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Awareness on the Evolving of Bacterial Resistance Bacteria Isolated in Joint Fluids Received at the Centre Pasteur Du Cameroun from 2016 To 2021

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Abstract **Introduction:** Septic arthritis is a serious medical emergency because of the morbidity and functional risks it entails. Variations in the microbiological profile and the emergence of antimicrobial resistance remain a challenge. This study was undertaken to determine the bacterial aetiologies of septic arthritis and their antimicrobial resistance trends, in order to update data and contribute to better therapeutic management of patients suffering from this pathology. **Methods:** A retrospective and prospective study was carried out on joint fluids received at the Centre Pasteur du Cameroun Laboratory between 2016 and 2021. Cytobacteriological analysis was performed on these joint fluids according to the medical microbiology guidelines. **Results:** 471 joint fluids were received and tested, of which 83 (17.6%) were culture positive. The main bacteria isolated were *Staphylococcus aureus* (n=33; 38.8%) followed by *Streptococcus pneumoniae* (n=5; 5.9%), *Klebsiella pneumoniae* (n=5; 5.9%) and *Streptococcus pyogenes* (n=4; 4.7%). Vancomycin, teicoplanin, linezolid and nitrofuran were 100% active on gram-positive cocci, while imipenem and amikacin were active on gram-negative bacteria, with sensitivity frequencies of 92.3% and 80.8% respectively. We also observed a significant upward trend in the frequency of isolation of MDR bacteria from joint fluids during the study period. **Conclusion:** Although the frequency of joint fluid infections is not very high, there is still a predominance of staphylococcus aureus. We therefore suggest combining these antibiotics during empirical treatment until definitive culture results are available

Keywords: *Septic arthritis , antimicrobial resistance (AMR), Joint Fluids,*

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1. Introduction

Infectious arthritis or septic arthritis is a direct invasion of a joint space by various microorganisms. This leads to very intense pain and a sudden continuous appearance of one to two weeks of a feeling of heat in the affected joint [1,2].

Infection occurs via the haematogenous route of the synovial vascular membrane during a bacterial episode or direct inoculation (penetrating trauma) [3,4]. This is a medical emergency which leads to cartilage and bone lesions, with consequences such as disability, requests for health care, social and even death in the absence of early management [5]. According to the recent French recommendations on the management of septic arthritis in native joints in adults, probabilistic antibiotic therapy consists of administering cefazolin (C1G injection) in patients aged up to 70 years (to cover at least *Staphylococcus aureus* and *Streptococcus spp*) and preferring a C3G injection (Ceftriaxone/cefotaxime) in patients over 70 years of age because of the higher frequency of BGN arthritis in this population [6].

Nevertheless, it is imperative to consider the clinical situation and the emergence of antimicrobial-resistant bacterial strains [7].

Incidence of septic arthritis ranges from 4 to 10/100,000/year in the general population, from 5.5 to 12/100,000/year in children, from 28 to 38/100,000/year in patients with rheumatoid arthritis (RA) and from 40 to 68/100,000/year in patients with joint replacements [8]. In Cameroon, there are very few published data on arthritis, much less on septic arthritis and the resistance of isolated bacteria. A study conducted in Cameroon on rheumatic diseases by Singwe et al., on a population of 536 patients at the Yaoundé General Hospital from October 2000 to October 2001 showed that 9.3% of arthritis was due to an infection [9].

This study was conducted to determine the profile and evolution of antimicrobial resistance in bacterial isolates from joint fluids received at the Centre Pasteur du Cameroun between 2016 and 2021.

2. Materials and Methods

This study was carried out at the Centre Pasteur du Cameroun in Yaoundé, which is a reference and public health laboratory, accredited according to ISO 15189 standard.

The study population consisted of patients whose joint fluids were received at the Centre Pasteur du Cameroun (CPC).

This study was a descriptive, retrospective and prospective cross-sectional carried out over a period of 6 years: from 1st January 2016 to 31 December 2021. It consisted of carrying out cytobacteriological analysis and microcrystal searches on the joint fluids received, extracting data from the CPC Laboratory information system and completing missing data. The microbiological analysis of the samples was performing according to the medical microbiology guidelines (REMIC) of the Société française de microbiologie, and medical bacteriology: standard techniques [10,11]. Identification and antimicrobial susceptibility testing were performed using the VITEK2 COMPACT automated system (BioMerieux, Marcy–L'Etoile, France). The results of the antimicrobial susceptibility tests were interpreted in accordance with the recommendations set forth by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). [12].

