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Analysis of the results of bacteriological study of wounds in patients with implant-associated spinal infection

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Introduction Results of bacteriological study of the pathological material taken from sinus exits and surgical wounds in 105 patients with implant-associated spinal infection (IAI) who were surgically treated at the bone infection clinic of the RISC for RTO in the period from 2011 to 2017 were analyzed. **Purpose** Identify the main pathogens of the IAI of the spine. **Materials and methods** Patients were divided into three groups: group 1 had fistulous forms of peri-implant infection: paravertebral and epidural abscesses and phlegmon (n = 18, subgroup 1); suppuration of soft tissues around metal structures (n = 20, subgroup 2); spondylitis / discitis / osteomyelitis (n = 26, subgroup 3), infectious complications associated with postoperative liquorrhea (n = 5, subgroup 4); group 2 suffered peri-implant infection that developed due to postoperative instability of the metal structure (n = 26); group 3 had infection that developed after implantation of the systems for chronic electrical stimulation (n = 10). Identification of the isolated bacterial cultures was carried out both by the traditional method and by using panels for gram-positive (PBBCS 20) and gram-negative microorganisms (NBC 44) on the WalkAway-40 Plus bacteriological analyzer (Siemens). **Results and discussion** According to the analysis of the bacteriological results, gram-positive microorganisms (65.7 %) significantly prevailed among the pathogens of implant-associated spinal infection, of which the major ones were staphylococci (52.9 %). Isolation of *S. aureus* was 34.8 %. Among the methicillin-resistant strains, *S. epidermidis* was dominant. Among gram-negative microorganisms, both non-fermentative (16.8 %) and enterobacteria (20.9 %) were found. Most frequently identified non-fermenting bacteria were *P. aeruginosa* and *A. baumannii*. Enterobacteria were mainly represented by strains of *K. pneumoniae* and *E. coli*. Mixed cultures, consisting of two or more microorganisms, were significant. **Conclusion** Difficulties in the treatment of IAI of the spine require the development of methods for predicting these complications and measures to reduce them. Microbiological monitoring is an integral part of infection control in hospitals, where patients with IAI of the spine are treated. Timely identification of infection pathogens enables to choose correct antibiotic therapy and arrest the infection process in a short time.
Keywords: implant-associated spinal infection, microbial panel, associations of microorganisms, biofilms

INTRODUCTION

To date, the treatment of implant-associated infection (IAI) of the spine is challenging [1, 2]. IAI develops due to several reasons: violation of implant sterility; non-compliance with aseptic and antiseptic rules during surgery; adsorption on the implant of bacterial cells circulating in the blood and lymph; improper use of antibacterial agents and antibiotics, etc. [3, 4].

According to published data, staphylococci colonize on the surfaces of implants most frequently: *Staphylococcus aureus, Staphylococcus epidermidis*, etc., which are present in the normal human microflora [5, 6]. Adhesion of microorganisms on the surface of implants promotes the formation of biofilms [7]. Microorganisms that compose such conglomerates become resistant to aggressive environmental factor and reduce the effectiveness of antibiotic therapy. The growth of biofilms on implant surfaces results in the development of tissue necrosis around the implant and further spreading of infection [1]. Removal of spinal instrumentation, on the one hand, enables to thoroughly debride the area of surgical intervention, but on the other hand, is associated with the risk of destabilizing of the spine, which will lead to deterioration in the patient's quality of life.

Despite the diversity of the methods proposed, there is no differentiated approach to the treatment of patients with spinal IAI. There are few works devoted to the analysis of the results of microbiological studies of the affected areas of the spine, depending on the location of the infectious process and the characteristics of the disease course.

The aim of our work was to analyze the microbial panel of spinal IAI and to identify its main pathogens.

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MATERIAL AND METHODS

The results of a microbiological study of the pathological material harvested from sinus exits and surgical wounds in 105 patients with implant-associated infection of the spine who were surgically treated at the purulent osteology clinic of the RISC for RTO in the period from 2011 to 2017 were analyzed.

