

ORIGINAL ARTICLES

Positive blood culture and neonatal sepsis – A five-year study

Hemocultura positiva e sépsis neonatal – Casuística de cinco anos

Adriana Ferreira¹, Eulália Sousa¹, Joaquim Freitas², Mariana Viana², Filipa Miranda³, Francisco Pereira da Silva³

ABSTRACT

Introduction: Neonatal sepsis remains a major cause of morbidity and mortality in pediatric age. Since the predominant causative microorganisms vary between regions and over time, it is crucial to know the local epidemiology. The aim of this study was to characterize patients with positive blood culture and clinical presentation of sepsis admitted to a Neonatology Unit and identify possible risk factors and implicated microorganisms and respective antimicrobial susceptibility patterns.

Methods: This was a retrospective descriptive study of clinical data of patients admitted to the Neonatology Unit of a level II hospital with positive blood culture and clinical presentation of sepsis over five years (2014-2018).

Results: Seventy-three culture-proven sepsis cases were identified, 51 (69.9%) of which corresponded to low-birth-weight neonates and 52 (71.2%) to preterm newborns. Most cases (60; 82.2%) concerned late-onset sepsis. The most frequent microorganisms identified were coagulase-negative *Staphylococcus* (55; 75.3%), mainly associated with late-onset sepsis. Group B *Streptococcus* and *Escherichia coli* were the most common microorganisms isolated in early-onset sepsis. No cases of methicillin-resistant *Staphylococcus aureus* were identified. Coagulase-negative *Staphylococcus* presented high resistance rates to beta-lactam antibiotics.

Conclusions: The results retrieved from this study document the local epidemiology of neonatal sepsis and show a high frequency of late-onset sepsis associated with nosocomial pathogens. Coagulase-negative *Staphylococcus spp.* are resistant to the most commonly used antibiotics, with these cases requiring the use of vancomycin. It is crucial to implement effective guidelines to control and prevent nosocomial infections and reduce the incidence, morbidity, and mortality of neonatal sepsis, as well as the need for broad-spectrum antibiotics.

Keywords: antimicrobial susceptibility; blood culture; newborn; sepsis

RESUMO

Introdução: A sépsis neonatal continua a ser uma das principais causas de mortalidade e morbilidade neonatal. Dado que os microrganismos etiológicos responsáveis predominantes variam entre regiões e ao longo do tempo, torna-se essencial conhecer a epidemiologia local. O objetivo deste estudo foi caracterizar os doentes com hemocultura positiva e apresentação clínica compatível com sépsis internados numa Unidade de Neonatologia e identificar possíveis fatores de risco e agentes microbianos envolvidos e respetivas sensibilidades.

Métodos: Estudo retrospectivo descritivo de dados clínicos de doentes com hemocultura positiva e apresentação clínica compatível com sépsis internados na Unidade de Neonatologia de um hospital de nível II ao longo de cinco anos (2014-2018)..

1. Department of Pediatrics and Neonatology, Centro Hospitalar do Tâmega e Sousa, Penafiel. 4564-007 Portugal. adriana.amf1@gmail.com, eulaliapatricia1@hotmail.com
2. Department of Clinical Pathology, Centro Hospitalar do Tâmega e Sousa, Penafiel, Portugal. 4564-007 Portugal. 73912@chts.min-saude.pt; 60565@chts.min-saude.pt
3. Neonatology Unit, Department of Pediatrics and Neonatology, Centro Hospitalar do Tâmega e Sousa. 4564-007 Portugal. filiparmiranda@gmail.com; jofpsilva@gmail.com

Resultados: Foram identificados 73 casos de sépsis confirmados por hemocultura, 51 (69,9 %) dos quais correspondentes a recém-nascidos com baixo peso e 52 (71,2%) a recém-nascidos pré-termo. A maior parte dos casos era relativa a sépsis tardia (60; 82,2%). Os microrganismos mais frequentemente identificados foram *Staphylococcus* coagulase-negativos (55; 75,3%), principalmente associados a sépsis tardia. Os principais responsáveis por sépsis precoce foram *Streptococcus* do Grupo B e *Escherichia coli*. Não foram isolados *Staphylococcus aureus* resistentes a meticilina. Os *Staphylococcus* coagulase-negativos apresentaram elevadas taxas de resistência aos antibióticos beta-lactâmicos.