All data were merged in the excel software version 2019: the data collected were: age, sex, infected joint, Gram result, culture result, hospital, name of the microorganism isolated and the antimicrobial susceptibility test respectively.

Statistical analysis

All statistical analyses were performed with R studio software version 4.1.2.

Continuous variables were expressed as means and standard deviations. The categorical variables were subjected to statistical analysis using the chi-square test or the exact Fisher test. A p-value of less than 0.05 was deemed to be statistically significant.

Ethical consideration

An ethical clearance was obtained from the Institutional Ethical Review Board of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I and a research authorization was obtained from the Centre Pasteur du Cameroun.

3. Results

During the study period, 471 joints fluid samples were collected, among which 83 were culture positive; a prevalence of 17.6% (Table 2).

The mean age of patients with confirmed septic arthritis was 35.3 (±24.9) with a predominance of males (59%) (Table 1). The most infected joint was the knee (82.9%). The age group most affected by septic arthritis were children aged 0-10 (20.5%) followed by those aged 11-20 (15.7%) (Table 1). Approximately half of the confirmed septic arthritis had a positive Gram stain (49.4%).

Table 1. Distribution of sociodemographic parameters

Variable		n	(%)
Age, mean ± SD	35,3(±24,9)		
Sex ratio	1,4		
	Male	49	59,0
	Female	34	41,0
Infected Joint			
	Ankle	2	2,9
	elbow	5	5,7
	knee	69	82,9
	clavicle	5	5,8
	Hip	2	2,9
Age scale			
	[0-10]	17	20,5
	]10-20]	13	15,7
	]20-30]	10	12,0
	]30-40]	8	9,6
	]40-50]	9	10,8
	]50-60]	8	9,6
	]60-70]	9	10,8
	]70-80]	7	8,4
	>80	2	2,4

Most specimens had a monomicrobial culture (94%); only 5 (6%) were polymicrobial samples, 2 types of microorganisms were isolated from 4 (4.8%) samples, and 3 types of microorganisms were isolated from 1 (1,2%) sample (table2).

Table 2. microbiological parameters

Variables		n	(%)
Gram stain		41	49,4
Positive Culture		83	17,6
	monomicrobial	78	94,0
Characteristic Culture	Polymicrobial	5	6,0
	2 bacterial types	4	4,8
	3 bacterial types	1	1,2

Figure 1 shows the distribution of organisms isolated from the joint fluid samples. *Staphylococcus aureus* was the most common microorganism (38.8%), followed by *Streptococcus pneumoniae* (5.9%) and *Klebsiella pneumoniae* (5.9%). Overall, Gram-positive cocci represented 64.2% (42.4% belonging to the genus *Staphylococcus*, 16.2% to the genus *Streptococcus*, *3.5%* to the genus *Enterococcus)* while Gram-negative bacilli represented 28.2% (23.5% belonged to the large family *Enterobacterales*, and 4.7% belonged to the large group of Gram-negative non-fermentative bacilli (Figure 1).

The associations of the different isolates are shown in Table 3. *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterobacter cloaceae* were the most common bacteria found in a polymicrobial culture.

Table 3. combination of different polymicrobial samples

number of microorganisms	combination of microorganisms
2	*S. aureus.+Str. Pyogenes*
	S. aureus +P. mirabilis
	E. faecalis+ P. stutzeri
	E. aerogenes + E. cloacae
	Neisseria gonorrhoeae + BG- anaérobie
3	*K. terrigena + E. cloacae + E. faecalis*

