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MICROBIOLOGICAL PARAMETERS IN PATIENTS WITH INFLAMMATORY COMPLICATIONS AFTER KNEE AND HIP JOINTS ENDOPROSTHESIS REPLACEMENT AND THEIR DIAGNOSTIC EVALUATION

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Introduction

Presently in the inflammatory joint diseases diagnosis and treatment microbiological examination plays a leading role. This is due to the infectious diseases frequency general increase, the hospital infections incidence rise risk, the widespread use of antimicrobial agents in medical practice and the change in the infectious foci microbiocenosises structure. Microbiological and, if necessary, serological studies of articular fluid are fundamental components of diagnosis and inflammatory joint diseases effective treatment selection [1].

Etiological agents of inflammatory processes in joints can be microorganisms of different groups. According to the literature, up to 80% of bacterial arthritis cases cause gram-positive cocci, among which *S.aureus* predominates. At the same time, the number of methicillin-resistant strains of this pathogen (MRSA) increases annually. Less commonly, from the affected joints β-hemolytic streptococcus group A and other groups streptococcus, gram-negative rods, microscopic fungi and anaerobic bacteria are isolated [2].

In view of the microorganisms biological nature characteristic, microbiological studies do not always make it possible to isolate the causative agent of infection. A major problem in bacteriological diagnostics is the periprosthetic and hematogenic infections low-grade causative microorganism, as well as subacute and chronic processes course presence. These include coagulase-negative staphylococcus (for example, *Staphylococcus epidermidis*) and anaerobic bacteria. Diagnostic and therapeutic difficulties can also be due to the pathogens ability to form antimicrobial therapy resistant microbial biofilms [3, 4, 5].

It is reported that an antibacterial drugs uncontrolled intake, the biofilms formation, errors in the collection and transportation of biological material, can cause a situation when the joint infection infectious agent can not be detected in approximately (10-20)% of the cases [6].

The inflammatory joint diseases microbiological diagnostics is a complex procedure, and a single standard for its implementation has not been developed to date. The "gold standard" of microbiological diagnostics is the causative agent isolation and identification in culture from the joint fluid and the samples of periprosthetic tissues. The effectiveness of the bacteriological diagnostic method can be improved by using various methods of biofilms formed on the prosthetic implant components destruction. In order to accelerate the diagnostic testing and to specify the results it is possible to conduct molecular diagnostic procedures [4, 6, 7].

The purpose of the studies was to identify the microbial spectrum of the synovial fluid in patients with hip and knee joints inflammatory diseases and to determine the isolated bacteria sensitivity to antibacterial drugs.

Materials and methods

The material for the studies were synovial fluid samples collected from 64 patients of the SE "Sytenko Institute of Spine and Joint Pathology, NAMS Ukraine" clinic. The patients' diagnosis were status after knee and hip joints endoprosthesis replacement with inflammatory complications. The biological material was tested in the 2015-2017 period.

The synovial material collection was conducted by the attending physician by the joint puncture method. The articular fluid withdrawn into the syringe was immediately got to a microbiological laboratory. The biological samples inoculation was carried out into a fluid thioglycollate storage medium, then to obtain the aerobic and facultative-anaerobic microorganisms pure cultures the isolate passage were conducted to Columbia blood agar, salt agar and Endo medium.

Further isolated microorganisms identification was performed by standard methods in accordance with current guidelines [8].

The microorganisms cultures were observed for 14 days. In the absence of microflora's growth, a preliminary negative result for all synovial material was given after 5-7 days. If there was a based on the disease anamnesis and clinic suspicion on the slowly growing pathogens presence the timing of the studies was increased.

The isolates sensitivity to antimicrobial agents was determined by the disc-diffusion method [9].

In determining the microorganism's sensitivity 29 antibacterial drugs from 8 chemical groups were used: β-lactams, fluoroquinolones, macrolides, aminoglycosides, tetracyclines, lincosamides, glycopeptides, oxazolidinones, glycylcyclines.

Results and discussion

As a result of the microorganisms' identification, 68 cultures of facultative-anaerobic bacteria and microscopic fungi were isolated from the joint fluid (Figure 1).

