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Pathogens causing septic complications in patients of the Liver Transplantation Unit in a multidisciplinary hospital

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Abstract

Background. *Despite the advances achieved in transplantation, the problem of septic infection in this field still remains crucial. This can largely be attributed to the immunocompromised status of patients, and the constant growth in the number of resistant strains of pathogens causing septic infection.*

The objective of the study *was to identify the spectrum of septic infection pathogens and their antibiotic sensitivity in patients of the Liver Transplantation Department of the N.V. Sklifosovsky Research Institute for Emergency Medicine.*

Material and methods. *The microbiology study results of 2,324 samples obtained from 236 patients who had been treated from 01.01.2023 to 30.06.2024 were analyzed. A total of 879 strains of microorganisms were isolated. In cases when carbapenem-resistant strains were isolated from the blood of patients, the carbapenemase genes were identified using an immunochromatography method.*

Results. *Among pathogens, Gram–negative rods accounted for 54% of the total number of strains, Gram–positive bacteria accounted for 43%,*

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yeasts of Candida spp. made 3%. Enterobacterales dominated among Gram-negative bacteria. Klebsiella pneumoniae strains were the absolute leader (33.0% of the total number of strains). Non-fermenting rods accounted for 11.3% of the isolated strains. Among the Gram-negative pathogens, the prevailing ones were the strains multidrug-resistant to the main classes of antibacterial drugs used in medical practice. Of all K. pneumoniae strains, the percentage of strains resistant to amikacin, ciprofloxacin, imipenem, and meropenem made 72.4%, 95.5%, 89.3%, and 87.9% respectively. Coagulase-negative staphylococci (18%) and enterococci (19.5%) prevailed among the Gram-positive cocci flora. Vancomycin, linezolid, and daptomycin retained antistaphylococcal effect. In carbapenem-resistant K. pneumoniae and E. coli strains isolated from patients' blood, the NDM metallo-beta-lactamases and serine carbapenemases of the OXA-48 group prevailed.

Conclusion. *The prevailing pathogens that cause septic infection in patients with surgical diseases of the hepatobiliary zone include K. pneumoniae strains among the Gram-negative microflora, and coagulase-negative staphylococci and enterococci among Gram-positive ones. The study results have confirmed the global trend towards an increase in the number of resistant strains among the pathogens of septic infections.*

Keywords: pathogens causing septic complications, antibiotic resistance, carbapenemases, hepatobiliary zone, liver transplantation

Conflict of interests Authors declare no conflict of interest

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SI, septic infection

Introduction

In recent years, significant advances in transplantation have made it possible to substantially increase the number of operations performed to transplant vital organs in patients previously considered hopeless. One of the complex problems in the rehabilitation of patients after organ transplantation is septic infections (SI), which significantly aggravate the course of the disease and reduce the possibility of a favorable outcome. The demand for high-tech organ transplant operations makes the solution to the problem of diagnosing and preventing SI in this group of patients a priority.

The immunocompromised status of patients with chronic diseases of solid organs (including the liver) contributes to the development of infections (bacterial, fungal, viral) even before immunosuppressive therapy. The growth in the number of resistant strains observed in recent years significantly complicates the treatment of patients with developed SI. In addition to therapeutic tasks, the need to prevent the spread of nosocomial infections remains relevant, which includes, among other things, identifying the sources of nosocomial infections, studying the mechanisms of resistance formation, and studying the routes of infection transmission. The development of effective measures to prevent the circulation of hospital strains and combat it is based, first of all, on the results of monitoring the main pathogens of SI in patients of transplant departments.

The aim of the study was to investigate the structure of pathogens causing septic infections and their antibiotic sensitivity in patients of the Liver Transplantation Department of the N.V. Sklifosovsky Research Institute for Emergency Medicine.

