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ETIOLOGICAL AND RESISTANCE PROFILE OF BACTERIA ISOLATED FROM PRIMARY BLOODSTREAM INFECTIONS ASSOCIATED WITH PERIPHERALLY INSERTED CENTRAL CATHETER IN NEONATES

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ABSTRACT

Introduction: The Peripherally Inserted Central Catheter (PICC) is an intravenous peripherally inserted device with characteristics of central venous access, with bloodstream infection being the main complication associated with the presence of this catheter. The main pathogens involved are: Candida sp., gram-positive cocci of the genus Staphylococcuscoagulase-negative and gram-negative bacilli. Objective:To identify the main risk factors for newborns reported with laboratory-confirmed Primary Bloodstream Infection (IPCSL) associated with PICC, as well as the etiological profile and resistance of the most prevalent bacteria. Methodology: A descriptive study with a quantitative approach was carried out from January 2015 to December 2016 in the ICU of a maternal and child hospital. The sample was composed of cases reported with IPCSL associated with the PICC. Data collection was carried out from the notification forms, blood culture results, and antibiograms attached to the medical records. The data were tabulated and analyzed in the light of descriptive statistics. Results: The main risk factors associated with blood infection were: prematurity, prolonged hospitalization, catheter permanence time, use of antibiotics and parenteral nutrition. Regarding the microbiological profile, S predominated. epidermidiswith 71% of oxacillin-resistant strains. Conclusion: The planning of care by the interdisciplinary team for the appropriate management of the PICC, as well as the monitoring, evaluation and discussion of the IPC indices associated with this catheter will enable the adequacy of practices that minimize the rates of nosocomial infections.

Keywords: PICC. Hospital Infection. Newborn.

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INTRODUCTION

The *Peripherally Inserted Central Catheter* (PICC) consists of an intravenous peripherally inserted device with characteristics of central venous access, due to its location up to the middle third of the superior vena cava or inferior vena cava1. It is considered an important therapeutic resource in Intermediate Care Units (ICU) and neonatal Intensive Care Units (ICU), which is performed by a graduated and previously trained nursing professional, as provided for by Resolution No. 258/20012 of the Federal Nursing Council2.

Among the benefits of the PICC are the reduced number of punctures; long-term venous access; used for the administration of parenteral nutrition, antibiotic therapy, infusion of vasoactive drugs and hyperosmolar solutions4,5,6.However, scientific studies have pointed to a set of non-infectious complications such as: obstruction, rupture, limb edema, extravasation, accidental traction; and infectious diseases such as: infectious phlebitis, infection of the insertion site and bloodstream infection7,5.

According to Pinheiro et al. (2009)[8], infectious complications stand out as an important cause of morbidity and mortality in the neonatal population. In a comparative analysis of the incidence and etiological profile of nosocomial infection in neonates admitted to the NICU and classified according to place of birth, a cumulative incidence of 30.6% of neonates with systemic infection was observed during the period of stay in the hospital.

Based on this context, Anvisa (2013)[9] highlights Primary Bloodstream Infection (PBSI) associated with Central Venous Catheter (CVC) as the main infection in neonatal ICUs. It also states that PBSI are classified as associated with CVC (umbilical, PICC, among others) if they are present at the time of diagnosis of the infection or up to 48 hours after their removal.

PCIs are defined as infections with severe systemic consequences such as bacteremia or sepsis, with no identifiable primary focus, and it is difficult to determine the involvement of the central catheter in their occurrence9. It is considered one of the main infections that affect premature infants weighing less than or equal to 1,500g, which reinforces the high vulnerability of this group10,11,12.

Also according to Anvisa (2013)[9], a primary bloodstream infection in the newborn can be classified as Primary Laboratory Bloodstream Infection (IPCSL) or Primary Clinical Bloodstream Infection (IPCSC).