82.3% of bacterial isolates were obtained in monoculture (n = 56) (Figure 2). Of these, 25.0% of the cultures (n = 14) were staphylococcus species with ability to coagulate the blood plasma (*S. aureus* (n = 9), *S. intermedius* (n = 5)), other staphylococcus isolation rate was 60.7% (*S. epidermidis* (n = 21), *S. haemolyticus* (n = 9), *S. simulans* (n = 4)). Pathogenic streptococcus species was isolated from 5.4% of the samples (*S. pyogenes* (n = 3)). The Gram negative bacteria *K. pneumonia* cultures were isolated from 8.9% of biological material samples (n = 5).

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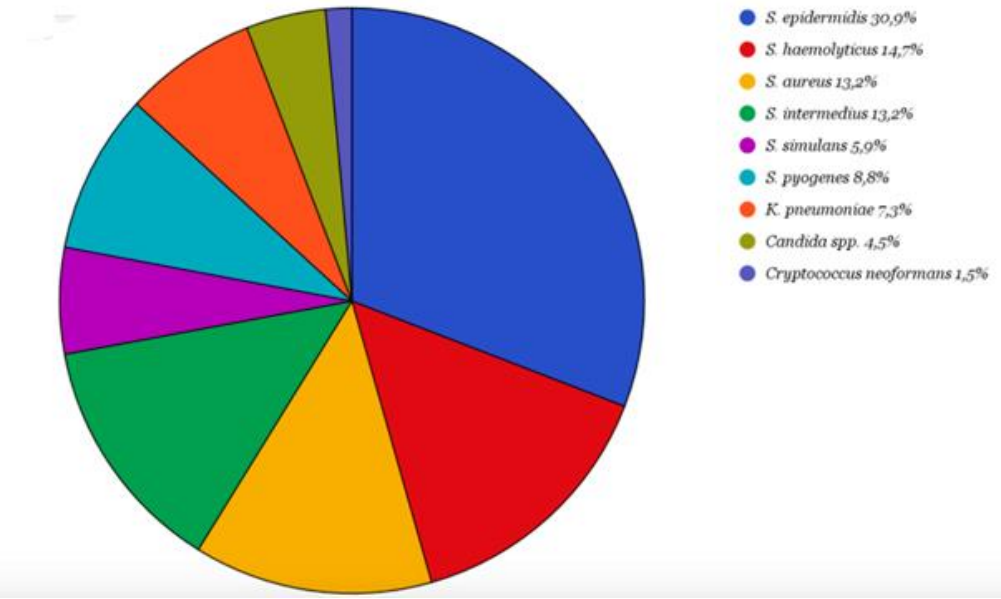

Figure 1. Microorganisms isolated from articular fluid quantitative ratio,%

Mixed microcenosises were detected in 6 samples of the biomaterial. The cultures associations consisted of two microorganisms species with the associations *S. intermedius* - *S. pyogenes*, *C. lusitania* and C. *neoformans* (n = 4) prevailing. Two other microbiocenoses were represented by *Candida* fungi with *S. pyogenes* and *S. haemolyticus*.

