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Factors Affecting the Course and Prognosis of Implant-Associated Infection Caused by *Klebsiella* spp.

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Abstract

Background. The outcome of complex treatment of implant-associated infection (IAI) depends on various factors, but one of the most important is the etiology of the inflammatory process. Treatment of orthopedic infection caused by Gram-negative microorganisms in general and representatives of the family *Enterobacteriaceae* in particular causes many difficulties, one of which is the rapid growth of antibiotic resistance.

Aim of the study — to evaluate the factors affecting the course and prognosis of implant-associated infection caused by *Klebsiella* spp.

Methods. We performed a retrospective analysis of case histories of 85 patients who underwent treatment of IAI caused by *Klebsiella* spp. in the clinical departments of the center from January 1, 2017 to December 31, 2021. According to the results of the telephone survey, the patients were divided into 2 main groups depending on the outcome of the 2-year follow-up period determined in accordance with the Delphi criteria.

Results. It was found that the prognosis was significantly worsened by the number of sanitizing surgical interventions in the anamnesis ($p = 0.022$), the need for sanitizing intervention in the early postoperative period ($p<0.001$) and the presence of *Klebsiella* spp. growth in postoperative culture tests ($p = 0.002$), hypoalbuminemia on 7-14 days after the surgery ($p = 0.008$). The administration of trimethoprim-sulfamethoxazole for the outpatient treament stage significantly improved the outcome ($p = 0.038$), which is most likely due to a high proportion of polymicrobial associations — 69.5%.

Conclusions. There is a statistically significant direct relationship between the probability of an unfavorable treatment outcome of patients with IAI caused by *Klebsiella* spp. and the number of sanitizing surgical interventions in the anamnesis, low serum albumin (g/l) on 7-14 days after the operation, revision intervention in the early postoperative period, positive growth of *Klebsiella* spp. in postoperative culture tests. The probability of a favorable outcome was increased by the prescription of trimethoprim-sulfamethoxazole for oral administration at the outpatient stage.

Keywords: periprosthetic infection, osteomyelitis, enterobacteria, *Klebsiella*, antibacterial therapy, trimethoprim-sulfamethoxazole.

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Факторы, влияющие на течение и прогноз имплантат-ассоциированной инфекции, вызванной *Klebsiella* spp.

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Реферат

Введение. Исход комплексного лечения имплантат-ассоциированной инфекции (ИАИ) зависит от различных факторов, однако одним из важнейших является этиология воспалительного процесса. Лечение ортопедической инфекции, вызванной грамотрицательными микроорганизмами вообще и представителями семейства энтеробактерий в частности, вызывает множество сложностей, одной из которых является стремительный рост антибиотикорезистентности.

Цель исследования — оценить факторы, влияющие на течение и прогноз имплантат-ассоциированной инфекции, вызванной *Klebsiella* spp.

Материал и методы. Выполнен ретроспективный анализ историй болезни 85 пациентов, находившихся на лечении с 1 января 2017 г. по 31 декабря 2021 г. по поводу лечения ИАИ, вызванной *Klebsiella* spp. По результатам телефонного анкетирования пациенты были поделены на две основные группы в зависимости от исхода двухлетнего периода наблюдения, определенного согласно критериям Делфи.

Результаты. Статистически значимо ухудшало прогноз исходов: число санирующих хирургических вмешательств в анамнезе ($p = 0{,}022$), необходимость санирующего вмешательства в раннем послеоперационном периоде ($p<0{,}001$) и наличие роста *Klebsiella* spp. в послеоперационных посевах ($p = 0{,}002$), гипоальбуминемия на 7–14-е сут. после проведенной операции ($p = 0{,}008$). Назначение на амбулаторный период триметоприма-сульфаметоксазола статистически значимо улучшало исход ($p = 0{,}038$), что, вероятнее всего, связано с высокой долей полимикробных ассоциаций — 69,5%.

Заключение. Выявлена статистически значимая прямая связь между вероятностью неблагоприятного исхода лечения пациентов с ИАИ, вызванной *Klebsiella* spp., и числом санирующих хирургических вмешательств в анамнезе, низким уровнем сывороточного альбумина на 7–14-е сут. после проведенной операции, проведением ревизионного вмешательства в раннем послеоперационном периоде, положительном ростом *Klebsiella* spp. в послеоперационных посевах. Вероятность благоприятного исхода увеличивало назначение триметоприма-сульфаметоксазола для перорального приема на амбулаторном этапе.

Ключевые слова: перипротезная инфекция, остеомиелит, энтеробактерии, *Klebsiella*, антибактериальная терапия, триметоприм-сульфаметоксазол.

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BACKGROUND

Over the last 10 years, there has been a steady increase in the incidence of infections caused by *Enterobacteriaceae* producing extended-spectrum beta-lactamases, which raised by 53.3% between 2012 and 2017 [1]. Despite the high prevalence, the true incidence of osteomyelitis and implant-associated infection (IAI), including periprosthetic joint infection (PJI), caused by *Enterobacteriaceae* is not reliably known [2].