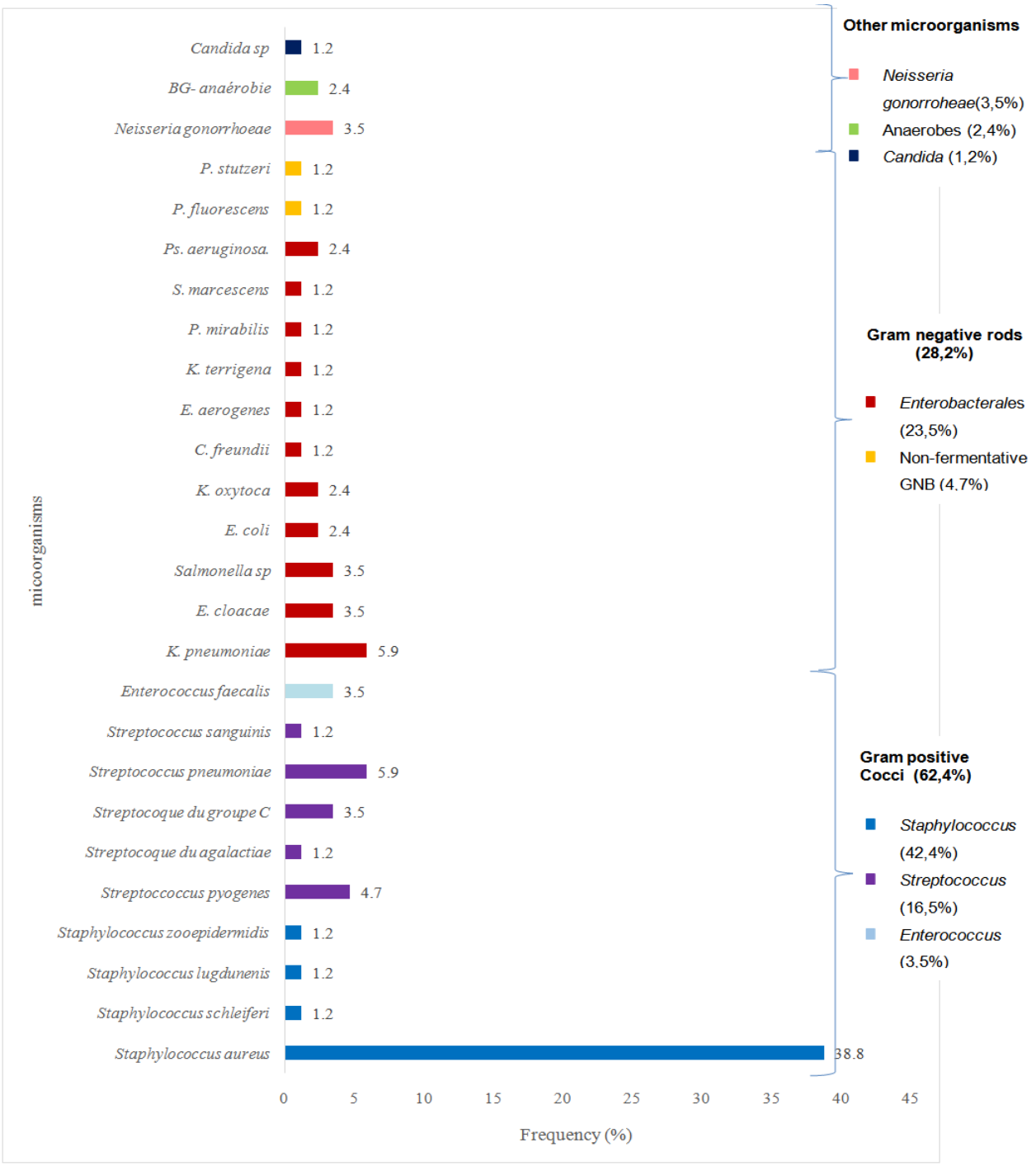


Figure 1. Distribution of microorganisms isolated from joint fluids

High levels of resistance were observed to penicillin G (97.1%), cotrimoxazole (85.3%) Approximately half of the *Staphylococcus aureus* strains isolated were resistant to oxacillin (52,9%), kanamycin (58,8%) and tobramycin (50%). The frequency of MRSA among the isolated *S. aureus* was 52.9%. No resistance was observed for the following antibiotics: nitrofurantoin, linezolid, vancomycin, teicoplanin pristanmycin, quinupristin / dalfopristin.

In streptococci and enterococci, we did not observe resistance for the following antibiotics: vancomycin, teicoplanin, linezolid, nitrofuran. However, high resistance was observed for tetracycline (82.4%) and low resistance to other antibiotics tested (Table 5).

