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MICROORGANISMS ANTIBIOTIC SENSITIVITY DETERMINATION IN URINARY TRACT INFECTIONS

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Introduction

Nowadays Urinary tract infections (UTI) are considered to be the most common bacterial infections, ranked second in the world after the respiratory tract infections. Specific subpopulations at increased risk of UTI include infants, pregnant women, the elderly, patients of intensive care units, patients with catheters, underlying urological abnormalities, associated with diabetes, oncopathology, multiple sclerosis, AIDS [1, 2].

UTI are dangerous because they can increase the risk of renal dysfunction in patients of different age, premature delivery, and fetal mortality among pregnant women.

The etiology of UTI has been well studied, it varies depending of the type of pathogen, the predisposing factors presence, previous antimicrobial therapy, its community-acquired or nosocomial nature.

Diagnosis of UTI is based on the presence of symptoms and the results of bacteriological research. *Escherichia coli* is the most frequently implicated uropathogen as reported by all epidemiological studies worldwide (70-95% of cases). Other microorganisms of the genera *Enterococcus, Klebsiella, Enterobacter, Proteus, Morganella, Citrobacter, Serratia, Pseudomonas, Streptococcus, Staphylococcus* or fungi, such as *Candida spp*., are also isolated with variable frequency [3, 4, 5]. Uncomplicated urinary tract infection is often treated with antibiotics, resulting in increasing resistance levels [6]. In many countries in recent years there has been a decreasing tendency of the causative agents of UTI sensitivity to various antibiotics, which causes growth of an inefficiency treatment risk. The high incidence of UTI, frequent unsuccessful results in the empiric antibiotic treatment order in addition to the causative agent species accurate identification are required in most cases to establish their susceptibility to the maximum possible number of antibacterial drugs. In this case a necessary components of the antimicrobial agents rational choice are the uropathogens resistance and the possibility of its changes over the years.

In connection with the above the investigations were carried out with the purpose to identify the actual causative agents of bacteriuria and their sensitivity to antibiotics and antifungal drugs.

Materials and methods

Bacteriological examination of urine was performed at 42 patients of SE "Sytenko Institute of Spine and Joint Pathology, AMS Ukraine" clinic. The bacteriological method for determining the number of bacteria in the test material, cultural and bacterioscopic methods for identifying microorganisms and disk-diffusion method for sensitivity of microorganisms to antibiotics determining were used. The clinical material for the study was an average portion of the morning urine or urine collected by catheter. The biological material collection and bacteriological examination was carried by quantitative method, the isolated microorganisms identification and their sensitivity to antibiotics determining was performed by standard methods in accordance with current guidelines [7, 8, 9]. During the work we used the following antibiotics group to determine the microorganisms sensitivity: penicillin - amoxicillin, ampicillin, amoksiklav, benzylpenicillin, carbenicillin; cephalosporin - cefazolin, cefuroxime, ceftriaxone, ceftriaxone+sulbactam; karbapenems - meropenem; monobactam - aztreonam; tetracyclines - tigecycline; aminoglycoside - gentamicin, amikacin; fluoroquinolones - norfloxacin, ofloxacin, gatifloxacin, moxifloxacin, pefloxacinum, lomefloxacin, levofloxacin; oxazolidinones - linezolid; macrolides - azithromycin, erythromycin; lincosamides - lincomycin; glycopeptides - vancomycin; antifungal antibiotics - fluconazole, intrakonazol, clotrimazole.

We took into account the following criteria to the antibiotics selection:

- the bacteria species sensitivity nature;
- antibiotics, which have a natural activity for identified microorganisms;
- clinically proven antibiotics effectiveness in infections treating;
- drugs used in the hospital clinical practice.

Results and discussion

In order to differentiate bacteriuria, which may occur due to the urine contamination by distal urinary tract normal microflora, from the true bacteriuria, which occurs at the urinary system infectious processes, we used quantitative research methods to determine the bacteriuria degree which was expressed by the number of microbial cells in 1 ml of urine [8]. During the biological material study 55 isolates of bacterial and fungal pathogens were obtained. As the identification result they were assigned to different taxonomic groups.