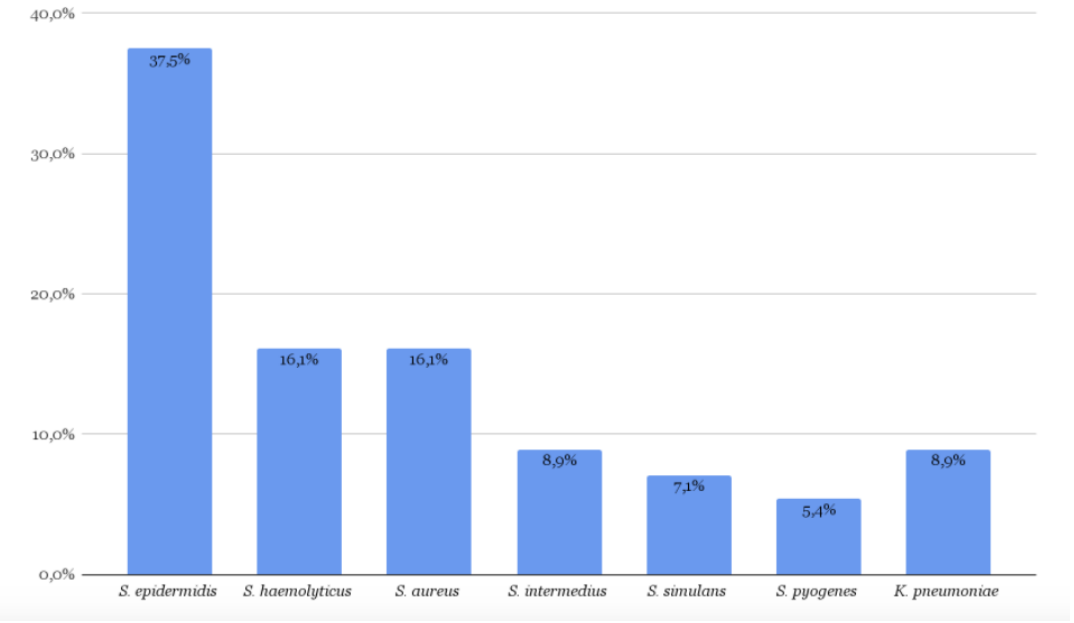

Figure 2. The proportion of microorganisms isolated in monoculture, %

Thus, in case of knee and hip joints inflammatory diseases, the synovial fluid microflora is mainly represented by Gram-positive bacteria, of which 77.9% are five species of staphylococcus. Coagulase-positive staphylococcus species *S. aureus* and *S. intermedius* are 24.1% of them.

Mixed articular fluid microcenosises are identified in 9.4% of patients. They are mainly represented by associations of gram-positive cocci with microscopic fungi of the *Candida* and *Cryptococcus* genera.

The bacterial cultures sensitivity to antimicrobial agents analysis showed that 100% of *S. aureus* isolates (n = 9) were sensitive to linezolid, levofloxacin and the ceftriaxone-sulbactam combination. At the same time, ceftriaxone showed an inhibitory effect only on 55.5% of isolates.

On average, the combination of third generation cephalosporins with sulbactam against pathogenic staphylococcus was 2.7 times more active than the corresponding cephalosporins.

77.8% of pathogenic staphylococcus isolates were sensitive to tigecycline, and 74.6% of cultures to 7 fluoroquinolone group preparations.

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The degree of an aminopenicillins activity was 25%. Amoxicillin and amoksiklav showed the same activity in relation to 44.4% of cultures.

8 out of 9 *S. aureus* isolates (88.9%) were resistant to benzylpenicillin, ampicillin and cefixime.

S. intermedius (n = 9), the second species of coagulase-positive staphylococcus, showed the greatest sensitivity degree to meropenem and linezolid - 8 out of 9 cultures (88.9%).

Aminoglycosides were less active, only 72, 2% of the isolates were susceptible to them. Fluoroquinolones and tigecycline inhibited the growth of only 66.7% *S. intermedius* cultures.

The lowest activity had cefixime and erythromycin, to which 77.8% of isolates were resistant.

The skin normoflora resident *S. epidermidis* (n = 21) under vegetation in the articular fluid is very difficult to be eliminated. Of all the 28 drugs tested, only linezolid and tigecycline exerted the inhibiting effect on 90.5% of the cultures. Aminoglycosides showed activity against 83.3% of isolates. The activity of cephalosporins, amoxiclav and fluoroquinolones was 26.2%, 19.5%, and 14.3%, respectively.

According to our data, all isolates of epidermal staphylococcus were resistant to aminopenicillins.

The antimicrobial preparations activity degrees varied for the coagulase-negative staphylococcus *S. haemolyticus* (n = 10) and *S. simulans* (n = 4).

All isolates of hemolytic staphylococcus were sensitive to linezolid. Aminoglycosides exerted an effect on 80.0% of the cultures, with amikacin maximal activity, which inhibited the growth of 90% of the isolates. Fluoroquinolones had a suppressive effect on the growth of 67.1% of isolates. Cephalosporins inhibited the growth of only 30% of cultures, while 9 of 10 isolates were resistant to cefixime.