Table 4. Antimicrobial susceptibility test of Staphylococcus aureus

Antimicrobial	%R	%I	%S	%R 95% of C.I.	%S 95% of C.I.
Penicillin G	97,1	0,0	2,9	83.4-99.9	0.1-16.6
Oxacillin	52,9	0,0	47,1	35.4-69.8	30.2-64.6
Gentamicin	26,5	2,9	70,6	13.5-44.7	52.3-84.3
Kanamycin	58,8	0,0	41,2	33.5-80.6	19.4-66.5
Tobramycin	50,0	5,6	44,4	26.8-73.2	22.4-68.7
Rifampicin	5,3	36,8	57,9	0.3-28.1	34.0-78.9
Levofloxacin	33,3	3,7	63,0	17.2-54.0	42.5-79.9
Trimethoprim/Sulfamethoxazole	85,3	0,0	14,7	68.2-94.5	5.5-31.8
Lincomycin	13,3	0,0	86,7	4.4-31.6	68.4-95.6
Erythromycin	25,0	0,0	75,0	10.6-47.1	52.9-89.4
Nitrofurantoin	0,0	0,0	100,0	0.0-18.5	81.5-100
Linezolid	0,0	0,0	100,0	0.0-13.3	86.7-100
Vancomycin	0,0	0,0	100,0	0.0-12.3	87.7-100
Teicoplanin	0,0	0,0	100,0	0.0-12.3	87.7-100
Pristinamycin	0,0	0,0	100,0	0.0-14.6	85.4-100
Quinupristin/Dalfopristin	0,0	0,0	100,0	0.0-14.6	85.4-100
Tetracyclin	39,4	0,0	60,6	23.4-57.8	42.2-76.6

Table 5. Antimicrobial susceptibility test of Streptococcus-Enterococcus

Antimicrobial	%R	%I	%S	%R 95% de C.I.	%S 95% de C.I.
Penicillin G	13,3	20,0	66,7	2.3-41.6	38.7-87.0
Ampicillin	11,8	0,0	88,2	2.1-37.7	62.3-97.9
Cefotaxime	13,3	0,0	86,7	2.3-41.6	58.4-97.7
Gentamicin (High)	15,4	38,5	46,2	2.7-46.3	20.4-73.9
Kanamycin (High)	33,3	33,3	33,3	9.0-69.1	9.0-69.1
Rifampin	11,1	0,0	88,9	0.6-49.3	50.7-99.4
Levofloxacin	0,0	5,6	94,4	0.0-21.9	70.6-99.7
Trimethoprim/Sulfamethoxazole	10,0	0,0	90,0	0.5-45.9	54.1-99.5
Clindamycin	20,0	0,0	80,0	1.1-70.1	29.9-98.9
Erythromycin	27,8	5,6	66,7	10.7-53.6	41.2-85.6
Telithromycin	11,1	0,0	88,9	0.6-49.3	50.7-99.4
Nitrofurantoin	0,0	0,0	100,0	0.0-34.5	65.5-100
Linezolid	0,0	0,0	100,0	0.0-26.8	73.2-100
Vancomycin	0,0	0,0	100,0	0.0-20.9	79.1-100
Teicoplanin	0,0	0,0	100,0	0.0-20.9	79.1-100
Pristinamycin	7,7	0,0	92,3	0.4-37.9	62.1-99.6
Quinupristin/Dalfopristin	25,0	0,0	75,0	4.5-64.4	35.6-95.5
Tetracycline	80,0	0,0	20,0	29.9-98.9	1.1-70.1

In the Gram-negative bacilli group, relatively high resistance was observed for the following antibiotics: amoxicillin (85.7%), cephalotin (81%), Trimethoprim/Sulfamethoxazole (78.3%), ticarcillin (68%), piperacillin (62.5%), and also amoxicillin-clavulanic acid (61.9%).

We observed high sensitivity to imipenem (92.3%), amikacin (80.8%), fosfomycin (78.6%), piperacillin-tazobactam (76%), Cefepime (69.2%), ciprofloxacin (68%) Gram-negative bacilli had a relatively moderate sensitivity to the following antibiotics: gentamicin (57.7%), tobramycin (61.7%), ceftazidime (57.7%), cefotaxime (52.4%); ofloxacin (61.9%). No resistance was observed for colistin (Table 6).