Material and methods

The analysis of the microbiological investigation results from 2324 samples of various clinical material obtained from 236 patients treated from 01.01.2023 to 30.06.2024 was performed, including 153 patients after orthotopic liver transplantation (64.8%), 46 from the liver transplant waiting list (19.5%), 23 after liver and pancreas resections of various extents (9.7%) and 14 patients having a liver graft dysfunction in the late post-transplant period (5.9%). The clinical material included blood, urine, wound discharge, bronchoalveolar lavage content, and endotracheal aspirate. Indications for microbiological investigations were determined by the patient's attending physician. The collection and transportation of clinical material samples were performed in accordance with Methodological Guidelines 4.2.203905 [1]. Primary inoculation was performed in accordance with generally accepted standards on 5% blood agar, chocolate agar, mannitol salt agar, Endo, Sabouraud and Uriselect agar media. The set of nutrient media depended on the type of clinical material being studied. Blood culture was performed using blood culture systems: Bactec™ FX (BD, USA) and Labstar 100 (Scenker, China). Pathogens and their antibiotic sensitivity were identified using Vitek MS and Vitek 2 Compact automated microbial identification systems (BioMerieux, France). When analyzing the data on the sensitivity of microorganisms, the data on the strains falling into the "resistant" category were taken as a basis. In cases when carbapenem-resistant strains were isolated from the blood of patients, the genes of carbapenemase groups VIM, IMP, NDM, KRS, OXA-48 were identified by the immunochromatographic method using the NG-Test CARBA 5 kits (NG Biotech Z.A., France).

When isolating several microorganisms from one clinical material sample, the subsequent analysis covered all etiologically significant pathogens, while generally accepted standards of etiological significance were used [2]. As a result, 879 strains of microorganisms were identified for this study.

Results

The structure of SI pathogens in patients of the Liver Transplant Department of the N.V. Sklifosovsky Research Institute for Emergency Medicine is presented in Fig. 1.

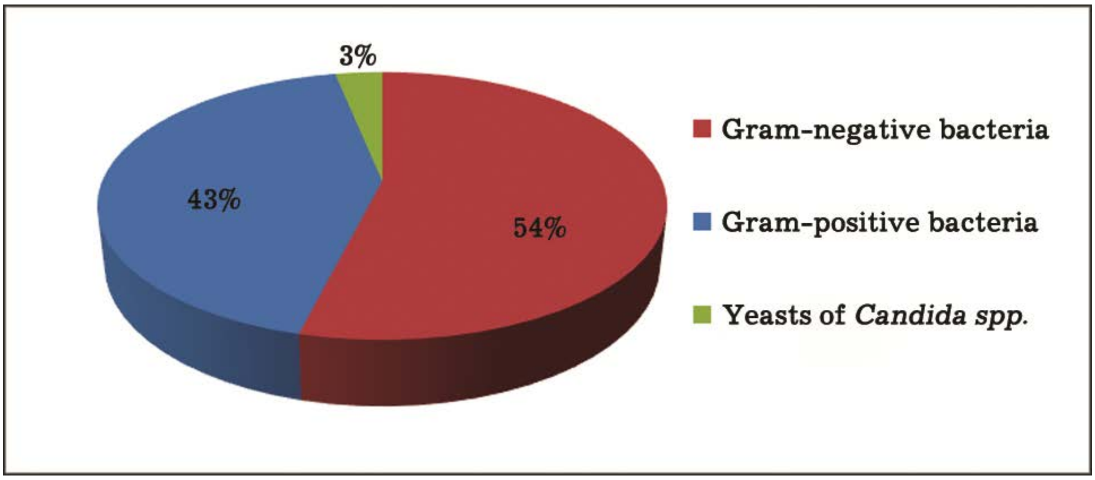

Fig. 1. The most common pathogens of septic infections in the Liver Transplantation Department of a multidisciplinary hospital

As can be seen in Fig. 1, Gram-negative pathogens accounted for 54% of the total number of strains, Gram-positive bacteria accounted for 43%, yeasts of Candida spp. made 3%.

The absolute leadership belonged to *Klebsiella pneumoniae* strains (33.0% of the total number of strains) (Fig. 2). *Klebsiella pneumoniae spp.* were followed by *Escherichia coli* (5.6%), *Proteus mirabilis* (2.5%), *Enterobacter spp.* (0.8%), and other enterobacteria (0.8%). Non-fermenting rods accounted for 11.3% of the isolated strains, among which *Pseudomonas aeruginosa* (7.3%) and *Acinetobacter baumannii* (2.7%) traditionally dominated.

