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MICROBIOLOGICAL MONITORING OF CAUSATIVE AGENTS OF NOSOCOMIAL INFECTION IN THE UROLOGICAL CLINIC

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The analysis of the results of microbiological examination of urine samples of 1022 patients (559 women and 463 men) who were hospitalized at the urological clinic of the I.P. Pavlov First Saint Petersburg State Medical University in period from 2018 to 2020 was performed. The age of the patients varied from 18 to 88 years (average 63.1 ± 17.6 years). In 587 (57.5%) patients, gram-negative microflora was detected, in 355 (34.7%) – gram-positive microflora, and in 80 (7.8%) – mixed microflora. *Escherichia coli* (28.2%), *Enterococcus faecalis* (20.9%), *Klebsiella pneumoniae* (14.1%) and bacteria of the *Staphylococcaceae* family (11.6%) were prevailed in the structure of uropathogens. The share of other microorganisms did not exceed 5%. A high level of microflora resistance to ampicillin, cephalosporins of the 2nd and 3rd generations, fluoroquinolones was noted. The highest level of antibiotic resistance was observed in *K. pneumonia*. In general the results obtained correspond to the general trends in the dynamics of the etiological structure and the level of antibiotic resistance of nosocomial urinary tract infections. This study confirms the need for local microbiological monitoring to develop optimal regimens for empiric antibiotic therapy and perioperative antibiotic prophylaxis.

Keywords: nosocomial infections; antibiotic resistance; catheter-associated infection; antibiotic therapy.

МИКРОБИОЛОГИЧЕСКИЙ МОНИТОРИНГ ВОЗБУДИТЕЛЕЙ НОЗОКОМИАЛЬНОЙ ИНФЕКЦИИ В УРОЛОГИЧЕСКОЙ КЛИНИКЕ

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Проведен анализ результатов микробиологического исследования образцов мочи 1022 пациентов (559 женщин и 463 мужчин), находившихся на стационарном лечении в урологической клинике ПСбГМУ им. И.П. Павлова в период с 2018 по 2020 г. Возраст пациентов варьировал от 18 до 88 лет (в среднем 63,1 ± 17,6 года). У 587 (57,5 %) человек выявлена грамотрицательная, у 355 (34,7 %) — грамположительная, и у 80 (7,8 %) — смешанная микрофлора. В структуре уропатогенов преобладали *Escherichia coli* (28,2 %), *Enterococcus faecalis* (20,9 %), *Klebsiella pneumoniae* (14,1 %) и бактерии семейства *Staphylococcaceae* (11,6 %). Доля остальных микроорганизмов не превышала 5 %. Отмечен высокий уровень резистентности микрофлоры к ампициллину, цефалоспоринам 2-го и 3-го поколений, фторхинолонам. Среди уропатогенов наиболее высокий уровень антибиотикорезистентности отмечена у *K. pneumoniae*. В целом полученные результаты соответствуют общим тенденциям динамики этиологической структуры и уровня антибиотикорезистентности нозокомиальных инфекций мочевых путей. Настоящее исследование подтверждает необходимость локального микробиологического мониторинга для выработки оптимальных режимов эмпирической антибактериальной терапии и периоперационной антибиотикопрофилактики.

Ключевые слова: нозокомиальные инфекции; антибиотикорезистентность; катетер-ассоциированная инфекция; антибиотикотерапия.

INTRODUCTION

The World Health Organization (1979) described nosocomial (hospital, intrahospital) infection as any clinically recognizable infectious disease that affects individuals as a result of their admission to the hospital or seeking medical help or hospital employees due to their work in this medical institution, regardless of the onset of the disease symptoms during or after a hospital stay.

