BJPS

Brazilian Journal of Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902022e19664

Article

Coagulase-negative staphylococci isolates from blood cultures of newborns in a tertiary hospital in southern Brazil

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Neonatal sepsis continues to be a major cause of morbidity and mortality worldwide. Coagulase-negative staphylococci (CoNS), commonly found on the skin, being the main agents isolated. The aim of this study was to evaluate CoNS isolated from blood cultures of newborn (NB) infants. The study took place between 2014 and 2016/2017 in a tertiary hospital in southern Brazil. Using the VITEK 2 system (bioMérieux, Marcy l'Etoile, France), the microorganisms were identified and had their sensitivity profiles determined. The minimum inhibitory concentrations of linezolid, tigecycline, and vancomycin were also determined. The clinical parameters and mortality rates of NBs were evaluated. From January to December 2014, 176 CoNS isolates were obtained from 131 patients and from June 2016 to July 2017, 120 CoNS isolates were obtained from 79 patients. *Staphylococcus epidermidis* was most prevalent in both periods. Resistance rates increased between 2014 and 2016/2017, especially against ciprofloxacin (52.27% and 73.11%, p = 0.0004), erythromycin (51.40% and 68.07%, p = 0.0054), gentamicin (50.59% and 67.23%, p = 0.0052), and penicillin (71.3% and 99.17%, p = 0.0001), respectively. With 100% susceptibility to linezolid, tigecycline, and vancomycin in both periods and methodologies tested. In 2014, 53.44% of the NBs received antibiotic therapy, and of these, 77.14% used a catheter; in 2016/2017, these were 78.48% and 95.16%, respectively. Regarding laboratory tests, a hemogram was ineffective, since patients with sepsis presented normal reference values. In 2014 and 2016/17, 15.71% and 17.74% of the NBs died, respectively. *S. epidermidis* was the predominant microorganism, related to catheter use in most cases. The resistance rates have increased over time, demonstrating the importance of adopting control and prevention measures in this hospital. CoNS are responsible for a significant neonatal sepsis mortality rate in infants.

Keywords: *Staphylococcus epidermidis*. Blood culture. Newborn. Sepsis. Microbial sensitivity tests. Mortality.

INTRODUCTION

Despite the advances in prenatal care, neonatal sepsis continues to be a major cause of morbidity and mortality, with more than 400.000 annual estimated deaths worldwide (Liu *et al.*, 2017; Arayici *et al.*, 2019). Its incidence varies geographically, reflecting differences in resources, maternal and child risk factors, and prevention strategies. It is one of the most common neonatal diseases, even in highly developed countries (Shane, Sanchez, Stoll, 2017).

The precise identification of neonatal sepsis is a challenge, owing to the nonspecific signs and symptoms that can be confused with other conditions among

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infants (Tzialla *et al*., 2015; Arayici *et al*., 2019; Shane, Sanchez, Stoll, 2017). Blood culture is considered the gold standard for the identification of sepsis; however, there are limitations for early diagnosis, such as the time to diagnosis (48 to 72 h), and may be influenced by various factors, including blood volume and antimicrobial treatment (Arayici *et al*., 2019; Memar *et al*., 2019). Standardized collection of blood cultures, as well as the use of biomarkers, such as blood counts, lactate, and C-reactive protein (CRP), are important to improve the diagnosis and therapeutic management of sepsis (Arayici *et al*., 2019; Memar *et al*., 2019).

Coagulase-negative staphylococci (CoNS), a common part of the skin microbiome, are found in 50%–80% of cases in the neonatal period, including neonatal intensive care unit (NICU) cases (Namvar *et al*., 2017; Cantey *et al*., 2018; Dong, Speer, Glaser, 2018; Pereira *et al*., 2020). Medical intervention is usually required with infections caused by CoNS and the use of invasive medical devices is a risk factor for other problems, such as bacterial resistance (Tzialla *et al*., 2015; Jiang *et al*., 2019).