It was found that the microorganisms’ concentration in urine was in the range of $3,0x10^6$ CFU/ml to $3,0x10^9$ CFU/ml, and the most bacterial isolates content was equal $1,5x10^9$ CFU/ml (Fig. 1). Among them 40% of isolates (n = 22) were identified in amount $1,0x10^9$ CFU/ml; 29% (n = 16) - $5,3x10^8$ CFU/ml; 15% (n = 8) - $3,0x10^8$ CFU/ml; 7% (n = 4) - $3,0x10^9$ CFU/ml; 5% (n = 3) - $3,0x10^6$ CFU/ml; 4% (n = 2) - $1,5x10^8$ CFU/ml.

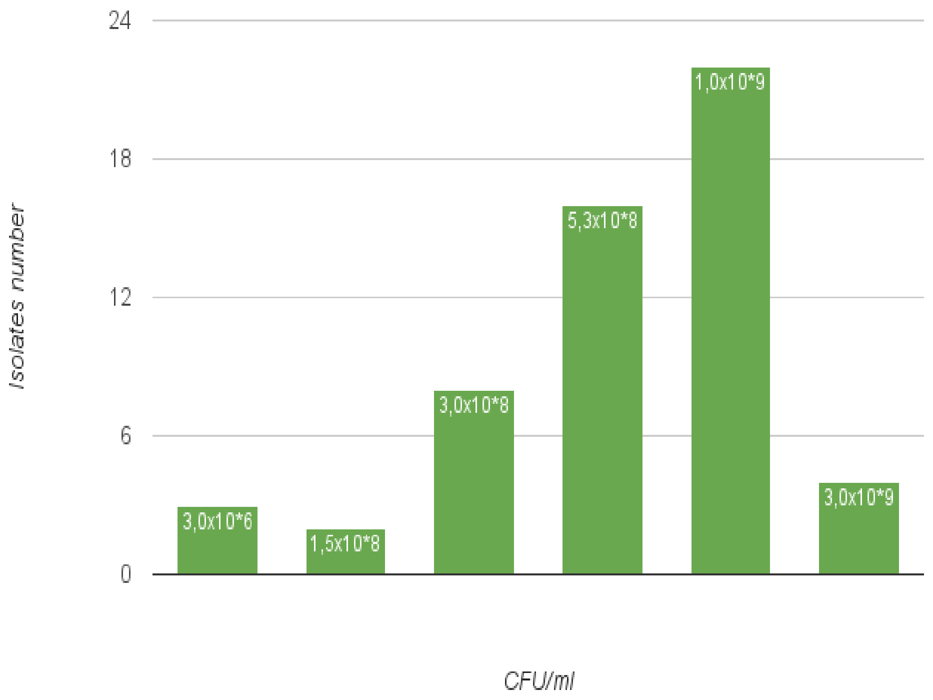

Figure 1. Quantitative characteristics of microorganisms that were isolated from urine (CFU/ml)

As a result of the microorganisms' identification and quantitative estimation the following species were determined: *Escherichia coli*, *Staphylococcus spp.*, *Streptococcus spp.*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Candida spp*. The studies showed that among the most commonly identified microorganisms *Escherichia coli* and *Staphylococcus spp*. were often detected in 5,3x10^{8} CFU/ml concentrations. For *Streptococcus spp*. and *Proteus mirabilis* that value was 1,5x10^{9} CFU/ml; for *Klebsiella pneumonia* - 3,0x10^{8} CFU/ml; for *Candida spp*. - 3,0x10^{6} CFU/ml respectively.

While the obtained isolates bacterioscopic and cultural identification it was found that the most often occurred microorganisms relate to the following families and genus: *Micrococcaceae* (n = 24, (43,6±6,7)%), *Staphylococcus* (n = 24); *Streptococcaceae* (n = 7, (12,7±4,5)%), *Streptococcus* (n = 7); *Enterobacteriaceae* (n = 16, (29,1±6,1)%), *Escherichia* (n=8), *Klebsiella* (n = 3), *Proteus* (n = 5); *Saccharomycetaceae* (n = 3, (5,4±3,0)%), *Candida* (n=3).