Thus, IPCSL is defined based on microbiological confirmation through the presence of one or more positive blood cultures paired (one peripheral vein and one central vein) with

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culture of non-contaminating microorganisms of the skin and that the microorganism is not related to infection in another site. It may also be considered as IPCSL if *coagulase-negative Staphylococcus* is cultured in at least one peripheral blood culture of a patient with a central venous catheter (CVC).

According to Bonvento (2007)[13], catheter colonization usually occurs through the external surface of the catheter, the subcutaneous tunnel and the surrounding skin, which can be colonized by the skin's own microbiota, the hands of the professionals and the contaminated antiseptics; and/or the internal surface of the catheter, which can occur through two mechanisms: 1. Inappropriate manipulation of the catheter; and 2. Contamination of infusion solutions by direct handling of the administered substance during the industrial manufacturing process of the solution or during the installation process.

Prematurity and clinical factors such as ICU admission, type of catheter material, insertion site, and non-compliance with the catheter insertion and maintenance technique may be related to PBSI associated with central venous catheter. While catheter permanence time, parenteral nutrition infusion, blood transfusion, more than one indication for device use and femoral insertion site can increase susceptibility to bloodstream infections14,5.

The main pathogens involved in PBIs associated with the presence of CVC *are Candidasp., gram-positive cocci of the genus Staphylococcuscoagulase-negative and gram-negative bacilli such as Escherichia coli, Enterobacterspp*, *Pseudomonasaeruginosa*, *Enterococcus, and Klebsiella pneumoniae13,15,16,3*.

According to the *Center for DiseasesControl and Prevention* (CDC)[17], the six bacteria with the greatest resistance to antibiotics associated with nosocomial infections are: those of the *carbapenem-resistant Enterobactereaceacea family and Extended Spectrum Beta-lactamases (ESBL); Methicillin-resistant Staphylococcus aureus* (MRSA); *Vancomycin-resistant enterococcus; Pseudomonase Enterobacter-resistant to multiple drugs.*

In a study carried out for the detection of slime production by coagulase-negative staphylococci (NEC) isolated from central venous catheters, it was observed that most of the *coagulase-negative Staphylococcus species* had an association between slime production and reduced sensitivity to antimicrobials, suggesting the pathogenic potential of these organisms in the hospital environment. And that the species *S. epidermidis,* the most

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frequent, showed maximum resistance against penicillin G, followed by erythromycin, oxacillin and gentamicin; and sensitivity to vancomycin18.

Tenover (2006)[19] describes that bacterial resistance can occur intrinsically - as a function of resistance genes present in the original chromosome of microorganisms; and extrinsically or acquired - through mutations, genetic recombinations or acquisition of resistance genes from other organisms, which occur through inversions, duplications, insertions or transpositions from one site of the bacterial chromosome to another, acquisition of resistance genes carried by plasmids or transposons20.

Considering the complexity of bloodstream infections, it is speculated that microorganisms can adhere and colonize the devices used to perform CVC, forming biofilms, thus enhancing their pathogenicity21,22.

However, with the increase in the resistance profile of microorganisms to antimicrobials, the susceptibility of neonates to bloodstream infections related to CVC insertion makes it relevant to define the microbial and resistance profile of these bacteria isolated from PICC-associated IPCs in neonates.

OBJECTIVE

To identify the main risk factors for neonates reported with IPCSL associated with PICC, as well as the etiological profile and resistance of the most prevalent bacteria.

METHODOLOGY

This is a descriptive and documental study in the form of a quantitative approach carried out in a maternal-child reference hospital in the city of Petrolina, located in the Pernambuco hinterland, between January 2015 and December 2016. Research project approved by the Research Ethics Committee, respecting Resolution No. 466/12 with CAAE 69962017.7.0000.5207.

