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Original Article

ESBL in positive hemoculture of a Southern-Brazil Teaching Hospital's Intensive Care Units

ESBL em hemoculturas positivas das Unidades de Terapia Intensiva de um Hospital Universitário do sul do Brasil

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Abstract

Bloodstream infections are among the most serious and frequent infections, and the people most exposed are patients in the Intensive Care Unit (ICU). ESBL (extended-spectrum beta-lactate) are resistant bacteria to penicillins, cephalosporins and monobactams. It´s necessary to know how often and which microorganisms are involved, checking their susceptibility. This study was carried out at the University Hospital. Data collection was performed in the Adult and Newborn ICUs, with assessment of microorganisms and their resistance profile. During six-month period, 156 samples were studied, and 42 were positive with microorganism isolation. Isolated species include *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Klebsiella pneumoniae*. Many resistant to carbapenem.

Keywords: ICU, bacteria, infection, antimicrobial susceptibility.

Resumo

ESBL in Positive Hemoculture of a Southern-Brazil Teaching Hospital's Intensive Care Units

As infecções da corrente sanguínea estão entre as infecções mais graves e frequentes, e os indivíduos mais expostos são os pacientes da Unidade de Terapia Intensiva (UTI). As ESBL (Beta-Lactamase de Espectro Estendido) são bactérias resistentes a penicilinas, cefalosporinas e monobactâmicos. Se faz necessário saber com que frequência e quais microrganismos estão envolvidos, verificando sua suscetibilidade. Este estudo foi realizado no Hospital Universitário. A coleta de dados foi realizada nas UTIs Adulto e Neonatal, com avaliação dos microrganismos e seu perfil de resistência. Durante o período de seis meses, foram estudadas 156 amostras, sendo 42 positivas com isolamento dos microrganismos. As espécies isoladas incluem *Staphylococcus aureus*, *Staphylococcus epidermidis* e *Klebsiella pneumoniae*. Muitos resistentes aos carbapenêmicos.

Palavras-chave: UTI, bactéria, infecção, suscetibilidade antimicrobiana.

1. Introduction

Bloodstream infections are among the most serious and stand out for their mortality rates and prolonged hospitalization time. In adults, bloodstream infection is the seventh leading cause of death in the United States and Europe (Ruth et al., 2014).

The identification of microorganisms that cause blood infection plays an important role in the diagnosis of a patient with fever, determining the presence of infection, reassuring the physician with the chosen empirical therapy and providing updated information on etiology and sensitivity to antibiotics, guiding the physician in the management of patients. The emergence of antibiotic-resistant bacteria is a problem that has worsened over the years. Since the discovery of beta-lactam antibiotics, penicillin, it has been known that bacteria have the ability to respond quickly to environmental changes. Beta-lactam is among the most prescribed class of antimicrobials, due to its therapeutic efficacy, low cost, low toxicity and clinical safety (Patel and Bonomo, 2011). Four are the main subgroups available for clinical use: penicillins, cephalosporins, monobactamics and carbapenems. ESBL bacteria (extended-spectrum beta-lactate) are resistant to penicillins, cephalosporins and monobactams (Santos et al., 2015).

Due to the increase in ESBL-producing strains, carbapenems are increasingly used as a therapeutic option for the treatment of serious infections, being the last line of defense against resistant lineage infections. Consequently, KPC (*Klebsiella pneumoniae* Carbapenemase) appeared, providing resistance to almost all beta-lactam

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and beta-lactam inhibitors. Bacteria with the KPC enzyme hydrolyze cephalosporins, monobactamic penicillins and carbapenems. The treatment was increasingly restricted to the use of polymyxins, tigecycline and aminoglycosides combined with imipenem (Pal and Sujatha, 2016).

Resistance to antibiotics, therefore, has clinical and economic consequences. The restriction of access to microbiological tests affects 35% of Brazilian hospitals, leading to the use of empirical therapies and, repeatedly, to the unnecessary use of antibiotics, postponing hospital discharge and increasing mortality rates and public health costs (Adam et al., 2011).

This study aimed to collect data in the Itensive Care Units (ICUs) of Adults and Newborns who sent material to the Clinical Analysis Laboratory at UFPel, to identify microorganisms and evaluate their profile of antibiotic resistance for a period of six months, investigating the frequency of microorganisms in blood cultures of patients at UFPel University Hospital.