The bacteria and fungi isolates identification results, which were obtained at appropriate nutrient media with its biochemical activity testing the following species were found: *Escherichia coli* 14,5% (n=8), *Streptococcus agalactiae* and *Staphylococcus haemolyticus* 10,9% (n=6); *Staphylococcus aureus* and *Proteus mirabilis* 7,3% (n=4); *Staphylococcus epidermidis, Klebsiella pneumoniae*, *Candida spp.*, *Staphylococcus hominis* 5,4% (n=3); *Pseudomonas aeruginosa*, *Staphylococcus intermedius*, *Staphylococcus saprophyticus*, *Staphylococcus capitis*, *Acinetobacter haemolyticus* 3,6% (n=2); *Streptococcus pyogenes*, *Proteus vulgaris*, *Bacillus subtilis*, *Staphylococcus simulans* and *Staphylococcus cohnii* 1,8% (n=1).

Thus, it was found that the most common microorganisms (which frequency of occurrence in urine was ≥ 5%) were: *Escherichia coli* (14,5±4,7)%, *Streptococcus agalactiae* and *Staphylococcus haemolyticus* (10,9±4,2)%; *Staphylococcus aureus* and *Proteus mirabilis* (7,3±3,5)%; *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Staphylococcus hominis*, *Candida spp*. (5,4±3,0)% (Fig. 2).

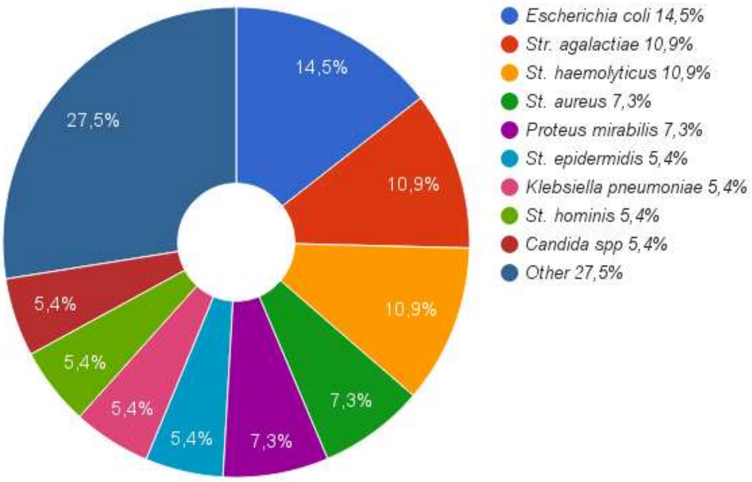

Figure 2. The proportion of microorganisms detected in urine, %

To determine the bacterial pathogens sensitivity to antibiotics we used disco-diffusion method. Testing objects were identified in quantities greater than 10^3 CFU/ml isolates only. Results below are presented by the microorganisms' nature and the frequency of its detection.

All identified *Staphylococcus haemolyticus* (n=6) isolates were sensitive to four drugs (amikacin, vancomycin, gatifloxacin, tigecycline). 83,3% of them have sensitivity to meropenem, cefazolin, moxifloxacin.

The most effective drug that inhibited the *Staphylococcus aureus* (n = 4) growth was norfloxacin. 100% of this species isolates were sensitive to it. 75,0% of isolates have sensitivity to levofloxacin, pefloxacin, lomefloxacin.

All *Staphylococcus epidermidis* (n = 3) isolates had a sensitivity to linezolid and vancomycin. 66,6% of its were sensitive to moxifloxacin, cefuroxime, tigecycline.

100% *Staphylococcus hominis* (n = 3) cultures were sensitivity to three drugs (gatifloxacin, linezolid, tigecycline). 66,6% isolates were sensitive to meropenem, cefuroxime, cefazolin, amikacin, vancomycin.

Gatifloxacin was the most effective drug against 80,0% of *Streptococcus agalactiae* (n=6) isolates. 66,6% of its were sensitive to amoxiclav, ampicillin, carbenicillin, cefazolin, amikacin, gentamicin, moxifloxacin, levofloxacin, norfloxacin, linezolid.

In determining the *Escherichia coli* (n = 8) cultures sensitivity we found that gatifloxacin was the most effective drug to 87,5% of its. 75,0% of cultures had sensitivity to four drugs (meronem, levofloxacin, norfloxacin, tigecycline).