The ability of CoNS to develop resistance to antibacterial agents has increased considerably in recent years. There is growing concern for public health, since treatment options are increasingly restricted owing to the rapid emergence and spread of multidrug-resistant (MDR) microorganisms (Tzialla *et al*., 2015; Namvar *et al*., 2017; Jiang *et al*., 2019; Pereira *et al*., 2020). Neonatal units are common sites for the development and transmission of MDR pathogens, with antibiotics being commonly prescribed (Tzialla *et al*., 2015). Among the resistances developed by CoNS, methicillin/oxacillin-resistant coagulase-negative staphylococci (MRCoNS) are most frequent, which may confer resistance to all β-lactam antibiotics. However, microorganisms with reduced susceptibility and even resistance to vancomycin and linezolid have been described, drugs commonly prescribed for the treatment of MRCoNS infection (Gu *et al*., 2013; Pinheiro *et al*., 2016).

The objective of this study was to evaluate CoNS isolates from NB blood cultures collected between 2014 and 2016/2017 in a tertiary hospital in southern Brazil, as well as to evaluate certain clinical parameters of these patients.

MATERIAL AND METHODS

Place and duration of study

This study was conducted at the Laboratório de Bacteriologia, Departamento de Análises Clínicas e Toxicológicas, Centro de Ciências da Saúde of Universidade Federal de Santa Maria (UFSM), Santa Maria, Rio Grande do Sul. Samples were taken in a tertiary hospital in southern Brazil over a two-year period (January to December 2014 and July 2016 to July 2017).

Isolates

The isolates were obtained from blood cultures of hospitalized NBs in a tertiary hospital in southern Brazil over a two-year period (January to December 2014 and July 2016 to July 2017). The samples were processed according to the standard operating procedure of the Clinical Analysis Laboratory of the hospital, and the guidelines of the Infectious Diseases Society of America (IDSA) (Miller *et al*., 2018). The isolates were processed using the automated BACTEC 9240 system (BD Biosciences, NJ, USA). Specieslevel identification was performed using the automated VITEK 2 system (bioMérieux, Marcy l'Etoile, France).

CoNS-positive blood cultures were subsequently sent to Laboratório de Bacteriologia, Departamento de Análises Clínicas e Toxicologicas, Centro de Ciências da Saúde at UFSM, where they were plated on tryptone soy agar (TSA) and incubated at 35 ±2 °C for 18 to 24 h. The colonies were stored in tryptone soy broth (TSB) with 15% glycerol and stored at −80 °C for subsequent testing.

Sensitivity profile

Automated methodology

Antibiotic sensitivity profiles were determined using the automated VITEK 2 system (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. Clindamycin, ciprofloxacin, erythromycin, gentamicin, linezolid, oxacillin, penicillin, sulfamethoxazole/trimethoprim, teicoplanin, tigecycline,

and vancomycin were tested. Clinical isolates were classified as sensitive, intermediate, or resistant according to the Clinical and Laboratory Standard Institute (CLSI) guidelines, which in effect at the time of the study (CLSI, 2014, 2016, 2017), with intermediate profiles considered resistant for this study.

Broth microdilution

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) for tigecycline, linezolid, and vancomycin according to CLSI document M07-A9 (CLSI, 2012). The results were classified as sensitive, intermediate, or resistant according to CLSI guidelines in effect at the time of the study (CLSI, 2014, 2016, 2017), with intermediate profiles considered resistant for this study.

Biomarkers insepsis

Blood count

Blood counts were performed using the Sysmex XE-5000 apparatus (Sysmex, Kobe, Japan), as recommended by the manufacturer. Whole blood containing EDTA was evaluated by automated cell counting, and complementary microscopy was performed when necessary. Data were collected through analysis of results obtained from the hospital records, and were evaluated according to the microorganism and not per patient, since blood count is always performed on samples for blood culture. The interpretation was performed using the hematological scores of Rodwell, Leslie, and Tudehope (1988). We considered a normal blood count as "without sepsis", leukocytosis as a "frequent factor" in sepsis, leucopenia a "poor prognosis", neutrophilia, left shift and granulations a "severe infection".

C-reactive protein (crp)

CRP values were measured using the Dimension Xpand Plus device (Siemens, Munich, Germany) according to the manufacturer's instructions at the referred hospital. Serum was evaluated by an immunoturbidimetry assay (reference value for inflammatory processes: < 0.30 mg/dL). Data were collected through analysis of hospital records and were also evaluated according to the microorganism, since in the request of blood culture, CRP is always requested.

Clinical significance and mortality rates

Data were collected through the analysis of the vernix caseosa of each NB, which was requested through each patient's identification number. It can be observed that the isolation of CoNS in blood culture was considered a colonization or infection, in relation to the use of a catheter. In addition, the patient death rate was recorded.