We obtained only 4 *S. simulans* isolates. All isolates grew in monoculture and were sensitive to gentamicin. The other groups antimicrobial preparations majority activity level was about 50%.

Tigecycline and vancomycin were most active against *S. pyogenes* (n = 6). The 83.3% of pyogenic streptococcus isolates had sensitivity to them. Linezolid was active against 66.7% of cultures, lincomycin - 50% of isolates.

The β-lactam preparations (inhibitor-protected aminopenicillin - amoxiclav, cephalosporins, and carbapenems) had the minimum activity at a rate of 16.7%. None of the different groups 28 drugs tested showed 100% inhibitory activity against S. pyogenes All K. pneumoniae isolates (n = 5) were sensitive to gentamicin. Aminoglycosides showed maximal activity against 90% of isolates. 80% of isolates were sensitive to carbapenems, 65.7% to fluoroquinolones.

Of all the 19 antibacterial drugs investigated, the second and third generations cephalosporins had the lowest active against 35.0% of the *K. pneumoniae* cultures.

All gram-negative rods isolates were resistant to cefixime and ceftriaxone and cefoperazone combination with sulbactam.

Thus, it was found that in the joint fluid in case of knee and hip joints inflammatory diseases, monocultures of gram-positive cocci are predominant. *S. epidermidis* and *S. haemolyticus* are prevalent.

These species isolates show a different degree of resistance to β-lactam antibiotics, which indicates the presence of β-lactamases. Some cultures are also resistant to inhibitor-protected aminopenicillins and the third generation cephalosporins combinations with sulbactam. Such strains may present certain difficulties in the patients treatment.

β-lactams also did not have high efficacy against *S. pyogenes*.

The most active drugs for gram-negative rods species *K. pneumoniae* infection elimination are aminoglycosides, carbapenems and fluoroquinolones. In this case all cultures were sensitive to gentamicin and showed resistance to cefixime and the third generation cephalosporins combinations with sulbactam.

Of particular concern is the presence of resistance to vancomycin in 44.4% of isolates of coagulase-positive staphylococcus, including 28.6% of the *S. aureus* isolated strains and 28.5% of other staphylococcus species cultures and 16.7% of *S. pyogenes* isolates.

The antimicrobial agents effectiveness against different systematic groups bacterial isolates determination results analysis allows us to conclude that the most effective drugs for gram-positive cocci are (Figure 3): linezolid, to which 88.1% of the studied isolates are sensitive, including all *S. aureus* and *S. haemolyticus* cultures, and tigecycline a preparation from the glycylcyclin group, which has activity against 78.0% of gram-positive cocci isolates. The estimated aminoglycosides efficacy is 73.7%. The fluoroquinolones, carbapenems and the third generation cephalosporins with sulbactam combinations antimicrobial activity is manifested for 50% of all isolates obtained. About 30% of cultures were sensitive to lincomycin and the third generation cephalosporins - ceftriaxone, cefixime and cefoperazone.

Conclusions

According to the research results obtained, the following conclusions were made.

1 Microflora isolated from synovial fluid in case of the knee and hip joints is inflammatory diseases is represented by gram-positive cocci (86.8%) in most cases, gram-negative rods amount is 7.3% and fungi of *Candida* and *Cryptococcus* genera are made 5.9%.

2 The isolated microorganisms species antimicrobials sensitivity is characterized by individual diversity with a tendency to vancomycin resistance increasing in 44.4% of coagulase-positive staphylococcus isolates, of which 28.6% are *S. aureus* strains; 28.5% o are other staphylococcus species cultures and 16.7% are *S. pyogenes* isolates. This indicates the exactly appropriate antibiotic therapy conducting necessity

3 When choosing antibiotic therapy in patients in case of coxarthrosis and gonarthrosis it is recommended to take into account the bacterial isolates antibiotic resistance formation actual trends.

Consequently, the knee and hip joints inflammatory processes treatment success is largely determined by timely and effective microbiological diagnostic. Bacteriological testing of synovial fluid allows to identify the pathogen in the culture and to determine its

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sensitivity to antibacterial drugs, which is necessary for the therapeutic approach correct choice.

