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Analysis of six-year monitoring of common pathogens causing periprosthetic joint infection of major joints and the tendency to resistance

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Abstract

The objective was to determine most common pathogens causing periprosthetic joint infection (PJI) of major joints, to identify the tendency to antibiotic resistance over the period from 2015 to 2020. **Material and methods** Microbiological culture results of 354 patients with PJI of major joints treated at our department were retrospectively analyzed. The spectrum of the leading pathogens causing PJI and the antibacterial resistance were explored and the prevalence of common organisms depending on the type of PJI demonstrated. **Results** 354 patients were examined and 317 microbial isolates identified. Gram-positive bacteria was isolated in 70.7 % (224 microorganisms) of cases, Gram-negative bacilli isolated in 28.1 % (89 organisms) and *Candida sp*. identified in 1.2 % (4 isolates). Microbial associations were identified in 15% of cases. **Discussion** Most common pathogens causing PJI included *S. aureus* identified in 31.9 %; *S. epidermidis*, in 20.2 %; *E. faecalis*, in 8.5 %; *P. aeruginosa*, in 7.9 %; *A. baumannii*, in 7.3 %. PJI associated MRSE strains increased from 12.1 % to 26.7 % and *S. haemolyticus* (MR) increased from 2 % to 11.6 %. *S. aureus* and Gram-negative bacilli were most common for early acute and hematogenous acute PJI. There were no significant differences in the prevalence of *S. aureus* and *S. epidermidis* in early/delayed and late chronic PJI. *Enterococcus* species and Gram-negative bacilli were detected less frequently with PJI. There was an increasing antibiotic resistance of *A. baumannii* and *P. aeruginosa*. Vancomycin-resistant strains and linezolid-resistant strains were newly found among Gram-positive bacilli and pan drug-resistant *A. baumannii* strains. **Conclusion** The six-year microbiological monitoring showed *S. aureus, S. epidermidis, P. aeruginosa*, and *A. baumannii* as most common pathogens causing PJI. The growing antibiotic resistance of Gram-positive and Gram-negative bacilli and the increasing role of the latter in the pathogenesis of early acute PJI require changes in empirical antibiotic therapy regimens.

Keywords: periprosthetic joint infection, implant associated infection, common pathogens, antibiotic resistance

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INTRODUCTION

The most common surgical intervention for end-stage degenerative diseases and consequences of joint injuries is total joint arthroplasty [1]. Worldwide, approximately 1.5 million primary arthroplasties of major joints are performed annually [2]. With the increasing utilization of arthroplasty and revision interventions [3–5] the proportion of complications also increases [6] with periprosthetic joint infection (PJI) being most devastating. Although arthroplasties have high success rates, 1.5 % to 2.5 % are complicated by PJI after primary replacements [7] and 40 % of revision procedures are performed for PJI [1, 2]. In oncoorthopedics, PJI can occur in 8.5–10 % of cases [8].

PJI is caused by various types of microorganisms that form microbial biofilms on the metal and polymer surfaces of the implant [9–12] synthesized from the extracellular matrix [6, 7, 13, 19, 20]. The most common causative agents in more than half of infections are representatives of gram-positive microflora [11, 14] including aureus (12–23 %) and coagulase-negative staphylococci (30–43 %). Less frequent microorganisms are streptococci (9-10 %), enterococci (3-7 %), anaerobic bacteria (2–4 %) and Candida sp. (1–3 %) [15]. Gram-negative microflora (Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae, etc.) account for 5 %–23 % of PJI [16]. However, PJI caused by Gram(-) bacteria is of great clinical importance and is difficult to treat and leads to an unfavorable outcome of surgical intervention due to the high virulence of the microorganisms and the growing resistance to many antibacterial drugs used [14, 16, 17].

An infectious complication would lead to longer inpatient period, require a multi-stage approach, knowledge of diagnostic algorithms, treatment and practical surgical skills as well as prolonged and very expensive antibiotic therapy [16, 18] involving additional economic costs [5]. N. Benito reported the cost of PJI associated treatment amounting to $20,000–40,000 per patient [10].

With annual increase in the resistance of pathogenic strains to antibiotics, the treatment of PJI becomes more and more difficult with costs increasing many times, which requires the search for new effective methods of treatment

and drugs, and can endanger the efficacy of antibiotics for pandrug-resistant bacteria [21]. This problem is an important component of the overall economic, medical and social burden of diseases and requires in-depth study and identification of risk factors for the development of resistance of microorganisms to antibacterial agents.

The objective was to determine most common pathogens causing periprosthetic joint infection (PJI) of major joints, to identify the tendency to antibiotic resistance over the period from 2015 to 2020.