Improvement on efficiency of prevention and treatment of nosocomial infections is one of the main aims of current medicine. The difficulties of nosocomial infection treatment are largely associated with antibiotic resistance of the main infectious agents. Antibiotic resistance of microorganisms that cause nosocomial infections worsens the results of treatment and increases the duration of hospitalization and significantly the cost of treatment, which poses a serious economic burden on the healthcare system. The presence of antibiotic-resistant strains of microorganisms in the patient's biomaterials has been shown to increase the duration of hospitalization by at least 1.5 times and mortality by 5 times [1, 2]. In turn, the duration of hospitalization and hospital stay before and after surgery correlate significantly with the probability of nosocomial infection occurrence [3, 4]. Thus, in patients with a preoperative period of less than 2 days, nosocomial urinary tract infections develop 5 times less frequently than in patients with a longer preoperative bed-day period [5]. Approximately 90% of all nosocomial infections are of bacterial origin, and the remaining 10% are caused by viruses, fungal pathogens, and protozoa.

Antibiotic resistance and the associated decreased antibiotic therapy efficacy are recognized to be a problem worldwide [6]. To prevent antibiotic resistance and improve the efficiency of treatment of infectious complications in the 2011 Russian Federation, the program "Antimicrobial therapy control strategy" (ATCS) was adopted [7]. It includes a set of measures aimed at rational use of antimicrobial drugs, containment of antibiotic resistance, and control of nosocomial infections. In the implementation of the ATCS program, microbiological monitoring plays a crucial role, which is mainly aimed at justifying the use of etiotropic therapy for the treatment and prevention of infectious complications, the formation of a strategy and approach for the use of antimicrobial drugs in individual medical institutions, and the justification for the implementation of infection control measures [7].

Urinary tract infections account for at least 40% of all nosocomial infections [8, 9]. The implication of urinary infections is due not only to their high prevalence but also to the insufficient effectiveness of treatment and the frequent development of severe complications [10, 11].

The etiological structure of nosocomial urinary tract infections differs from community-acquired infections and is characterized by a lower incidence of *Escherichia coli*, a higher proportion of gram-negative non-fermenting bacteria, and a higher frequency of antibacterial multidrug resistance of uropathogens [12, 13]. In uncomplicated urinary tract infections, *E. coli* is detected in 64–76% of cases; however, in complicated infections, including catheter-associated urinary tract infection, the detection rate of *E. coli* decreases to 30–40% [12–16]. Along with the lower frequency of *E. coli* as a cause of nosocomial infection, a tendency towards an increase in the role of *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Enterococcus faecalis* in its occurrence has recently been noted [3, 16, 17]. Multidrug resistance to antimicrobial agents is typical for both gram-positive and gram-negative uropathogens. A significant cause of antibiotic resistance is the production of extended-spectrum β-lactamases (ESBL) by bacteria. The results of studies conducted in recent years showed that in the Russian Federation, *E. coli*, producing ESBL, is the causative agent of urinary infections in 27–44% of cases [18]. In Russia, the resistance of hospital strains of enterobacteria to cephalosporins already exceeds 70%, which is mainly due to the proliferation of extended spectrum β-lactamase producers [19].

The use of antibiotic prophylaxis prior to urological manipulations and surgeries is a routine practice. In such cases, antibacterial drugs are most often prescribed empirically. The choice of starting empiric antibiotic therapy should be based on knowledge of the likely spectrum of pathogens and their possible resistance. Local microbiological monitoring enables the creation of standards for empirical antimicrobial therapy with the need to revise them annually. These measures optimize the approach of antibiotic prophylaxis and antibiotic therapy, which leads to an increase in the treatment efficiency and a decrease in pharmacoeconomic costs.

The present study *aimed* to investigate the species composition of uropathogens and their sensitivity to antibacterial drugs to select the optimal empirical prophylaxis and therapy of urinary tract infections in a urological hospital.