100% of *Proteus mirabilis* (n = 4) isolates had sensitivity to three drugs (meropenem, amikacin, gatifloxacin). The sensitivity to levofloxacin was identified in 75,0% of *Proteus mirabilis* cultures.

All *Klebsiella pneumoniae* (n=3) cultures were sensitive to six drugs (ofloxacin, moxifloxacin, levofloxacin, pefloxacin, meropenem, gatifloxacin). Sensitivity to ceftriaxone, cefuroxime, norfloxacin, tigecycline was detected in 66,6% of isolates.

Microscopic fungi of the genus *Candida* (n = 3) had the sensitivity to fluconazole. 66,6% of them turned out to be sensitive to clotrimazole and itraconazole. All fungi isolates have no resistance to antimycotics.

All *Pseudomonas aeruginosa* (n = 2) cultures have sensitivity to meropenem.

While analyzing obtained results we concluded that meropenem was the most effective drug, 78,7% of all cultures had the sensitivity to it. The sensitivity to gatifloxacin had 73,7% of cultures; to tigecycline – 71,1% of isolates; to amikacin -67,3% of cultures; to moxifloxacin – 59,6% of isolates; to two drugs (levofloxacin and pefloxacin) - 55,8% of isolates respectively (Fig. 3).

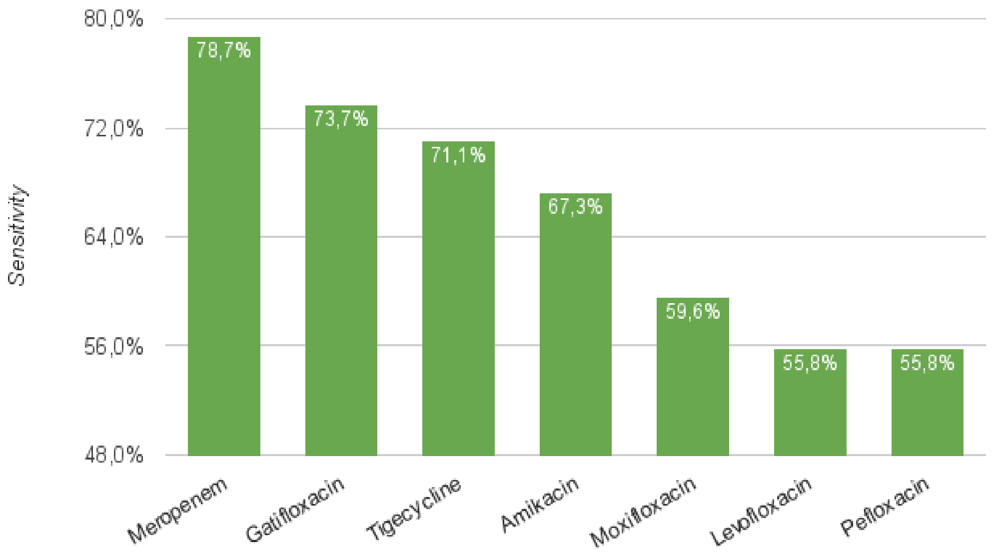

Figure 3. The most active drugs against microorganisms isolates in bacteriuria

Taking into account the pathogens resistance problem importance we conducted the isolated cultures resistance analysis.

As a result of studies, it was found that all genus *Staphylococcus* family Micrococcaceae isolates (n = 24) proved to be resistant to benzylpenicillin. 95,8% of isolated cultures were resistant to ampicillin. 87,5% of isolates had no amoxicillin and cefuroxime sensitivity and 83,3% of isolates - to azithromycin, erythromycin and lincomycin. It was also found that *Staphylococcus aureus* (n = 4) appeared to be resistant to vancomycin, ampicillin, cefuroxime, cefazolin, azithromycin, linezolid. This species and *Staphylococcus epidermidis* (n=3) and *Staphylococcus hominis* (n = 3) isolates were multi-drug resistant to four or more drugs from different groups. Thus, *Staphylococcus epidermidis*, that is human skin normal microflora member, can be difficult to eliminate in the urinary tract due to acquisition of resistance to many modern drugs that are recommended for the bacteriuria treatment.