MATERIAL AND METHODS

Microbiological culture results of 354 inpatients with PJI of major joints of the upper and lower limbs treated at the department of the consequences of musculoskeletal injuries and bone and joint infection, the Federal State Budgetary Institution "N.N. Priorov National Medical Research Center of Traumatology and Orthopedics" between January 2015 and December 2020 were retrospectively analyzed. There were 207 (58.5 %) female and 147 (41.5 %) male patients. The average age is 61.3 years (range, 18 to 92 years) and 149 (42 %) patients ≥ 65 years of age. 185 (52.3 %) patients were diagnosed with PJI of the hip, 151 (42.7 %) patients had PJI of the knee joint, 9 (2.5 %) patients of the shoulder and 9 (2.5 %) of the elbow joints. 131 (37 %) patients had had revision arthroplasty. The Coventry-Fitzgerald-Tsukayama grading system was used to assign patients to 4 groups according to the time the infection developed. The classification, unlike other existing ones can allocate an additional group of patients with no symptoms of inflammation with a positive culture identified intraoperatively (Table 1).

The cases of PJI were divided into four time-based groups: early acute, early acute delayed, late chronic and acute hematogenous infections shown in Fig. 1. Patients of the first group (n = 153 (43.2 %)) developed an infection within 2 weeks after surgery including 133 patients (87 %) after primary arthroplasty, 20 (13 %) after revision procedures. 11 % of patients developed an infectious complication more than 3 years after implantation of the prosthesis. Two (0.6 %) patients developed infection of the hip joint after 21 years and 1 (0.3 %) had PJI occurred after 33 years of primary arthroplasty.

Distribution of patients according to the duration of PJI is shown in Fig. 2. Duration of PJI ranged from 10 to 26 years in 15 patients (4.2 %).

Table 1

Distribution of patients by the timing of PJI and localization of the prosthesis using the Coventry-Fitzgerald-Tsukayama classification

Manifestations seen	Hip joint		Knee joint		Shoulder joint		Elbow joint		Total	
	n	%	n	%	n	%	n	%	n	%
Within 4 weeks	85	24	91	25.7	5	1.4	6	1.7	187	52.8
1 month to 3 months	11	3.1	21	5.9	1	0.3	1	0.3	34	9.6
Over 3 months	87	24.6	38	10.7	3	0.8	2	0.6	130	36.7
PIC	2	0.6	1	0.3	–	–	–	–	3	0.9
Total	185	52.3	151	42.6	9	2.6	9	2.6	354	100

Note: PIC, positive intraoperative culture.

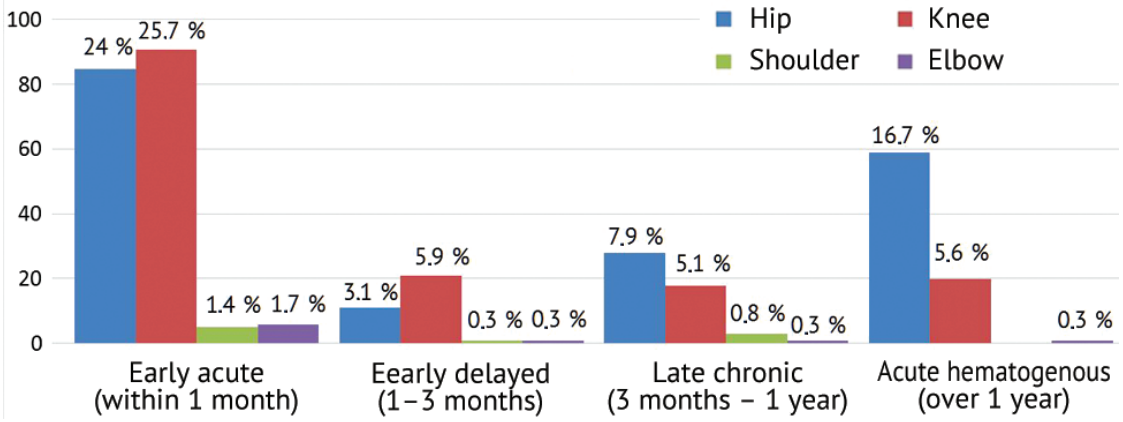

Fig. 1 Diagram showing time-based distribution of patients with various localization of PJI