Table 6. Antimicrobial susceptibility test of Gram-negative bacilli

Antimicrobial	%R	%I	%S	%R 95% de C.I.	%S 95% de C.I.
Amoxicillin	85,7	0,0	14,3	62.6-96.2	3.8-37.4
Piperacillin	62,5	0,0	37,5	40.8-80.5	19.5-59.2
Amoxicillin/Clavulanic acid	61,9	0,0	38,1	38.7-81.0	19.0-61.3
Ticarcillin	68,0	0,0	32,0	46.4-84.3	15.7-53.6
Ticarcillin/Clavulanic acid	40,0	0,0	60,0	7.3-83.0	17.0-92.7
Piperacillin/Tazobactam	16,0	8,0	76,0	5.3-36.9	54.5-89.8
Cefalothin	81,0	4,8	14,3	57.4-93.7	3.8-37.4
Cefotaxime	42,9	4,8	52,4	22.6-65.6	30.3-73.6
Ceftazidim	26,9	15,4	57,7	12.4-48.1	37.2-76.0
Cefepime	11,5	19,2	69,2	3.0-31.3	48.1-84.9
Imipenem	7,7	0,0	92,3	1.3-26.6	73.4-98.7
Amikacin	3,8	15,4	80,8	0.2-21.6	60.0-92.7
Gentamicin	42,3	0,0	57,7	24.0-62.8	37.2-76.0
Tobramycin	38,5	0,0	61,5	20.9-59.3	40.7-79.1
Ciprofloxacin	24,0	8,0	68,0	10.2-45.5	46.4-84.3
Ofloxacin	38,1	0,0	61,9	19.0-61.3	38.7-81.0
Trimethoprim/Sulfamethoxazole	78,3	0,0	21,7	55.8-91.7	8.3-44.2
Fosfomycin	21,4	0,0	78,6	5.7-51.2	48.8-94.3
Colistin	0,0	0,0	100,0	0.0-53.7	46.3-100

Multi-drug resistant bacteria (MDR) are bacteria that combine several resistance mechanisms to several families of antibiotics (more than 3 families), which limits the therapeutic possibilities in case of infection [13]. Of the bacteria isolated, 43.3% (36/83) were multidrug resistant bacteria (MDR). The figure below shows the different resistance mechanisms (inner ring), as well as the bacteria involved in each resistance mechanism (outer ring). The resistance mechanisms observed were methicillin resistance and extended spectrum beta lactamase production by Gram-negative bacilli. MRSA represented 21.7%, while ESBL-producing GNB represented 10.8% and Carbapenem-resistant Gram-negative bacilli represented 2.4% of all isolated bacteria. *Klebsiella pneumoniae* was the predominant MDR bacteria in GNB (Figure2).