According to the data of the AMRmap online platform for antimicrobial resistance analysis in Russia, the share of *Enterobacteriaceae* in the spectrum of pathogens of bone and joint infections for the period 1997-2021 was 18.72%, with *Klebsiella pneumoniae* being the leading representative (44.2% of all *Enterobacteriaceae*). In dynamics there is an increase in the share of representatives of this group in the structure of orthopedic infection from 12.2% for the period 2000-2010 to 19.9% for the period 2011-2021 [3]. According to L. Drago et al., the share of Gram-negative microorganisms in the structure of orthopedic infection pathogens was 13.3%, 9.4% of which were representatives of the *Enterobacteriaceae* family [4]. Basing on the 6-year monitoring of the structure of pathogens of orthopedic infection, there is also a tendency towards an increase in the frequency of occurrence of the *Enterobacteriaceae* representatives from 6.6 to 8.7% for the period from 2012 to 2018 [5].

When infections caused by *Enterobacteriaceae* are associated with the orthopedic implants, the problem of providing efficient medical care is particularly urgent. Physicians need to remove the infected implant and prescribe long-term antibiotic therapy for a successful treatment outcome [6]. However, the problem of treating IAI caused by these pathogens is due to their ability to form multilevel microbial biofilms on the implant surface, which in combination with growing antibiotic resistance significantly complicates the choice of an adequate surgical strategy and the possibility of administering long-term etiotropic antibiotic therapy [7].

Studies on the influence of various factors on the course and outcomes of IAI caused by *Enterobacteriaceae* in general and *Klebsiella* spp. in particular are very few and represent mainly small series of clinical cases (3-10 patients) or individual examples. The probability of a favorable outcome, even with optimal surgical tactics and etiotropic antibiotic therapy, is on average estimated as 50% [6, 8].

When carrying out studies, various factors influencing the prognosis are identified, related both to the patient himself and to the features of the IAI course, the nature of the surgical intervention performed and the peculiarities of antibiotic therapy. In most studies, a two-year follow-up period is used for assessment, which was determined by the participants of the international interdisciplinary consensus held in Delphi. According to its materials, a favorable outcome of PJI treatment is understood as the absence of clinical and laboratory signs of recurrence of the infectious process, debridement surgical interventions and fatal outcome within 2 years after the performed operation [9].

Aim of the study is to evaluate the factors influencing the course and prognosis of implant-associated infection caused by *Klebsiella* spp.

METHODS

Study design

Type of study — single-center retrospective study.

Treatment of patients with IAI in the clinical departments of the center from January 1, 2017 to December 31, 2021 was analyzed.

Inclusion criteria:

– musculoskeletal infection, IAI and/or diagnosed osteomyelitis and performed surgical intervention in the focus of infection (index surgery);

– growth of *Klebsiella* spp. (either as an isolated strain or as a part of a polymicrobial association) from one or more samples of pre- and/or intraoperative materials; the results of the examination of preoperative materials were taken into account: tissue biopsies from fistula, wound discharge, synovial fluid from previous interventions, tissue biopsies taken intraoperatively, removed hardware, synovial fluid.

Exclusion criteria:

– localization of the infectious process in the vertebrae or within the soft tissues;

– readmission with recurrence of infection after index surgery caused by *Klebsiella* spp.;

– no information on the outcome of the disease.

We performed a retrospective analysis of the results of microbiological studies of biomaterial from patients operated on in the hospital departments in 2017-2021 using the microbiological monitoring program "Microbe-2". According to the local microbiological monitoring data, the average share of *Klebsiella* spp. in the IAI spectrum for 2017-2021 was 4.6%.

A total of 135 cases of *Klebsiella* spp. growth from patients' biomaterials were analyzed. Following the inclusion/exclusion criteria, 92 patients were included in the study. All of them were surveyed by phone. Seven patients could not be contacted by phone and therefore were excluded from the study. Thus, the number of patients enrolled in the study was reduced to 85. Patients were asked standard questions aimed at determining their adherence to the recommendations received at discharge for outpatient treatment and disease outcome at 2 years after the treatment. According to the results of the survey, the patients were divided into two clinical groups: group 1 — patients with unfavorable treatment outcome, group 2 — patients with favorable outcome according to the Delphi criteria [9].

The following data on each patient were gathered: anthropometric data, anamnesis data (duration of infection, number of debridement operations in anamnesis), type of surgical intervention, surgery duration, intraoperative blood loss, laboratory tests data (WBC, ESR, CRP, total protein and albumin before surgical intervention and on the 7-14th days after the surgery (before discharge), results of microbiological examination of pre-, intra- and postoperative material, features of antibiotic therapy at the inpatient and outpatient stages and features of the course of the postoperative period (repeated surgical intervention at the same admission).