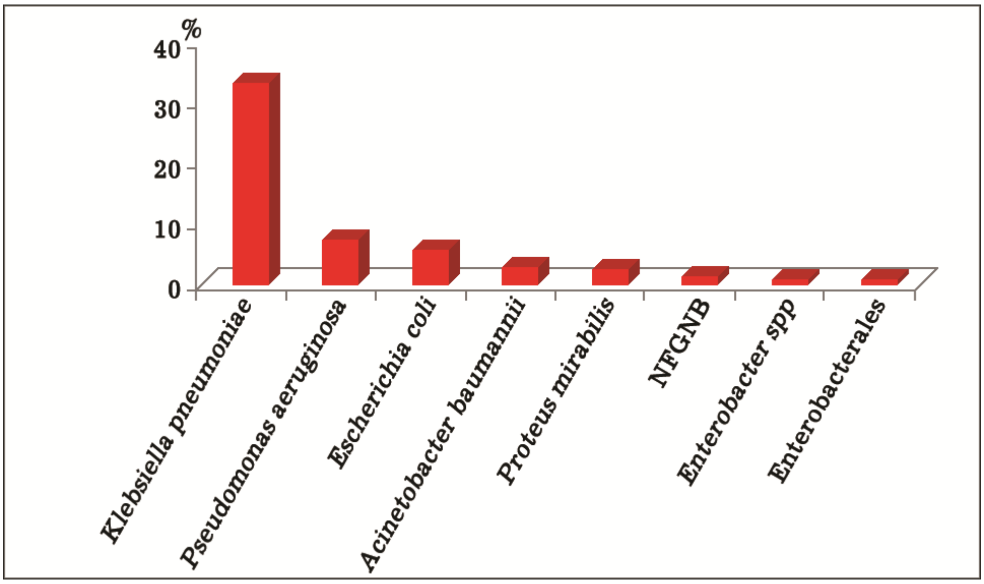

Fig. 2. Spectrum of Gram-negative pathogens

NFGNB, non-fermenting Gram-negative bacteria

The group of Gram-positive microorganisms was represented mainly by coccal microflora, among which coagulase-negative staphylococci predominated (18%, of which *S. epidermidis* accounted for 2%) and enterococci *(*19.5 %) represented by the *E. faecium spp. (*14.8 %) and *E. faecalis spp.* (4.7%) (Fig. 3). *Staphylococcus aureus* accounted for 2.4%, *Streptococcus spp. accounted* for 0.8%, other Gram-positive microorganisms in total made 2.4%.

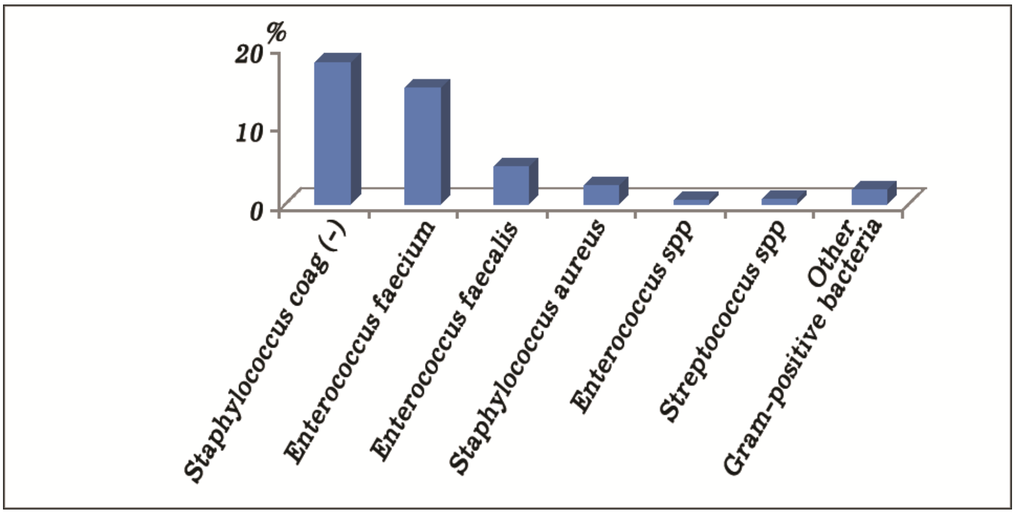

Рис. 3. Представители грамположительной микрофлоры

Fig. 3. Gram-positive bacteria

Among the Gram-negative pathogens of SI, the strains multidrug-resistant to the main classes of antibacterial drugs used in medical practice predominated (Table 1). As can be seen from Table 1, of all *K. pneumoniae* strains, the prevalence of strains resistant to amikacin, ciprofloxacin, imipenem, and meropenem was 72.4%, 95.5%, 89.3%, and 87.9% respectively. The antimicrobial potential was retained by tigecycline (7.6% of resistant Klebsiella strains) and colistin (11.2%). As for other enterobacteria, from 67.3% to 90.0% of strains resistant to ciprofloxacin were detected. Almost every fourth strain *of Proteus mirabilis* was resistant to amikacin (22.7%). Carbapenem group agents retained their activity against other *Enterobacteriaceae spp.* (the number of strains resistant to meropenem did not exceed 9.1%, those resistant to imipenem 8.2%).