2. Materials and Methods

The study was carried out with data from the samples sent for blood culture at the Intensive Care Units for Adults and Newborns at the University Hospital of the Federal University of Pelotas - Pelotas-RS / Brazil (HE UFPel). It is a public hospital, with free medical care via the Unified Health System (SUS) and offers coverage to 28 cities in the southern region of Rio Grande do Sul. Currently, the hospital has 175 beds, however there are 6 for adult ICUs and 9 for ICU newborns. The study was carried out over a six-month period in the second half of 2017.

The samples were obtained by venous or arterial puncture, or by central or umbilical catheterization, totaling 156 blood cultures during this period. Following the instructions of the Hospital Infection Control Committee (CCIH), which requires that, before each sample collection, antisepsis be performed with 70% alcohol gel or chlorhexidine in the puncture area, to minimize the possibility of contamination of the sample.

This study was submitted to and approved by the Ethics Committee of the Faculty of Medicine of the Federal University of Pelotas, under registration number 2,377,130.

The analysis of the samples was carried out at the Clinical Analysis Laboratory of HE UFPel, with the Bactec system, used to detect the growth of microorganisms in blood samples. The methodology is based on the fluorescence emitted by the sensor in flasks with culture medium. BD BactecTM 9050 equipment monitors, shakes and incubates the bottles simultaneously and continuously, with a capacity of 50 bottles.

After sampling, the flasks with BD BactecTM culture medium were incubated for 5 days (or until positive), at 35ºC. The equipment automatically monitored the samples every 10 minutes. If an average turbidity were shown, a beep would be emitted and positive samples taken from the equipment. The samples were then sown on Macconkey / Blood agar and Sabouraud agar and incubated for another 24 hours.

After this period, colony micromorphology and Gram stain were visualized. In the case of identification of fungal structures, the result was immediately labeled "Fungal growth". In the case of gram-positive or gram-negative bacteria, the BD PhoenixTM equipment performed the identification and antibiogram. This equipment works with panels in which, on the ID (identification) side, there are 51 wells, and on the AST (Antimicrobial Sensitivity Test) side, there are 85. The ID side contains 45 wells with dehydrated biochemical substrates and two wells for fluorescence control. This way, in less than 24 hours, the equipment provides bacteria identification, as well as the antibiogram.

In case of gram-negative bacteria growth, the antibiotics tested were: sulfamethoxazole + trimethoprim, ampicillin, cefalotin, gentamicin, amikacin, ceftriaxone, cefuroxime, ciprofloxacin, ceftazidime, cefepime, piperacillin + tazobactam, levofloxacin, imipenem, meropenem and colistin.

When gram-positive bacteria growth was shown, antibiogram was performed with sulfamethoxazole + trimethoprim, ampicillin, gentamicin, amikacin, ciprofloxacin, levofloxacin, oxacillin, penicillin, erythromycin, clindamycin, vancomycin and rifampicin.

3. Results

During the six months, 156 blood culture samples from the ICU of HE UFPel were analyzed, with 93 samples from adult ICU and 63 from neonatal ICU. 114 were negative and 42 showed microbiological growth. Bacterial and fungal infections have been detected. More than 90% of the positive samples analyzed contained bacterial growth (Table 1).

In two cases, two microorganisms grew concomitantly. In the first, *Klebsiella pneumoniae* and fungus. In the second, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

All samples with bacterial growth had their antibiogram performed and a high average percentage of resistance was verified. No vancomycin-resistant bacteria were detected. The percentages of resistance found are frightening, since several standard antibiotics used by the hospital do not demonstrate efficacy against most of the identified bacteria.

The uncontrolled use of antibiotics gives rise to the so-called "super-resistant" or "multi-resistant bacteria; *Klebsiella pneumonia* (Pal and Sujatha, 2016) presented KPC and *Staphylococcus aureus* (Patel and Bonomo, 2011), *Klebsiella pneumonia* (Ruth et al., 2014) *and Enterococcus cloacae* (Ruth et al., 2014) presented EBSL.

4. Discussion

This is the first data collection of this type to be carried out in the south of RS and the percentage of positivity of blood cultures was 27% (42 positive / 156 total samples), a relevant amount. Similar studies carried out in other Brazilian hospitals showed different positivity profiles. In Recife/PE was observed 19,61% positive hemoculture in a retrospective study (185 positive / 943 total samples); in a hospital in Rasht, North of Iran, a survey in education hospitals showed 6.85% positivity (225 positive/ 3283 total samples), while in a large European teaching hospital, 13.9% (541 positive/ 3349 total samples) blood cultures were positive for one or more microorganisms (Oliveira, 2019; Keihanian et al., 2018; Nannan Panday et al., 2019). This diversity of places and percentages shows the importance of regional studies to assess the local reality and define preventive strategies.