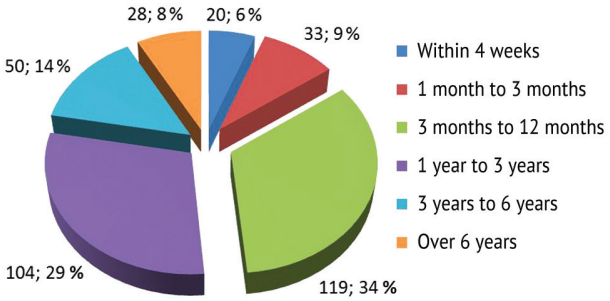

Fig. 2 Distribution of patients according to the duration of PJI

PJI developed after primary arthroplasty in 276 (78 %) patients and after revision arthroplasty in 78 (22 %). Recurrence of infection occurred in the interval between two stages of treatment in 22 (6.2 %) patients. 184 (52 %) patients underwent multiple surgeries (3 times or more), of which 116 (33 %) underwent repeated debridement prior to admission to our hospital. Articular procedures had been performed in 68 (19 %) cases prior to arthroplasty. Fistulas were detected in 186 (52.5 %) patients, a wounded postoperative scar was noted in 29 (8.2 %) patients, hyperemia and hyperthermia in 139 (39.3 %) patients. 26 (7.3 %) patients presented with pain in the operated joint. Concomitant diseases such as diabetes mellitus, systemic diseases, obesity, HIV infection, hepatitis B and C as risk factors for implant-associated infection (IAI) were observed in 201 (56.8 %) patients. Patients underwent a complete clinical, radiological, laboratory, microbiological examinations preoperatively. A fistula or a wound was bacteriologically explored. Then 5-6 wound swabs were taken intraoperatively and three postoperatively. The histology of the intraoperative wound swabs was explored to confirm chronic inflammatory process and to identify the latency.

The culturing of pathogens was carried out according to the standard method [22]. Generic and species identification of isolated microorganisms and determination of antibiotic resistance were carried out using a VITEK 2 COMPACT automated microbial identification system (BioMerieux, France).

The study was performed in accordance with ethical principles for medical research involving human subjects stated in the Declaration of Helsinki developed by the World Medical Association as revised in 2013. Statistical analysis was performed with computer software (IBM SPSS Statistics 22). Pearson's χ^2 test was applied for qualitative variables to identify the reliability of the data obtained. For calculations, a significance level of < 0.05 was adopted.

RESULTS

Among 354 TJR patients, 21 (6 %) from 43.2 % with early PJI could retain the implant through surgical debridement and in combination with a course of antibiotic therapy termed DAIR (debridement, irrigation, antibiotics and implant retention). Among 354 patients with PJI of major joints, 317 microbial isolates were identified during the study period. Gram-positive bacteria was isolated in 70.7 % (224 microorganisms) and 28.1 % (89 samples) were gram-negative. There were 1.2 % (4 isolates) fungi isolates.

Staphylococcus aureus was most common identified in 31.9 % of gram-positive microflora Staphylococcus. Methicillin-sensitive (MSSA) and resistant (MRSA) staphylococci were 21.5 % and 10.4 %, respectively. Then *Staphylococcus epidermidis* followed with a detection rate of 20.2 % with 2.2 % methicillin-sensitive strains (MSSE) and 18 % resistant (MRSE). *Enterococcus faecalis* was detected in 8.5 %, Staphylococcus sp. in 6.9 %, with 2.5 % microisolates of Staphylococcus hominis and 2.8 % Staphylococcus haemolyticus. The Streptococcaceae family accounts for 2 % of the total microorganisms.

P. aeruginosa was the leading pathogen among gram-negative bacteria identified in 7.3 % of isolates, followed by *A. baumannii* which accounted for 7.9 %. Representatives of the *Enterobacteriaceae* family accounted for 8.2 %, among them *E. coli* verified in 42.6 %, *K. pneumoniae* in 38.5 % and *E. cloacae* in 15.4 %. Other Gram-negative bacteria including *Serratia marcescens, Morganella morganii* and *Proteus sp*. were found in 5.3 % of cases. The infecting pathogens profile causing PJI in TJR patients are shown in the diagram (Fig. 3). Dynamics of frequency of Gram(+) and Gram(-) rod occurrence is shown in Fig. 4 and Fig. 5, respectively.

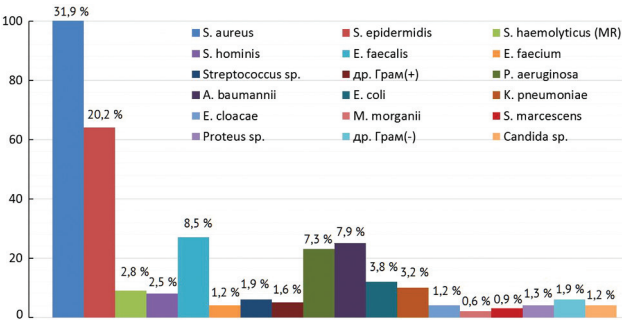

Fig. 3 Infecting pathogens profile causing PJI in TJR patients

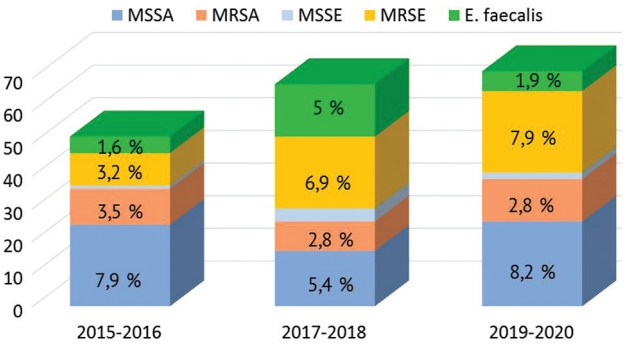

Fig. 4 Dynamics of frequency of Gram (+) bacteria occurrence in the studied periods