Statistical analysis

The Kappa index was used to evaluate the agreement between the methodologies (Vaz, Takei, Bueno, 2007).

To show the difference between the two years of study (2014 and 2016/2017), a comparison test of the two periods was carried out, using a 5% level of significance.

Ethical concepts

The study was approved by the Research Ethics Committee of UFSM and is registered under the Certificado de Apresentação para Apreciação Ética (CAAE) [Certificate of Presentation for Ethical Appreciation] (No. 38850614.4.0000.5346).

Ethical considerations

This study was approved by the Ethical Research Committee of the Universidade Federal de Santa Maria (No. 38850614.4.0000.5346).

RESULTS

In 2014, 131 patients were admitted to the referred hospital, of which 176 CoNS were collected. In 2016/2017, these rates were of 79 patients and 120 isolates. Among the CoNS, in 2014 the most prevalent species was *Staphylococcus epidermidis* (56.82%, 100/176), followed

by *Staphylococcus warneri* (9.66%, 17/176) (Table I). In 2016/2017, *S. epidermidis* (66.67%, 80/120) was the most prevalent species, followed by *Staphylococcus haemolyticus* (10%, 12/120) and *Staphylococcus hominis* (10%, 12/120) (Table I).

TABLE I – Distribution of coagulase-negative staphylococci (CoNS) species isolated from blood cultures of newborns in 2014 and 2016/2017 in a tertiary hospital in southern Brazil

Species	2014	%	2016/2017	%	p-value
	n		n		
S. epidermidis	100	56.82	80	66.67	0.088
S. warneri	17	9.66	8	6.67	0.459
S. capitis	13	7.39	4	3.33	0.151
S. haemolyticius	13	7.39	12	10	0.552
S. hominis	13	7.39	12	10	0.430
CoNS	8	4.55	1	0.83	0.068
S. saprophyticus	6	3.41	1	0.83	0.148
S. lugdunensis	2	1.14	0	0	-
S. xylosus	2	1.14	1	0.83	0.797
S. equorum	1	0.57	0	0	-
S. auricularis	1	0.57	0	0	-
S. lentus	0	0	1	0.83	-
Total	176	100	120	100	

CoNS = Coagulase-negative staphylococci that the specie were not identified by automated system.

In 2014, 6.87% (9/131) of the patients presented with coinfection by CoNS and other microorganisms including: *Acinetobacter baumannii*, *Acinetobacter complex*, *Enterococcus faecalis*, *Citrobacter freundii, Proteus mirabilis*, *Serratia marcescens*, *Stenotrophomonas maltophilia* (n = 2), and *Streptococcus pneumoniae*. In the 2016/2017 period, coinfections made up 13.92% (11/79) of NB CoNS infections with: *A. baumannii*, *E. faecalis*, *Escherichia coli, Klebsiella pneumoniae* (n = 2), *S. marcescens, S. maltophilia, S. maltophilia,* and *Streptococcus agalactiae, S. agalactiae*, *Stenotrophomonas maltophilia, K. pneumoniae*, *Acinetobacter baumannii,* and *S. marcescens.*

With respect to the hospital units where NBs were admitted, in 2014, The NICU had the highest rate of CoNS infection in NBs out of the hospital units included in this study (61.36%, 108/176), followed by the Obstetric Center (OC) (18.75%, 33/176). In 2016/2017, these rates were 80.83% (97/120) and 9.17% (11/120), respectively (Table II). Data were based on the number of isolates, since patients were could be transferred between hospital units.

TABLE II – Distribution of blood cultures in which there was the isolation of coagulase-negative staphylococci (CoNS) and respective hospitalization units, in 2014 and 2016/2017, in a tertiary hospital in the southern region of Brazil

Hospitalization Units	2014		2016/2017	
	n	%	n	%
NICU	108	61.36	97	80.83
PICU	3	1.70	4	3.33
RR	2	1.14	0	0
PPEC	1	0.57	2	1.67
GO	27	15.34	4	3.33
OC	33	18.75	11	9.17
SB	1	0.57	0	0
AMB	1	0.57	2	1.67
Total	176	100	120	100

AMB = Ambulatory; SB = Surgical block; OC = Obstetric Center; GO = Gynecology and Obstetrics; PPEC = Pediatric Prepared Emergency Care; RR = Recovery room; PICU = Pediatric Intensive Care Unit; NICU = Neonatal Intensive Care Unit.