As of the *Streptococcaceae* family, all *Streptococcus agalactiae* (n = 6) isolates turned out to be insensitive to cefuroxime.

For the family *Enterobacteriaceae* (n=16) it was found that 100% of isolates were insensitive to carbenicillin; 87,5% - to cefuroxim and gentamicin, 81,2% - to amoxicillin and ampicillin, 75,0% - to cefazolin, 50,0% - to ceftriaxonum and aztreonam.

All *Escherichia* cultures (n = 8) and *Proteus* cultures (n = 5) were resistant to cefuroxime, *Proteus* to ampicillin and cefazolin. That is not protected penicillins and 1 and 2 generations cephalosporin antibiotics had no activity against these microorganisms. This can cause problems in patients with bacteriuria. In addition, *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae* had multi-drug resistance.

All *Pseudomonas aeruginosa* (n = 2) and *Acinetobacter haemolyticus* (n=2) isolates also appeared to be multi-drug resistance.

Thus, we found *Enterobacteriaceae* and non-fermenting gram-negative bacteria isolates that had a beta-lactamase resistance to corresponding drugs.

In addition, (73,1 ± 6,1)% of all 52 bacterial isolates obtained were resistant to protected beta-lactam drug amoxiclav.

Conclusions

Today the bacteriuria and choosing the right treatment problem becomes increasingly important in medicine. In the bacteriuria treatment the key factors are the pathogen identification and its sensitivity to antibiotics. Because of the correct pathogen and its sensitivity to drugs determination the right choice of antibiotic therapy depends, that will prevent the microorganisms antibiotic resistance development and will lead to patients' rapid recovery.

The following conclusions were made on the results of the research.

1. The most common microorganisms (which incidence in urine were ≥ 5%) are: *Escherichia coli* (14,5±4,7)%, *Streptococcus agalactiae* and *Staphylococcus haemolyticus* (10,9±4,2)%; *Staphylococcus aureus* and *Proteus mirabilis* (7,3±3,5)%; *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Staphylococcus hominis*, *Candida spp*. (5,4±3,0)%.
2. The most effective antibacterial drug is meropenem. 78,7% of all isolates have sensitivity to it. The sensitivity to gatifloxacin have 73,7%; to tigecycline 71,1%; to amikacin – 67,3%, to moxifloxacin – 59,6% of cultures, to two agents (levofloxacin and pefloxacin) – 55,8% of cultures respectively.

3. 100% of the family *Micrococcaceae* isolates were resistant to benzylpenicillin, 95,8% - to ampicillin, 87,5% - to amoxicillin and cefuroxime, 83,3% - to azithromycin, erythromycin and lincomycin. Members of the *Streptococcaceae* family were resistant to cefuroxime. 100% of the family *Enterobacteriaceae* isolates had no sensitivity to carbenicillin, 87,5% of isolates - to cefuroxim and gentamicin, 81,2% - to amoxicillin and ampicillin, 75,0% - to cefazolin, 50,0% - to ceftriaxonum and aztreonam.
4. *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter haemolyticus* isolates were multi-drug resistant.

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Introduction. Nowadays Urinary tract infections (UTI) are considered to be the most common bacterial infections. *Escherichia coli* is the most frequently uropathogen. Other microorganisms of the genera *Enterococcus, Klebsiella, Enterobacter, Proteus, Morganella, Citrobacter, Serratia, Pseudomonas, Streptococcus, Staphylococcus*, *Candida* are also isolated with variable frequency. In recent years there has been a decreasing tendency of the causative agents of UTI sensitivity to various antibiotics, which causes growth of an inefficiency treatment risk. In connection with the above the investigations were carried out with the purpose to identify the actual causative agents of bacteriuria and their sensitivity to antibiotics and antifungal drugs. **Materials and methods.** Bacteriological examination of urine was performed at 42 patients of SI "Sytenko Institute of Spine and Joint Pathology, AMS of Ukraine" clinic. The bacteriological method for determining the number of bacteria in the test material, cultural and bacterioscopic methods for identifying microorganisms and disk-diffusion method for sensitivity of microorganisms to antibiotics determining were used. The clinical material for the study was an average portion of the morning urine or urine collected by catheter. The biological material collection and bacteriological examination was carried by quantitative method, the isolated microorganisms identification and their sensitivity to antibiotics determining was performed by standard methods in accordance with current guidelines. We used the following antibiotics group to determine the microorganisms sensitivity: penicillin, cephalosporin, karbapenems, tetracyclines, aminoglycoside, fluoroquinolones, oxazolidinones, macrolides, lincosamides, glycopeptides, antifungal antibiotics.