Methods of study

In the period of hospital stay, during pre-, intra- and postoperative microbiological examination of the material, the isolation of clinical isolates was performed by standard methods in accordance with international Standards for Microbiology Investigations (UK SMI). Species identification was performed on Microlatest panels (Erba Lachema) using iEMS Reader MF (Labsistems, Finland). Antibiotic sensitivity of cultures was studied by diffusion methods (Oxoid disks and test strips, England) and by serial dilutions using an automated analyzer VITeK 2 compact (BioMerieux, France) according to EUCAST requirements (2016-2020, v.6-v.10). The ESBL production by Gram-negative strains was determined by the "double disc" method on Mueller-Hinton agar and on CHROMagar ESBL. The carbapenemase production was detected by molecular genetic methods. Bacterial DNA was isolated using DNA-Sorb-AM kit according to the manufacturer's instructions (FBIS Central Research Institute of Epidemiology, Russia). Genes of acquired carbapenemases KPC/OXA-48 and metallo-beta-lactamases of NDM group were detected by Real-time PCR using reagent kits with hybridization-fluorescence detection "AmpliSense MDR KPC/OXA-48-FL" and "AmpliSense MDR MBL-FL" (Interlabservice, Russia) on "Amplifier Real-time CFX96 Touch" device (BioRAD, USA).

All patients received starting antibacterial therapy with two drugs from the day of surgery. It was administered either empirically, according to the local protocol adopted in the center (vancomycin, cefoperazone + [sulbactam]), or taking into account the results of microbiological test performed before the surgical intervention. Antibacterial therapy consisted of the drugs of the following groups in various combinations: cephalosporins (including inhibitor-protected ones), inhibitor-protected amino-penicillins, carbapenems, monobactams, fluoroquinolones, aminoglycosides, as well as fosfomycin, polymyxin B, tigecycline, dioxidine, sulfamethoxazole-trimethoprim. After the results of intraoperative bacterial inoculation were obtained, therapy was either left unchanged until hospital discharge or adjusted. The average course of treatment with intravenous antibiotics was 10-14 days. After that, 6-8-week course of oral antibacterial therapy was administered to patients, considering the presence or absence of sensitivity of the isolated strain to antibacterial drugs of fluoroquinolones or sulfamethoxazole-trimethoprim.

Statistical analysis

Acquired data were registered in form of spreadsheets, visualization of data structure and subsequent analysis were performed using MS Office Excel 2007 (Microsoft, USA) software, IBM SPSS STATISTICS v. 26 software. Quantitative data were evaluated for correspondence to normal distribution using the Kolmogorov-Smirnov test (if the number of subjects was more than 50). In the case of non-normal distribution, quantitative data were described using median, lower and upper quartiles: Me [Q1-Q3]. Categorical data were described with absolute values and percentages. Quantitative variables with a distribution different from normal were compared between the two groups using the Mann-Whitney U-test. The odds ratio (OR, 95% CI) was calculated to assess risk in the comparison groups. Comparison of percentages in the analysis of 2x2 contingency tables was performed using Pearson's chi-square test (χ^2) (for expected values of more than 10) or Fisher's exact test (for expected values of less than 10), and the association was assessed using Cramér's V. The level of correlation was evaluated using the Chaddock scale (ρ). Differences between groups were considered statistically significant at $p<0.05$.

RESULTS

A total of 85 patients were included in the study. Group 1 consisted of 41 (48.3%) patients with unfavorable outcome, of which 12 (29.3%) were fatal. Group 2 consisted of 44 patients (n = 51.8%). Among the patients of group 2, in 22 cases (50%) further orthopedic treatment was not performed due to the absence of indications or patients' unwillingness due to satisfaction with the quality of life. The rest 22 patients (50%) underwent another stage of surgical intervention, after which no evidence for recurrence of infection was found.

There were no statistically significant differences in gender, age, and anthropometric data between the comparison groups (Table 1).

In 11 (13%) patients included in the study, *Klebsiella* spp. with the sensitivity similar to that taken from the intraoperative material was isolated during urine microbiological examination due to the presence of leukocyturia. However, the isolation of *Klebsiella* spp. in urine did not statistically affect the outcome (p = 0.752).

There was a statistically significant difference between the groups in the number of debridement surgeries (p = 0.022). In the group with an unfavorable outcome, the median for the surgeries performed was 2 [1-3], while in the group with a favorable outcome 1 surgery was performed [0-2]. Moreover, the maximum amount of debridement in the medical history of patients in group 1 was 7, while in group 2 it was 5 (Fig. 1).

No statistically significant results were obtained when assessing the effect of duration of the infectious process on the outcome (p = 0.302). The median duration of the infectious process in group 1 was 455 [226-943] days and in group 2 was 488 [102.5-782.0] days.

The type of surgical intervention performed did not influence the outcome (p = 0.234). In more than half of the cases, patients underwent implantation of an antimicrobial spacer with gentamycin (with vancomycin, meropenem, or fosfomycin added according to the results of preoperative microbiological testing). The second and the third most frequent types of surgical intervention in both groups were debridement with prosthesis components retention and resection arthroplasty with vascularized muscle flap grafting (Table 2).