Conclusões: Os resultados obtidos documentam a epidemiologia local de sépsis neonatal, caracterizada por elevada frequência de sépsis tardia associada a microrganismos nosocomiais. Os *Staphylococcus* coagulase-negativos são resistentes à maioria dos antibióticos mais frequentemente utilizados, sendo necessária a utilização de vancomicina nestes casos. É urgente implementar protocolos eficazes de prevenção e controlo de infeções nosocomiais para diminuir a incidência, mortalidade e morbilidade de sépsis neonatal e a utilização de antibióticos de largo espectro.

Palavras-chave: hemocultura; recém-nascido; sensibilidade antimicrobiana; sépsis

INTRODUCTION

Neonatal sepsis remains a major cause of mortality and morbidity, including long-term complications such as poor developmental outcomes.⁽¹⁾

Neonatal sepsis is defined by a systemic infection affecting normally sterile body fluids, such as blood and cerebrospinal fluid (CSF), associated with hemodynamic changes and other clinical manifestations. It is mainly caused by bacteria and viruses but also by fungi and parasites.^(2,3) Despite their low sensitivity, blood, urine, and CSF culture remain the gold standard for sepsis diagnosis.⁽³⁾

Sepsis can be classified as early- or late-onset according to the age of onset. Early-onset sepsis (EOS) occurs in the first 72 hours of life, although some authors also consider the first seven days if it is caused by Group B *Streptococcus* (GBS). The transmission may occur *in utero* through the placenta, by ascension of vaginal microorganisms after membrane rupture, or during newborn passage through the birth canal. Late-onset sepsis (LOS) occurs after the first 72 hours or seven days of life and is usually caused by hospital- or community-acquired microorganisms during the post-natal period.^(2,3)

A decrease in the incidence of EOS has been observed due to GBS screening during pregnancy and intrapartum antibiotic prophylaxis for GBS infection.⁽⁴⁾ Conversely, the incidence of LOS due to commensal microorganisms has increased due to a higher incidence of prematurity and extended need for intravascular devices, parenteral nutrition, and mechanical ventilation.⁽⁵⁾ The emergence of multidrug-resistant microorganisms and lack of effective antibiotics have become a global problem and represent a challenge in the treatment of neonatal sepsis.⁽⁶⁾ Predominant microorganisms vary between regions and over time. Therefore, it is of utmost importance to investigate the local epidemiology to define preventive strategies and optimize empirical antibiotic treatment.

The main aim of this study was to characterize patients with positive blood culture and clinical presentation of sepsis admitted to

the Neonatology Unit of a level II hospital in the North of Portugal between January 1, 2014, and December 31, 2018. Secondary aims were to identify possible risk factors and implicated microorganisms and respective antimicrobial susceptibility patterns.

MATERIAL AND METHODS

This was a retrospective descriptive study of clinical data of neonates with culture-proven sepsis admitted to the Neonatology Unit of a level II hospital between January 1, 2014, and December 31, 2018. Patients were admitted immediately after birth and had moved from the nursery, another hospital, or the community. Until 2017, this Neonatology Unit received newborns with a minimal gestational age of 28 weeks. Currently, it admits newborns with a minimal gestational age of 30 weeks and birth weight higher than 1000 g.