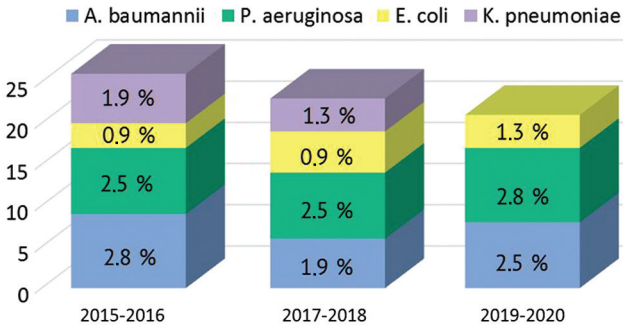

Fig. 5 Dynamics of frequency of Gram (-) bacteria occurrence in the studied periods

Microbial associations were observed in 48 (15.1 %) cases of the total number of microorganisms, of which 70.8 % represented the coexistence of Gram(+) and Gram(-) rods. Other communities consisted of either Gram-positive (18.8 %) or Gram-negative (10.4 %) pathogens. Associations consisting of 3 or more pathogens were identified in the results of microbiological studies in 9 (19 %) patients (Table 2).

Table 2

The frequency of pathogen occurrence in microbial communities of the total pathogens identified

Microorganism	Total number	In microbial associations	%
MRSA	32	11	34
MSSA	67	14	21
MRSE	54	10	19
E. faecalis	27	16	59
E. faecium	4	1	25
S. haemolyticus	9	2	22
S. hominis	8	2	25
Streptococcus sp.	6	3	50
Staphylococcus sp.	5	3	60
P. aeruginosa	25	15	60
A. baumannii	23	14	61
E. coli	12	7	58
K. pneumoniae	10	6	60
E. cloacae	4	3	75
M. morganii	2	2	100
S. marcescens	3	1	33
Other Gram(-)	6	5	83

Despite the clear clinical picture of the infectious process in the implant site, intraoperative biopsy specimens showed negative results in 99 (28 %) patients. Among the total number of MSSAs, susceptibility analysis to the tested antibiotics revealed 23.5 % of strains resistant to one or more antibiotics. Resistance to lincomycin was revealed in 4 strains, to fluoroquinolones in 8 isolates, clindamycin in 2 microorganisms and there was a single case of resistance to tigecycline.

MSSE compared to MSSA showed almost complete sensitivity to the studied antibacterial drugs, with the exception of a single case of resistance to clindamycin. Comparative dynamics of the frequency of resistant strains of S. aureus and S. epidermidis identified is shown in Fig. 6. In 2018, 2 vancomycin-resistant MRSE isolates were identified for the first time with persistent sensitivity to linezolid, teicoplanin.

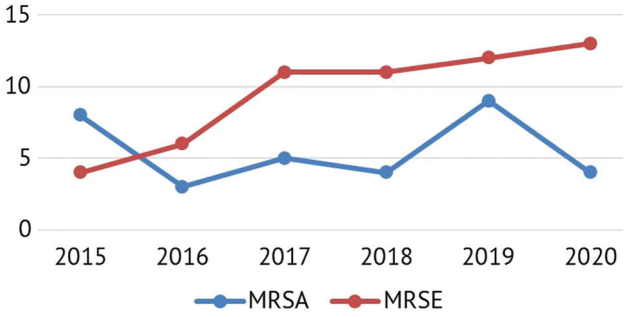

Fig. 6 Dynamics of detection of resistant strains of S. aureus and *S. epidermidis* in 2015–2020

11 % of *E. faecalis* isolates were resistant to ampicillin. The peak of ampicillin-resistant strains falls on 2017. Sensitivity to linezolid, vancomycin and tigecycline remained unchanged throughout the entire period. In 2019, 1 strain of vancomycin-resistant (*VRE*) and 2 strains of linezolid-resistant microorganism were detected. Of the antibiotics used in clinical practice, E. faecalis showed the greatest resistance to clindamycin (84 %). *Enterococcus faecium* were mostly sensitive to glycopeptides (vancomycin, teicoplanin), oxazolidinones (linezolid) and glycylcycline derivatives (tigecycline). In 2015, one case of the *VRE* strain was detected.

Two vancomycin-resistant strains of *Staphylococcus lentus* (2015) and *S. hominis* (MR) (2018) were also identified over the entire period of the retrospective study. 39.1 % of *A. baumannii* isolates were sensitive to aminoglycosides and carbapenems, 26.1 % were sensitive to cefoperazone/sulbactam and ceftazidime. The strains remained highly sensitive to polymyxin (91.3 %) and tigecycline (73.9 %) throughout the study. Two (8.7 %) *A. baumannii* microorganisms, sensitive only to polymyxin were first detected in 2019 and 2 (8.7 %) pan-resistant isolates were detected in 2020.