Table 1

Comparison of groups in terms of gender, age, anthropometric parameters

Parameter	Group 1 (n = 41)	Group 2 (n = 44)	p
Male, n (%)	24 (58.6)	25 (56.8)	0.873
Age, years old, Me (IQR)	59 (38-68)	61.5 (47.5-69.5)	0.526
Weight, kg, Me (IQR)	76 (67-92)	82 (71-89.5)	0.673
Height, cm, Me (IQR)	173 (162-178)	170 (164.5-176)	0.989

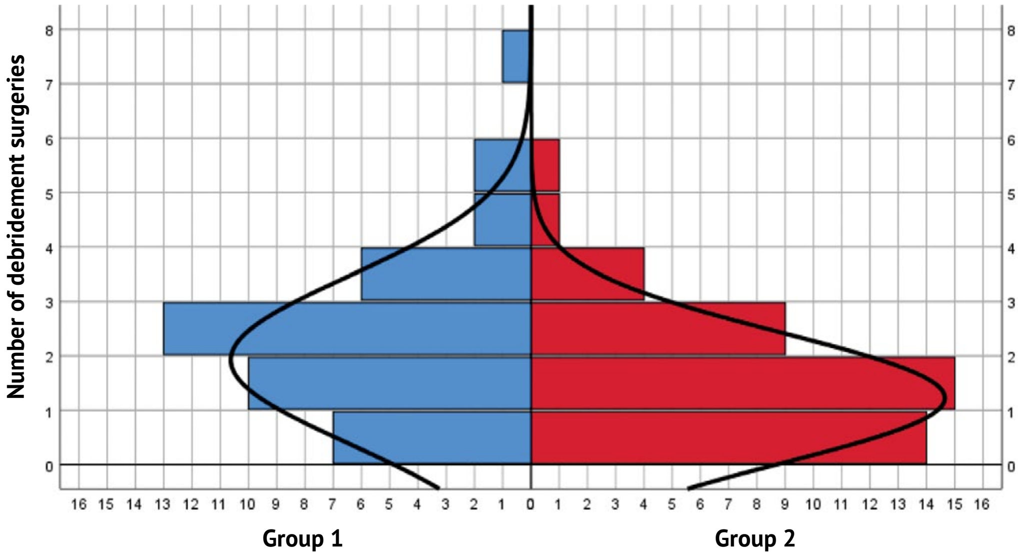


Fig. 1. Distribution of patients by the number of debridement surgeries in the medical history in the comparison groups

Table 2

Types of surgeries in the study groups, n (%)

Type of surgery	Group 1 (n = 41)	Group 2 (n = 44)	Total
Antibiotic spacer implantation	24 (58.5)	22 (50.0)	46
Hardware retaining debridement	3 (7.3)	5 (11.4)	8
Muscle grafting	3 (7.3)	4 (9.1)	7
Debridement with arthroplasty	4 (9.8)	2 (4.5)	6
Debridement without hardware removal and implantation	4 (9.8)	0 (0)	4
Debridement with hardware removal	1 (2.4)	3 (6.8)	4
Second stage of two-stage PJI treatment	0 (0)	3 (6.8)	3
Arthrodesis	1 (2.4)	2 (4.5)	3
Amputation	1 (2.4)	1 (2.3)	2
Hardware replacement	0 (0)	2 (4.5)	2

The studied groups were comparable in terms of the localization of the infectious process (p = 0.241), which in the vast majority of cases (96%) affected the lower extremity: hip joint — 54%, knee joint – 20%, long bones (femur, tibia and fibula) – 16.5% (Table 3).

Before the surgical intervention, no significant differences were found between the groups in laboratory parameters, which are markers of anemia, protein deficiency, and infectious inflammation (Table 4). The median level of CRP and ESR at the admission in both groups was higher than normal, indicating the presence of inflammatory process. At the same time, in group 1 CRP, WBC and ESR were initially higher than in group 2.

Postoperatively, the levels of laboratory markers of inflammation in the groups were comparable, which probably reflects the laboratory pattern of the early postoperative period. However, albumin levels in group 1 were statistically significantly lower than in group 2 (p = 0.008), suggesting that a decrease in this marker is a possible predictor of an unfavorable outcome.