According to the unit's protocol, a 2% chlorhexidine and 70% isopropyl alcohol solution was used for skin antisepsis before blood sample collection. A minimum of one milliliter of blood was collected and injected into BACTEC PedsPlus™ (Becton Dickinson and Company, USA) culture vials. Culture vials were then incubated into BACTEC 9240 automated system for five days according to the manufacturer's instructions. If a vial was identified as positive during this period, cells were subcultured onto chocolate agar, Macconkey agar, and chromID™ CPS® (bioMérieux) agar plates, and a smear was prepared for Gram staining. When Gram-positive cocci were identified, cells were also subcultured onto Columbia Blood agar to detect hemolytic reactions. Antibiotic susceptibility test was performed using the Viktek II automated system (bioMérieux), following The European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

Patients' blood culture data were retrieved from the hospital laboratory database. Blood cultures were generally performed in newborns with clinical suspicion of sepsis or with risk factors, such as

premature birth (<37 weeks), premature and prolonged (>18 hours) membrane rupture time, and maternal peripartum infection.

Neonatal sepsis was defined as a positive blood culture for a potentially pathogenic organism associated with one or more clinical symptoms (fever, hypothermia, temperature instability, apnea, bradycardia, respiratory distress, increased oxygen requirement, feeding intolerance, lethargy, or hypotonia) and/or abnormal laboratory findings (white blood cell count $\geq 20 \times 10^3/\mu\text{L}$ or $< 5 \times 10^3/\mu\text{L}$, immature/mature neutrophil ratio > 0.20 , platelet count $< 100.000/\mu\text{L}$, or C-reactive protein ≥ 10 mg/L). Coagulase-negative *Staphylococcus* (CNS) was considered pathogenic if one positive blood culture was associated with elevated C-reactive protein within two days of blood culture collection or if a patient with one positive blood culture was treated with vancomycin for five or more days.

Data retrieved from patients' clinical files included maternal risk factors (obstetric history, prolonged membrane rupture, GBS screening, prenatal antibiotic administration), type of labor, gestational age, Apgar score, birth weight, length of hospital stay, ventilatory support, parenteral nutrition duration, presence of intravascular catheter, and type and duration of antibiotics used. Retrieved laboratory data included identification of isolated microorganisms and respective antimicrobial susceptibility patterns, and whether the patient was submitted to lumbar puncture and/or urine culture and respective results. Cases of positive blood culture with no clinical presentation of sepsis and positive blood culture of the same sepsis episode were excluded.

EOS was considered when a positive blood culture result was retrieved within the first 72 hours of life. Positive blood culture obtained after that period was considered LOS.

Statistical analysis was conducted on SPSS® (IBM) version 20. The annual incidence of sepsis was calculated by dividing the number of inborn infants with sepsis by the number of live births in the hospital maternity and expressed per 1000 live births. This study was approved by the Ethics Committee of the study hospital.

RESULTS

Blood culture was performed for 1650 samples during the study period. Seven percent (119/1650) were positive, 20 (16.8%) of which referred to contaminant organisms. After excluding positive blood cultures of the same sepsis episode (26/119), a total of 73 episodes were included in the analysis. The incidence of culture-proven sepsis was 5.5 cases per 1000 births, corresponding to 3.9% of admitted newborns. Eight outborn infants were identified, all with LOS. The incidence of sepsis in the five-year study period is depicted in **Figure 1**.

Most isolates (90.4%) referred to gram-positive bacteria, and only 9.6% referred to gram-negative bacteria. The vast majority of sepsis cases were caused by *Staphylococcus epidermidis* (45.2%) and other CNS (30.1%) and referred almost exclusively to LOS. GBS

was the most commonly isolated microorganism in EOS, followed by *Escherichia coli*. GBS and *Escherichia coli* were only identified in full-term newborns, while CNS prevailed in preterm newborns. **Table 1** summarizes the isolated microorganisms and respective frequencies according to the age of presentation and gestational age.