MATERIALS AND METHODS

The results of microbiological studies of non-sterile urine samples of 1022 patients (559 women and 463 men), who received inpatient treatment at the urology clinic of Pavlov State Medical University of Saint Petersburg between 2018 and 2020, were analyzed. The age of the patients varied from 18 to 88 years (average, 63.1 ± 17.6 years). Furthermore, 48.4% of the patients were older than 61 years, and 33.7% were 41–60 years old. Hospitalization was due to urolithiasis in 327 (32%) patients, and 135 (13.2%) patients were diagnosed with benign prostatic hyperplasia, 125 (12.2%) patients renal cysts and hydronephrosis, and 90 (8.8%) patients bladder cancer; additionally, kidney cancer was diagnosed in 75 (7.3%) patients, prostate cancer in 56 (5.5%) patients, neurogenic urinary disorders in 51 (5%) patients, and acute pyelonephritis in 39 (3.8%) patients, and other different diagnoses were found in 124 (12.1%) patients. During the last year before hospitalization, 257 (25.1%) patients took antibacterial drugs. Moreover, $\geq 10^3$ CFU/ml indicated a positive result of bacteriological examination of urine.

The material for the study was the mid-stream specimen of urine, and in the presence of an indwelling urethral catheter, biomaterial was taken after replacing the drainage/catheter or by sterile suprapubic puncture. The material was collected during the first 2 days after the patient was admitted to the hospital. Urine was collected in sterile disposable containers and delivered to the bacteriological laboratory no later than 2 hours after sampling. At the laboratory stage, the bacterial agent was isolated and identified with the determination of its concentration in the urine sample (degree of bacteriuria) and sensitivity to antimicrobial drugs. Inoculation on nutrient media and isolation and identification of pure cultures were performed according to standard methods. The sensitivity of microorganisms to antibacterial drugs was determined by the disc-diffusion method. Microbiological examination of urine was performed in accordance with the current rules set out in the Order of the Ministry of Health of the USSR No. 535 (April 22, 1985) “On the unification of microbiological (bacteriological) research methods used in clinical diagnostic laboratories of medical and preventive institutions” and guidelines for clinical and laboratory diagnostics “Bacteriological analysis of urine” approved by the Ministry of Health of the Russian Federation (December 25, 2013) [20].

The systematization, processing, and statistical analysis of the research materials were performed using the Statistica 10 En computer program (StatSoft, Inc.) using the χ^2 test. Differences were considered significant at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Microbiological examination revealed the presence of gram-negative microorganisms in the urine of 587 (57.5%) patients, gram-positive microorganisms in 355 (34.7%) patients, and mixed microflora in 80 (7.8%) patients. In 277 (27.1%) cases, the growth of microflora was obtained from the enrichment medium.

Among the isolated gram-negative microorganisms, *E. coli* prevailed in frequency and amounted to 28.2%, the proportion of *K. pneumoniae* was 14.1%, *Pseudomonas aeruginosa* was detected in 4.8% of cases, and *Proteus mirabilis* was found in 2.9% of cases. The frequency of detection of *Acinetobacter* spp. (2.4%) is noteworthy. In recent years, a tendency toward an increase in the role of non-fermenting gram-negative microorganisms of the genus *Acinetobacter* in the development of nosocomial infections has been observed. Microorganisms of the genus *Acinetobacter* are part of the skin microflora (inguen, axillary region, toes). A high contamination with resistant strains of the microorganism and infection of the patient is noted when infected with hospital strains of *Acinetobacter* spp. Gram-positive bacteria were most often represented by *E. faecalis* (20.9%) and bacteria of the *Staphylococcaceae* family (11.6%).

Most patients with positive results of bacteriological analysis of urine had no clinical symptoms of urinary tract infection; thus, asymptomatic bacteriuria was detected in 595 (58.2%) cases. The most frequent manifestation of nosocomial infection among the patients examined was acute pyelonephritis in 256 patients (25%) and acute cystitis in 161 patients (15.8%). Urethritis was diagnosed in 10 (1%) patients.