Table 3

Surgery localization in groups, n (%)

Localization	Group 1 (n = 41)	Group 2 (n = 44)	Total
Hip	20 (48.8)	26 (59.1)	46
Knee	6 (14.6)	11 (25)	17
Tibia and fibula	5 (12.2)	2 (4.5)	7
Femur	6 (14.6)	1 (2.3)	7
Ankle	2 (4.9)	1 (2.3)	3
Shoulder	1 (2.4)	1 (2.3)	2
Humerus	1 (2.4)	0 (0)	1
Elbow	0 (0)	1 (2.3)	1
Calcaneus	0 (0)	1 (2.3)	1

Table 4

Laboratory markers before and after the surgery

Laboratory marker	Group 1 (n = 41)		Group 2 (n = 44)		p
	Me	IQR	Me	IQR	
Before surgery					
WBC, 10^9/l	8.65	6.6-10.7	7.5	6.2-9.1	0.314
ESR, mm/h	52	30-62	40.5	22-63	0.268
Hb, g/l	111	95-126	117.5	100-131	0.371
CRP, mg/l	45.5	11-76	27	27-37.5	0.097
Total protein, g/l	74.5	63-79	74	68.5-81	0.275
Albumin, g/l	35	33-43	41	35-43	0.102
On the 7-14th days after the surgery (before discharge)					
WBC, 10^9/l	7.0	5-8.5	6.5	5.5-8	0.566
ESR, mm/h	30	21-44	34.5	22.5-52.5	0.365
Hb, g/l	102.5	91-114	105	95-115.5	0.751
CRP, mg/l	20	9-39	20	8.5-41	0.846
Total protein, g/l	62	56-67	63	59-70	0.211
Albumin, g/l	32	30-35	34.5	32-42.5	0.008*

* — differences are statistically significant ($p<0,05$).

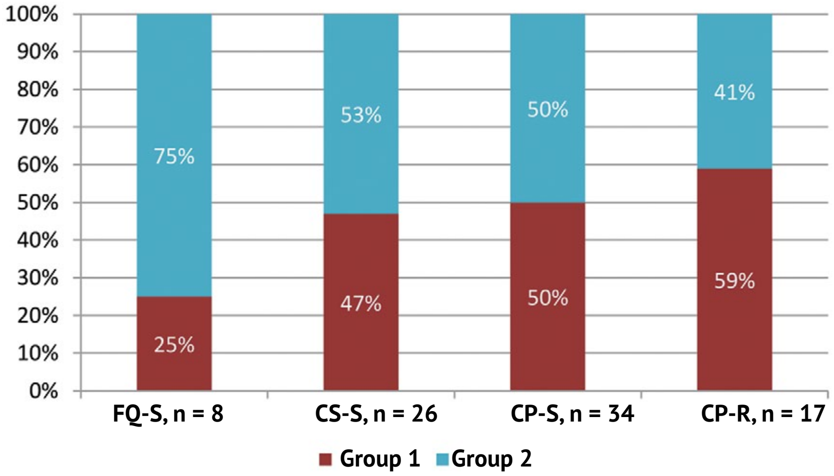
In 93% of patients (n = 79) the etiologic agent of IAI was *K. pneumoniae*, in 7% (n = 6) − *K. oxytoca*. The isolate belonging to a particular *Klebsiella* spp. had no statistically significant effect on the outcome (p = 0.445).

In the studied cohort of patients, *Klebsiella* spp. was isolated as a part of microbial associations in 69.5% of cases. Polybacterial infection with *Klebsiella* spp. was 2.27 times more frequent than monobacterial one. In 16.5% of patients (n = 14) *Klebsiella* spp. was a part of microbial associations with other Gram-negative and Gram-positive microorganisms, in 18.9% (n = 16) − only with Gram-negative (most often *Pseudomonas aeruginosa*, *Acinetobacter baumanii*) and in 34.5% (n = 29) − only with Gram-positive (most often representatives of the genus *Staphylococcus* spp.). Intergroup analysis showed that monobacterial infection was more frequent in group 2, while polybacterial – in group 1.

The share of *Klebsiella* spp. strains resistant to fluoroquinolones was 90%, resistant to cephalosporins – 60%, resistant to carbapenems – 20%. Even though in the analyzed groups the shares of strains with sensitivity/resistance to certain groups of antibiotics were comparable (p = 0.461), a certain tendency towards worse outcomes with increasing resistance was established (Fig. 2).

Klebsiella spp. strains producing different carbapenemases (NDM, OXA-48, KPC) were isolated in 30% (n = 26) of patients. However, carbapenemase production by the infectious agent had no statistically significant effect on the outcome (p = 0.482). At the same time, *Klebsiella* spp. isolates producing different carbapenemases were 7% more frequent among patients of group 1 than among patients of group 2.

The clinical outcome did not depend on the features of antibacterial therapy at the inpatient stage (Table 5). Antibacterial therapy was started prior to surgery in 15 (17.7%) patients in the entire cohort because of septic state or previous surgery. Among them, the share of patients with unfavorable outcome was 1.5 times higher than with a favorable one, but statistical significance was not noticed (p = 0.315).



FQ-S — fluoroquinolone-sensitive strains of *Klebsiella* spp.,
CS-S — cephalosporin-sensitive strains of *Klebsiella* spp.,
CP-S — carbapenem-sensitive strains of *Klebsiella* spp.,
CP-R — carbapenem-resistant strains of *Klebsiella* spp.