Patients were divided into three groups: group 1 had fistulous type of peri-implant infection: paravertebral and epidural abscesses and phlegmon (n = 18, subgroup 1); suppuration of soft tissues around metal structures (n = 20, subgroup 2); spondylitis/discitis/osteomyelitis (n = 26, subgroup 3), infectious complications associated with postoperative liquorrhea (n = 5, subgroup 4); group 2 suffered peri-implant infection that developed due to postoperative instability of metal structured (n = 26); group 3 had infection that developed after implantation of the systems for chronic electrical stimulation (n = 10).

Identification of bacterial cultures was carried out both by the traditional method and by using panels for gram-positive (PBBCS 20) and gram-negative microorganisms (NBC 44) on the WalkAway-40 Plus bacteriological analyzer (Siemens).

RESULTS AND DISCUSSION

Bacteriological study of smears taken from the sinus exits and wounds of patients with implant-associated spinal infection, 196 isolates belonging to 19 taxa were identified: *Staphylococcus aureus* (n = 65), *Staphylococcus epidermidis* (n = 26), *Staphylococcus haemolyticus* (n = 3), *Staphylococcus saprophyticus* (n = 3), *Staphylococcus hominis* (n = 1), *Staphylococcus simulans* (n = 1), *Enterococcus faecalis* (n = 17), *Streptococcus sp*. (n = 3), *Corynebacterium sp*. (n = 3), *Bacillus sp*. (n = 1), *Pseudomonas aeruginosa* (n = 17), *Acinetobacter baumannii* (n = 15), *Acinetobacter lwoffii* (n = 1), *Enterobacter cloacae* (n = 10), *Klebsiella pneumoniae* (n = 11), *Escherichia coli* (n = 10), *Morganella morgannii* (n = 1), *Proteus mirabilis* (n = 7), *Proteus vulgaris* (n = 1).

In a subgroup of patients with a fistulous type of peri-implant infection such as paravertebral and epidural abscesses and phlegmon (n = 18), the microbiological study of pathological material identified 39 strains of microorganisms in four individuals, among which there were 8 associations: *P. aeruginosa* + *A. baumannii* + *E. faecalis, S. aureus* + *S. epidermidis MRSE* (methicillin-resistant staphylococcus) (n = 2), *E. cloacae* + *S. haemolyticus MRSH, S. pyogenes* + *S. aureus, A. baumannii* + *E. faecalis, S. saprophyticus MRSS* + *E. faecalis* + *A. baumannii, P. aeruginosa* + *E. coli*, the remaining microorganisms were monoculture (n = 21) в монокультуре: *S. aureus* (n = 9)*, S. epidermidis* (n = 5, including 4 – *MRSE*), *S. haemolyticus MRSH* (n = 1), *S. saprophyticus MRSS* (n = 1), *P. mirabilis* (n = 3), *E. cloacae* (n = 1), *M. morgannii* (n = 1). Bacteriological cultures of three patients did not show growth of pathogen microflora.

Ten associations were identified in the smears taken from the sinus passages and from the wounds of patients with implant-associated infection presenting as suppuration of soft tissues around metal structures (n = 20): *P. mirabilis* + *E. coli, P. mirabilis* + *P. aeruginosa, S. aureus* + *E. faecalis* (n = 2), *E. cloacae* + *S. epidermidis MRSE, P. aeruginosa* + *S. aureus, K. pneumoniae* + *S. epidermidis MRSE, Streptococcus sp.* + *S. hominis MRSH*, 15 isolates in monoculture: *S. aureus* (n = 6, including 1 – *MRSA*), *S. epidermidis MRSE* (n = 1), *E. faecalis* (n = 2), *A. baumannii* (n = 1), *E. coli* (n = 1), *K. pneumoniae* (n = 2), *Corynebacterium sp.* (n = 1), *Bacillus sp.* (n = 1). No pathogenic microflora was found in smears of four patients.