The sample consisted of notifications of IPCUs associated with PICC with laboratory confirmation in neonates admitted to the ICU, according to the Diagnostic Criteria for Infection Related to Health Care in Neonatology9. Inclusion criteria were defined as neonates admitted to the ICU notified with PICC-associated PCIs with laboratory confirmation, and cases of PBSI reported according to clinical criteria, inconsistency of the information contained in the notification forms, as well as cases that did not have the results of blood culture and antibiogram attached to the medical records. Data collection was

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carried out using the notification forms used by the Hospital Infection Control Commission (CCIH) and the medical records of the institution's Medical Archive and Statistics Service (SAME). The data were recorded in an instrument containing sociodemographic, clinical, and laboratory variables contained in items 1, 2, 5, 7, 8, and 9 of the notification form used by the CCIH and in the results of blood cultures and antibiograms attached to the medical records. The etiological profile was defined based on the results of blood cultures, and bacterial resistance was described for the most prevalent strains for which the results of the antibiograms were used.

The data obtained through the notification forms and medical records were tabulated and analyzed in the light of descriptive statistics, using the Microsoft Office Excel® program, and the results were presented in the form of graphs and tables.

RESULTS

From January 2015 to December 2016, 190 Care-Associated Infections (ARIs) were reported, of which 80 were Primary Bloodstream Infections with Laboratory Confirmation (IPCSL) associated with *PeripherallyInserted Central Catheter* (PICC). According to the exclusion criteria, 19 notifications were not contabilizadas.Com relation to the main sociodemographic and clinical variables related to neonates, 100% (61) were born with a gestational age (GA) ≤ 36 weeks and six days, 33 females and 28 males; and 73%(51) weighed less than 1,500g.

The mean length of hospital stay at the time of notification of PICC-associated IPCUS was 19 days. Regarding the use of Parenteral Nutrition (PN), 75%(46) used this infusion, with an average of 16.5 days of therapy use; 16% (10) did not receive it; and 8%(5) were not accounted for, due to lack of information in the medical records.

Regarding the diagnostic hypotheses of newborns (NB) admitted and hospitalized in the ICU, 100% (61) of the sample had a diagnosis of prematurity, 31% (59) of which were pathologies associated with the respiratory system, 19% (36) possibility of metabolic decompensation, 15% (28) possibility of maternal-fetal blood incompatibility, 25% (13) possibility of nonspecific infection, 12% (22) infectious risk, 6% (12) possibility of specific infection, 12% (3) perinatal hypoxia, 1% (2) congenital syphilis, 1% (1) risk of syphilis, and 1% (1) congenital heart disease.

Regarding the variables associated with the PICC, a mean catheter stay of 23.5 days was observed. Regarding the most commonly used blood vessel, the right cephalic vein

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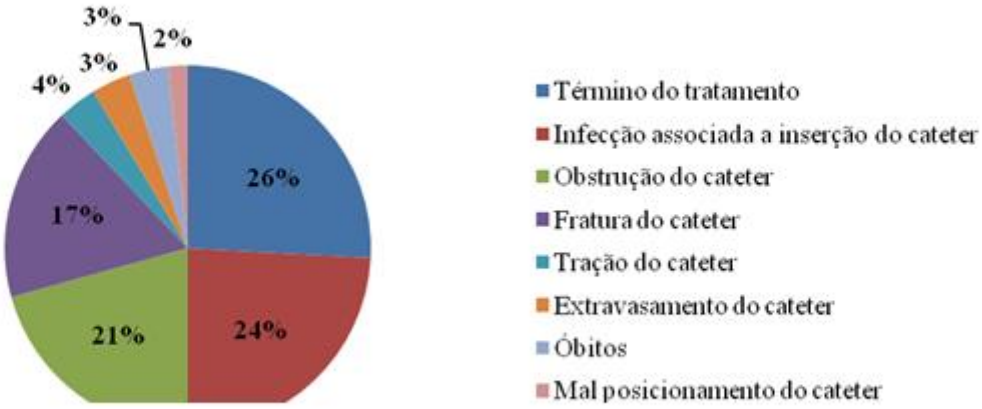
(27%) stands out, followed by the left cephalic vein (22%) and the right basilic vein (17%), as shown in Graph 1. The reasons for catheter removal were 26% (15) due to the end of treatment, 24% (14) infection associated with catheter insertion, 21% (12) catheter obstruction, 17% (10) catheter fracture, 3% (2) catheter traction, 3% (2) catheter leakage, 3% (2) deaths, and 2% (1) catheter malposition (Graph 2).

