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Adverse Trends in the Etiology of Orthopedic Infection: Results of 6-Year Monitoring of the Structure and Resistance of Leading Pathogens

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Abstract

Osteomyelitis remains one of the most intractable diseases. The nature of the pathogen and its resistance to antibiotics significantly affect the outcome and cost of treatment. ***The aim of the study:*** to analyze the dynamics of the spectrum and antibiotic resistance of the leading pathogens of orthopedic infection for the period 2012–2017. ***Material and Methods.*** The structure of pathogens isolated from the focus of infection from 2774 patients with periprosthetic infection and chronic osteomyelitis was retrospectively analyzed. Antibiotic resistance of the leading pathogens that occupied more than 4% in the species structure was studied. Comparative analysis of changes in the spectrum of pathogens and antibiotic resistance was carried out for the periods 2012-2013, 2014–2015 and 2016-2017. Epidemiological analysis was performed in the program „microbiological monitoring system" Microbe-2. Statistical processing of the obtained data was carried out using the Z-criterion. ***Results.*** From 2774 patients with orthopedic infection have been isolated 4359 strains, in the structure of which about 73.5% were occupied by *S. aureus, S. epidermidis, E. faecalis, E. faecium, P. aeruginosa, Acinetobacter sp.* representatives of the family Enterobacteriacea. In 27% of the cases, microorganisms of other species were identified. Microbial associations were identified in 19.4% of cases. In the structure of the leading gram-positive (Gram (+)) pathogens, a significant decrease in the incidence of *S. aureus* was detected, while the share of *S. epidermidis* increased significantly. Among the leading gram-negative (Gram (-)) microorganisms, a significant increase in the proportion of representatives of the fam. Enterobacteriacea was found, against the background of a decrease in the share *of Acinetobacter* sp. and *P. aeruginosa*. The level of resistance of MSSA to the studied antibiotics ranged from 0.1 to 8.8%, for MSSE the spread was from 1.9 to 16.7%. Negative dynamics of growth of resistance of non-fermenting bacteria is established. The strains of *Acinetobacter* sp. demonstrated greater resistance to tested antibiotics in comparison with *P. aeruginosa*. ***Conclusion.*** An increase in the role of *S. epidermidis* and *K. pneumoniae* in the etiology of orthopedic infection was established. The revealed increase in the resistance of microbial pathogens to most tested and used antibiotics should be taken into account in the appointment of empirical antibiotic therapy. The extremely high frequency of resistance of gram-negative bacteria to cephalosporins and fluoroquinolones excludes the possibility of their empirical use, which requires the management of carbapenems in the starting treatment regimens. High resistance to fluoroquinolones limits the ability of oral antibiotic therapy in patients with periprosthetic infection.

Keywords: periprosthetic infection, osteomyelitis, leading pathogens, antibiotic resistance.

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Background

Despite the fact that the first description of bone infection was given in the era of Hippocrates, osteomyelitis still remains one of the most intractable diseases. The increasing medical and social significance of this pathology is largely determined by the increase in the number of orthopedic surgeries using implants due to expansion in the number of high-energy injuries associated with open fractures due to road accidents and military injuries [1–3]. Primary total hip and knee endoprosthetics (EP) are among the most common operations in orthopedic surgery. It is predicted that the demand for these interventions will increase significantly in the next two decades [4, 5]. One of the most devastating complications of endoprosthetics is a deep infection of the surgical area — a periprosthetic infection (PI), which is a special case of an implant-associated infection (IAI). The development of this complication significantly increases the period of hospitalization, leads to additional financial costs for treatment, in some cases ends with the chronicity of the infectious process and the development of osteomyelitis. Currently, researchers note that the infection accounts for 15% in the spectrum of causes of revision arthroplasty of large joints [6], and in the structure of early revisions of the hip joint, this measure reaches 64% [7]. While the incidence of periprosthetic infection after primary operations is less than 2%.

Staphylococcus aureus and coagulase-negative staphylococcus (CNS) in more than half of cases are the causes of IAI; gram-negative bacteria are responsible for 5–23% of cases of orthopedic infection, especially among the elderly [8–10]. The pathogenesis of infection caused by gram-negative (Gram (-)) and gram-positive (Gram (+)) pathogens is associated with the formation of biofilms on the components of the endoprosthesis that protect bacteria from antimicrobial agents and the host immune system [11]. It is known that the clinical outcomes of the prosthetic joint infections caused by Gram (-) bacteria are less favorable [12, 13, 14]. The isolation of antibiotic resistant strains of Gram (-) bacteria from patients with periprosthetic infection also cause serious concern. For example, acute PPI, caused by a pathogen resistant to fluoroquinolones, is associated with failure of rehabilitation and the need to remove the endoprosthesis [15].

Monitoring of infectious agents and their antibiotic sensitivity is one of the main tools that allow timely correction of empirical antibiotic therapy schemes, develop means of control of resistance and monitor their effectiveness.

The purpose of this study was to analyze the dynamics of the spectrum and antibiotic resistance of the leading causative agents of orthopedic implant-associated infection for the period 2012–2017.

Materials and Methods

A retrospective analysis of the etiological structure of IAI was performed in 2,774 patients due to periprosthetic infection (73.5%) and chronic postoperative and post-traumatic osteomyelitis (26.5%) from January 1, 2012 to December 31, 2017. As a result the spectrum of the leading causative agents of IAI was determined. Positive growth of microorganisms was obtained in 68.7% of cases.

