *S. aureus* isolates. When compared with recent reports of the microbiology of OAI in children locally, the identification of MRSA has ranged between 8.3 - 28% (19,22,23,31). In South America, reports of MRSA as a cause of OAI vary between 7.7 - 63.3% according to some authors (7-11). The data suggest an increase in OAI cases caused by MRSA, with a highly variable distribution even at the local and regional levels. Finding a MRSA proportion of 48.3% in the studied group would indicate that initial therapeutic management should consider an effective antibiotic against MRSA (1,9,10,17,24,32).

When comparing different types of cultured samples, we found a very high proportion of primary cultures (bone, bone pus, synovial fluid, and tissue) and positive blood cultures (100%). Synovial fluid culture in conventional media had a lower positivity rate (68.1%). In OAI, cultures are typically positive in 40 - 60% of cases (6,10). In acute cases, blood cultures reach a positivity rate of 60% (9). Atehortua S *et al*. reported that, in confirmed cases of septic arthritis, 49% of blood cultures were positive, and in 36.9% of cases, synovial fluid cultures were negative, indicating the importance of routinely ordering blood cultures (19). Zuluaga AF *et al*. demonstrated, in cases of chronic osteomyelitis, that bone biopsy had a high diagnostic yield (94/100; 94%) and was superior to cultures from samples other than bone (21). Recently, it was reported that, in confirmed cases of IOA in children (n: 39), bone, bone pus or synovial fluid cultures in blood culture bottles, and synovial tissue culture, showed the highest positivity rates at 96%, 87.5%, and 66.5%, respectively (23). The high proportion of positive cultures in our study could be explained by several reasons. First, the CS is an institution that receives patients referred from distant rural areas with complex and prolonged infectious processes that may take a long time to receive medical attention. Second, the orthopedic surgical team has prioritized the collection of samples with the best diagnostic performance in surgery, such as bone pus, bone and synovial tissue. Third, empirical antibiotics are initiated after blood cultures are taken in acute IOA cases. It has been mentioned that the use of antibiotics before sample collection in surgical culture may reduce diagnostic yield. Ratnayake K *et al*. in a study on acute osteomyelitis in children found that in 42/50 (84%) of cases, a positive primary culture was obtained after antibiotics were initiated (33). Other authors have reported similar findings (4,23,34). These data suggest that antibiotics in acute IOA could be initiated before surgical procedures, ensuring prior blood culture collection, proper sample collection in surgery, and the use of appropriate culture media. To increase microbiological recovery, the use of blood cultures to inoculate with pus or synovial fluid could be considered (19,28,35).

The study has various limitations, including a small number of patients from a single institution. Including patients retrospectively may mean that individuals with osteomyelitis or septic arthritis registered with different diagnoses may not have been identified. Information on the characteristics of the synovial fluid cytological study was not included in cases of septic arthritis, and microbiological cultures were not uniformly taken in all patients.

The lack of molecular microbiological diagnostic tests may limit the detection of difficult-to-isolate microorganisms such as *K. kingae*. The number of isolates and susceptibility to different antibiotics was low for microorganisms other than *S. aureus*, preventing the generalization of any recommendations for empirical treatment. However, this did not significantly impact the objectives and results of the research.

CONCLUSIONS

In this cohort, osteoarticular infections (IOA) predominated in school-age children and adolescents. *Staphylococcus aureus* was the most common etiological agent, with a significant proportion of MRSA. MRSA showed high susceptibility to trimethoprim-sulfamethoxazole and vancomycin. Enteric and environmental gram-negative bacilli predominated in open fractures. The high positivity of primary cultures suggests the importance of always obtaining them, prioritizing the collection of bone and joint tissue and bone pus. The results highlight the need for studies with a larger number of patients to better characterize the etiology of IOA in children, monitor antibiotic susceptibility over time, and establish clinical differences between infections caused by MSSA and MRSA.

FUNDING

None.

CONFLICT OF INTEREST

None to declare.

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