Table 1. Sensitivity of Gram-negative rods to antibacterial drugs

Microorganisms	**Proportion of strains (%) resistant to:**					
	Tigecycline	**Colistin**	**Amikacin**	**Meronem**	**Imipenem**	**Ciprofloxacin**
Acinetobacter baumannii	n/a	0	54.1	87.5	75	n/a
Escherichia coli	4.1	4.1	8.2	8.2	6.1	67.3
Klebsiella pneumoniae	7.6	11.2	72.4	87.9	89.3	95.5
Proteus mirabilis	n/a	100	22.7	9.1	4.5	90.9
Pseudomonas aeruginosa	n/a	3.1	50	65.6	73.4	75

Note: n/a, non-available data

Among non-fermenting bacteria (*Pseudomonas aeruginosa* and *Acinetobacter baumannii*), multidrug-resistant strains also predominated. The percentage of *P. aeruginosa strains* resistant to imipenem, meropenem, or ciprofloxacin made 73.4%, 65.6% and 75.0%, respectively. Among *A. baumannii strains*, the prevalence of those resistant to imipenem was 75.5%, those to meropenem was 87.5%.

The dominance of multidrug-resistant strains was also revealed among the Gram-positive microflora (Table 2), with the proportion of oxacillin-resistant strains being MRSE from 80.9% (*S. epidermidis*) to 85.1% of strains (other coagulase-negative *Staphylococcus spp.*), MRSA making 33.3%. The antistaphylococcal activity was retained by vancomycin (4.8–9.5% of resistant strains), linezolid (0–9.5%), and daptomycin (4.8–9.5%).

Table 2. Antibiotic susceptibility of staphylococci

Microorganisms	Proportion of strains (%) resistant to:					
	Ciprofloxacin	Tigecycline	Linezolid	Oxacillin	Vankomycin	Daptomycin
Staphylococcus aureus	28.3	0	9.5	33.3	9.5	9.5
Staphylococcus coag (-)	84.4	2.8	9.2	85.1	4.96	4.96
Staphylococcus epidermidis	88.9	0	0	80.9	4.8	4.8

Among enterococci (Table 3), *E. faecium* strains predominated. (76% of strains of the total number) with a high level of resistance to beta-lactam antibiotics (100% of strains resistant to ampicillin and 90% to imipenem), as well as to vancomycin (46.1%). Linezolid (2.3% of resistant strains) and tigecycline (23.1%) retained a certain antibacterial potential against *E. faecium*. *E. faecalis* revealed high susceptibility rates to all antimicrobials tested.

Table 3. Antibiotic susceptibility of enterococci

Microorganisms	Proportion of strains (%) resistant to:				
	Tigecycline	Imipenem	Linezolid	Vancomycin	Ampicillin
Enterococcus faecalis	0	0	0	2.4	2.4
Enterococcus faecium	23.1	90	2,3	46.1	100

In our study, we attempted to identify the genes of the most common types of carbapenemase in imipenem- or meropenem-resistant strains of Gram-negative rods isolated from blood. Carbapenemase genes were identified in 16 strains (14 strains of *K. pneumoniae* and 2 strains of

E. coli). Metallo-beta-lactamase genes of NDM group were detected in 8 strains o*f K. pneumoniae* and 2 strains of *E. coli,* as well as one IMP-producing strain of *K. pneumoniae* were revealed. The genes of serine carbapenemases OXA-48 groups have been identified in 8 strains *of K. pneumoniae* (6 strains of *K. pneumoniae* simultaneously contained genes of OXA-48 and NDM groups) and three KPC-producing strains of *K. pneumoniae* were also revealed.