Gráfico 1. Vaso sanguíneo utilizado para a inserção do PICC em neonatos internados na UCI entre o período de janeiro de 2015 a dezembro de 2016. Petrolina-PE, 2017.

Fonte: Prontuários arquivados ao SAME da instituição.

Gráfico 2. Motivos para a retirada do PICC em neonatos internados na UCI entre o período de janeiro de 2015 a dezembro de 2016. Petrolina-PE, 2017.



Fonte: Prontuários arquivados ao SAME da instituição.

The microbiological analysis used by the institution's laboratory was based on the method of automated and/or manual techniques with reference to the Clinical Laboratory Standards Institute – CLSI - M100 S25 (2015). A mixed etiological profile of the isolated microbiota was observed. Of the bacterial findings, the prevalence was for Gram positive

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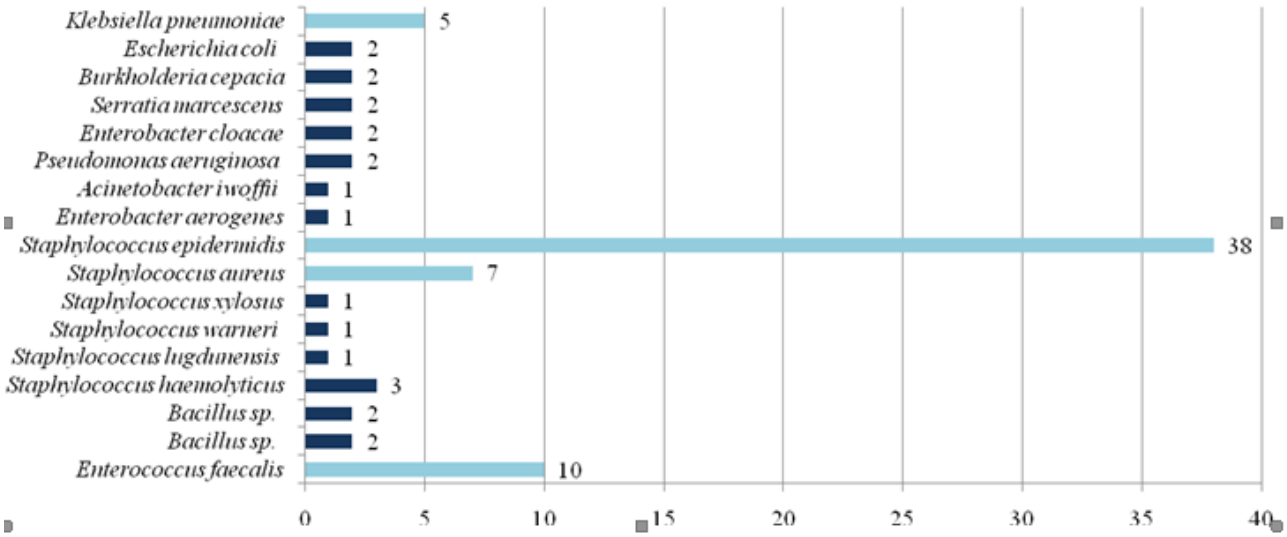
cocci, 46% (38) *Staphylococcus epidermidis*, 12% (10) *Enterococcusfaecalis* and 9% (7) *Staphylococcus aureus.* Regarding Gram-negative bacilli, *Klebsiellapneumoniae* had a frequency of 6% (5) (Graph 3).

Gráfico 3. Perfil etiológico das IPCS associadas à PICC em neonatos internados na UCI entre o período de janeiro de 2015 a dezembro de 2016. Petrolina-PE, 2017.



Fonte: Hemoculturas anexadas aos prontuários arquivados ao SAME da instituição.