Fig. 2. Share of strains of *Klebsiella* spp. with different sensitivity to antibacterial drugs isolated from patients of the comparison groups

Table 5

Impact of antibiotic therapy on treatment outcome at the outpatient stage, n (%)

Factor	Group 1 (n = 41)	Group 2 (n = 44)	Total, n	p	OR (95% CI)
Administration of fluoroquinolones at the outpatient stage	22 (45)	27 (55)	49	0.473	0.729 (0.308-1.728)
Administration of trimethoprim-sulfamethoxazole at the outpatient stage	5 (26)	14 (74)	19	0.038*	0.298 (0.096-0.922)
Simultaneous administration of trimethoprim-sulfamethoxazole and fluoroquinolones	2 (25)	6 (75)	8	0.268	0.325 (0.062-1.711)
Administration of other antibiotic drugs	4 (57)	3 (43)	7	0.707	1.477 (0.310-7.042)
No administration of antibiotics	12 (66)	6 (33)	18	0.111	2.617 (0.897-7.812)

* — differences are statistically significant (*p*<0,05).

The analysis of the influence of the necessity to correct antibacterial therapy in the postoperative period showed that delaying the prescription of etiotropic therapy against *Klebsiella* spp. until the results of microbiological examination (on the 5-9th days after surgery) increased the chances of unfavorable outcome 2.2 times as compared to administration of etiotropic antibacterial therapy from the day of surgical intervention (p = 0.193; OR = 2.187; 95% CI: 0.764-6.258).

Patients with favorable outcome who took fluoroquinolones were 1.2 times more than those who did not, but their administration at the outpatient stage had no statistically significant effect on outcome (p = 0.473). However, administration of trimethoprim-sulfamethoxazole at the outpatient stage statistically significantly reduced the risk of unfavorable outcome (p = 0.038; V = 0.235). Also noteworthy was the correlation between trimethoprim-sulfamethoxazole prescription at the outpatient stage and the presence of polymicrobial association (p = 0.006; ρ = 0.295). The absence of antibiotic administration at the outpatient stage increased the odds of unfavorable outcome by 2.6 times (p = 0.111).

It was found that the need for revision surgery in the early postoperative period (p<0.001; OR = 15.86; 95% CI: 3.5-77.2), as well as repeated isolation of *Klebsiella* spp. strains in the postoperative period (p = 0.002; OR = 4.96; 95% CI: 1.72-14.29) significantly worsened the long-term treatment results (Table 6). There was a positive correlation of medium strength between the above-mentioned factors (p<0.001, ρ = 0.515). The presence or absence of hardware at the time of patient's discharge had no significant effect on the prognosis of the course of IAI (p = 0.307).

Table 6

Features of the postoperative period in the comparison groups, n (%)

Factor	Group 1 (n = 41)	Group 2 (n = 44)	Total, n	p	OR (95% CI)
Surgical intervention in the early postoperative period	18 (90%)	2 (10%)	20	<0.001*	15.8 (3.5-77.2)
Klebsiella spp. growth in the postoperative cultures	18 (75%)	6 (25%)	24	0.002*	4.96 (1.72-14.29)

* — differences are statistically significant (*p*<0,05).

DISCUSSION

Various factors influence the outcome of complex treatment of IAI, and one of the leading roles belongs to the etiology of the infectious process. According to M. Beck et al., the risk of recurrence after debridement surgery on the knee or hip joint is 25-67%, but the probability of an unfavorable prognosis depends on the etiologic agent [10]. In our study, we evaluated the outcome in patients with IAI caused by *Klebsiella* spp., and the probability of unfavorable outcome at two-year follow-up was 48%. This figure is consistent with the results of individual observations describing unfavorable outcomes of IAI caused by Gram-negative pathogens in an average of 50% of cases [6].

For many years, Gram-positive microorganisms (*S. aureus, S. epidermidis, E. faecalis, E. faecium*) have remained the leading causative agents of IAI [11, 12, 13]. Gram-negative microorganisms (representatives of *Enterobacteriaceae*, *Acinetobacter* spp., *P. aeruginosa*) total 17.5-18.6% of the spectrum of PJI pathogens [5]. The share of representatives of *Enterobacteriaceae* in the structure of the spectrum of orthopedic infection, according to the 6-year monitoring conducted by S.A. Bozhkova et al. in 2012-2020, increased from 6.6 to 8.7%, which is an extremely unfavorable trend [5]. According to A.V. Tsiskarashvili et al., it amounted to 8.2% for the period from 2015 to 2022 [14]. At the same time, the spectrum of pathogens depends on the continent: according to S. Sebastian et al. (India), the share of Gram-negative microorganisms in the spectrum of IAI pathogens reached 61% [15], which significantly differs from the data of European registers [18].

While the share of these microorganisms in the spectrum of IAI is relatively small, there are great difficulties associated with their eradication, as a result of which the infection itself often acquires a recurrent course [16]. The main reason is the development of various mechanisms of resistance to beta-lactam antibiotics and fluoroquinolones, for which reason the spectrum of antibacterial agents for their eradication is extremely narrow. In early 2017, carbapenemase-producing *Enterobacteriaceae* were included by the World Health Organization in the group of pathogens of critical priority, posing a serious threat to health and dictating the need for priority development of new drugs [17].