The leading pathogens were microorganisms, whose share in the species structure was more than 4%. The antibioticograms of the strains of the leading causative agents of IAI isolated from tissue biopsies, aspirates and remote metal structures (endoprostheses, screws, plates, cement spacers, etc.) were analyzed. The strains with identical sensitivity to antibiotics, isolated from different biological materials from one patient were counted only once.

Identification of pathogens was carried out in accordance with standard manual laboratory techniques and also the automatic identification was performed by Microlatest panels (Erba Lachema) using the iEMS

Reader MF. Determination of antibiotic sensitivity was performed by disco-diffusion method using Mueller-Hinton agar (Oxoid, United Kingdom) and disks with antibiotics (Oxoid, United Kingdom), and also by the method of minimum inhibitory concentrations using E-tests (Oxoid, United Kingdom) and automatic analyzer VITEK 2 Compact (BioMerieux, France). Evaluation of sensitivity to antibiotics was performed in accordance with the criteria of EUCAST (2012-2017). A comparative analysis of changes in the spectrum of pathogens and antibiotic resistance was carried out for the periods of 2012–2013, 2014–2015 and 2016–2017. Epidemiological analysis of the results of the study was performed using the program "Microbiological monitoring system "Microbe-2" (©2002–2016 MedProject-3).

Statistical analysis

Statistical analysis of the data obtained was carried out with MS Office Excel 2007 (Microsoft, USA) and the Z-criterion of the standard normal distribution to estimate the difference between the portions.

Results

4359 strains were isolated from 2774 patients with orthopedic infection during the studied period. About 73.5% (n = 3205) of these strains consist of *S. aureus, S. epidermidis, E. faecalis, E. faecium, P. aeruginosa, Acinetobacter sp.* and different species of Enterobacteriacea (*K. pneumoniae, E. coli and E. cloacae*), which have been classified as the leading pathogens. In 27% of cases, microorganisms of other species were identified, whose percent was less than 4% and which were not included in the further analysis. Microbial associations (combination of 2 to 4 pathogens) were founded in 19.4% of cases of these diseases.

In the structure of the leading Gram (+) causative agents of IAI, a significant (p<0.01) decrease in the frequency of *S. aureus* isolation from 34.5% in 2012–2013 up to 28.6% in 2016–2017 was detected, including methicillin-resistant strains (MRSA) (p<0.05) (Fig. 1).

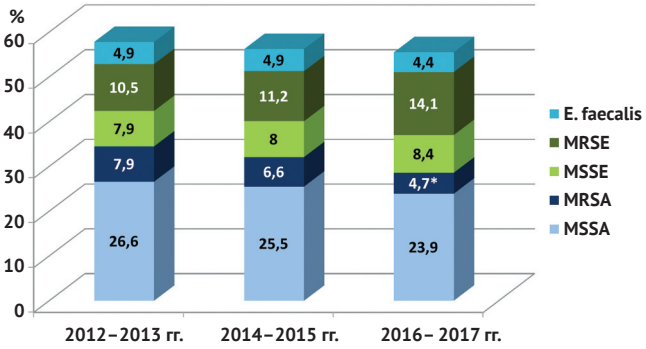


Fig. 1. Spectrum of the leading Gram(+) causative agents of IAI in the analyzed periods of time
* — p<0.05 compared with the period of 2012–2013

At the same time, the proportion of *S. epidermidis* increased significantly (p<0.01) from 18.4% to 22.5%, however, the increase in the frequency of methicillin-resistant isolates (MRSE) was insignificant. In the period 2016-2017 methicillin-resistant (MR) strains accounted for 16.4 and 62.7% of *S. aureus* and *S. epidermidis*, respectively. Significant changes in the dynamics of the percent of enterococci were not founded; this measure was 4.9–4.4% for *E. faecalis* during the entire observation period.

The analysis of the structure of leading Gram (-) pathogens revealed a significant (p<0.05) percent increase of enterobacterial strains from 6.6% in 2012–2013 to 8.7% in 2016–2017, at the same time the significant percent decrease of *Acinetobacter sp.* strains and the trend to reduce of *P. aeruginosa* percentage (Fig. 2).

The species analysis revealed a statistically significant increase (p<0.01) in the proportion of K. pneumoniae from 46.9 to 63.8% and a decrease in the proportion of *E. cloacae* from 36.7 to 12.6% in the spectrum of the leading representatives of fam. Enterobacteriaceae (Table 1).

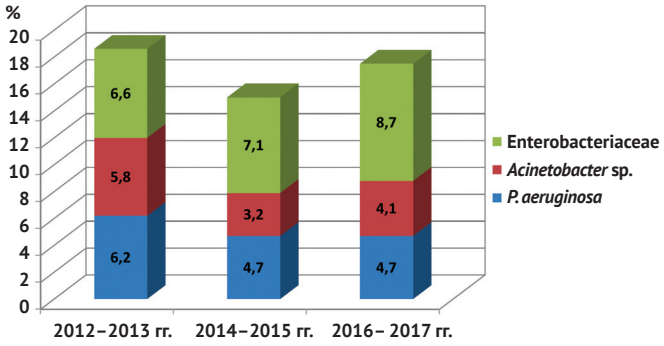


Fig. 2. Spectrum of the leading Gram(-) causative agents of bacteria IAI in the analyzed periods of time
* — p <0.05 compared with the period of 2012–2013
** — p <0.01 in comparison with the period of 2012–2013

A comparative analysis of antibiotico-grams of staphylococcal isolates that are sensitive and resistant to methicillin showed that the latter, regardless of species, are characterized by high cross-resistance to most of the tested antibiotics (Table 2). The level of resistance of the MSSA strains to the antibiotics under study was generally low and ranged from 0.1 to 8.8%; for MSSE isolates, the spread of this indicator ranged from 1.9 to 16.7%. Regardless of sensitivity to methicillin the isolates resistant to gentamicin, fluoroquinolones, co-trimoxazole, erythromycin, clindamycin, and fusidic acid were significantly more common (p<0.05) among *S. epidermidis* compared with *S. aureus*. In addition, MSSE isolates showed resistance to rifampicin and tetracycline significantly (p<0.05) more often, than MSSA.