Discussion

Numerous studies have shown that the increase in the number of multidrug-resistant pathogens of SI in solid organ (including the liver) transplant departments significantly worsens the prognosis for the course and outcome of the disease [3]. The incidence of infections after liver transplantation increases in the presence of SI in the preoperative period, which in turn increases the likelihood of a fatal outcome in the early postoperative period [4, 5]. Our study showed the leading role of *Enterobacteriales* in the development of SI. Among enterobacteria, the undisputed leaders were the multidrug-resistant strains of *K. pneumoniae*. In recent years, the etiologic role of *K. pneumoniae* in the development of bacteremia and sepsis, infectious diseases of the lower respiratory tract, wounds and other infectious complications in surgical patients has significantly increased. The problem of SI caused by *K. pneumoniae* in patients of liver transplant departments is especially crucial in the early postoperative period [5]. The presence of multidrug-resistant and even pandrug-resistant strains sometimes creates an insurmountable barrier to the successful treatment of infections caused by *K. pneumoniae*. The increase in the number of carbapenem-resistant strains *of K. pneumoniae* directly correlates with the high incidence of deaths in the early postoperative period [6, 7].

Currently, full-scale monitoring of SI pathogens and their antibiotic sensitivity cannot be done without the knowledge of antibacterial drug resistance mechanisms possessed by SI pathogens in a particular hospital. In recent years, the strains synthesizing extracellular carbapenemases that destroy beta-lactam antibiotics have been increasingly isolated. Genes encoding the synthesis of carbapenemases are part of mobile genetic elements (transposons, integrons, prophages, etc.) located both on chromosomes and on plasmids [8]. Plasmids are capable of quite easily transferring genetic information from one bacterial species to another [8]. Moreover, the same mobile genetic elements often contain genes of resistance to other antibacterial drugs [9]. The presence of horizontal spread of genetic determinants of resistance to drugs of several classes, in addition to intraclonal spread, carries the threat of involving practically all representatives of Gram-negative microflora in this “resistance epidemic”. Therefore, at present, in order to assess the risks of the emergence of new sources of hospital multidrug-resistant strains, it is considered necessary to monitor genetic determinants encoding the synthesis of enzymes that hydrolyze clinically significant antibiotics, including carbapenems. However, one should bear in mind that the resistance to carbapenems can also be realized with the mutually reinforcing action of several mechanisms at once, for example, modification of porin channels, oversynthesis of chromosomal beta-lactamases AmpC and production of extended-spectrum beta-lactamases (ESBL group CTX-M) [10]. In addition, *Klebsiella spp*. have such protection mechanisms against the antibiotics action as the synthesis of exopolysaccharides, which form a mucous coating that protects cells from external impacts (they hinder the contact with the antibiotic) [11]. In non-fermenting bacteria, the efflux mechanism (an active removal of the antibiotic from the cell) also operates [10]. Obviously, the possibility of simultaneous implementation of several resistance mechanisms should be taken into account when selecting genetic determinants for screening. There are examples of the rational use of such an approach. Thus, the use of express systems for detecting molecular determinants of carbapenemases in intensive care unit patients with bacteremia caused by carbapenem-resistant strains of enterobacteria made it possible to shorten the time till initiating an adequate therapy and to reduce mortality in the 30-day period after surgery by more than twice, i.e. from 37 to 16% [12].

The ratio of different types of carbapenemases varies in different hospitals [13]. In our study, metallo-beta-lactamases of NDM, IPM types and serine carbapenemases of OXA-48 and KPC groups were detected in carbapenem-resistant strains of gram-negative blood cultures.

There are currently no clear recommendations regarding the treatment tactics for SI caused by carbapenem-resistant strains of gram-negative bacilli. New drugs are being developed (this is a long and resource-intensive process), and attempts are being made to find successful combinations of existing ones. A number of current reviews have proven a high efficacy of ceftazidime-avibactam [14]. Scientific articles provide the evidence of highly efficacious using ceftazidime-avibactam together with colistin [15] or aztreonam [16]. It is assumed that combination therapy is more effective than monotherapy, especially targeted to strains producing metallo-beta-lactamases. There is evidence that therapy with drugs such as ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-cilastatin-relebactam is effective against serine carbapenemase producers of OXA-48-type and KPC groups [16]. New inhibitors such as taniborbactam, zidebactam and nacubatam, as well as the aztreonam-avibactam combination, show promise in combination with other β-lactams and are currently being evaluated in phase 3 clinical trials [16]. These drugs have not yet been registered in Russia. Interestingly, the emergence of strains resistant to some of these new drugs and their combinations has already been reported.

