

## Monitoring of Microbiological Flora Antibiotic Resistance in Oncohematological Patients

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### Abstract

At present, the resistance of microorganisms is confirmed for different classes of antibiotics applied for various infectious diseases treatment. Problem of multiple resistance may ultimately lead to fatal consequences. In this regard, the description of the microflora taxonomic structure in oncohematological patients during the period of 2017 with the detection of the resistance to the antibacterial drugs is of particular relevance. The taxonomic structure of various biotopes microflora in oncohematological patients is presented in the article. Received data indicate that the main causative agent of infectious complications in patients was gram positive bacteria. All types of isolated microorganisms revealed the greatest sensitivity to antibiotics of the carbapenem class and high sensitivity to the 3rd generation cephalosporins and fluoroquinolones.

**Keywords:** Antibiotic resistance; Biotope; Infectious complications; Microbial flora; Taxonomic structure

### Introduction

the problem of antibiotic resistance has existed for many years, but due to the irrational, simultaneous use of several classes of antibiotics becomes even more actual [1-3]. It is known that resistance of microorganisms to antibiotics can be original and acquired [1,4]. In clinical practice, the greatest problem is acquired resistance - the ability of individual strains of bacteria to remain viable when used concentrations of antibiotics suppress the bulk of the microbial population. The formation of acquired resistance in bacteria does not obligatory result in decrease of the clinical efficiency of the antibiotic [5-7]. Resistance formation, in all cases, is based on genetic factors: acquiring new genetic information, alteration of host gene expression level. The basic reasons of antibiotic resistance are as follows: uncontrolled antibiotic prescription, incorrect antibiotic therapy, incorrect doses of medications, the wrong combined therapy, incorrect therapy duration. However, in general, the formation of antibiotic resistance is associated with biochemical mechanisms developed during evolution. There are following ways of antibiotic resistance realization in bacteria: modification of the antibiotic action target, inactivation of the antibiotic itself by bacteria, decrease in permeability of bacterial cells, formation of new metabolic

pathways and active excretion of antibiotic from the bacterial cell. Different bacteria are characterized by their own mechanisms of resistance formation [8,9].

Currently, resistance of microorganisms is registered to antibiotics used for the treatment of meningitis, sexually transmitted diseases, hospital infections and even to a new class of antiretroviral drugs used for the treatment of HIV infection. This is a problem that affects both highly developed and industrialized countries, as well as developing countries, and ultimately poses a threat to humanity as a whole. Similar situation regarding antibiotic resistance is observed in the Scientific Center of Pediatrics and Pediatric Surgery. Our center treats patients with such diseases as hemoblastosis, oncology, orphan diseases, primary immunodeficiency, which require prolonged use the various classes antibacterial drugs. Thus, proceeding from the above mentioned.

Our work was aimed to determine the taxonomic structure of different biotopes microflora in oncohematological patients with the detection of resistance to antibacterial drugs for the period of 2017.

### Materials and Methods

Studies were performed in the microbiological laboratory of Scientific center of pediatrics and children surgery. 2.204 samples, isolated from different biotopes of oncohematological patients, were investigated in 2017. The following routine microbiological

methods of isolation were used: direct seeding and enrichment, with differential-diagnostic elective nutrient media for each group of microorganisms. Biochemical identification was executed using routine schemes and Berdshi classification keys [10]. Identification of microorganisms and determination of their antibiotic susceptibility was carried out both on the automatic bacteriological analyzer “Walkway” and by the disk-diffusion method. The evaluation of antibiotic resistance results was carried out according to the tables recommended by the WHO NCCLS using computer processing program WHONET-5 [11,12].

## Results

The number of studied tests (throat swabs, exudate from the wound and from the nose) made up 2,204 units. 1,420 (64.4%) samples gave a positive result, i.e. the presence of pathogenic/conditionally pathogenic microflora was shown, while the number of negative results (sowings did not reveal the presence of microorganisms) made up - 784 (35,6%). Opportunistic gram-positive *Streptococci* were detected in the most of the positive seeding - 911 (64,2%). The distribution by different types of gram-positive *Streptococci* was as follows: in the maximum percentage of cases (52,8%) *Str. dysgalactae* (group b-hemolytic *Streptococci*) was detected, then *Str.spp*-22,8%; *Str. saliv.* – 21,0% (a-hemolytic group) (Figure 1).