The median time to sepsis onset was 8.56 days (range 0-41.6; interquartile range [IQR] 5.14-11.29). Amongst newborns with culture-proven sepsis, 69.9% had a birth weight less than 2500 g, 71.2% were preterm, and 61.6% were born by cesarean section (**Table 2**).

In preterm newborns and LOS, neonatal sepsis was more prevalent in males (1.6:1 and 1.86:1, respectively). The incidence ratio of EOS in female versus male newborns was 2.25:1. EOS was identified in 17.8% of all cases. All EOS episodes were diagnosed within the first 24 hours of life, except for two cases of *S. epidermidis*, which were diagnosed later (at 25 and 55 hours).

Two patients reported two episodes of sepsis. The first had sepsis caused by CNS on the seventh day of life, followed by sepsis caused by *Enterococcus faecalis* 12 days later. The second had symptoms of sepsis on the sixth day of life, with isolation of *S. warneri* in blood culture, and experienced an episode of sepsis caused by *S. epidermidis* nine days later. In one newborn, the same two agents (*S. epidermidis* and *S. hominis*) were isolated in two blood cultures collected three days apart. *Enterobacteriaceae* were identified in seven newborns, two premature (33 and 36 weeks) and with low birth weight, four with prolonged membrane rupture time (>18 hours), and one with unknown membrane rupture time.

CSF culture was performed in 61 cases (six EOS and 55 LOS) and urine analysis in three, with no microorganisms isolated.

Noteworthy, all newborns with EOS were full-term. Among full-term newborns, 13 (61.9%) had EOS. Of these, seven presented infectious risk factors, including prolonged membrane rupture time in two patients, maternal intrapartum fever in three, positive GBS screen result and incomplete intrapartum antibiotic prophylaxis in one, and low birth weight in another one.

GBS obstetric screening was not performed in 58.9% of cases due to premature delivery (<35 gestation weeks) and had no available data in 4.1% of cases. Only one GBS case had a positive screening result, corresponding to a patient who did not complete the intrapartum antibiotic prophylaxis.

Clinical and laboratory characteristics of the study population collected at the time of Neonatology Unit admission are summarized in **Table 3**.

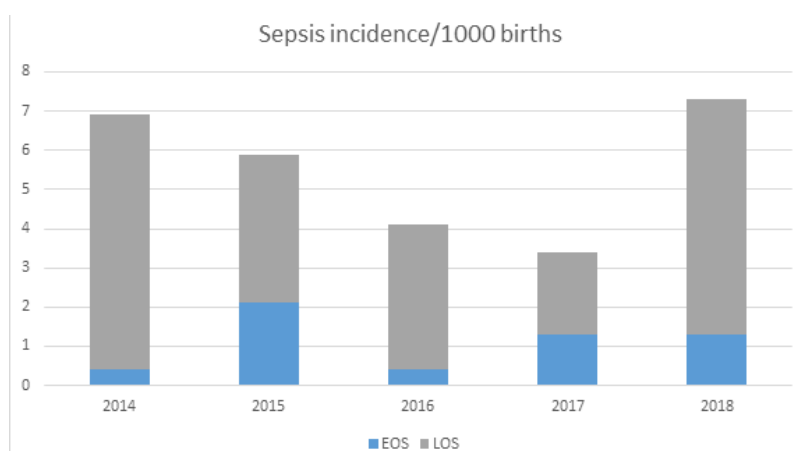
The most used initial antibiotic therapy in EOS cases was ampicillin and gentamicin, and in LOS cases vancomycin and cefotaxime. Antibiotics were adjusted according to clinical presentation and results of the antibiogram (if required).