Vancomycin- and linezolid-resistant staphylococci strains were not detected during the observation period. In addition to these antibiotics, the most active antibiotics for MR strains were fusidic acid and fosfomycin.

Analysis of the dynamics of the resistance level of staphylococcal strains showed that the frequency of MRSA isolation significantly decreased from 22.9 to 16.5% (p<0.05) over the observation period, while for MRSE a trend to increase this indicator from 56.6 up to 63.3% (p>0.05) was detected. Generally, the resistance level of MRSA changed statistically insignificantly, however, the increase of rifampicin resistance from 29.8% to 39% is noteworthy. With respect to all tested antibiotics, the resistance of MSSA strains did not exceed 4% (Table 3), with the exception of tetracycline and erythromycin, for which this indicator ranged from 7.2 to 10.4% and 6.7–7.8%, respectively.

From 2012-2013 to the end of the observation period, the resistance of MSSE to gentamycin decreased significantly (18.3% to 10%) (Table 4) (p<0.05). A similar, but not so significant trend was identified for the MRSE: from 83.8 to 72.1 (p>0.05). The resistance of MSSE to moxifloxacin (from 2.5 to 10%) and fosfomycin (from 3.8 to 15.2%) increased significantly (p<0.05), moreover activity of fosfomycin against MRSE isolates was significantly reduced (p<0.05).

Table 1

The dynamics of the share of leading pathogens from fam. Enterobacteriaceae

Species	2012–2013	2014–2015	2016–2017
Klebsiella pneumoniae	46 (46,9%)	46 (44,7%)	81 (63,8%)[1*, 2*]
Escherichia coli	16 (16,4%)	24 (23,3%)	30 (23,6)%
Enterobacter cloacae	36 (36,7%)	33 (32,0%)	16 (12,6%)[1*, 2]
Total	98 (100%)	103 (100%)	127 (100%)

1* — p<0,01 in comparison with the period of 2012–2013; 2 — p<0,05 in comparison with the period of 2014–2015; 2* — p<0,01 in comparison with the period of 2014–2015.

Table 2

Level of resistance of *S. aureus* and *S. epidermidis* depending on their sensitivity to methicillin

Antimicrobial agent	MSSA, n = 1102	MRSA, n = 283	MSSE, n = 341	MRSE, n = 507
Cefoxitin	0	100	0	100
Oxacilline	0	100	0	100
Gentamycine	2.5	74.1[s*]	15.7[a]	77 [s*]
Moxifloxacin	1.1	81.1[s*]	5.8[a]	44 [s*.a*]
Levofloxacin	1.6	80.0 [s*]	16.7[a]	59.3 [s*.a]
Ciprofloxacin	2.6	86.9 [s*]	10.3[a]	61.2 [s*.a*]
Co-trimoxazole	0.0	5.0 [s*]	14[a*]	39.1 [s*.a*]
Rifampicin	2.5	31.9 [s*]	4.2	19.2 [s*.a*]
Tetracycline	8.8	45.0 [s*]	12.5	35.7 [s*.a]
Erythromycin	6.4	50.0 [s*]	35[a*]	62.5 [s*.a*]
Clindamycin	2.0	48.9 [s*]	5.2[a*]	29.3 [s*.a*]
Fusidic acid	0.0	0.0	1.9[a]	15 [s.a*]
Fosfomicin	0.1	10.5 [s*]	11.5	11.6
Linezolid	0.0	0.0	0	0
Vancomycin	0.0	0.0	0	0

s — p <0.05 compared with methicillin-sensitive (MS) strains of this species; s* — p<0.01 compared with methicillin-sensitive (MS) strains of this species; a — p<0,05 compared with *S. aureus*; a* — p<0,01 compared with *S. aureus*.

Table 3

Dynamics of the resistance level of *S. aureus* depending on the sensitivity to methicillin, %

Antimicrobial agent	MSSA			MRSA		
	2012–2013, n = 390	2014–2015, n = 374	2016–2017, n = 338	2012–2013, n = 116	2014–2015, n = 100	2016–2017, n = 67
Gentamycine	1.5	3.2	3	77.6	75.8	65.7
Co-trimoxazole	0	0	0	5.4	6.1	3
Tetracycline	7.2	9.1	10.4	45.1	41	50.7
Erythromycin	6.7	4.8	7.8	48.2	49.5	53.7
Clindamycin	0.8	1.8	3.6	51.4	47.4	47
Ciprofloxacin	3.3	1.9	2.7	87.5	87.6	84.8
Moxifloxacin	1	0.8	1.5	77.7	85.6	80.3
Levofloxacin	NA	NA	1.6	NA	NA	79.1
Fosfomicin	0.3	0	0	8.3	13.4	9.4
Rifampicin	2.6	1.6	3.6	29.8	29.9	39
Fusidic acid	NA	NA	0	NA	NA	0

NA — no data available.