After isolation and identification of uropathogens, their sensitivity to antimicrobial drugs was determined (Table 1). *E. coli* was most often detected in urine. It was noted that *E. coli* had the highest resistance to ampicillin (69.4%) and ciprofloxacin (52.5%). Among the cephalosporin drugs, *E. coli* was least resistant to cefepime (21.2%). Resistance of *E. coli* was significantly higher in the second- and third-generation cephalosporins (cefuroxime, cefotaxime, ceftazidime, ceftriaxone), ranging from 36.6 to 41.6%, and its resistance level to gentamycin was 25.7% and to the combination of amoxicillin and clavulanic acid was 22.8%. *E. coli* was most sensitive to amikacin (resistance in 6.9% of cases) and meropenem (resistance in 0.7% of cases). In addition, there was a low incidence of resistance of *E. coli* to nitrofurantoin (5.9%).

The frequency of detection of resistant *K. pneumonia* strains exceeded that of *E. coli*. Thus, 89.5% of isolated *K. pneumonia* strains were resistant to ampicillin, 69.4% to ciprofloxacin, 49.3–66.7% to cephalosporins of the second and third generations, 59.7% to amoxicillin/clavulanic acid, 50% to gentamicin, and 48.7% to amikacin. The lowest frequency of resistance was noted to meropenem (28.2%).

The high level of *P. aeruginosa* resistance to gentamicin (63.9%) and meropenem (44.8%) should be noted. In general, the detection rate of resistant *P. aeruginosa* strains is slightly lower than that of *K. pneumonia*; however, it was not less than 30% for any of the antibacterial drugs under study.

Thus, the results of the study demonstrated an extremely high level of resistance of uropathogens to ampicillin. This drug does not have acceptable activity against the main causative agents of urinary tract infections. The increasing level of resistance of the main causative agents of nosocomial infections to antibiotics of the cephalosporin series of the second and third generations is alarming. Attention should be drawn to the problem of high antibiotic resistance of *K. pneumonia*. *P. aeruginosa* turned out to be somewhat more sensitive to most antibiotics; however, the antibiotic resistance of this uropathogen remains quite high.

A significant level of resistance of the main causative agents of urinary infection was noted in fluoroquinolone antibacterial drugs. Aminoglycosides retain a high level of activity against the main uropathogens. In this case, the sensitivity of gram-

Table 1 / Таблица 1

Resistance of etiologically significant causative agents of nosocomial infection to antimicrobial drugs
Резистентность этиологически значимых возбудителей нозокомиальной инфекции к антимикробным препаратам

Drug	Pathogen, %				
	Escherichia coli (*n* = 288)	*Klebsiella pneumoniae* (*n* = 144)	*Pseudomonas aeruginosa* (*n* = 49)	*Proteus mirabilis* (*n* = 30)	*Enterococcus faecalis* (*n* = 214)
Ampicillin	69.4	89.5	–	51.7	28.3
Amoxicillin/Clavulanic acid	22.8	59.7	39.8	43.2	7.5
Cefuroxime	41.6	57.3	–	28.6	–
Cefotaxime	38.6	66.7	–	34.3	–
Ceftazidime	36.6	63.9	31.4	21.7	–
Ceftriaxone	38.6	65.28	–	24.3	–
Cefepime	21.2	49.3	42.9	29.6	–
Ciprofloxacin	52.5	69.4	36.1	52.9	53.1
Levofloxacin	–	47.3	33.3	–	49.6
Gentamycin	25.7	50.0	63.9	21.4	43.7
Amikacin	6.9	48.7	35.3	7.1	31.6
Meropenem	0.7	28.2	44.8	–	–
Vancomycin	–	–	–	–	2
Nitrofurantoin	5.9	–	–	–	3.2

negative flora to the third-generation aminoglycosides (amikacin) depends on the type of pathogen. Thus, the sensitivity of *E. coli* was 93.1%, and that of *K. pneumoniae* and *P. aeruginosa* was >50%. Regarding *E. faecalis*, the main representative of gram-positive microorganisms, high activity was noted in vancomycin (98%) and nitrofurantoin (96.8%).