Tables 1, 2, 3 and 4 characterize the isolated microorganisms with the highest prevalence in terms of resistance profile. It was observed that 100% of the S . *epidermidis strains were resistant to penicillin and ampicillin; 95% to oxacillin and gentamicin; 86% to levofloxacin; 84% ciprofloxacin; 76% trimethoprim+sulfamethoxazole and 5% intermediate resistance to vancomycin. For four strains of S. epidermidis, the following antibiotics were also tested: teicoplanin, rifampicin, erythromycin and clindamycin (Table 1).*

For the S. *aureus* isolates, 100% of the strains showed resistance to penicillin and ampicillin; 71% to oxacillin, gentamicin, ciprofloxacin and levofloxacin; 43% to trimethoprim + sulfamethoxazole and 14% tetracycline (Table 2), while the E. *faecalis strains showed only 70% of intermediate resistance to ciprofloxacin (Table 3).*

Of the K . *pneumoniae isolates, 33% were resistant to ampicillin and 20% to amoxicillin + clavulanic acid, gentamicin, tobramycin, ciprofloxacin, levofloxacin, ceftazidime, cefepime, cefotaxime, and trimethoprim + sulfamethoxazole (Table 4).*

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Table 1. Resistance profile of *S. epidermidis* strains isolated from IPCs associated with PICC in neonates admitted to the ICU between January 2015 and December 2016. Petrolina-PE, 2017.

Antibiotics Tested	Number of resistant/tested samples	Resistance (%)
Penicillin	38/ 38	100
Ampicillin	38/ 38	100
Oxacillin	36/ 38	95
Gentamicin	36/ 38	95
Ciprofloxacino	32/ 38	84
Levofloxacino	32/ 37	86
Trimethoprim+Sulfamethoxazole	29/ 38	76
Vancomycin	2/ 38	5*
Teicoplanin	0/ 4	0
Linezolid	0/ 37	0
Tetracycline	2/ 34	6
Rifampicin	0/ 4	0
Erythromycin	4/ 4	100
Clindamycin	4/ 4	100

*Intermediate Resistance/Source: Antibiograms attached to the medical records filed with the institution's SAME.

Table 2. Resistance profile of *S. aureus strains isolated from PICC-associated IPCs in neonates admitted to the ICU between January 2015 and December 2016. Petrolina-PE, 2017.*

Antibiotics Tested	Number of resistant/tested samples	Resistance (%)
Penicillin	7/ 7	100
Ampicillin	7/ 7	100
Oxacillin	5/ 7	71
Gentamicin	5/ 7	71
Ciprofloxacino	5/ 7	71
Levofloxacino	5/ 7	71
Trimethoprim+Sulfamethoxazole	3/ 7	43
Vancomycin	0/ 7	0
Linezolid	0/ 7	0
Tetracycline	1/ 7	14

Source: Antibiograms attached to the medical records filed with the institution's SAME.

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Table 3. Resistance profile of *E. faecalis* strains isolated from IPCs associated with PICC in neonates admitted to the ICU between January 2015 and December 2016. Petrolina-PE, 2017.

Antibiotics Tested	Number of resistant/tested samples	Resistance (%)
Penicillin	0/ 10	0
Ampicillin	0/ 10	0
Streptomycin	0/ 10	0
Gentamicin	0/ 10	0
Ciprofloxacino	3/ 10	30*
Vancomycin	0/ 10	0
Linezolid	0/ 10	0
Tetracycline	0/ 10	0

*Intermediate Resistance/Source: Antibiograms attached to the medical records filed with the institution's SAME.

Table 4. Resistance profile of *K. pneumoniae strains isolated from IPCs associated with PICC in neonates admitted to the ICU between January 2015 and December 2016. Petrolina-PE, 2017.*

Antibiotics Tested	Number of Displays Resistant/Tested	Resistance (%)
Amoxicilina+Ác. Clavulânico	1/ 5	20
Ampicillin	1/ 3	33
Piperacilina+Tazobactam	1/ 5	20*
Gentamicin	1/ 5	20
Amicacin	0/ 5	0
Tobramycin	1/ 5	20
Ciprofloxacino	1/ 5	20
Levofloxacino	1/ 5	20
Ceftazidima	1/ 5	20
Cefepime	1/ 5	20
Cefotaxime	1/ 5	20
Trimethoprim+Sulfamethoxazole	1/ 5	20
Imipenem	0/ 5	0
Meropenem	0/ 5	0
Ertapenem	0/ 1	0

*Intermediate Resistance/Source: Antibiograms attached to the medical records filed with the institution's SAME.