Table 4

Dynamics of the resistance level of (%) *S. epidermidis* depending on the sensitivity to methicillin

Antimicrobial agent	MSSE			MRSE		
	2012–2013, n = 114	2014–2015, n = 114	2016–2017, n = 113	2012–2013, n = 148	2014–2015, n = 164	2016–2017, n = 195
Gentamycine	18.3	18.3	10*	83.8	76.4	72.1
Co-trimoxazole	17.6	11	13	41.4	37	39.1
Tetracycline	18.2	8.9	9.9	38.8	34.6	34.2
Erythromycin	33.3	34	38	61.1	61.1	64.9
Clindamycin	4.6	3.9	7	29.7	24.4	33.2
Ciprofloxacin	10.9	6.9	13	60.7	57.7	64.6
Moxifloxacin	2.8	5	10*	38.5	47	45.6
Levofloxacin	NA	NA	16.7	NA	NA	60
Fosfomicin	3.8	17.8*	15.2*	5.7	15.5*	12.9*
Rifampicin	4.6	3.9	4	23.5	18.6	16.3
Fusidic acid	NA	NA	1.9	NA	NA	14.5

* — p<0,05 compared with the period 2012–2013; NA — no data available.

All the *E. faecalis* strains (n = 102) isolated during the observation period were sensitive to ampicillin, imipenem, linezolid, and tigecycline. In the period 2016–2017, only one vancomycin-resistant *E. faecalis* strain was isolated. Additional testing showed that the MIC of vancomycin for this strain was more than 256 μg/ml. There was a statistically insignificant increase in resistance to co-trimoxazole from 32.4% to 51.7% and a decrease in resistance to ciprofloxacin from 64.5% to 49.2%.

The most active antibiotic against representatives of non-fermenting bacteria was colistin. All isolates of *P. aeruginosa* and Acinetobacter sp., included in the study, were sensitive to this antibiotic. Negative growth dynamics of the resistance of *P. aeruginosa* strains to all tested antibiotics was detected with the exception of colistin (Fig. 3).

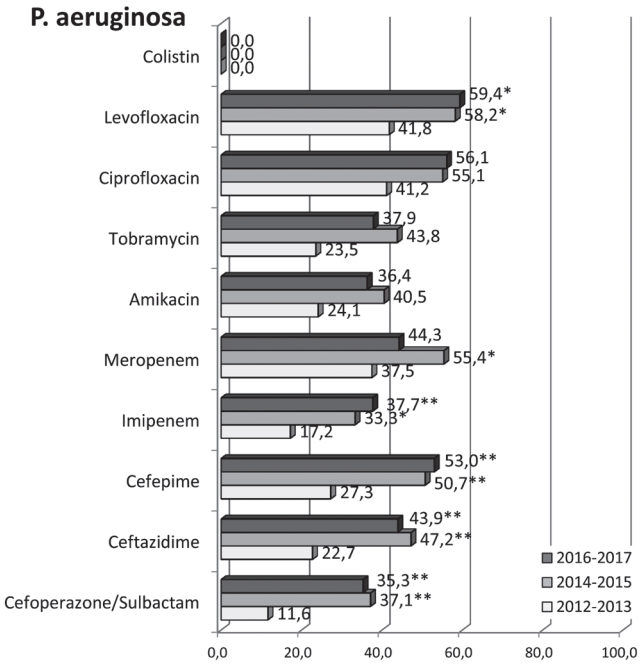
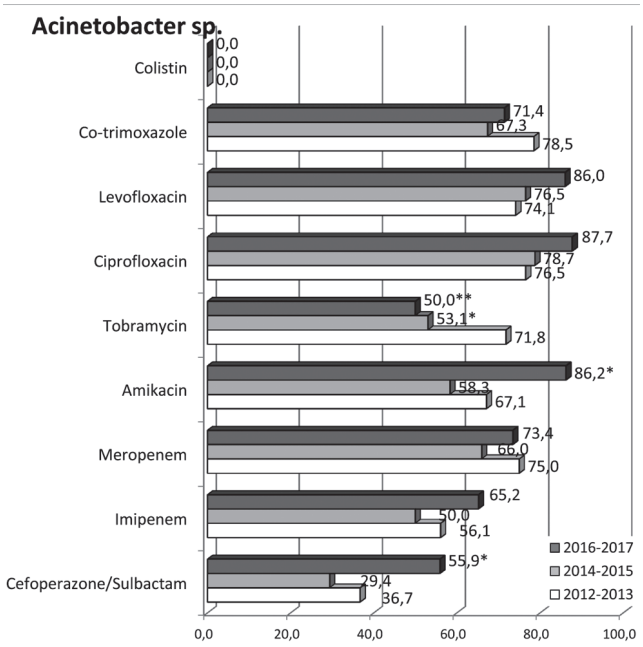


Fig. 3. The dynamics of the level of resistance *P. aeruginosa*
[1] — *p*<0,05 in comparison with the period 2012–2013
[1*] — *p*<0,01 in comparison with the period 2012–2013

An increase in the proportion of resistant isolates to cephalosporins of 3–4 generations, imipenem, meropenem, levofloxacin was statistically significant (p<0.05). By the end of the study, about 63–65% of *P. aureginosa* strains remained sensitive to cefoperazone/sulbactam, imipenem, amikacin and tobramycin, 56–57% to ceftazidime, meropenem, and only about 40–45% to fluoroquinolones and cefipime.