In patients of the third subgroup (spondylitis/discitis/osteomyelitis), 10 associations of microorganisms were isolated: *S. aureus MRSA* + *P. aeruginosa* (n = 2), *P. aeruginosa* + *E. coli, P. aeruginosa* + *A. baumannii, K. pneumoniae* + *A. baumannii* (n = 2), *E. coli* + *K. pneumoniae, A. baumannii* + *S. epidermidis MRSE, K. pneumoniae* + *E. faecalis, S. epidermidis* + *E. faecalis*, 30 strains as monoculture: *S. aureus* (n = 19, with 5 – *MRSA*), *S. epidermidis MRSE* (n = 2), *E. faecalis* (n = 1), *Corynebacterium sp.* (n = 1), *A. baumannii* (n = 1), *E. cloacae* (n = 3), *P. aeruginosa* (n = 1), *P. vulgaris* (n = 1). *E. coli* (n = 1).

In patients with peri-implant infection with infectious complications associated with postoperative liquorrhea (n = 5), two associations were identified according to the results of the microbiological study: *K. pneumoniae* + *E. faecalis, E. cloacae* + *S. simulans*, 7 isolate as monoculture: *S. epidermidis* (n = 2, out of which one was *MRSE*), *Streptococcus sp*. (n = 1), *A. baumannii* (n = 1), *E. cloacae* (n = 2), *S. haemolyticus* (n = 1).

Eleven associations were isolated in IAI group 2 (n = 26) developed due to postoperative instability of the metal structures: *S. aureus* + *S. epidermidis* (n = 3, including 1 *MRSE*), *S. aureus* + *A. baumannii, P. aeruginosa* + *S. aureus, K. pneumoniae* + *S. aureus* + *E. faecalis, S. aureus* + *P. mirabilis* + *E. faecalis, P. aeruginosa* + *E. coli, S. epidermidis* + *E. coli* + *E. cloacae, P. aeruginosa* + *A. baumannii* + *E. faecalis, P. mirabilis* + *A. baumannii* + *E. faecalis*, 25 microorganisms as monoculture: *S. aureus* (n = 10, out of which 3 was *MRSA*), *S. epidermidis* (n = 5, out of which 2 – *MRSE*), *E. faecalis* (n = 1), *Corynebacterium sp*. (n = 1), *P. aeruginosa* (n = 3), *A. baumannii* (n = 2), *K. pneumoniae* (n = 1), *E. coli* (n = 2). The growth of pathogenic microflora was absent in one person.

In the third group of patients (n = 10) with an infection that developed after implantation of systems for chronic electrical stimulation, 3 associations were found in the studied smears: *S. aureus* + *S. epidermidis, S. aureus* + *A. lwoffii, P. aeruginosa* + *S. saprophyticus*, four strains as monoculture: *S. aureus* (n = 3), *P. aeruginosa* (n = 1). In 2 patients, no pathogenic microflora was found in their bacteriological material.

Gram-positive microorganisms prevailed among the causative infectious agents in the first group, mainly *S. aureus* (33.3 %) and *S. epidermidis* (12.7 %) (Table 1). The numbers of *MRSA* and *MRSE* were 8 and 13, respectively. Among other *CoNS* (coagulase-negative staphylococci), there was high percentage of methicillin-resistant strains. Both non-fermentative (15.1 %) and enterobacteria (23.7 %) were found among the gram-negative microflora, and, mainly, enterobacteria were in monocultures. Gram-negative organisms were most often found in associations with gram-positive bacteria (60.7 % of all associations).

Gram-positive microorganisms, mainly *S. aureus* (34 %), prevailed among the causative agents of infection in the second study group. The ratio of MRSA to the total number of isolated strains of *S. aureus* was 1:6, and the *MRSE* to *S. epidermidis* was 1:3. A high percentage of isolation of strains of *Enterococcus sp*. was among gram-negative microorganisms, the number of enterobacteria (18 %) and non-fermentative bacteria (18 %) was equal. Ninety percent of associations were mixed cultures of gram-positive and gram-negative microorganisms.