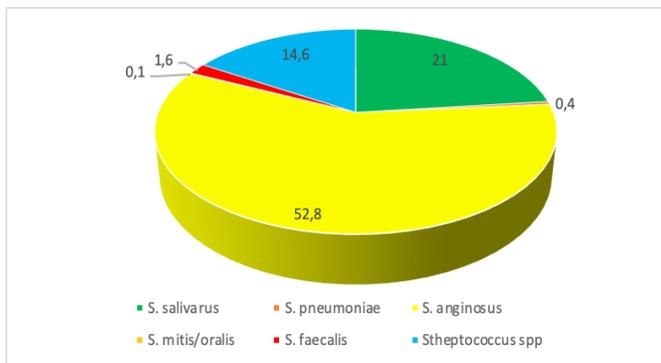


Figure 1: Distribution of *streptococci* by type.

Analysis the antibiotic sensitivity of these microorganisms, it should be noted that the complete resistance to all used in hospital antibiotics was determined in 54 patients (5,9%).

Total antibiotic resistance may be caused by the empirical prescription of drugs as well as the prolonged duration of therapy courses due to severity of the pathology (severe cytopenia after chemotherapy, etc.). Interesting in our opinion, is the fact that a more pronounced antibiotic resistance by *Streptococci* was detected when combined with the growth of fungal flora. The most obvious reason, is the development of immunosuppressive condition due to the use of both cytostatics and long-term courses of combined antibacterial therapy. The high sensitivity *Streptococci* were

detected to Vancomycin- in 642 cases (70,5%), Ceftriaxone in 624 (68,5%), ofloxacin - 594 (65,2%), carbopenems – 58,2% (Figure 2).

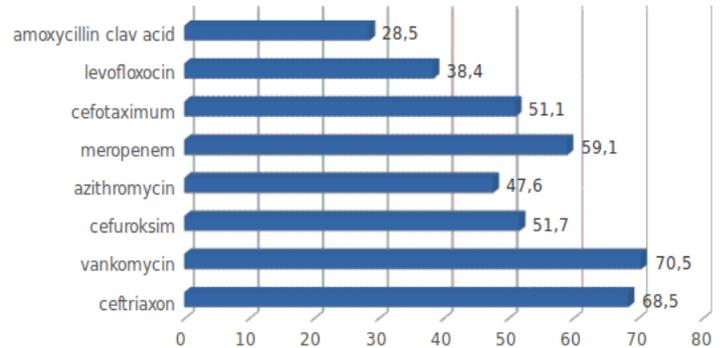


Figure 2: *Streptococcus* sensitivity to antibiotics.

Azithromycin, levofloxacin and amoxicillin were active against *streptococci* in a lower percentage of cases - 47,6%, 38,4% and 28,5%, correspondingly. The high activity of vancomycin may be explained by not so frequent use of this antibiotic, compared to other medications, as it shows the effect on a more limited range of microorganisms. Analyzing the frequency of antibiotic resistance, it was shown that in 491 samples (54,2 %) *Streptococci* were resistant to levofloxacin, in 414 cases (45,4%) - to azithromycin and in 363 (39,8%) - cefuroxime (Figure 3).

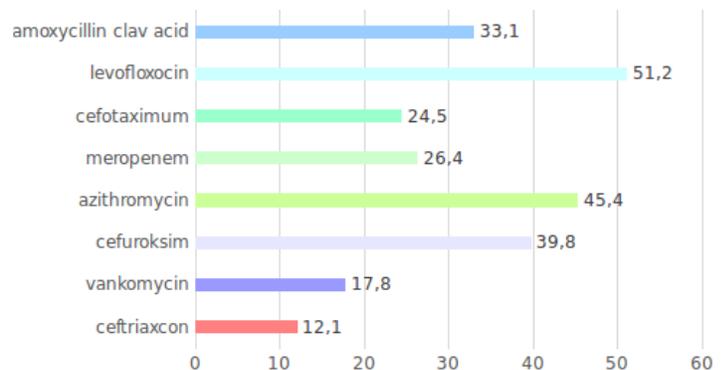
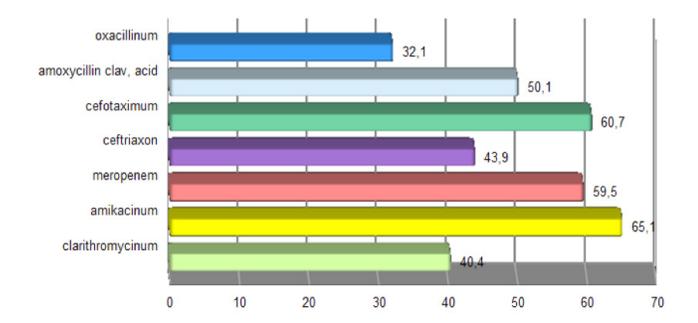


Figure 3: Analysis of *streptococcal* resistance to different classes of antibiotics.