In medical institutions, the incidence of bacteriuria after insertion of a urethral catheter is known to increase by 3–8% per day [21]. In a study by Maki et al. [22], it is shown that the probability of a clinically significant urinary tract infection increases 6.8 times if a urethral catheter is used for more than 6 days. Moreover, the use of three-way catheters with continuous bladder irrigation has become a significant risk factor for the development of nosocomial infection in a urological hospital [4].

In the present study, bacteriological examination of urine was conducted in 114 patients with suprapubic urinary drainage on days 3–5 after hospitalization. Ninety-nine (86.8%) patients were found to have bacteriuria $\geq 10^5$ CFU/ml. The structure of uropathogens in this category of patients was significantly different from that in patients without drains. In patients with epicystotomy, microbiological examination of urine most often revealed *K. pneumonia* (29.3%) and *Staphylococcus epidermidis* (28.3%), whereas *E. faecalis* (17.2%) was detected more rarely. The prevalence of *E. coli* (7.1%), *Candida albicans* (5.1%), and *P. mirabilis* (2%) in the biomaterial has been found to be insignificant.

Our data differ from the results presented by Kuzmenko et al. [23], who, upon bacteriological examination of 119 urine samples from a urethral catheter, revealed an increase in microflora in a diagnostically significant titer in 30 (25.2%) patients treated in a urological hospital. Moreover, among the isolated strains of gram-negative microorganisms, *Klebsiella* spp. was the most frequent (36.7%), followed by *E. coli* (16.6%) and *P. aeruginosa* (6.6%), and in one case, *Proteus vulgaris* (3.3%) was noted. *E. faecalis* prevailed among the gram-positive flora in 33.3% of cases. Thus, it can be stated that the structure of the causative agents of nosocomial infections is unique for each hospital. The factors influencing it are the nosological profile of patients, their age and gender, the duration of the patient's stay in the hospital, and the frequency and nature of invasive instrumental diagnostic and therapeutic interventions, antibacterial drugs, and disinfectants used, as well as compliance with the sanitary regime. Further, in a study by Kuzmenko et al. [23], the antibiotic resistance of isolated microorganisms was assessed. A high frequency of resistance of uropathogens to ceftriaxone (66%) and ciprofloxacin (70%) was observed, which were used as drugs for empiric antibiotic therapy in patients with a urethral catheter. Much less resistance was detected for amikacin and doxycycline, which were prescribed after the results of bacteriological examination of urine were obtained.

In AntiMicrobial Resistance Map (AMRmap) [24], which combined the results of several multicenter studies conducted in various regions of Russia, *E. coli* (32.6%), *K. pneumoniae* (25.4%), and *P. aeruginosa* (10.4%) prevailed in the structure of causative agents of nosocomial urinary infection in the Russian Federation, whereas the detection rate of other pathogens was low. However, it should be known that each hospital has its own microbiological ecosystem and the pathogens released differ both in species composition and in sensitivity to antibacterial drugs. Additionally, in our study, we noted a high prevalence of *E. coli* and *K. pneumoniae*, whereas we revealed a significantly higher frequency of gram-positive pathogens, namely, *E. faecalis* (20.9%) and bacteria of the *Staphylococcaceae* family (11.6%).

This study warrants local microbiological monitoring to develop optimal regimens for empiric antibiotic therapy and perioperative antibiotic prophylaxis. It is unreasonable to prescribe the antibacterial drugs for this purpose if the resistance level to them of the main causative agents of urinary infection exceeds 20%. Compliance with these rules is a critical component of the implementation of the national program Antimicrobial Therapy Control Strategy.

CONCLUSION

The results of our study correspond to the general trends in the dynamics of the etiological structure and the level of antibiotic resistance of nosocomial urinary tract infections. Awareness of the etiological structure of pathogens and their level of antibiotic resistance largely determines the efficiency of empirical antibiotic therapy. Based on the range of bacterial pathogens and their antibiotic sensitivity, it is recommended to start antimicrobial treatment with the drugs to which the hospital microflora of a particular hospital has the least resistance.

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