The susceptibility of P. aeruginosa to aminoglycosides (amikacin, tobramycin) is 52 %, to carbapenems: 56 % to imipenem and 48 % to meropenem. Ciprofloxacin were less active showing 45 %, ceftazidime and cefoperazone / sulbactam with 44 %. In 2019, 12 % of the total number of *P. aeruginosa* microorganisms were multidrug-resistant with retention of sensitivity to polymyxin only. Changes in the activity of the latter to the pathogens were not recorded throughout the entire period of the study. All strains of *E. coli* and *K. pneumoniae* were producers of extended-spectrum beta-lactamase (ESBL), no

carbapenemase-resistant strains were identified during the entire study period. Table 3 shows the frequency of occurrence of the total number of Gram(+) and Gram(-) microflora depending on the timing of PJI. The frequency of pathogen occurrence is presented in Table 4.

Table 3

The frequency of occurrence of Gram(+) and Gram(-) rods from the total number (n = 317) depending on the type of PJI

The timing of the development of PJI	Infecting pathogens					
	Gram(+) rods		Gram(-) rods		Total	
	n	%	n	%	n	%
Early acute (within 1 month)	114	36	47	14.8	161	50.8
Early delayed (1-3 months)	24	7.6	9	2.8	33	10.4
Late chronic (3 months – 1 year)	32	10.1	5	1.6	37	11.7
Acute hematogenous (over 1 year)	54	17	28	8.9	82	25.9
Total	224	70.7	89	28.1	313	98.8

Table 4

Infecting pathogens profile causing PJI in TJR patients (n=317) depending on the type of PJI

Infecting pathogen	Onset of PJI							
	Early acute		Early delayed		Late chronic		Acute hematogenous	
	n	%	n	%	n	%	n	%
S. aureus	52	16.4	12	3.8	14	4.4	23	7.3
S. epidermidis	27	8.5	10	3.2	11	3.5	16	5.0
Enterococcus	20	6.3			2	0.6	9	2.8
S. haemolyticus	7	2.2			1	0.3	1	0.3
Streptococcaceae	1	0.3			2	0.6	3	0.9
Other Gram(+)	7	2.2	2	0.6	2	0.6	2	0.6
A. baumannii	13	4.1	2	0.6			8	2.5
P. aeruginosa	13	4.1	6	1.9			6	1.9
E. coli	4	1.3			3	0.9	5	1.6
K. pneumoniae	5	1.6			1	0.3	4	1.3
E. cloacae	4	1.3						
Other Gram(-)	8	2.5	1	0.3	1	0.3	5	1.6

DISCUSSION

A detailed analysis of the records of the patients we treated showed the onset of PJI with a sudden pain that the attending physician diagnosed as aseptic loosening which was primarily associated with difficult diagnosis and latency of IAI in many cases. Postoperative microbiological findings suggest that 45 % of unstable implants are of an infectious nature [1].

Only 6 % of patients could retain the implant through surgical debridement in combination with a course of antibiotic therapy (DAIR) due to timely referral to specialized institutions. However, the majority of patients (85 %) failed to undergo DAIR due to late diagnosis or late referral to a specialist. In those cases where PJI was recognized at the initial stage, the reason for such, which subsequently leads to the implementation of a radical surgical intervention, was the wrong tactics Inadequate treatment strategy for patients diagnosed with PJI at the initial stage resulted in a long course of infection and the transition to a chronic form. Unreasonable multiple debridement followed by antibiotic therapy using empirically and etiotropically inappropriate drugs was performed for 37 % (n=116) of our patients in attempts to retain the implant. The approach indicated to the recommendations of the International Consensus Meeting (ICM) on periprosthetic joint infection in 2013 and 2018 being ignored with ignorance of the basics of antibiotic therapy for PJI. Recurrence of infection occurred in the interval between two stages of treatment in 6.2 % of patients and 22 % developed PJI after revision arthroplasty.

As seen from Fig. 1, 52.8 % of patients develop PJI within the first month after surgery with early postoperative suppuration (up to 2 weeks) observed in 43.2 %. Hematogenous infection was seen in 22.6 %. Late chronic PJI occurred in 14.1 % of cases and early delayed infection developed in 9.6 % that is not in line with the data of two scientific works by N. Benito et al. published with a difference of 3 years [10, 23]. They

reported [10] late chronic PJI seen in 47.4 %, early acute PJI in 35.7 % and hematogenous infection in 11.6 %. The authors referred to late infection occurring more than 1 month after the operation. Given this fact, the early acute form of PJI was most common in our series.