Gram-positive *Staphylococci* were detected in 647 patterns (45,5%) from all positive results. In 545 samples (84,2%) coagulase-negative *Staphylococci* were revealed, exhibiting less pathogenicity, while more pathogenic coagulase-positive *Staphylococci* were determined in 2 smears (15,8%): *St. Aureus* - 85 smears (13,2%), *Staph. intermedius*, *St. hyicus* - in 17 smears (2,6%). The sensitivity of these microorganisms was determined in a minimal percentage of cases to the penicillin series antibiotics. It is necessary to note that the high sensitivity of *staphylococci* to the other antibiotic classes was manifested in more than 50-60% of the

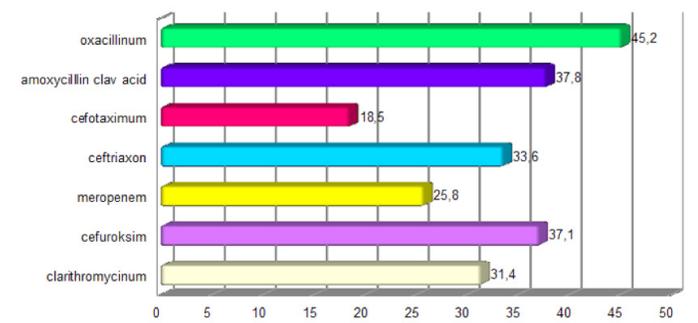
samples, with the maximum response for amikacin (aminoglycoside III generation)- in 421 cases (65,1%) and cefotaxime in 395 cases (61%) (Figure 4).



**Figure 4:** Screening of antibiotic sensitivity of the *Staphylococcus* family.

It is well known that combined therapy aminoglycosides together with cephalosporines results in synergism related to both gram-negative and gram-positive microorganisms. High *Streptococci* sensitivity was revealed to carbapenems series of antibiotics (in 60% of cases). In clinical practice this class of antibiotic is considered to be the final medication reserve but may be used as emergence therapy in extreme life-threatening infection.

Analyzing the staphylococcus's resistance to antibiotics, it was shown that in 211 samples (45,2%) oxacillin resistance has been revealed, probably due to the predominance of methicillin-resistant *staphylococci* strains, the resistance of which, according to scientific data [13-15] is associated with the appearance of atypical penicillin-binding MRSA proteins, as well as the production of methicillin-resistant protein PBP [16]. For other classes of antibiotics resistance was observed in approximately equal numbers of cases (30-35%) (Figure 5).

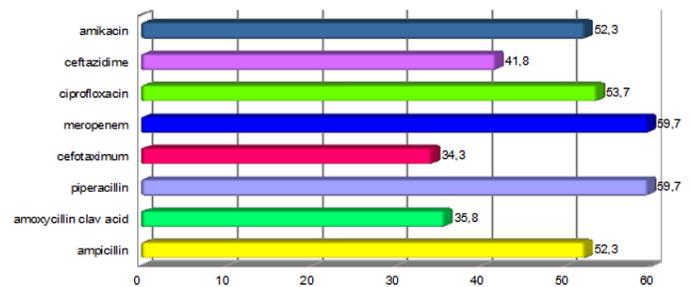


**Figure 5:** *Staphylococci* resistance to antibiotics.

For amikacin and Cefotaxime resistance was found in 18,5% of cases. In our opinion, a higher percentage of *staphylococci* resistance to antibiotics demands particular attention as the main reason for such situation, according to opinions of many authors,

may be the unjustified use or premature termination of courses, prior to sanitation of foci, insufficient daily dosage or gradual withdrawal of the drug, leading to its insufficient concentration in the focus, as well as the widespread use of antimicrobial medication.