DISCUSSION

Infectious complications are usually severe in premature newborns, due to the peculiarities of this stage of life, and are the main cause of morbidity and mortality in this population. Thus, the immature immunological condition, prolonged hospitalizations, and invasive procedures are factors that contribute to the exposure and invasion of neonates by a variety of maternal and hospital microorganisms, increasing the risks of hospital infection and death23,24,8.

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The use of antimicrobials is frequent in the care of premature newborns, justified by the susceptibility to infections and because they are commonly submitted to invasive procedures during the hospitalization period. However, the indiscriminate use of antibiotics has contributed to the growing bacterial resistance, which is considered a public health problem worldwide25.

For the results observed in this study, the epidemiological profile of neonates notified with IPCSL associated with PICC corroborates those described in the literature, where 100% of newborns are characterized as premature, most of whom weigh less than 1,500g. However, it differs in relation to the gender variable, with a higher incidence of female neonates described here5,16,8,11.Regarding the main risk factors associated with blood infection, prematurity, length of hospital stay at the time of PBSI, catheter permanence time, antibiotic therapy, and parenteral nutrition infusion were identified. Similar data are described in the literature14,5.

Regarding the variables related to the PICC, we obtained a mean length of stay of 23.5 days, with the predominant insertion site being the cephalic vein, these findings differ from other studies, which report a lower mean length of stay than the one mentioned above and the main insertion site in the basilic vein26,7,27,16,28,11.In a study conducted by Camargo (2007)[27] The first indication for catheter removal was bloodstream infection, in our study we observed an almost equivalent number with 26% (15) at the end of treatment and 24% (14) due to blood infection. However, recent findings show a variation in relation to the indication for catheter removal, with indications similar to the results found in this study15,7,16,28.

Regarding the microbiological profile, the sample was characterized by a mixed microbiota, with a predominance of *S. epidermidis* (46%), *E. faecalis* (12%), *S. aureus* (9%) and *K. pneumoniae* (6%). A broad spectrum of infection-causing microorganisms is common in high-risk neonatal units and educational institutions, which mainly include coagulase-negative Staphylococcus and *Candida* sp., while for low-risk neonatal units, *S. aureus,* enteropathogens, and respiratory viruses stand out24.

According to Rangel (2013)[28], analyzing isolates from peripheral blood cultures, he observed a high frequency of S. epidermidis, *S. aureus*, *E. coli* and *Serratiamarcescen*. Catarino, et al. (2013)[15 describe] *S. epidermidis* and *S. aureus* as the most prevalent strains in bloodstream infections in neonatesis in a study developed by Silva (2015)[29] the main bacteria isolated in biological samples were *coagulase-negative Staphylococcus, S. aureus,*

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Enterobacterspp, *Burkholderiacepacia, Pseudomonasaeruginosa* and *E. coli.* Thus, the aforementioned studies corroborate the findings of the present study.

The frequency of infections associated with *Coagulase-Negative Staphylococcus (NEC) has increased over time. Because they belong to the human skin microbiota, these bacteria are recognized as important pathogens associated with nosocomial infections, presenting high resistance to oxacillin. It is known that NEC isolates have a pattern of resistance to antibiotics used in clinical routine, and are considered the main microorganisms associated with late bloodstream infections in neonatal ICUs30*.