Results and discussion. During the biological material study 55 isolates of bacterial and fungal pathogens were obtained. The microorganisms' concentration in urine was in the range of $3,0x10^6$ CFU/ml to $3,0x10^9$ CFU/ml, and the most bacterial isolates content was equal $1,5x10^9$ CFU/ml. Among the most commonly identified microorganisms *Escherichia coli* and *Staphylococcus spp*. were often detected in $5,3x10^8$ CFU/ml concentrations. For *Streptococcus spp*. and *Proteus mirabilis* that value was $1,5x10^9$ CFU/ml; for *Klebsiella pneumonia* - $3,0x10^8$ CFU/ml; for *Candida spp*. - $3,0x10^6$ CFU/ml respectively. The most common microorganisms (which frequency of occurrence in urine was ≥ 5%) were: *E. coli* (14,5±4,7)%, *Str. agalactiae* and *St. haemolyticus* (10,9±4,2)%; *St. aureus* and *Pr. mirabilis* (7,3±3,5)%; *St. epidermidis*, *Kl. pneumoniae*, *St. hominis*, *Can-*

dida spp. (5,4±3,0)%. While analyzing obtained results we concluded that meropenem was the most effective drug, 78,7% of all cultures had the sensitivity to it. The sensitivity to gatifloxacin had 73,7% of cultures; to tigecycline – 71,1% of isolates; to amikacin -67,3% of cultures; to moxifloxacin – 59,6% of isolates; to two drugs (levofloxacin and pefloxacin) - 55,8% of isolates respectively. It was found that *Staphylococcus aureus* appeared to be resistant to vancomycin, ampicillin, cefuroxime, cefazolin, azithromycin, linezolid. This species and *St. epidermidis* and *St. hominis* isolates were multi-drug resistant to four or more drugs from different groups. All *Streptococcus agalactiae* isolates were insensitive to cefuroxime. All *Escherichia* cultures were resistant to cefuroxime, *Proteus* - to cefuroxime, ampicillin and cefazolin. *E. coli, Pr. mirabilis, K. pneumoniae*, *Ps. aeruginosa, Ac. haemolyticus* had multi-drug resistance. In addition, (73,1 ± 6,1)% of all 52 bacterial isolates obtained were resistant to amoxiclav.

Conclusions. 1. The most common microorganisms (which incidence in urine were ≥ 5%) are: *Escherichia coli* (14,5±4,7)%, *Streptococcus agalactiae* and *Staphylococcus haemolyticus* (10,9±4,2)%; *Staphylococcus aureus* and *Proteus mirabilis* (7,3±3,5)%; *Staphylococcus epidermidis, Klebsiella pneumoniae, Staphylococcus hominis, Candida spp*. (5,4±3,0)%. 2. The most effective antibacterial drug is meropenem. 78,7% of all isolates have sensitivity to it. The sensitivity to gatifloxacin have 73,7%; to tigecycline 71,1%; to amikacin – 67,3%, to moxifloxacin – 59,6% of cultures, to two agents (levofloxacin and pefloxacin) – 55,8% of cultures respectively. 3. 100% of the family *Micrococcaceae* isolates were resistant to benzylpenicillin, 95,8% - to ampicillin, 87,5% - to amoxicillin and cefuroxime, 83,3% - to azithromycin, erythromycin and lincomycin. Members of the *Streptococcaceae* family were resistant to cefuroxime. 100% of the family *Enterobacteriaceae* isolates had no sensitivity to carbenicillin, 87,5% of isolates - to cefuroxim and gentamicin, 81,2% - to amoxicillin and ampicillin, 75,0% - to cefazolin, 50,0% - to ceftriaxonum and aztreonam. 4. *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter haemolyticus* isolates were multi-drug resistant.