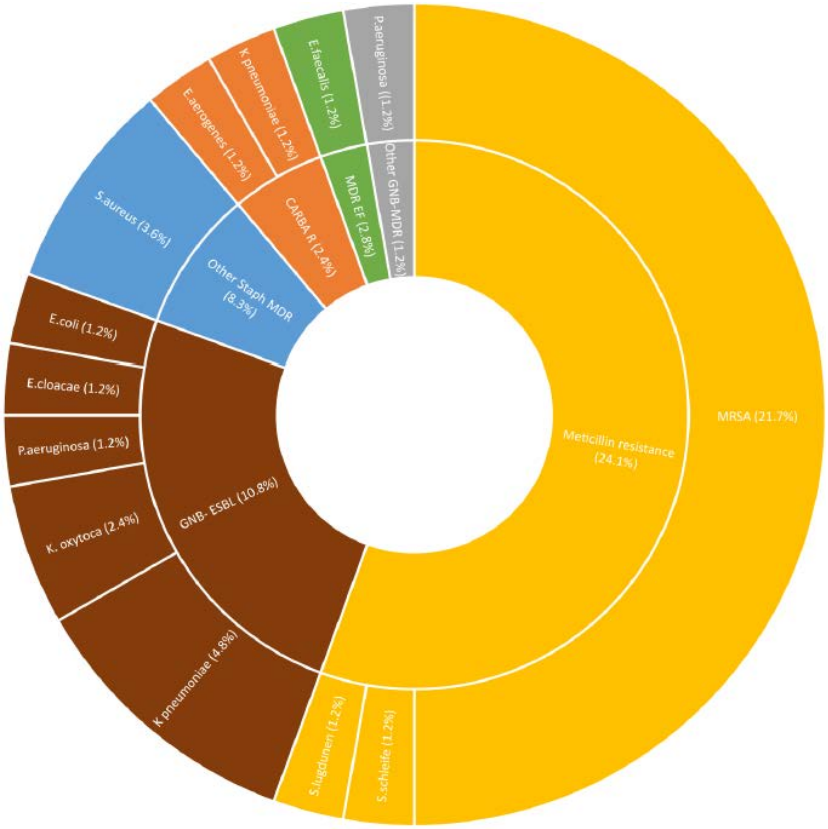
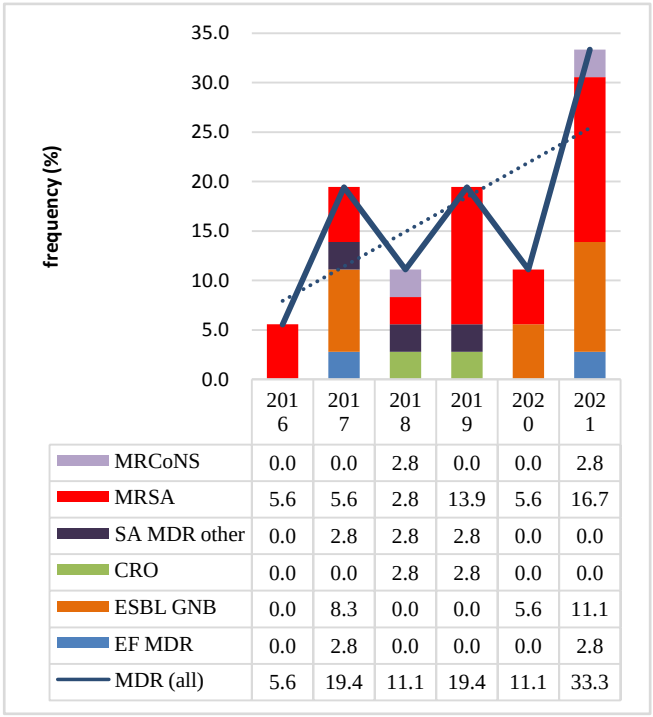


Figure 2. Distribution of MDR bacteria and their resistance mechanism.



	2016	2017	2018	2019	2020	2021
MRCoNS	0.0	0.0	2.8	0.0	0.0	2.8
MRSA	5.6	5.6	2.8	13.9	5.6	16.7
SA MDR other	0.0	2.8	2.8	2.8	0.0	0.0
CRO	0.0	0.0	2.8	2.8	0.0	0.0
ESBL GNB	0.0	8.3	0.0	0.0	5.6	11.1
EF MDR	0.0	2.8	0.0	0.0	0.0	2.8
MDR (all)	5.6	19.4	11.1	19.4	11.1	33.3

Legend: MRCoNS=Methicillin resistant Coagulase-negative *Staphylococci*; MRSA=Methicillin Resistant *Staphylococcus aureus,* SA MDR= *S. aureus* Multidrug resistant (other mechanisms), CRO= Carbapenem-resistant organisms, ESBL GNB= Extended-spectrum beta-lactamase Gram negative bacilli producer, EF MDR= *Enterococcus faecalis* Multidrug resistant*;* MDR (all)= Multidrug resistant organisms (all)

Figure 3. Evolution of MDR bacteria from 2016 to 2021

Overall, we observed a significant upward trend in the frequency of isolation of MDR bacteria in joint fluids from 2016 to 2021. This went from 5,6% in 2016 to 33.3% in 2021 ($p<0.0001$). Among MDRs, MRSA in particular have increased from 5.6% in 2016 to 16.2% in 2021 ($p<0.0001$) (Figure 3).

4. Discussion

The main objective of this study was to determine the profile and evolution of antimicrobial resistance in bacterial isolated from joint fluids. However, we were not able to study the associated risk factors and antibiotic use patterns.

Patients with suspected arthritis were predominantly male. This is similar to several previous studies such as those by de Mue D et al., George et al., Bileckot et al., who observed high frequencies of arthritis in male patients [14,15,16]. There is no obvious reason for this gender variation, but it may be that men are more likely to be involved in activities that result in repetitive minor joint trauma.