In this study, the isolates presented resistance to penicillin (71.26% in 2014; 99.17% in 2016/2017) and oxacillin (76% in 2014; 84.17% in 2016/2017), and increased significantly when comparing 2014 to 2016/2017 for clindamycin (23.12% to 57.50%, p = 0.0001), ciprofloxacin (52.27% to 73.11%, p = 0.0004), erythromycin (51.40% to 68.07%, p = 0.0054), gentamicin (50.59% to 67.23%, p = 0.0052), and sulfamethoxazole/trimethoprim (41.62% to 66.67%, p = 0.0001) (Table III). In 2014 and 2016/2017, 100% sensitivity to linezolid, tigecycline, and vancomycin was observed (Table III). In addition, high sensitivity to teicoplanin was shown (98.09% in 2014; 97.96% in 2016/2017).

TABLE III – Antimicrobial susceptibility profile of the 176 and 120 coagulase-negative staphylococci (CoNS) samples isolated from blood cultures in 2014 and 2016/2017, respectively, in a tertiary hospital in the southern region of Brazil

Tested antimicrobials	2014					2016/2017					p-value
	Number of samples tested	Sensitive		Resistant		Number of samples tested	Sensitive		Resistant		
		n	%	n	%		n	%	n	%	
Clindamycin	173	133	76.88	40	23.12	120	51	42.50	69	57.50	0,0000
Ciprofloxacin	176	84	47.73	92	52.27	119	32	26.89	87	73.11	0,0004
Erythromycin	167	81	48.50	86	51.50	119	38	31.93	81	68.07	0,0054
Gentamicin	170	84	49.41	86	50.59	119	39	32.77	80	67.23	0,0052

TABLE III – Antimicrobial susceptibility profile of the 176 and 120 coagulase-negative staphylococci (CoNS) samples isolated from blood cultures in 2014 and 2016/2017, respectively, in a tertiary hospital in the southern region of Brazil

Tested antimicrobials	2014					2016/2017					p-value
	Number of samples tested	Sensitive		Resistant		Number of samples tested	Sensitive		Resistant		
		n	%	n	%		n	%	n	%	
Linezolid	173	173	100	0	0	120	120	100	0	0	---
Oxacillin	175	42	24	133	76	120	19	15.83	101	84.17	0,0898
Penicillin	171	49	28.65	122	71.3	120	1	0.83	119	99.17	0,0000
Sulfamethoxazole/ Trimethoprim	173	101	58.38	72	41.62	120	40	33.33	80	66.67	0,0000
Teicoplanin	157	154	98.09	3	1.91	98	96	97.96	2	2.04	0,9420
Tigecycline	165	165	100	0	0	120	120	100	0	0	----
Vancomycin	176	176	100	0	0	120	120	100	0	0	-----

*Test for the difference between two population proportions, significance level adopted 5%.

The susceptibility profile of the 176 CoNS isolated in 2014 to linezolid, tigecycline, and vancomycin using the broth microdilution method demonstrated MIC values of ≤ 2 μg/ml, ≤ 0,25, and ≤ 2 μg/ml, respectively; and of the 120 isolates from 2016/2017, the MIC values were ≤ 2 μg/ml, ≤ 0,5, and ≤ 4 μg/m, respectively, showing sensitivity to all isolates.

When comparing the broth microdilution method with the automated system, the MIC values presented by the automation were generally higher than those obtained by the microdilution method, however all results were in agreement (kappa = 1).

Regarding clinical significance, of the 131 patients admitted in 2014 in the ambulatory (AMB), OC, and gynecology and obstetrics (GO) units; 46.56% (61/131) were not treated and did not use invasive devices. Of the 70 treated patients, 77.14% (54/70) of the infections were related to catheter use. Of the 79 patients admitted to the AMB, OC, and GO units in 2016/2017, 21.52% (17/79) did not undergo treatment and did not use invasive devices. Of the NBs treated (n = 62), 95.16% (59/62) of the infections were related to the use of invasive devices.