Detailed antibiotic susceptibility and resistance data of the most commonly identified microorganisms are shown in **Table 4**. All GBS were susceptible to penicillin and all *E. coli* were susceptible to gentamicin. During the study period, no methicillin-resistant

Staphylococcus aureus (MRSA) was identified. CNS showed high resistance rates to beta-lactam antibiotics, aminoglycosides, and fluoroquinolones, except when associated with EOS, where susceptibility to oxacillin and gentamicin was reported. The only case of *S. aureus* isolated in EOS setting was also susceptible to gentamicin and oxacillin, with no resistances detected. All CNS cases were sensitive to vancomycin, linezolid, and daptomycin. Antimicrobial susceptibility analysis was not performed for the remaining microorganisms associated with EOS (*Micrococcus spp* and *Bacillus spp*). Both *Enterobacter* strains were resistant to ampicillin, amoxicillin, and clavulanic acid, with one showing susceptibility

to gentamicin and the other to cefotaxime and cotrimoxazole. *Enterococcus faecalis* showed resistance to erythromycin and tetracycline and susceptibility to penicillin, ampicillin, levofloxacin, and nitrofurantoin. The only case of *Klebsiella pneumoniae* identified referred to an extended-spectrum beta-lactamase-producing bacteria and was only sensitive to carbapenems.

The most relevant complications registered during hospital stay were three episodes of necrotizing enterocolitis and one episode of disseminated intravascular coagulation with hepatic dysfunction. On ambulatory follow-up, four cases of developmental delay were reported. No deaths occurred during the study period.



EOS, early-onset sepsis; LOS, late-onset sepsis

Figure 1 - Evolution of cases of sepsis with blood culture in the five years of the analysis

Table 1 - Distribution of microorganisms detected in blood cultures and respective frequency according to age of presentation and gestational age

Microorganism	Number (%)	EOS	LOS	Preterm	Term
		n=13	n=60	n=52	n=21
<i>Staphylococcus epidermidis</i>	33 (45.2%)	2	31	29	4
Other coagulase-negative <i>Staphylococcus</i>	22 (30.1%)	0	22	19	3
Group B <i>Streptococcus</i>	5 (6.8%)	5	0	0	5
<i>Escherichia coli</i>	3 (4.1%)	3	0	0	3
<i>Staphylococcus aureus</i>	3 (4.1%)	1	2	1	2
<i>Enterobacter cloacae</i> complex	2 (2.7%)	0	2	1	1
<i>Bacillus spp.</i>	1 (1.4%)	1	0	0	1
<i>Citrobacter braakii</i>	1 (1.4%)	0	1	1	0
<i>Enterococcus faecalis</i>	1 (1.4%)	0	1	1	0
<i>Klebsiella pneumoniae</i>	1 (1.4%)	0	1	0	1
<i>Micrococcus spp.</i>	1 (1.4%)	1	0	0	1

EOS, early-onset sepsis; LOS, late-onset sepsis

Table 2 - Characteristics of the study population

Characteristic	EOS	LOS	Total	Percentage
	n=13	n=60	n=73	
NEONATAL population				
Gender				
Male	4	39	43	59
Female	9	21	30	41
Gestational age				
< 28 weeks	0	1	1	1.4
28 to 31 weeks	0	11	11	15.1
32 to 33 weeks	0	24	24	32.9
34 to 36 weeks	0	16	16	21.9
37 to 41 weeks	13	8	21	28.8
Birth weight				
< 1000 g	0	1	1	1.4
1000 to 1499 g	0	9	9	12.3
1500 to 2499 g	1	40	41	56.2
≥ 2500g	12	10	22	30.1
Type of delivery				
Eutocic	6	17	23	31.5
Vacuum-assisted	4	1	5	6.8
Cesarian section	3	42	45	61.6
Twin pregnancy	0	14	14	19.4
MATERNAL population				
Maternal fever	3	2	5	6.9
Time of membrane rupture				
≤ 18 hours	10	46	56	82.4
> 18 hours	2	10	12	17.6
GBS screen result				
Negative	10	4	14	19.2
Positive	3	10	13	17.8
Not performed	0	43	43	58.9
Not reported	0	3	3	4.1