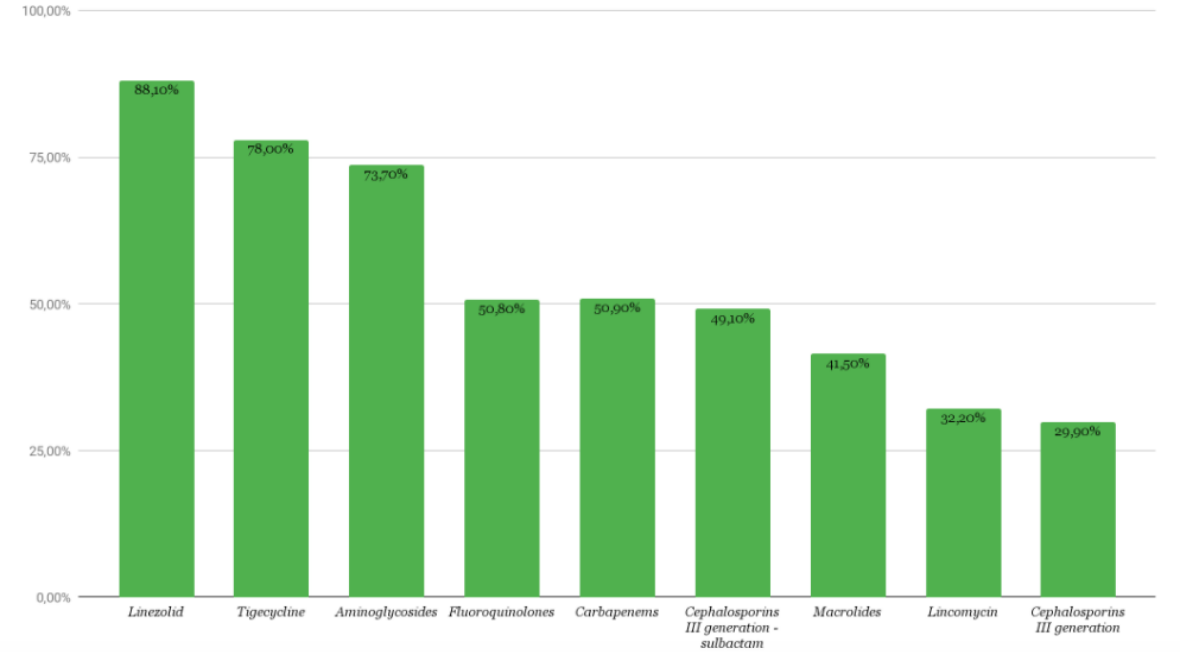

Figure 3. The gram-positive cocci sensitivity to antimicrobial agents, % of isolates

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Introduction. Presently in the inflammatory joint diseases diagnosis and treatment microbiological examination plays a leading role. This is due to the infectious diseases frequency general increase, the hospital infections incidence rise risk, the widespread use of antimicrobial agents in medical practice and the change in the infectious foci microbiocenosises structure. Microbiological and, if necessary, serological studies of articular fluid are fundamental components of diagnosis and inflammatory joint diseases effective treatment selection. Etiological agents of inflammatory processes in joints can be microorganisms of different groups. According to the literature, up to 80% of bacterial arthritis cases cause gram-positive cocci, among which *S.aureus* predominates. At the same time, the number of methicillin-resistant strains of this pathogen (MRSA) increases annually. Less commonly, from the affected joints β-hemolytic streptococcus group A and other groups streptococcus, gram-negative rods, microscopic fungi and anaerobic bacteria are isolated. In view of the microorganisms biological nature characteristic, microbiological studies do not always make it possible to isolate the causative agent of infection. A major problem in bacteriological diagnostics is the periprosthetic and hematogenic infections low-grade causative microorganism, as well as subacute and chronic processes course presence. These include coagulase-negative staphylococcus (for example, *Staphylococcus epidermidis*) and anaerobic bacteria. Diagnostic and therapeutic

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Keywords: periprosthetic infections, synovial fluid, microbiological examination

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