The high diversity of microorganisms found is comparable to other studies, as in Oliveira (2019) and Keihanian et al. (2018). The genus *Staphylococcus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterobacter cloacae* and *Proteus mirabilis* appear among the main microorganisms in all studies. It was found that the *Staphylococcus* genus represents 60% of frequency in relation to the other microorganisms found. This is probably due to the fact that this genus is easily found in the skin and in the nasal cavities and, with the imbalance of the microbiota-host relationship, they become dangerous pathogens.

Klebsiella pneumoniae, another high frequency microorganism in this study, is part of the natural oropharynx and intestine microbiota. The frequency of outbreaks of hospital infections by *K. pneumoniae* is increasingly high due to its high resistance to available antimicrobials, which highlights the importance of its control and monitoring in a hospital environment.

Infection prevention and control is a practical approach and is based on evidence that prevents patients and healthcare professionals from being harmed by preventable infections. A study carried out in a hospital in Porto Alegre / RS found that the frequency of appearance of multidrug-resistant bacteria is 70% higher, with the majority being the ESBL (Meyer and Picoli, 2011). This percentage is approximately three times higher than that obtained in the data of the present study. In this study, 100% of *Klebsiella pneumoniae* cases were resistant to multiple drugs (Table 2).

In 2001, Winokur et al. (2001) evaluated the frequency of ESBLs among the Enterobacteriaceae family isolated from several geographic regions. *Klebsiella pneumoniae* was found to be the most commonly found enterobacterium, with all isolated cases showing resistance to multiple drugs, confirming research carried out in Latin America (45.4%), Western Pacific region (24.6%), Europe (22%), Europe (22%), the United States (7.6%) and Canada (4.9%). In the diversity of microorganisms found, our results corroborate the findings of other authors, demonstrating that there are several species of microorganisms to be fought. In addition, the intensification of the work of UFPel's CCIH is of vital importance. Bacterial resistance and the increasing incidence of multidrug-resistant microorganisms lead to serious consequences, such as increased hospital stay and deteriorating health conditions. Thus, knowing the

Table 1. Total number and isolated microorganisms' frequency in hemocultures from HE UFPEL Adult and Newborn ICUs' patients.

Isolated Microorganism	***Number of Samples***	***Percentage %***
Acinetobacter sp.	2	1.28
Aerococcus viridans	1	0.64
Bacillus circulans	1	0.64
Burkholderia sp.	1	0.64
Cellulomonas turbata	1	0.64
Enterobacter cloacae	1	0.64
Enterococcus faecalis	1	0.64
Fungo	3	1.92
Klebsiella pneumoniae	5	3.2
Proteus mirabilis	2	1.28
*Pseudomonas aeruginosa**	1	0.64
Staphylococcus aureus	7	4.48
Staphylococcus spp. (coagulase negativa)	7	4.48
Staphylococcus epidermidis	5	3.2
Staphylococcus haemolyticus	2	1.28
Staphylococcus hominis	1	0.64
Staphylococcus saprophyticus	1	0.64
Staphylococcus warneri	1	0.64
SubTotal	42*	27
Fungus	3	1.9
Negative	111	71.1
Total	156	100

**Pseudomonas aeruginosa* only growth with other bacteria.

Table 2. Bacterial resistance percentage in hemoculture samples from HE UFPel's Adult and Newborn ICUs.

Antibiotics	***Bacterial Resistance %***
Amikacin	26.6
Ampicillin	88.5
Cephalothin	77.8
Ceftazidime	61.5
Ceftriaxone	83.3
Cefuroxime	80
Cefepime	76.9
Ciprofloxacin	66.7
Clindamycin	63.1
Gentamicin	78.6
Erythromycin	84.2
Imipenem	42.8
Levofloxacin	38.8
Meropenem	42.8
Oxacillin	78.9
Penicillin	94.4
Pipetacycline + Tazobactam	64.3
Rifampicin	5.3
Sulfamethoxazole + Trimethoprim	62.5
Vancomycin	0

regional microbiota and its characteristics of sensitivity to antibiotics becomes vital for the maintenance of the health of the population as a whole.

This result may lead to a discussion about new antibiotic use protocols, as well as the need to standardize others in addition to the drugs currently used.

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