EOS, early-onset sepsis; GBS, Group B Streptococcus; LOS, late-onset sepsis

Table 3 - Clinical and laboratory characteristics of the study population

Variables	EOS			LOS		
	n	Median	IQR	n	Median	IQR
Length of stay (days)	13	9.6	8.2-15.0	60	25.6	18.5–33.1
Invasive ventilation days	0	-	-	13	1.0	1.0–2.8
Parenteral nutrition days	0	-	-	56	15.0	1.0–20.5
Epicutaneous catheter days	0	-	-	31	9.0	6.0–17.0
Central catheter days	0	-	-	3	14.0	-
Without CRP elevation	0	-	-	6	-	-
Elevated CRP value (mg/L)	13	51.0	28.0–63.2	54	56.5	3.5–112.2

CRP, C-reactive protein; EOS, early-onset sepsis; IQR, interquartile range; LOS, late-onset sepsis

Table 4 - Antimicrobial resistance of isolated microorganisms

Antibiotic	Staphylococcus epidermidis		Other CNS		Streptococcus agalactiae		Escherichia coli			
	n=33		n=22		n=5		n=3			
	R/(R+S)	% R	R/(R+S)	% R	R/(R+S)	% R	R/(R+S)	% R	R/(R+S)	% R
Beta-lactam										
Penicillin	5/5	100	15/15	100	0/5	0	-	-	1/1	100
Ampicillin	-	-	-	-	-	-	1/3	33.3	-	-
Oxacillin	29/33	87.9	20/20	100	-	-	-	-	0/3	0
Non-beta-lactam										
Erythromycin	12/15	80.0	19/20	95	3/3	100	-	-	0/1	0
Fosfomicin	0/3	0	18/19	94.7	-	-	-	-	0/1	0
Gentamicin	26/33	78.9	21/21	100	-	-	0/3	0	0/3	0
Ciprofloxacin	-	-	-	-	-	-	0/2	0	-	-
Ofloxacin	-	-	1/1	100	-	-	-	-	0/2	0
Levofloxacin	7/8	87.5	11/16	68.8	0/1	0	-	-	-	-
Moxifloxacin	4/5	80.0	7/15	46.7	0/1	0	-	-	-	-
Clindamycin	14/23	60.9	1/3	33.3	2/3	66.7	-	-	0/1	0
Linezolid	0/11	0	0/19	0	0/1	0	-	-	-	-
Vancomycin	0/30	0	0/21	0	0/2	0	-	-	0/1	0
Daptomycin	0/11	0	0/18	0	-	-	-	-	-	-
Teicoplanin	10/13	76.9	11/16	68.8	0/1	0	-	-	-	-
Tetracycline	4/4	100	-	-	4/4	100	-	-	-	-
Cotrimoxazole	11/32	34.4	8/21	38.1	0/3	0	0/2	0	0/3	0

CNS, coagulase-negative Staphylococcus; R, resistant; S, susceptible

DISCUSSION

Neonatal sepsis is a significant cause of morbimortality in the Neonatal Intensive Care Unit. In this study, cases of positive blood culture associated with neonatal sepsis were retrospectively assessed. The positivity rate of blood cultures was 7%, similar to that reported in 2005 by another Portuguese hospital unit.⁽⁷⁾

In a study conducted in 13 Portuguese Neonatology units, the incidence of sepsis with positive blood culture ranged between 1.8 and 16.1% of patients admitted.⁽⁸⁾ In the present study, the incidence of sepsis was 3.9%, which can be explained by the fact that the considered Neonatology Unit is located in a level II hospital, and patients admitted have a higher gestational age and birth weight, and hence fewer risk factors for neonatal sepsis.