Microbiological monitoring showed the predominant representation of the Gram (+) rods in the etiology of PJI (70.7 %). The leading position continued to be occupied by S. aureus for the entire period of the study excluding resistance to methicillin with frequency of 32 % which was 1.6 times more compared to *S. epidermidis* (20.2 %). Our data are consistent with those reported of some authors [5, 16, 23, 24] and not in line with findings of others [25]. Yu Y. et al. report 21.1 % versus 25.5 %, respectively [26]. The frequency of occurrence of the two pathogens over the years demonstrated the ratio of verification of *S. aureus* and *S. epidermidis* being 4:1 in 2015 that amounted to 1:1 by 2020. Although there was a trend towards a decrease in the frequency of identification of S. aureus from 39.5 % to 25.7 % in 2017–2018 compared to 2015–2017 there was no difference in the frequency by 2019-2020 compared with the first analyzed period of time that amounted to 38.6 %. The frequency of *MRSA* occurrence showed no significant differences by 2019–2020 ($p = 0.531$) as compared to 2015–2016 ($p = 0.235$) and 2017–2018 ($p = 0.714$). The incidence of resistant strains of *S. aureus* ranged from 30.5 to 34.6 % during the study period.

The incidence of *S. epidermidis* causing PJI is gradually and steadily increasing. The frequency of detection of epidermal staphylococcus significantly increased from 12.1 % (2015) to 26.7 % (2020) ($p = 0.018$) with *MRSE* isolated in 90.9 and 92.6 %, respectively. The double rise of the isolate incidence occurred mainly due to an increase in the indices of methicillin-resistant strains, which accounted for 89 % of the total number of *S. epidermidis*. K. Becker et al. suggested that the annual increase in the resistance of the pathogen to methicillin was associated with the adaptive ability [5, 27]. C. Triffault-Fillit et al. reported *MRSA* in 16.1 % of cases ($n = 164$), *MRSE* in 59.1 % ($n = 162$) in bacteriological cultures from 567 patients with PJI [16, 28]. We obtained a similar ratio of data from the total number of *S. aureus* and *S. epidermidis* – 32.7 and 89 %, respectively in our series which is generally consistent with the results reported by the above and other authors [5, 16, 28].

Antibiotic susceptibility patterns of methicillin-susceptible *S. aureus* showed a gradual increase in the resistance to the antibiotics tested. A single strain of *MSSA* identified in 2015 was resistant to 3 antibacterial agents, the number of isolates being inactive to 3 or more drugs increased to 15 % (10 isolates) of the total number of *MSSA* in 2018. The organisms were resistant to fluoroquinolones in 12 % of cases. MSSE demonstrated less resistance to antibiotics with one case out of 7 available. Resistance was found only to clindamycin which differs significantly from the data of other authors [16]. Our result could be associated with the small sample of the pathogen. The review of antibiotic susceptibility patterns indicated administration of cephalosporins of the first or second generation in therapeutic doses for MSSA or *MSSE*.

We did not consider the sensitivity of the organisms to drugs from other antibacterial groups due to standard doses of vancomycin, linezolid and teicoplanin used for the treatment of methicillin-resistant strains. However, 4 cases of vancomycin-resistant strains of *MRSE* and *Staphylococcus sp*. detected during the study were alarming with three verified in 2018. The microisolates were isolated from biopsy specimens of patients with a history of multiple surgical interventions and subsequent antibacterial therapy with various drugs. Linezolid or teicoplanin were used at therapeutic doses for the scenario. There was a significant increase in the frequency of *S. haemolyticus* (MR) identified in 2019–2020 that reached 11.6 % ($p = 0.005$) as compared with 2 % in 2015–2016.

The incidence of *E. faecalis* (87.1 %) for 2015–2016 and 2019–2020 was almost identical with – 5.5 and 5.9 %, respectively. The peak of detection of the strains was recorded in 2017–2018 and amounted to 15.8 %. The incidence of *E. faecium* remained unchanged throughout the study period (no more than 1 strain per year). Glycopeptides and a derivative of glycylcyclines, tigecycline remained active antimicrobial drugs for *E. faecalis* throughout the study. One strain resistant to vancomycin and 2 strains resistant to linezolid were identified in 2017–2018 when there was a sudden increase in the frequency of isolation of the isolate. Ampicillin-resistant *E. faecalis* were isolated in 11 % of microbiological cultures.

No trend towards increasing resistance to ampicillin was observed at the end of the study period. Despite a small proportion (12.9 %) of *E. faecium* among Enterococcus representation, they were more resistant to the majority of antibiotics tested with the exception of vancomycin, linezolid and tigecycline. In 2015, 1 vancomycin-resistant strain of *E. faecium* was verified. Antibacterial therapy was carried out with ampicillin or amoxicillin / clavulanic acid in therapeutic doses with the sensitivity confirmed, with glycopeptides, oxazolidinones used for other cases and with glycylcyclines with the latter being reserve drugs for *A. baumannii* in rare cases. Gram-

negative rods causing PJI were significantly inferior to Gram-positive rods with the incidence of 28.1 % in our series which is within the range of values reported in Russian and foreign literature [16, 23]. There was a trend towards an insignificant decrease in the incidence ($p > 0.05$) of *A. baumannii* from 9.9 % ($p = 0.167$) in 2015–2016 to 7.9 % ($p = 0.947$) in 2019–2020.