Positive results on the *Enterobacteria* group were shown in 67 cases (4%). Non-fermentative gram-negative bacteria were visualized in 21 (31,3%) smears, and in 16 cases *Pseudomonas aeruginosa* was determined, which has a pronounced pathogenic action. The data obtained are fully similar to the results of published studies [15], which show an active increase of non-fermentative-gram-negative bacteria growth compared to previous years. Among the group of the fermenting spp. prevailed in almost half of the samples. *E. coli.* and *Klebsiella spp.* cultures were determined in 7,5%. In 60% of patient high sensitivity of this species was determined to a wide spectrum of antibiotics, including: some penicillin series medications, aminoglycosides carbapenems, fluoroquinolones (Figure 6). At the same time, amoxicillin (penicillin) sensitivity was detected only in 35,8%, that, in our opinion, is associated with the frequent prescription of this antibiotic, and therefore, the risk of developing resistance to it increases. Cephalosporin sensitivity is registered only in 35-40% of patients.



**Figure 6:** Analysis of gram-negative microorganism antibiotic sensitivity.

It is proved that some particular representatives of *Enterobacteriaceae* (*Enterobacter spp.*, *Providencia spp.*, as well as *Pseudomonas aeruginosa*) reveal the ability to produce inducible chromosomal cephalosporinases, characterized by high affinity for III generation cephalosporins. Secretion of these chromosomal beta lactamases during the period of treatment with the 3rd generation cephalosporins may eventually resulting to all available cephalosporins resistance. This form of resistance increases in cases of treatment with broad spectrum cephalosporins the infections caused mainly by *Enterobacter cloacae* and *Pseudomonas Auer* [16]. The resistance to imipenem was detected in a few cases (13,4%), that is significantly lower even in comparison with meropenem (23,8%) and clearly demonstrates the occurrence of stable forms when frequent use of the drug (in our center meropenem is prescribed more often).

It should not be forgotten that the usage of carbapenems can lead to the increase the risk of infections caused by carbapenem producing strains of *enterobacteria*, *Pseudomonas aeruginosa* and other non-fermenting rods, showing resistance to both carbapenems and other classes of antibiotics. Our data also show the presence of *Enterobacteriaceae* resistance to tobramycin in a high percentage of cases (55,2%) (Figure 7).

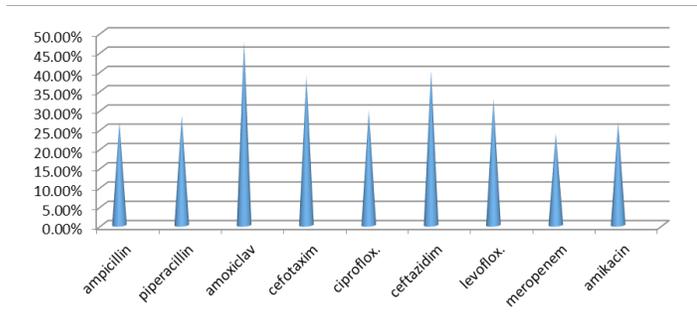


Figure 7: Analysis of gram-negative microflora antibiotic resistance.

According to scientific publications, aminoglycosides activity may be reduced while the bacteria production the group of enzymes that reduce positive charges on these poly cationic antibiotics [17,18]. *Enterobacteria* resistance to other antibacterial drugs was registered in approximately equal percentage of cases (from 28 to 38%). Fungal infections were caused by fungi *Candida* spp. - 83 smears (5,8%) and *Aspergillus* spp. - 3 cases (0,2 %). Resistance to Itraconazole was shown in 98% of cases, while nystatin and amphotericin B were more effective against the identified fungal infections.

## Conclusion

- The taxonomic structure of the studied microflora biotopes in oncohematological patients was distributed as follows: *Streptococci* - 64,2%, *Staphylococci* - 45,5%, gram negative bacteria - 4,2%, fungi - 6,1%, i.e. the main causative agent of infectious complications were gram-positive bacteria.
- Out of the overall number of *Staphylococci*, methicillin-resistant strains characterized by greater pathogenicity were detected in 15,2% of cases.
- The percentage of *Pseudomonas aeruginosa* among from the total number of positive results smears was 1,1%, although out of the total Non-fermentative gram-negative Bacteria (21 patients), *Ps. aeruginosa* was detected in 6,2% (16 patients).
- All types of isolated microorganisms showed the greatest sensitivity to carbapenem class antibiotics and high sensitivity to 3rd generation cephalosporins and fluoroquinolones.

- Dynamic observation and monitoring of the microbial flora enables the opportunity to establish our own antibiotic resistance database that will allow to control the level of major pathogens resistance to commonly used antibiotics.

**Conflict of Interest:** None to declare.

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