Most sepsis cases in this study occurred in premature newborns with low birth weight, with 71.2% and 69.9% of cases occurring in newborns with less than 37 weeks of gestational age and with birth weight less than 2500 g, respectively. These data are in agreement with studies in the literature stating that prematurity and low birth weight are major risk factors for neonatal infection due to immunological immaturity associated with the frequent need for invasive procedures.⁽²⁾

Most positive blood cultures referred to LOS, with an incidence of 4.4 cases per 1000 births. On the other hand, EOS had an incidence ratio of one case per 1000 births, corresponding to 17.8% of studied cases. These data agree with those reported by Bizarro *et al.* (2005), who investigated the incidence of neonatal sepsis over 75 years and observed a decline in the incidence of EOS and an increase in the incidence of LOS, mainly attributed to commensal microorganisms. More recently reported incidence rates agree with data from the present study.^(5,9,10) This fact is explained by the broad implementation of GBS screening in pregnancy and associated intrapartum antimicrobial prophylaxis.⁽⁵⁾

In this study, EOS cases were mostly caused by GBS (5/13) and *E. coli* (3/13). Contrary to reports in the literature suggesting that *E. coli* is the main etiological agent of EOS in preterm newborns, in this study, the three EOS cases caused by *E. coli* occurred in full-term newborns.^(2,10) Sepsis caused by *E. coli* was thus rare in the present study, and only one case was resistant to ampicillin, indicating that intrapartum GBS antimicrobial prophylaxis does not increase the number of sepsis cases caused by other agents like *E. coli*. These results confirm those previously reported by Morioka *et al.*⁽¹¹⁾

Most LOS cases occurred in preterm newborns with low birth weight. The most common organisms associated with LOS were CNS, responsible for 75.3% of cases. These results agree with reports of a higher incidence of CNS in Portugal and other developed countries.^(5,8,12) This can be explained by the higher survival rate of preterm newborns with immunological immaturity and by an increased need for intravascular catheters and mechanical ventilation. More than half of LOS cases in this study had an epicutaneous catheter, and 21.7% required mechanical ventilation. However, as skin commensals, CNS are frequently labeled as contaminants, making it hard to distinguish sepsis from contamination. This can lead to overestimation of sepsis cases and unnecessary treatment in some cases.^(5,9)

Few *S. aureus* were isolated (4.1%), and no methicillin-resistant strains were identified among these. Portuguese data show an incidence of MRSA ranging from 6 to 37.5%.^(7,8,12) A wide variation in the incidence of MRSA can also be observed in other countries, with this being the main etiological agent of LOS in Japan.⁽¹¹⁾ In England and USA, the incidence of *S. aureus* has been reported to be 18 and 8%, respectively.^(9,13) Gram-negative bacteria, such as *Klebsiella*, *Enterobacter*, and *Citrobacter*, only accounted for isolated LOS cases (6.7%). It is noteworthy that no *Listeria* or fungi were isolated throughout the five years of this study.

No microorganisms were identified in the 61 cases submitted to CSF analysis. These data are in accordance with the decreasing incidence of meningitis reported in other studies. In addition, CNS, the most frequently reported agent in this study, has shown a low probability of causing meningitis.⁽¹³⁾ Nonetheless, CSF analysis is still recommended in the presence of neonatal sepsis as a way of avoiding neonatal meningitis underdiagnosis and inadequate treatment.^(14,15)

This study's results allowed to conclude that agents responsible for EOS agents are susceptible to ampicillin and/or gentamicin. Although no antibiogram was performed in two cases in which different agents were identified (*Micrococcus* and *Bacillus spp*), good clinical response to ampicillin and gentamicin was observed in both. Therefore, this antibiotic scheme can be considered acceptable. As reported by another Portuguese unit, the two *S. epidermidis* and one *S. aureus* cases detected in association with EOS showed different susceptibility in LOS, probably due to their acquisition in community versus nosocomial setting.⁽¹⁶⁾

CNS is resistant to most daily used antibiotics, with vancomycin required in most cases. In this study, CNS showed high resistance rates to beta-lactams, aminoglycosides, and fluoroquinolones. Its susceptibility to ceftaroline was not tested. On the other hand, a 100% susceptibility to vancomycin, linezolid, and daptomycin was reported. Some LOS cases did not respond well to vancomycin and cefotaxime, improving their response after the introduction of an aminoglycoside. Furthermore, cefotaxime is a broad-spectrum antibiotic, and its use is associated with the emergence of resistant strains, with susceptibility analysis not justifying that. Therefore, the empirical scheme currently used in hospital setting to treat LOS is vancomycin and one aminoglycoside.