Among the factors preventing the spread of multidrug-resistant strains of SI, the leading role is undoubtedly belong to the rational, justified use of antimicrobial drugs, based on the knowledge of the real spectrum of SI pathogens in a particular hospital. However, a long-term intensive use of a particular drug in medical practice inevitably leads to the formation of strains resistant to it (or its group) [6]. Thanks to using microbiological monitoring of SI pathogens, it was shown that the most important sources of SI pathogens are the patients themselves, in whose bodies the resistant strains of potential SI pathogens persist. Thus, a number of studies have shown the carriage of carbapenem-resistant *K. pneumoniae* and *E. coli* strains in the intestines of patients with chronic liver diseases who underwent inpatient antibiotic therapy with third-generation cephalosporins in combination with beta-lactamase inhibitors or fluoroquinolones for 3 months prior to hospitalization [6, 7].

Many countries in Europe, America, and Asia have created unified national electronic databases containing information on resistant strains circulating in a particular region. In Russia, a multicenter study has also been conducted for a long time (over 20 years) to identify resistant strains of SI pathogens [18]. An electronic database AMRmap (https://amrmap.ru) has been created, which is updated annually. This database contains a complete description of resistant strains (in-hospital and outpatient); for some isolates, there is also available information on molecular determinants of resistance. These data are being used with varying efficacy to create systems for protecting against the spread of resistant hospital strains in medical in-hospital facilities in Russia.

Conclusion

As can be seen from the presented material of the article, the most common Gram-negative pathogens causing septic infections in patients of the Liver Transplantation Department of the N.V. Sklifosovsky Research Institute for Emergency Medicine were strains of *K. pneumoniae*, the Gram-positive ones - coagulase-negative staphylococci and enterococci.

Monitoring of causative agents of septic infections is the basis for developing the schemes of empirical antibacterial therapy in liver other vital organ transplant departments. The results of our study have once again confirmed the global trend to the increased number of multidrug-resistant strains among causative agents of septic infections. A serious threat is posed by multidrug-resistant strains of carbapenem-resistant gram-negative bacteria, which eradication is a difficult task for practical medicine due to the constantly shrinking range of therapeutic drugs. Additional difficulties are also created by the spread of multi-drug resistant gram-positive coccal microflora. Vancomycin, linezolid, and daptomycin retain their activity against it, but there is an increase in the number of strains resistant to them.

An important component of microbiological monitoring is screening the carriers of the molecular determinants of resistance, studying the mechanisms and routes of their spread among septic infection pathogens in each specific hospital. In our study, the carbapenem-resistant *K. pneumoniae* and *E. coli* strains isolated from the blood of patients in the Liver Transplantation Department of the N.V. Sklifosovsky Research Institute for Emergency Medicine were dominated by NDM metallo-beta-lactamase and OXA-48-type serine carbapenemase groups. KPC serine carbapenemase and IPM metallo-beta-lactamase groups were also found. Given the ease with which the genes for these carbapenemases are transferred from one bacterial species to another by mobile genetic elements, the possibility of the emergence of new strains of Gram-negative rods resistant to carbapenems should be assumed.

Data from monitoring of pathogens causing septic infections are the basis for developing the methods to prevent the spread of their hospital pathogen strains among the patients in transplant departments. The main tool to fight the accumulation of resistant strains is a rational use of antibacterial drugs in the treatment of septic infections at all stages of patients' stay in hospital. Possible long-term persistence of multidrug-resistant strains in a patient body creates a risk of spreading the multi-drug-resistant pathogens of septic infections beyond hospitals ("resistance pandemic").

Our analysis of the currently available database is associated with a number of limitations, the main ones being the retrospective observational nature of the study and the heterogeneity of the sample. In our study, we did not take into account the heterogeneity of patient sample and types of surgery, the presence and nature of complications, the identification of patients with contamination, since the purpose of the study was to identify the peculiar structure of septic infection pathogens, their sensitivity to antibiotics in patients of a liver transplant department of a large multidisciplinary hospital.

At the same time, we should note that in all cases, the microbiological analysis was performed in accordance with generally accepted criteria for assessing the etiological significance of the isolated microorganisms, which allows us to confidently operate with the concept of "pathogen". In addition, we have taken into account the fact that in relation to patients in transplant departments, any conditional contaminant, if neglected, can become a pathogen, so it is important to evaluate the results changing over time (in repeated samples). The limited dataset allows us only suggests a trend, and final conclusions will be made on the basis of further studies. Global practice shows that in a multidisciplinary hospital, there is always a risk of hospital strains circulating within the entire hospital, that is, the spectra of pathogens can overlap in different departments [19].

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