In the present study, we observed that the strains of *S. epidermidis presented 100% resistance to penicillin and ampicillin; 95% to oxacillin and gentamicin; 86% to levofloxacin; 84% to ciprofloxacin; 76% trimethoprim + sulfamethoxazole and 5% to vancomycin, as observed in studies carried out from the microbiological analysis of blood cultures31,30,32,33*. For four strains, the following antibiotics were also tested: teicoplanin, eryfampicin, to which they were sensitive; erythromycin and clindamycin to which they were resistant34.

It is known that *S. aureusus at the end of the 50's acquired resistance to practically all parenteral antibiotics, including erythromycin and tetracycline. With the introduction of methicillin, used in the treatment of staphylococcal infections, methicillin-resistant strains called MRSA (methicillin-resistantStaphylococcus aureus) also emerged*, and in 1997 samples of *S. aureus* resistant to vancomycin/VRSA (VancomycinResistantStaphylococcus aureus) were discovered, strains that are of great concern to health professionals35.

The isolates of S. aureus in this study showed a resistance profile similar to the isolates from blood cultures in newborns described by VIANA (2011)[34], except for oxacillin, in which none of the isolates showed resistance to this drug, but in relation to our findings, 71% of the strains were resistant to oxacillin.

AK.pneumoniae is a bacterium that has important virulence factors associated with its pathogenic potential. It is considered one of the main bacteria that produce Extended Spectrum β-lactamases (ESBL), and is often resistant to clinically important antimicrobials, such as penicillins, cephalosporins, aminoglycosides, and quinolones. It is important to note that the *K.pneumoniae*, which produces the enzyme carbapenemase (KPC), caused concern in several parts of Brazil, such as the Federal District in 2010, with 187 notifications of infection and 18 deaths36.

For the isolates of *K.pneumoniae,* analyzed in this study, only one strain showed a

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resistance profile to the antibiotics tested. The strains of *K. pneumoniaeisolated by Gonçalves (2012)*[37] From blood cultures of neonates, where variables such as frequency of isolation, etiology, and factors associated with late-onset sepsis were evaluated, the pattern of sensitivity to carbapenems was similar to those found in this study.

According to Murray (1990)[38] Enterococcus species are described as organisms of the normal microbiota of the intestinal tract, have low pathogenicity, and are related to nosocomial infections. They are characterized by having intrinsic and acquired resistance, of which the main resistance traits are to penicillin, apparently acquired from staphylococci, and vancomycin.

These bacteria are usually transmitted in a hospital environment, and may suggest that their isolation is the result of incorrect hand and hospital object hygiene techniques. *E. faecalis* was the second most isolated bacterium in this study, and only 30% showed intermediate resistance to ciprofloxacin. In a study carried out from isolates of bloodstream infection in neonates *, E. faecalis was not resistant to any of the antibiotics tested39*.

Healthcare-associated infections are a cause for concern, especially in premature infants, as they require intermediate and intensive care units. In these environments, the use of antimicrobials represents a highly consumed item, and the indiscriminate use of these drugs, as well as the lack of criteria in the choice of empirical treatment, are determining factors for the emergence of more multidrug-resistant microorganisms40,41.

CONCLUSION

In the present study, newborns notified with IPCSL associated with PICC were characterized as premature, weighing less than 1,500g and female, and the diagnoses established indications for hospitalization in an intermediate care unit. Regarding the PICC insertion site, the cephalic vein predominated, and the main reasons for catheter removal were the end of treatment and the presence of blood infection.

The main risk factors identified and associated with blood infection were: prematurity, prolonged hospitalization, catheter permanence time, use of antibiotics and parenteral nutrition. Regarding the microbiological profile, the sample was characterized by a mixed microbiota, with a predominance of *S. epidermidis* with 71% of the oxacillin-resistant strains.

Thus, the planning of care by the multidisciplinary and/or multiprofessional team for the appropriate management of the PICC during insertion, maintenance and removal, pre-established in protocols and/or bundles; the monitoring of this catheter during its permanence period; The discussion and evaluation of the PCI indices associated with the

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PICC by health professionals inserted in this context will enable the adaptation of practices that minimize the rates of hospital infections.

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