The frequent use of vancomycin can additionally lead to the emergence and dissemination of resistant strains. Therefore, measures should be taken to optimize the use of broad-spectrum antibiotics and shorten treatment duration whenever possible.

LOS was found to be more prevalent than EOS in this study. The low incidence of EOS has been attributed to higher rates of intrapartum GBS prophylaxis. On the other hand, the higher survival rates of premature newborns associated with a higher need for invasive procedures have increased the risk of nosocomial infections. CNS is a recognized pathogen in LOS, but also a contaminant. Moreover, neonatal sepsis signs and symptoms are often unspecific, and obtaining blood samples from newborns is a complex procedure, making it difficult to distinguish between real CNS infection and contamination. Bizarro *et al.* showed that it is possible to nearly eradicate CNS-associated sepsis through strict prevention and infection control measures, including not drawing blood samples for culture from preexistent catheter lines and introducing enteral nutrition earlier.⁽¹⁷⁾

Prevention of nosocomial infections in Neonatology units is challenging and requires continuous monitoring, strict antiseptic measures, and sensibilization and training of healthcare professionals. Hand hygiene and the use of breast milk have been shown to be very

effective measures.⁽¹⁸⁾ In addition, other behaviors and procedures are extremely relevant, such as cleaning and sanitizing surfaces and materials, following protocols for insertion and maintenance of intravascular catheters and removing them as soon as possible, and minimizing the length of invasive ventilation.⁽¹⁸⁾

It is therefore urgent to implement effective guidelines to control and prevent nosocomial infections and hence reduce neonatal sepsis morbimortality.

This study has the limitations of its retrospective design and small sample retrieved from one Neonatology unit of a level II hospital. Still, it allowed to investigate the local epidemiology of sepsis-causing microorganisms over the last five years and will hopefully contribute to improving neonatal care, especially regarding neonatal sepsis treatment.

CONCLUSIONS

The present study showed that coagulase-negative *Staphylococcus* was responsible for most sepsis cases and was almost exclusively associated with LOS. GBS remains the main etiological agent of EOS, followed by *Escherichia coli*. *Coagulase-negative Staphylococcus* displayed high resistance to beta-lactams, aminoglycosides, and fluoroquinolones, requiring the use of broad-spectrum antibiotics as empirical therapy for LOS. EOS etiological agents were shown to be susceptible to ampicillin and/or gentamicin, confirming that this antibiotic scheme remains effective and acceptable for this type of sepsis. Effective guidelines for the control and prevention of nosocomial infections should be implemented as a way to reduce the incidence, morbidity, and mortality of neonatal sepsis, as well as the need for broad-spectrum antibiotics.

AUTHORSHIP

Adriana Ferreira – Study design; Statistics; Writing – review & editing
 Eulália Sousa – Study design; Statistics; Writing – review & editing
 Joaquim Freitas – Writing – review & editing
 Mariana Viana – Writing – review & editing
 Filipa Miranda – Writing – review & editing
 Francisco Pereira da Silva – Writing – review & editing

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CORRESPONDENCE TO

Adriana Ferreira
Department of Pediatrics and Neonatology
Centro Hospitalar do Tâmega e Sousa
Avenida do Hospital Padre Américo 210
4564-007 Penafiel
Email: adriana.amf1@gmail.com

Received for publication: 10.03.2021

Accepted in revised form: 17.09.2021