The most active antibiotic (after colistin) against strains of *Acinetobacter* sp. was cefoperazone/sulbactam. However, resistance to cefoperazone/sulbactam significantly increased (p<0.05) compared with 2012–2013 and 2014–2015, and amounted to 55.9%. A similar dynamics was detected for resistance to amikacin, which had reached 86.2% by the end of the study. In general, representatives o*f Acinetobacter sp.* showed greater resistance to tested antibiotics compared to *P. aeruginosa.* Cefoperazone/sulbactam and tobramycin were active against 45–50% isolates of *Acinetobacter sp.* Less than 30% of the strains were sensitive to co-trimoxazole, meropenem, less than 20% were sensitive to fluoroquinolones and amikacin (Fig. 4).



Analysis of the dynamics of intraspecific resistance of representatives of fam. Enterobacteriaceae showed that the main problem is *K. pneumoniae* isolates, whose resistance significantly (p<0.01) increased even to reserve antibiotics: to cefoperazone/sulbactam from 30.4 to 54.1%, to imipenem from 6.5 to 29.6%, to Meropenem from 4.3 to 27.2% (Table 5). More than 90% of isolates isolated at the end of the observation period were resistant to ampicillin/sulbactam, fluoroquinolones, co-trimoxazole and tobramycin. Fosfomycin and colistin, sensitivity to which was additionally determined in 2017, showed activity against 63.3% (19 of 30) and 80% (16 of 20) of *K. pneumoniae* strains, respectively. At the same time, the proportion of amikacin-resistant strains decreased insignificantly from 51.1 to 35.5%. A similar trend was detected for *E. coli,* and all 16 *E. cloacae* isolates isolated in 2016–2017 were sensitive to this antibiotic.

A decrease in the activity of unprotected cephalosporins included in the study was founded with respect to *E. coli,* especially for cefepime (from 72.5 to 38.5%, p<0.05). By the end of the study period, carbapenems, cefoperazone/sulbactam and amikacin were most active against *E. coli* and *E. cloacae.* From 2017, the minimum inhibitory concentration of fosfomycin was determined for all multiresistant strains of enterobacteria (E-test, Oxoid, UK). According to the results of this study, 11 out of 30 (36.7%) K. pneumoniae isolates showed resistance to fosfomycin. All 5 strains of tested *E. cloacae* were sensitive to this antibiotic.

Fig. 4. The dynamics of the resistance level of *Acinetobacter* sp.
[1] — p<0,05 in comparison with the period 2012–2013
[2] — p<0,05 in comparison with the period 2014–2015
[1*] — p<0,01 in comparison with the period 2012–2013
[2*] — p<0,01 in comparison with the period 2014–2015

Table 5

The dynamics of the resistance level (%) of representatives of fam. Enterobacteriaceae

Antimicrobial agent	*Escherichia coli*			*Klebsiella pneumoniae*			*Enterobacter cloacae*		
	2012–2013, *n* = 16	2014–2015, *n* = 24	2016–2017, *n* = 30	2012–2013, *n* = 46	2014–2015, *n* = 46	2016–2017, *n* = 81	2012–2013, *n* = 36	2014–2015, *n* = 33	2016–2017, *n* = 16
Ampicillin/sulbactam	66.7	43.5[1*]	80.8[2*]	95.5	81.8	95.7	72.7	83.9	87.5
Cefoperazone/sulbactam	0.0	4.2	3.6	30.4	50.0	54.1[1*]	28.1	15.2	12.5
Ceftazidime	43.8	37.5	61.5	88.9	79.5	86.1	60.0	71.0	62.5
Ceftriaxone	46.7	34.8	61.5	90.9	81.8	85.5	61.8	71.0	62.5
Cefepime	37.5	33.3	61.5[2]	91.1	79.5	84.7	50.0	71.0	56.3
Imipenem	0.0	0.0	0.0	6.5	21.7[1]	29.6[1*]	2.9	0.0	6.3
Meropenem	0.0	4.2	0.0	4.3	21.7[1]	27.2[1*]	2.9	0.0	0.0
Ertapenem	NA	NA	0.0	NA	NA	46.9	NA	NA	0.0
Amikacin	6.3	16.7	3.3	51.1	37.0	35.5	33.3	29.0	0.0[1*.2]
Tobramycin	33.3	37.5	53.6	89.1	77.3	91.3[2]	47.1	74.2	56.3
Ciprofloxacin	43.8	41.7	69.2	87.0	73.9	93.1[2]	37.1	61.3[1]	37.5
Moxifloxacin	46.7	39.1	75.02	91.1	75.6	92.8[1.2*]	43.8	62.5	37.5
Co-trimoxazole	37.5	33.3	53.8	75.6	63.6[1*]	91.81[*.2*]	52.8	60.0	40.0

1 — p<0,05 in comparison with the period 2012–2013; 2 — p<0,05 in comparison with the period 2014–2015; 1* — p<0,01 in comparison with the period 2012–2013; 2* — p<0,01 in comparison with the period 2014–2015; NA — no data available.