In the third study group, *S. aureus* prevailed among the causative agents of infection (54.5 %), non-fermentative bacteria accounted for 27.3 % of gram-negative microorganisms. The remaining microorganisms were found in single quantities.

According to the analysis of the results of bacteriological study, gram-positive microorganisms (65.7 %) significantly prevail in the structure of the pathogens of the implant-associated spinal infection, of which staphylococci is a majority (52.9 %). The percentage of *S. aureus* isolation is 34.8 %. Among the methicillin-resistant strains, *S. epidermidis* dominates. Gram-negative microorganisms, both non-fermentative (14.7 %) and enterobacteria (14.8 %), are found. Non-fermentative bacteria are most often isolated in associations, and enterobacteria, as a rule, in monoculture or in association with gram-positive microorganisms.

Table 1

Rates of microorganisms isolated, %

Микроорганизм	Group 1 (n = 69)	Group 2 (n = 26)	Group 3 (n = 10)
S. aureus	33.3 (6.4 % *MRSA*)	34 (6 % *MRSA*)	54.5
S. epidermidis	12.7 (10.3 % *MRSE*)	18 (6 % *MRSE*)	9.1
Прочие *CoNS*	5.6 (5 % *MRCoNS*)	–	9.1
Enterococcus sp.	5.6	10	–
Streptococcus sp.	1.6	–	–
Other gram-positive microorganisms	2.4	2	–
Non-fermentative gram-negative microorganisms	15.1	18	27.3
Enterobacteria	23.7	18	–

n – number of strains isolated

Currently, bacteria of the genus *Staphylococcus* are the leading pathogens of nosocomial infections [6]. It is believed that infection caused by staphylococci prevails not so much due to their virulence as due to a large number of susceptible patients with weakened immunity. The appearance of coagulase-negative staphylococci among pathogens is associated with the widespread use of implants, artificial joints, vascular grafts, urinary catheters, etc. [5, 6].

Along with staphylococci, the *Enterobacteriaceae, Pseudomonadaceae* and *Moraxellaceae* families play an important role in the etiology of purulent diseases. For example, strains of *P. aeruginosa, Acinetobacter spp., K. pneumoniae, Enterobacter sp.* belong to the most common pathogens of intra-hospital infection [8, 9]. At the same time, P. aeruginosa, Acinetobacter spp., K. pneumoniae, Enterobacter sp. have a high affinity for biofilm formation [9, 10].

Microorganisms in bacterial associations can significantly influence the course of the infectious process by their joint pathogenic ability and mutual enhancement of the virulence of the associates. In a study of the biofilm-forming ability of the strains of *K. pneumoniae* and *S. aureus*, it was shown that the adhesive activity of a mixed culture (*K. pneumoniae* + *S. aureus*) is higher as compared to monocultures of these microorganisms [11]. According to published data, the number of agents resistant to antibiotics increases in mixed cultures [10, 11]. Standard antibiotic therapy may not bring proper results in the fight against mixed infection and biofilm microorganisms. On the contrary, irrational administration of etiotropic therapy can lead to suppression of the main factors of specific and nonspecific defense of the body, which, ultimately, will contribute to the attachment of a new infectious agent and secondary infection of the body.

The study showed that the leading role in the structure of the causative agents of implant-associated spinal infection belongs to staphylococci, mainly coagulase-positive. High percentage of methicillin-resistant strains of epidermal staphylococcus was found. Among non-fermenting bacteria, *P. aeruginosa* and *A. baumannii* are most often frequent. Enterobacteria are mainly represented by strains of *K. pneumoniae* and *E. coli*. Mixed cultures, consisting of two or more microorganisms, have a big portion. The causative agents of IAI of the spine can be both monocultures of microorganisms and bacterial associations.

CONCLUSION

Difficulties in the treatment of IAI of the spine require the development of methods predicting these complications and measures to reduce them. Microbiological monitoring is an integral part of infection control in hospitals, where patients with IAI of the spine are treated. Timely identification of infection pathogens enables to choose correct antibiotic therapy and to arrest the infection process in a short time.

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