The blood count in 2014 demonstrated that of the 131 patients (176 CoNS isolates), 61.36% (131/176) were normal, 23.30% (41/176) presented with leukocytosis, 7.95% (14/176) leucopenia, and 7.39% (13/176) neutrophilia, left shift and toxic granulations. In 2016/2017 the examinations of the 79 NBs (120 isolates), showed that 55% (66/120) of patients had leukocytosis, 35% (42/120) were normal, 8.33% (10/120) had neutrophilia, left shift and toxic granulations, and 1.67% (2/120) leucopenia.

Regarding CRP, the mean was 0.15±0.13 mg/dL for in untreated CoNS infections (61/176) and 1.41±2.89 mg/dL considering patients who had sepsis and received treatment (115/176). In 2016/2017 these rates were 0.27±0.34 mg/dL for untreated patients (17/120) and 1.82±2.94 mg/dL for patients receiving treatment (103/120).

In 2014, the mortality rate of NBs was 15.71% (11/70), and 84.29% (59/70) were discharged from hospital. In 2016/2017, these were 17.74% (11/62) and 82.26% (51/62), respectively. Of these, three patients died of coinfections associated with gram-negative microorganisms in 2014, and two in 2016/2017.

DISCUSSION

Coagulase-negative staphylococci (CoNS) are among the most identified microorganisms in blood cultures during the neonatal period (Namvar *et al*., 2017; Cantey *et al*., 2018; Dong, Speer, Glaser, 2018; Pereira *et al*., 2020). In this study, when comparing 2014 with 2016/2017, the number of isolates decreased (176 to 120), with *S. epidermidis* prevalence ranging from 56.82% to 66.67%, corroborating with other studies, since the occurrence rate of this species varied between 50% and 80% in infections (Namvar *et al*., 2017; Cantey *et al*., 2018; Dong, Speer, Glaser, 2018; Pereira *et al*., 2020). Freitas *et al.* (2019) evaluated NB bloodstream infections in a public maternity hospital located in Brasília, Brazil, between January 2014 and December 2016, and found 50% of sepsis cases to be caused by CoNS. A study carried out over 20 years, at Botucatu Clinics Hospital in the southern center of Brazil, analyzing CoNS isolated from blood cultures, also identified *S. epidermidis* as the predominant microorganism (57.4%) in NBs, followed by *S. haemolyticus* (37.9%) (Pereira *et al*., 2020). Dong, Speer and Glaser (2018) evaluated 96 cases of neonatal sepsis in the NICU of Bengbu Third People's Hospital in China from January 2010 to August 2014, and demonstrated that 70.10% (68/97) of blood cultures developed CoNS, of which *S. epidermidis* was responsible for 44.3% (43/97) of the cases, followed by *S. haemolyticus* at 14.4% (14/97) and *S. hominis* at 8.2% (8/97). Ertugrul *et al.* (2016) conducted a study with blood cultures of NBs at the NICU of Dicle University Hospital, Turkey, between January 2011 and December 2014, in which CoNS were the most prevalent agent in neonatal sepsis at 34.07% (46/135) of cases, with *S. epidermidis* comprising 60.87% (28/46) of these.

CoNS, especially *S. epidermidis*, are bacteria that are present in the skin, thus, a possible explanation for these findings is that the migration of these microorganisms to the bloodstream occurs, facilitated by the use of invasive devices (Berlak *et al*., 2018). Differences can still be observed, since their incidence varies geographically, and between health units (Shane, Sanchez, Stoll, 2017).

In relation to the hospital sectors, CoNS were isolated most frequently from the NICU at 61.36% of cases in 2014 and 80.83% in 2016/2017. Infections associated with medical intervention in the NICU are usually caused by CoNS, corroborating with data from this study (Bizzarro *et al*., 2015; Namvar *et al*., 2017; Cantey *et al*., 2018; Dong, Speer, Glaser, 2018).

The resistance rates increased in practically all antimicrobial agents when comparing 2014 to 2016/2017, including: ciprofloxacin (52.27% to 73.11%, $p = 0.0004$), erythromycin (51.40% to 68.07%, $p = 0.0054$), gentamicin (50.59% to 67.23%, $p = 0.0052$), penicillin (71.3% to 99.17%, $p = 0.0001$), and sulfamethoxazole/trimethoprim (41.62% to 66.67%, $p = 0.0001$). Dong, Cao and Zheng (2017) found similar high resistance rates for gram-positive bacteria, 93.2% for penicillin, 90.0% for ampicillin, 84.7% for oxacillin, and 81.1% for erythromycin.