Discussion

The study revealed several trends in the dynamics of the spectrum of leading pathogens and their antibiotic resistance. In the etiological structure of orthopedic infections, the proportion of *S. aureus* decreased in comparison with earlier periods of observation. In 2010–2012, the share of this species was 33.1%, 23.9% of them were MRSA strains [8], whereas in this study in the period 2016–2017 these indicators were respectively — 28.6 and 16.5%. At the same time, insignificant fluctuations or preservation of the level of MRSA resistance to the studied antibiotics were founded. The obtained results are consistent with the changes in the epidemiology of MRSA not only in Russia [16], but also in Europe and North America [17]. An increase in the frequency of *S. epidermidis* isolation from patients with orthopedic infection was revealed at the same time with a decrease in the etiological role of *S. aureus*. In 2010–2012, *S. epidermidis* was isolated in 16.8% of cases of implant-associated infection [8], and by 2016–2017 this measure reached 22.5%, while the share of MRSE was 56.6 and 63.3% respectively. Similar data were obtained in the study of Triffault-Fillit C with co-authors (2018). In an investigation of the etiology of 567 cases of PIP, they revealed that among staphylococci the frequencies of MRSA and MRSE were16.1% and 59.1% respectively [10]. Regardless of the sensitivity of staphylococcal strains to methicillin, *S. epidermidis* isolates were significantly more often resistant to most of the antibiotics studied. The vancomycin and linezolid retain high

activity against staphylococci (no resistant strains). Also the high activity of fusidic acid and fosfomycin was founded. With respect to *E. faecalis*, vancomycin, linezolid, imipenem, and tigecycline remain highly active. However, it is alarming to isolate the first vancomycin-resistant strain in our hospital.

Despite the fact that the most frequent causative agents of nosocomial infections in Russia are representatives of fam. Enterobacteriaceae, *P. aeruginosa* and *Acinetobacter* sp., which account for 43.1, 19.6 and 14.4% of all isolated bacterial pathogens of nosocomial infections [18, 19, 20], their participation in the etiology of orthopedic implant-associated infections does not exceed as a whole 10–35% according to the data of various authors, [9, 10, 21, 22]. In this study, a decrease in the frequency of isolation of Acinetobacter sp. by 29.3% ($p<0.05$) and P. aeruginosa by 24.2% ($p>0.05$) was shown by 2016-2017 compared with the initial period of the study (2012-2013). In this case, the share of fam. Enterobacteriaceae as a whole increased by 31.8% ($p>0.05$) by increasing the frequency of isolation of K. pneumoniae ($p<0.01$). In Western European countries, a significant increase in the number of cases of periprosthetic infection ($p = 0.024$) caused by aerobic Gram (-) rods is also noted: from 25% in 2003–2004. to 33.3% in 2011–2012 and reducing ($p<0.02$) the proportion of Gram (+) cocci from 80.3% to 74.3% [9].

In our opinion, this is an extremely dangerous tendency, since, despite the fact that Gram (-) bacteria share 17% in the etiological structure of IAI, the results of the analysis of the dynamics of antibiotic resistance indicate the growing resistance of *K. pneumoniae* and non-fermentative pathogens to most of the tested drugs. The most clinically significant problem is the resistance of Gram (-) causative agents of IAI to current cephalosporins, carbapenems and fluoroquinolones. At present, the strains of *P. aeruginosa, Acinetobacter* sp., and *K. pneumoniae* resistant to carbapenems are becoming an acute problem in the treatment of infectious diseases due to high mortality [18, 20, 23, 24]. Few existing publications indicate a significant decrease in the effectiveness of treatment of orthopedic IAI caused not only by carbapenem-resistant strains, but also Gram (-) bacteria in general [17, 25, 26].

A contemporary view the list of antibiotics with activity against bacteria in the composition of biofilms is limited to rifampicin (staphylococcal IAI), fluoroquinolones (Gram (-) pathogens) and fosfomycin, that highly active against enterococci [27]. In this regard, pathogens resistant to these antibiotics are referred to as the so-called difficult-to-treat DTT (Difficult-To-Treat) pathogens. Among all strains included in our study, 8.5% (112/1310) of *S. aureus* strains and 13.5% (109/810) *S. epidermidis* were resistant to rifampicin; 50% (110/220) *P. aeruginosa,* 78.9% (112/142) *Acinetobacter sp.* and 81.5% (141/173) *K. pneumoniae* were resistant to ciprofloxacin. Moreover, in 2016-2017, about 85–90% of isolates of *Acinetobacter sp.*, *K. pneumoniae* and *E. coli* showed resistance to fluoroquinolones. The colistin demonstrated the highest activity against Gram (-) bacteria among all the tested antibiotics. All isolates of *P. aeruginosa* and *Acinetobacter sp.* and 80% of *K. pneumoniae* strains were sensitive to this antibiotic. However, the prolongation of the course of antibiotic therapy (at least 4–6 weeks after release from the hospital) at the outpatient stage is almost impossible due to the high cost and the lack of an oral form of colistin, as well as carbapenems. Therefore the isolation of Gram (-) pathogens extremely unfavorable prognostic sign in the treatment of orthopedic IAI.

According to existing recommendations, the main risk factors for isolating multiresistant pathogens, regardless of the source of infection, are elderly age (over 65 years), comorbidity (including multiple), courses of

antibiotic therapy (in the previous 90 days) and previous hospitalizations in history [28]. In our opinion, for patients with IAI, risk factors are also a long period of infection with repeated attempts of conservative antibiotic therapy and previously performed non-radical surgical interventions with preservation of an infected implant [29]. However, this assumption requires further investigation.

Conclusion

Thus, the obtained results indicate an increase in the role of S. epidermidis and *K. pneumoniae* in the etiology of orthopedic infection. The detected increase in the resistance of microbial pathogens to the most of used antibiotics should be considered when it is necessary to prescribe antibiotic therapy before obtaining the results of bacterial studies. With respect to gram-positive pathogens, there remains a high activity of vancomycin, linezolid, fosfomycin, which can be used for empirical therapy of patients with IAI.