In the etiology of PJI, *K. pneumoniae* occupies one of the leading positions among Gram-negative microorganisms. According to a major study conducted by A. Papadopoulos et al. in 18 centers, dedicated to the evaluation of the treatment outcomes of patients with orthopedic infection caused by Gram-negative microorganisms with broad and multidrug resistance, *K. pneumoniae* was the etiologic agent in only 21.4% of cases, after *E. coli* and *P. aeruginosae* [16]. Russian researchers publish data indicating that this microorganism is predominant among *Enterobacteriaceae*: its share in the total spectrum of PJI pathogens for 2017 amounted to 5.5% [5]. The same authors draw attention to the fact that 6.8% of *K. pneumoniae* isolates identified in orthopedic patients in 2017-2019 produce various carbapenemases [19]. This group of patients represents a special problem, since, according to M.J. Schwaber et al., the infections caused by Gram-negative carbapenemase producers, especially representatives of *Enterobacteriaceae*, are associated with 50-90% lethality and cause one of the most serious problems for public health [20].

Analysis of the literature indicates that the risk factors for recurrent IAI are insufficiently studied [21]. Assessment of the outcomes of IAI caused by representatives of *Enterobacteriaceae* in general and *Klebsiella* spp. in particular is currently especially challenging. The majority of both Russian and foreign publications contain either individual clinical cases or descriptions of treatment of small samples of patients with this pathology. In our study, we evaluated the outcomes of treatment of IAI with bone and joint lesions of the lower and upper extremities caused by *Klebsiella* spp. in 85 patients who underwent various types of surgical interventions. This small number of patients refers to the limitations of the study; however, compared to similar studies, it represents a sufficiently large sample [18, 22].

Patients were allocated into two groups based on their outcome during a two-year follow-up period according to the Delphi criteria. A similar follow-up period was chosen by the research group led by E. Giannitsioti, who evaluated the influence of various parameters on the outcome of treatment of 57 patients with PJI caused by Gram-negative microorganisms (12% – *Klebsiella* spp.) [22].

The question of interpretation of the growth of microorganisms in postoperative cultures and the appearance of inflammation signs in laboratory tests in the early postoperative period has not been resolved to date. A number of authors stick to the opinion that this is a recurrence of IAI. However, by nature it is an exacerbation of infection, which is due to the presence of remaining pathogens and fragments of their biofilms in the area of surgical intervention and the bloodstream. It is caused by irrational or insufficient debridement, inadequate or insufficiently prolonged antibiotic therapy [21]. For this reason, we did not include those patients with *Klebsiella* spp. growth in the early postoperative period in the microbiological examination. At the same times, our study confirmed that this fact worsens the delayed treatment results ($p = 0.002$).

The duration of the infectious process in about half of the patients from both groups of our study was more than a year, with no statistically significant differences in this parameter ($p = 0.302$).

During the study, growth of *Klebsiella* spp. isolates with identical sensitivity from the wound area and from urine was detected in 13% of cases. This fact indirectly confirms the probability of hematogenous spread of urinary pathogens to prosthetic joints and the area of surgical intervention, as pointed out by other authors [23, 24].

The influence of the number of previous debridement surgeries on prognosis was shown both in our study ($p = 0.022$) and in studies by other authors, for example, E. Giannitsioti et al. However, in the same study, no statistical effect of the type of surgical intervention on the outcome of PJI caused by Gram-negative pathogens was shown (p = 0.292; OR = 1.902; 95% CI: 0.663-5.457) [22]. We obtained similar results in our work: in both groups, more than half of the patients underwent antimicrobial spacer placement, with hardware retaining debridement and muscle grafting ranking second and third, respectively.

Many researchers emphasize the fact that the spectrum of IAI pathogens depends on the localization of the infectious process. In particular, Chinese researchers Y. Tsai et al. concluded from their observation that Gram-negative microorganisms (in particular, *Enterobacteriaceae*) are more often the etiologic agent of PJI of the hip than of the knee [25]. Similar results were obtained in our study: the share of patients with hip joint infection was higher in both groups (54%). The knee joint was in the second place with a large lag (21%), and other localizations totalled 25%. However, the localization of IAI did not statistically affect the outcome of the disease (p = 0.241).

Hypoalbuminemia as a risk factor for unfavorable outcome in the case of both primary arthroplasty and IAI treatment has been described in the publications of various researchers [26, 27, 28]. In our study, we have found a statistically significant (p = 0.008) influence on the probability of recurrence of the infectious process over a two-year follow-up period of low serum albumin level at the patient's discharge from the hospital (on the 7-14th days after the surgical intervention). In our opinion, low albumin may adversely affect the metabolism of drugs, particularly antibiotics, which may reduce their efficacy and the treatment efficiency in general.