The prevalence of septic arthritis in our study was 17.6%. This result differs slightly from that of Singwe et al., where 9.3% of the arthritis observed was due to microorganisms [9]. This difference could be explained by the fact that this author was working on arthritis in general.

The knee was the most frequently involved joint (82.9%); which is similar with several previous studies such as those carried out by Mue D et al., Georges et al., Lavy et al., where the knee was the most affected [14,15,17]. This is because the joints of the lower limbs are more frequently involved in trauma than those of the upper limbs and therefore present more septic arthritis. Microtrauma at the capillary level may also locally reduce

oxygen tension and decrease the effectiveness of the humoral and cellular nerve defence response. Olney's work supports this theory that microtrauma in the presence of co-existing bacteremia makes joints susceptible to infection [18].

The frequency of septic arthritis was high in children (0-10 years: 39.5%,) adolescents (11-20 years: 24.5%,) and young adults (21-30 years: 20.4%,) and decreased with age. This result is consistent with studies by Monsalve et al., and de Souza Miyahara et al., who found a predominance of septic arthritis in children and adolescents [2,19]. This may be because septic arthritis in children is most often a haematogenous infection and the slow blood flow in the metaphyseal capillaries makes growing bones susceptible to infection by haematogenous inoculation; in addition, malnutrition and an inadequate immune response may contribute [17,20].

The majority of cultures were monomicrobial (94%). This result is similar to the study by Muñoz-Egea et al., who observed a mono-microbial growth of 92.6% in joint fluids [21].

Staphylococcus aureus was the most frequently isolated microorganism (38.8%). This result is consistent with several previous studies where *Staphylococcus aureus* was the predominant microorganism (2,13,21–24).

All isolated strains of *Staphylococcus aureus* were susceptible to glycopeptides (vancomycin, teicoplanin) and linezolid. These results are similar to those of Yadav S et al., and Kalantari et al., [22,23] where no resistance to these antibiotics was detected.

For streptococci, good activity was observed with most of the antibiotics tested. This was observed in the study by Kalantari et al. [76].

For Gram-negative bacilli, we observed good activity of imipenem (92.3%) and amikacin (80.8%) on them. These results are similar to those of Kalantari et al. [76].

Of the total bacteria isolated, 43.3% were multidrug resistant bacteria (MDR): The proportion of MRSA isolates was 21.7%, while that of Gram-negative ESBL bacilli was 10.3%. These results are respectively similar to those of Lim et al., who observed a rate of 22.6% of MRSA isolated in septic arthritis and Lin et al where 7.1% of Gram-negative bacilli were ESBL [24,25]. Our results differ from those of Georges et al. and Ben-Chetrit et al., who described a low rate of MRSA isolation from joint puncture fluid of 16.3% and 8% respectively. Furthermore Ben-Chetrit et al., did not observe any Gram-negative ESBL bacilli in their study [15,26].

Overall, from 2016 to 2021, the emergence of MDR was noted in our study. The main MDR were MRSA and GNB ESBL. These results are different from the results of the study by Ben-Chetrit et al., and Dubost et al., who observed no significant increase in MRSA over 16 years and 30 years respectively [26,27]. This difference would be related to the stricter prescription control of antibiotics in their context.

5. Conclusion

Septic arthritis is a common condition in our environment. The patients most affected are children, adolescents and young adults. Joint infections are usually mono-microbial. *Staphylococcus aureus* is the bacterium frequently isolated from joint fluids followed by *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Streptococcus pyogenes*. The frequency of multidrug-resistant bacteria, particularly MRSA and Gram-negative ESBL-producing bacilli, is high. Overall, we observed an increasing emergence of MDR Bacteria between 2016 and 2021. Antibiotics active on isolated Gram-negative cocci, including MRSA, were vancomycin, teicoplanin, linezolid and nitrofuran. Antibiotics active on Gram-negative bacilli including ESBL producers were imipenem and amikacin and colistin.

Disclosure

This study was carried out within the framework of the Master's degree requirement in Medical Microbiology in the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I

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Declaration

The authors declare no conflict of interests regarding the publication of this paper.

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