The extremely high frequency of resistance of Gram-negative bacteria to modern cephalosporins and fluoroquinolones eliminates the possibility of their empirical use, which requires maintaining carbapenems in starting treatment regimens. In addition, high resistance to fluoroquinolones significantly limits the possibilities of prolonged oral antibiotic therapy in patients with periprosthetic infection and chronic osteomyelitis, which must be considered when choosing the tactics of surgical treatment.

References

1. Del Pozo J.L., Patel R. Clinical practice. Infection associated with prosthetic joints. *N Engl J Med.* 2009;361(8):787-794. DOI: 10.1056/NEJMcp0905029.
2. Johnson E.N., Burns T.C., Hayda R.A., Hospenthal D.R., Murray C.K. Infectious complications of open type III tibial fractures among combat casualties. *Clin Infect Dis.* 2007;45(4):409-415. DOI: 10.1086/520029.
3. Lima A.L., Oliveira P.R., Paula A.P. Acinetobacter infection. *N Engl J Med.* 2008;358(26):2846.
4. Day J.S., Lau E., Ong K.L., Williams G.R., Ramsey M.L., Kurtz S.M. Prevalence and projections of total shoulder and elbow arthroplasty in the United States to 2015. *J Shoulder Elbow Surg.* 2010;19(8):1115-1120. DOI: 10.1016/j.jse.2010.02.009.
5. Peel T.N., Dowsey M.M., Daffy J.R., Stanley P.A., Choong P.F., Buising K.L. Risk factors for prosthetic hip and knee infections according to arthroplasty site. *J Hosp Infect.* 2011;79:129-133. DOI: 10.1016/j.jhin.2011.06.001.
6. Bozic K.J., Lau E., Kurtz S., Ong K., Rubash H., Vail T.P., Berry D.J. Patient-related risk factors for periprosthetic joint infection and postoperative mortality following total hip arthroplasty in Medicare patients. *J Bone Joint Surg Am.* 2012;94(9):794-800. DOI: 10.2106/JBJS.K.00072.
7. Tikhilov R.M., Shubnyakov I.I., Kovalenko A.N., Totoyev Z.A., Lyu B., Bilyk S.S. [The structure of early revisions after hip replacement]. *Travmatologiya i ortopediya Rossii* [Traumatology and Orthopedics of Russia]. 2014;(2):5-13. (In Russ.). DOI: 10.21823/2311-2905-2014-0-2-5-13.
8. Bozhkova S.A., Tikhilov R.M., Krasnova M.V., Rukina A.N. [Orthopedic implant-associated infection: the main etiological agents, local resistance and antimicrobial therapy recommendations]. *Travmatologiya i ortopediya Rossii* [Traumatology and Orthopedics of Russia] 2013;(4): 5-15. (In Russ.). DOI: 10.21823/2311-2905-2013-4-5-15.
9. Benito N., Franco M., Ribera A., Soriano A., Rodriguez-Pardo D., Sorlí L. et al. Time trends in the aetiology of prosthetic joint infections: a multicentre cohort study. *Clin Microbiol Infect.* 2016;22(8):732.e1–8. DOI: 10.1016/j.cmi.2016.05.004.
10. Triffault-Fillit C., Ferry T., Laurent F., Pradat P., Dupieux C., Conrad A. et al. Microbiologic epidemiology depending on time to occurrence of prosthetic joint infection: a prospective cohort study. *Clin Microbiol Infect.* 2018 May 25; pii:S1198-743X(18)30411-7. DOI: 10.1016/j.cmi.2018.04.035.
11. del Pozo J.L., Patel R. The challenge of treating biofilm-associated bacterial infection. *Clin Pharmacol Ther.* 2007;82(2):204-209. DOI: 10.1038/sj.clpt.6100247.
12. Aboltins C.A., Dowsey M.M., Buising K.L., Peel T.N., Daffy J.R., Choong P.F., Stanley P.A. Gram-negative prosthetic joint infection treated with debridement, prosthesis retention and antibiotic regimens including a fluoroquinolone. *Clin Microbiol Infect.* 2011;17(6): 862-867. DOI: 10.1111/j.1469-0691.2010.03361.x.
13. Hsieh P.H., Lee M.S., Hsu K.Y., Chang Y.H., Shih H.N., Ueng S.W. Gram-negative prosthetic joint infections: risk factors and outcome of treatment. *Clin Infect Dis.* 2009;49(7):1036-1043. DOI: 10.1086/605593.
14. Zmistowski B., Fedorka C.J., Sheehan E., Deirmengian G., Austin M.S., Parvizi J. Prosthetic joint infection caused by Gram-negative organisms. *J Arthroplasty.* 2011;(6 Suppl):104-108. DOI: 10.1016/j.arth.2011.03.044.
15. Martinez-Pastor J.C., Munoz-Mahamud E., Vilchez F., Garcia-Ramiro S., Bori G., Sierra J. et al. Outcome of acute prosthetic joint infections due to Gram-negative

bacilli treated with open debridement and retention of the prosthesis. *Antimicrob Agents Chemother*. 2009;53(11):4772-4777. DOI: 10.1128/AAC.00188-09.

16. Romanov A.V., Dekhnich A.V., Sukhorukova M.V., Skleenova E.Yu., Ivanchik N.V., Edelstein M.V., Kozlov R.S. and the „MARATHON" Study Group. [Antimicrobial resistance of nosocomial Staphylococcus aureus isolates in Russia: results of multicenter epidemiological study „MARATHON" 2013-2014]. *Klinicheskaya mikrobiologiya i antimikrobnaya khimioterapiya* [Clinical Microbiology and Antimicrobial Chemotherapy]. 2017;19(1):57-62. (In Russ.).