The frequencies of occurrence of *P. aeruginosa* was 8.8 % ($p = 0.719$) in 2015–2016, 7.9 % ($p = 0.114$) in 2017–2018 and 8.9 % in 2019–2020 ($p = 0.289$). The incidence of the microorganism fluctuates insignificantly throughout the period of time analyzed. Representatives of the *Enterobacteriaceae* family including *E. coli*, *K. pneumoniae, E. cloacae* were detected in 8.2 % of cases with insignificant differences in the detection of *A. baumannii* and *P. aeruginosa*. However, each type in the family has different occurrence. The frequency of detection of E. coli strains in our series ranged from 3.3 % to 5 % with the mean of 4.1 %. No significant difference was found in the identification of the pathogen for the period of time analyzed ($p = 0.395$). *K. pneumoniae* was isolated from patient biopsies in the first three years of the study ($n = 10$). In 2017, the incidence decreased from 6.6 ($p = 0.758$) to 3.9 % ($p = 0.106$) and was nil in 2018–2020. *E. cloacae* verifications were insignificant ($n = 4$) and amounted to 1.3 % of the total number ($n = 317$) of organisms. Two isolates of *E. cloacae* were isolated during the last period of the study analyzed and we did not focus on a specific species and explored the organisms with the incidence of pathogens of 3 % and over. The incidence of *A. baumannii* decreased ($p = 0.291$) was reported to decrease in 2012–2017 as the frequency of *P. aeruginosa* isolation ($p = 0.529$) with an increase in the incidence of *Enterobacteriaceae* representatives (from 6.6 to 8.7 %) [16]. We report different findings for patients with PJI only excluding other IAIs.

Despite the insignificant role of gram-negative rods in the etiology of joint PJI this is a serious problem worldwide due to the steadily increasing resistance to many antibacterial drugs [29]. Analysis of antibiograms for *A. baumannii* and *P. aeruginosa* over the past 2 years revealed a trend towards an increase in resistance to the majority of antibiotics. The involvement of Gram(-) bacteria in the etiology of PJI complicates and extends the treatment [30], and the strains of *A. baumannii* and *P. aeruginosa* resistant to carbapenems (60.9 and 52 %, respectively), ceforaperazone / sulbactam and ceftazidime (73.9 and 56 %), fluoroquinolones (56.5 and 55 %) and *A. Baumannii* 26.1 % of tigecycline-resistant isolates and 8.7 % of pan-resistant isolates interfere with the effectiveness of surgical treatment increasing the morbidity and mortality of patients with PJI.

In recent years, the growing resistance of *K. pneumoniae* to carbapenems has been observed due to the production of carbapenemases (NDM, OXA-48, KPC) [29]. The strains of *K. pneumoniae* isolated in our series were ESBL producers and etiotropic therapy with carbapenems was successful. The bacterial resistance to antimicrobial drugs is a natural process but their wide availability, irrational and uncontrolled use and administration of minimal therapeutic doses contribute to its acceleration. The fact is supported by vancomycin-resistant strains of *MRSE, E. faecalis, S. hominis, S. lentus*, linezolid-resistant *E. faecalis*, a high proportion of multi-resistant Gram(-) bacteria and 2 cases of pan-resistant strains of *A. baumannii* identified in our series.

51 % of Gram(+) rods ($n = 224$) with *S. aureus* being common and 53 % of Gram(-) rods ($n = 89$) were involved in the etiology of early acute PJI and hematogenous acute PJI (see Table 3 and 4). D. Morcillo et al. also reported the predominance of *S. aureus* and Gram-negative pathogens in early PJI [31]. In 2010 R. Sousa reported the prevalence of Gram(-) flora in patients with chronic and hematogenous infection [32], and recent publications indicated the frequent occurrence of the microbial agents in the pathogenesis of acute early PJI [10, 11] which correlates with our results. Chronic infections usually involve low-virulence microorganisms [6, 33] with *S. epidermidis* being common [10, 11]. Our series showed that most of *S. epidermidis* were involved in the pathogenesis of early PJI (42.2 %) and hematogenous PJI (25 %) of the total number ($n = 64$). There were no significant differences in the frequency of detection of *S. epidermidis* and *S. aureus*, with Gram (-) rods being uncommon in early delayed and late infections. *E. faecalis* and *E. faecium* were not detected in specific cases (Table 4). 64.5 % and 29 % of *Enterococcus* ($n = 31$) were verified in early acute and hematogenous PJI and 6.5 % in late chronic cases (Table 4). The discrepancy between the findings may be due to the descriptive experience of a hospital or a center with a certain geographic location, different sample sizes, the focus of studies on certain sites and types of PJI or surgical methods used in the treatment resulting in inadequate evaluation of the incidence of various organisms causing different types of PJI [10].