According to multiple researchers, treatment outcomes for IAI caused by Gram-negative microorganisms are generally worse than those caused by Gram-positive microorganisms. However, the prognosis is influenced, first of all, by the nature of the infectious agent, the presence of microbial associations and its sensitivity to the tested antibiotics [29]. It is noteworthy that in our study the polybacterial infection with *Klebsiella* spp. is 2.27 times more frequent than the monobacterial one. This fact is confirmed by the results of other authors [22].

According to A. Papadopoulos et al., in case of orthopedic infection caused by bacteria with multiple and broad drug resistance to antibiotics, the probability of favorable treatment outcome was significantly lower (p = 0.018) [18]. In our study, there was no statistically significant effect of antibiotic sensitivity of *Klebsiella* spp. on the outcome. However, a trend towards worse prognosis depending on the degree of resistance of the microorganism to the tested antibiotics was observed. Among patients in whom IAI was caused by *Klebsiella* spp. resistant to carbapenems, a favorable outcome was observed in only 41%.

Antibiotic therapy of orthopedic infection caused by *Klebsiella* spp. is a difficult task. In our study, the choice of antimicrobial drugs prescribed from the day of surgery is based on anamnesis data, results of microbiologic examination of material taken from the fistula, wound surface, joint cavity, tissue biopsy specimens, or hardware from previous surgeries. The exceptions were the patients who were admitted in a septic state, and antibiotics before surgery were prescribed for vital indications, or antibiotic therapy was administered from the day of the previous surgical intervention (17%). Patients with an unfavorable outcome were 1.5 times more likely to receive antibiotic therapy before surgery, but there was no statistically significant effect on outcome (p = 0.315).

Starting antibiotic therapy in 66% of patients was carried out with drugs to which the isolate of *Klebsiella* spp. identified in the patient remained sensitive, i.e. it was etiotropic. Adequate starting antibiotic therapy had no statistically significant effect on the outcome (p = 0.193). The share of patients with unfavorable outcome, who received etiotropic antibiotic therapy only after the results of intraoperative cultures (on the 6-9th days), was 8% higher than of those with favorable outcome. This allows us to conclude that a delay in prescribing an adequate antibiotic may be an additional predictor of treatment failure of periprosthetic infection.

According to current recommendations, antibiotic therapy at the outpatient stage should last at least 6-8 weeks. [30]. However, the choice of a specific antibacterial medication is one of the most difficult issues in the treatment of IAI caused by *Klebsiella* spp. The 6-year monitoring of the structure and resistance of the leading pathogens of PJI conducted by S.A. Bozhkova et al. showed that the resistance of *Klebsiella* spp. to ciprofloxacin was 84%, to moxifloxacin – 86%, and to trimethoprim-sulfamethoxazole – 76% [5]. In our study, only 8 patients (9.5%) had isolates sensitive to fluoroquinolones. The share of strains sensitive to trimethoprim-sulfamethoxazole is difficult to determine because sensitivity to this drug was not included in the standard panel and was determined only in the presence of resistance to two groups of antibacterial drugs (fluoroquinolones and cephalosporins) and, therefore, was not determined for all isolates.

Assessing the effect on outcomes of oral fluoroquinolones administered to patients at the outpatient stage, we did not reach the level of statistical significance ($p = 0.473$). A positive correlation between the probability of a favorable outcome and outpatient administration of trimethoprim-sulfamethoxazole was shown ($p = 0.038$). However, given the positive correlation between trimethoprim-sulfamethoxazole administration and the presence of a polymicrobial association in the patient, which included *Klebsiella* spp., it can be assumed that the favorable outcome was due to the antibacterial effect on other microorganisms in the association. According to some authors, this medication can be prescribed for oral administration at the outpatient stage as an alternative to fluoroquinolones (or along with them) in case of IAI caused by *Enterobacteriaceae* [6, 30]. We plan further studies of the effect of trimethoprim-sulfamethoxazole and fluoroquinolones on the outcome of complex treatment of IAI in a prospective study.

The most important statistically significant predictors of unfavorable outcome were revision surgery in the early postoperative period ($p<0.001$) and *Klebsiella* spp. growth in the postoperative cultures ($p = 0.002$). The presence of a direct correlation between these factors ($p<0.001$; $\rho = 0.515$) attracts attention, which allows us to conclude that insufficiently effective debridement of the focus of infection may lead to recurrence and another surgical intervention.

According to A. Papadopoulos et al., the presence of hardware is an independent risk factor for unfavorable outcome in the treatment of IAI caused by Gram-negative microorganisms (OR = 3.57; 95% CI: 1.68-7.58; $p<0.001$) [18]. However, these observations were not confirmed in our study.

CONCLUSIONS

According to the results of the study, there is a statistically significant direct correlation between the probability of unfavorable treatment outcome in patients with IAI caused by *Klebsiella* spp. and the number of debridement surgeries in the history, low serum albumin (g/l) on the 7-14th days after the operation, revision intervention in the early postoperative period, *Klebsiella* spp. growth in the postoperative cultures. The probability of a favorable outcome is increased by oral administration of trimethoprim-sulfamethoxazole at the outpatient stage.

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