17. Olearoa F., Albrichb W.C., Vernaza N., Harbartha S., Kronenbergc A. and the Swiss Centre for Antibiotic resistance (ANRESIS). Staphylococcus aureus and methicillin resistance in Switzerland: regional differences and trends from 2004 to 2014. *Swiss Med Wkly*. 2016;146:w14339. DOI: 10.4414/smw.2016.14339.

18. Sukhorukova M.V., Edelstein M.V., Skleenova E.Yu., Ivanchik N.V., Mikotina A.V., Dekhnich A.V., Kozlov R.S., and the „MARATHON" study group. [Antimicrobial resistance of nosocomial *Enterobacteriaceae* isolates in Russia: results of multicenter epidemiological study „MARATHON" 2013-2014]. *Klinicheskaya mikrobiologiya i antimikrobnaya khimioterapiya* [Clinical Microbiology and Antimicrobial Chemotherapy]. 2017;19(1):49-56. (In Russ.).

19. Edelstein M.V., Sukhorukova M.V., Skleenova E.Yu., Ivanchik N.V., Mikotina A.V., Sheck E.A., Dekhnich A.V., Azizov I.S., Kozlov R.S. and the „MARATHON" study group. [Antimicrobial resistance of nosocomial *Pseudomonas aeruginosa* isolates in Russia: results of multicenter epidemiological study „MARATHON" 2013-2014]. *Klinicheskaya mikrobiologiya i antimikrobnaya khimioterapiya* [Clinical Microbiology and Antimicrobial Chemotherapy]. 2017;19(1):37-41. (In Russ.).

20. Sukhorukova M.V., Edelstein M.V., Skleenova E.Yu., Ivanchik N.V., Shek E.A., Dekhnich A.V., Kozlov R.S. and the „MARATHON" study group. [Antimicrobial resistance of nosocomial Acinetobacter spp. isolates in Russia: results of multicenter epidemiological study „MARATHON" 2013-2014]. *Klinicheskaya mikrobiologiya i antimikrobnaya khimioterapiya* [Clinical Microbiology and Antimicrobial Chemotherapy]. 2017;19(1):42-48. (In Russ.).

21. Terekhova R.P., Mitish V.A., Paskhalova Yu.S., Skladan G.E., Prudnikova Blatun S.A., L.A. [Osteomyelitis agents of the long bones and their resistance]. *Rany i ranevye infektsii. Zhurnal im. prof. B.M. Kostyuchenka*. [Wounds and Wound Infections. The Prof. B.M. Kostyuchenok Journal]. 2016;3(2):24-30. (In Russ.). DOI: 10.17650/2408-9613-2016-3-2-24-30.

22. Drago L., De Vecchi E., Bortolin M., Zagra L., Romano C.L., Cappelletti L. Epidemiology and antibiotic resistance of late prosthetic knee and hip infections. *J Arthroplasty*. 2017;32(8):2496-2500. DOI: 10.1016/j.arth.2017.03.005.

23. Labarca J.A., Salles M.J., Seas C., Guzmán-Blanco M.. Carbapenem resistance in Pseudomonas aeruginosa and Acinetobacter baumannii in the nosocomial setting in Latin America. *Crit Rev Microbiol*. 2016;42(2):276-292. DOI: 10.3109/1040841X.2014.940494.

24. Rizek C., Fu L., Dos Santos L.C., Leite G., Ramos J., Rossi F. et al. Characterization of carbapenem-resistant Pseudomonas aeruginosa clinical isolates, carrying multiple genes coding for this antibiotic resistance. *Ann Clin Microbiol Antimicrob*. 2014;13:43. DOI: 10.1186/s12941-014-0043-3.

25. Bozhkova S., Tikhilov R., Labutin D., Denisov A., Shubnyakov I., Razorenov V., Artyukh V., Rukina A. Failure of the first step of two-stage revision due to polymicrobial prosthetic joint infection of the hip. *J Orthop Traumatol*. 2016;17(4):369-376. DOI: 10.1007/s10195-016-0417-8.

26. De Sanctis J., Teixeira L., van Duin D., Odio C., Hall G., Tomford J.W. et al. Complex prosthetic joint infections due to carbapenemase-producing *Klebsiella pneumoniae*: a unique challenge in the era of untreatable infections. *Int J Infect Dis*. 2014;25:73-78. DOI: 10.1016/j.ijid.2014.01.028.

27. Winkler T., Trampuz A., Renz N., Perka C., Bozhkova S.A. [Classification and algorithm of diagnosis and treatment of periprosthetic infection of the hip joint]. *Travmatologiya i ortopediya Rossii* [Traumatology and Orthopedics of Russia]. 2016;(1):33-45. (In Russ.). DOI: 10.21823/2311-2905-2016-0-1-33-45.

28. Shubnyakov I.I., Bozhkova S.A., Artyukh V.A., Liventsov V.N., Kochish A.A., Afanas'ev A.V. [Early treatment result in a patient with periprosthetic hip infection]. *Vestnik travmatologii i ortopedii imeni N.N. Priorova* [N.N. Priorov Journal of Traumatology and Orthopedics]. 2017;(4):52-55. (In Russ.). DOI: 10.32414/0869-8678-2017-4-52-55.

29. [Surgical skin and soft tissue infections Russian national guidelines. 2nd revised and expanded edition]. Moscow; 2015. 109 p. (In Russ.). Available from: http://nasci.ru/?id=3392&download=1.

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