Microbial associations in PJI were found in 15 % of cases with the majority (70.8 %) representing a mixed group of organisms. The incidence of Gram(+) and Gram(-) rods was 18.8 % and 10.4 % of the associations, respectively. The frequency of Gram(-) pathogens was higher in polymicrobial than in monomicrobial PJIs (57.3 %). The result is in line with the data from other

sources [5]. Polymicrobial infections caused acute early PJI in 54.2 %, acute hematogenous in 27.1 %, late PJI in 12.5 % and infection with an early delayed onset in 6.3 % which is comparable with data reported by N. Benito et al. indicating the predominance of polymicrobial pathogens in the etiology of early PJI (27.4 %) compared with the other types [10]. Cobo J. et al. reported the incidence of 32 % [34], de Vries L. et al. about 46 % [25], Aaron J. Tande et al. reported the range from 35 to 56 % of cases [11]. *E. faecalis*, *A. baumannii* and *P. aeruginosa* were most common in associations. There is a tendency to coexistence observed in *K. pneumoniae*, *E. coli* and *E. cloacae* (3 out of 4 isolates) (Table 2). Representatives of the *Enterobacteriaceae* family in the form of microbial associations are more common for prosthetic hip joint than for the knee (36.4 vs. 16.7 %) substantiating the fact by the proximity to the gastrointestinal tract [5]. *MSSA* and *MRSE* appeared to be more isolated in 21 and 19 %, respectively.

A negative result of bacteriological studies in the presence of obvious symptoms of PJI, does not indicate the "sterility" of cultures and absence of infection. The culture-negative results can be associated with the microbial biofilm which is not detected with traditional culturing methods (sensitivity is 20 %) [11, 12] and antibacterial therapy prior to culturing [5] does contribute to the eradication of planktonic forms of biofilm organisms. Insufficient number of intraoperative biopsy specimens [5], inadequate collection of samples and transportation to the laboratory, inappropriate culturing methods can affect the results of the study. The incidence of culture-negative PJIs ranges from 5 % to 35 % [10, 11]. Yifang Tsai et al. reported 27.2 % of negative microbiological cultures [5] and we obtained nearly identical incidence of 28 %. It is in these situations, as well as, when there is a possibility of retention of the endoprosthesis, The role of empiric antibiotic therapy is important for the scenario and at the initial stage of treatment of acute early PJI to allow implant retention.

Knowledge of the microbiological spectrum of the main causative agents of PJI in major joints would allow for the rational approach to antibiotic therapy narrowing the spectrum of antibiotics used which is of decisive importance in the outcome of surgical treatment of PJI and reducion of rapidly developing resistance to antimicrobial drugs. However, there is no consensus on which antibiotic should be used as empiric therapy due to geographical differences in antibacterial susceptibility spectra [24]. Various empirical schemes are used in different countries and medical institutions to include combinations of glycopeptides with third-generation cephalosporins, fluoroquinolones, clindamycin [26] or extended-spectrum beta-lactams [35] or monotherapy based on the local pathogenic spectrum and local treatment protocols. Piperacillin-tazobactam was considered to be included in the spectrum of antibiotics for empirical therapy but had to be excluded due to the high frequency of side effects (nephrotoxicity, especially in combination with vancomycin) [35].

First- or second-generation cephalosporins were used for initial empiric therapy in our series prior to culture results with Gram(+) rods amounting to 70.7 % of PJI cases with *Staphylococcaceae* being more than half of them. Glycopeptides (vancomycin, teicoplanin) or oxazolidinones (linezolid) were administered for a methicillin-resistant strain in the patient's history. Glycopeptide and fluoroquinolone were administered for patients with a history of repeated surgical interventions on the joint and continuous use of antibacterial drugs of different groups prior to culture results. Antibiotic treatment was adjusted after identification of the pathogen. Initial empiric therapy was not changed in cases of culture-negative PJI. The revealed trend towards an increase in the resistance of Gram(-) bacteria to carbapenems and the discovery of multidrug-resistant strains in our series required revision of the old schemes of empirical antibiotic therapy and the development of new ones based on local monitoring and pan-resistant pathogens *A. baumannii* required the creation of new antimicrobial agents.

CONCLUSION

S. aureus (31.9 %), S. epidermidis (20.2 %), *E. faecalis* (8.5 %), *P. aeruginosa* (8 %) and *A. baumannii* (7.4 %) were most common for the etiology of PJI with increased incidence of *MRSE*, *S. haemolyticus* (MR). *S. aureus* and representatives of Gram (-) bacteria prevailed in early acute and hematogenous forms of PJI, *S. aureus* and *S. epidermidis* were common early delayed and late chronic infection, representatives of *Enterococcus* and Gram (-) rods were less common. There was a trend towards a steady increase in antibiotic resistance of both Gram(+) and Gram(-) pathogens revealed. Strains of *MRSE*, *E. faecalis, E. faecium, S. hominis, S. lentus* resistant to vancomycin, linezolid-resistant *E. faecalis*, and pan-resistant strains of *A. baumannii* were detected for the first time. Annually increasing resistance to antimicrobial agents of Gram(+) and Gram(-) organisms and the increasing role of Gram(-) bacteria in the etiology of early acute PJI require a revision of existing empirical antibiotic therapy regimens.

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