Since most CoNS isolates show high resistance to beta-lactams, such as penicillin, vancomycin remains the drug for the mainstay treatment of these infections (Shane, Sanchez, Stoll, 2017). Dong, Cao and Zheng (2017) observed that all the isolates were sensitive to vancomycin, this was corroborated in our research, where the antimicrobials linezolid, tigecycline, in addition to vancomycin presented 100% sensitivity in the both periods of the study and in the different methodologies. However, Pereira *et al*. (2020) showed a reduced sensitivity to vancomycin (2.7%). To our knowledge, the isolation of CoNS with reduced susceptibility to vancomycin has not been identified in this hospital to date.

In relation to clinical significance, 53.44% (70/131) of the NBs were treated in 2014, of which 77.14% were related to catheter use. In 2016/2017, these were 78.48% (62/79) and 95.16% (59/62), respectively. This is relevant because, historically, CoNS were considered colonizers of the skin and not a serious health concern. However, over the years, they appeared as important nosocomial pathogens causing infections in patients with compromised or immature immune systems, such as NBs (Shane *et al*., 2017; Heilmann, Ziebuhr, Becker, 2019). They can migrate to the bloodstream, assisted by the use of invasive devices and the breakdown of the skin's natural barrier (Ertugrul *et al*., 2016; Heilmann, Ziebuhr, Becker, 2019).

Laboratory tests are an important tool to aid in the diagnosis of neonatal sepsis, which have a positive effect on reducing neonatal morbidity and mortality (Granzotto, Fonseca, Lindemann, 2012). The blood count of NBs usually presents valuable information, and its interpretation varies with age and other characteristics of the patient (Aguiar, Baldessar, Dal-Bó, 2015). Our research showed that in both 2014 and 2016/2017, the severity of the infection was not be related to the blood count, as infected patients who had received antibiotic therapy had blood counts with normal reference values.

CRP is a protein synthesized by the liver and is released rapidly after the onset of an inflammatory process. It has been used to monitor inflammatory and infectious diseases, and is the most used marker in neonatal care worldwide (Lobo, 2012; Perrone *et al*., 2018). In our study, the mean CRP in 2014 was 0.15±0.13 mg/dL for patients who did not receive treatment and who developed CoNS infections, and 1.41±2.89 mg/dL for patients who had sepsis and used antibiotics. In 2016/2017 these were 0.27±0.34 mg/dL and 1.82±2.94 mg/dL, respectively. Considering the two-year study, the NBs in which the isolation of CoNS was considered to be infection, CRP values were higher, above 0.30 mg/dL (RV), confirming that CRP can be used as a biomarker in NB sepsis. However, CRP used for the diagnosis of sepsis may present low specificity, since its levels may be elevated in other inflammatory processes (Perrone *et al*., 2018).

Regarding mortality rates, the indices in 2014 were 15.71% and in 2016/2017, 17.74% respectively. Of these, in 2014, three patients died of polymicrobial infections; and in 2016/2017, two patients died. In Brazil, studies involving CoNS and infant mortality rates are rare, as they are opportunistic pathogens. Freitas *et al*. (2019) reported a 13% mortality rate in NBs, including gram-positive and gram-negative bacteria. After evaluating microorganisms isolated from blood cultures of NBs, Al-Taiar *et al.* (2013) reported that 4.8% of newborns died owing to CoNS infection. Cantey *et al.* (2018) showed that the mortality rate due to CoNS in neonatal sepsis was 1.6%; however, in the multivariate analysis, this percentage was not significant. Lee, Chang, and Kim (2015) have shown that CoNS mortality rates can reach 9.4% in very low birth weight infants.

In this study, it was observed that in both 2014 and 2016/2017, *S. epidermidis* was the predominant microorganism in neonatal sepsis, and most cases was related to catheter use. The involvement of CoNS in infections is already well established in this hospital, with resistance against most antimicrobials increasing and the associated mortality rates becoming a major concern in NBs.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

We thank all the staff of the Archive and the Laboratory of Clinical Analysis of the Hospital Universitário de Santa Maria (HUSM).

FINANCIAL SUPPORT

CAPES

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Received for publication on 05th September 2019
Accepted for publication on 14th March 2021