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Coagulase-negative Staphylococcus and Antibiotic Susceptibility Patterns in Cases of Sepsis in the Neonatal Intensive Care Unit of Amiens-Picardie University Hospital (France)

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Abstract

Neonatal sepsis is the most serious disease encountered in the neonatal Intensive Care Unit, and is associated with high morbidity and mortality rates. Here, we evaluated coagulase-negative *Staphylococcus* strains and the corresponding antibiotic susceptibility profiles in clinically suspected cases of neonatal sepsis. The objectives of the study were to determine the significance of coagulase-negative *Staphylococcus* strains isolated from cases of neonatal sepsis, another isolation site for coagulase-negative *Staphylococcus* other than blood culture and their susceptibility pattern. In a prospective study of newborns admitted to our neonatal Intensive Care Unit between January 20th, 2017, and January 20th, 2019, we analyzed all cases of sepsis with a positive blood culture, and available stool, central venous catheter, tracheobronchial, and nasopharyngeal fluid. Strains were identified using Matrix-Assisted-Laser Desorption Ionization Time of Flight Mass Spectrometry. Antimicrobial susceptibility patterns were recorded and analyzed. Of 157 premature newborns enrolled in the study, 28 (17.8%) had a coagulase-negative *Staphyloccus*-positive blood culture. Eighteen (64.2%) presented with early onset sepsis and 10 (35.8%) presented with late onset sepsis. Based on the gestational age at birth, there were 10 (35%) extremely preterm newborns, 12 (42.8%) very preterm newborns, and 6 (21. 5%) moderately preterm newborns. The birth weight was extremely low in 13 (46.4%), very low in 9 (32.2%), low in 4 (14.2%), and normal 2 (7.2%). All coagulase-negative *Staphylococcus* isolates showed high levels of resistance to cefoxitin (100% of the strains), aminoglycosides (100%), fusidic acid and, ofloxacin (100%). The isolates were highly susceptible to pristinamycin (100% of the strains), vancomycin (100%) and trimethoprim-sulfamethoxazole (100%). Coagulase-negative *Staphylococcus* isolates constituted the most frequent cause of neonatal sepsis. The abundance of these strains may contribute to the emergence of multi-drug resistance.

Keywords: Coagulase-negative *Staphylococcus*, Neonatal sepsis, Antimicrobial resistance, Blood culture, Early-onset sepsis, Late-onset sepsis.

Abbreviations

APUH: Amiens Picardie University Hospital; BCA: blood Columbia

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agar; BW: birth weight; CFU: colony forming unit; CoNS: coagulase-negative *Staphylococcus*; CI: confidence interval; CRP: C-reactive protein; CVC: central venous catheter; EOS: early-onset sepsis; E.coli; *Escherichia coli*; FMS: French Society of Microbiology/EUCAST: European Committee on Antimicrobial Susceptibility Testing; GBS: group B *Streptococcus*; G-CSF: granulocyte colony-stimulating factor; GM-CSF: granulocyte macrophage colony-stimulating factor; LOS: late-onset sepsis; MALDI-TOF MS: matrix-assisted laser desorption ionization time flight mass spectrometry; MDR: multi-drug resistant; MSA: mannitol salt agar; nICU: neonatal Intensive Care Unit: NPF: nasopharyngeal fluid; NS: neonatal sepsis; OR: Odd ratio: SD: standard deviation; S. aureus: Staphylococcus aureus; SXT: trimethoprim-sulfamethoxazole; TBF: tracheobronchial fluid; TLC: total leukocyte agar; VLBW: very low-birth-weight.

Introduction

Coagulase-negative *Staphylococcus* (CoNS) strains are among the most frequent pathogens involved in late-onset sepsis (LOS), particularly in preterm infants born at a lower gestational age (GA) [1-3]. The risk of infection is inversely related to GA and birth weight (BW) approximately 1 in 6 very low-birth- weight (VLBW, <1500 g) newborns, develop an episode of CoNS bacteremia [2]. Neonatal CoNS infections in this setting are associated with high morbidity and mortality rates [3]. This infection is responsible for 30 to 50% of neonatal deaths [4].

Neonatal sepsis (NS) can be categorized into early-onset sepsis (EOS) (≤ 72 hours of life) and LOS (>72 hours of life) [1,3]. The types of pathogen depend on the time of onset: the per partum transmission involved in EOS includes group B *Streptococcus* (GBS), *Escherichia coli (E.coli),* CoNS*, Staphylococcus aureus (S. aureus), Listeria monocytogenes, Haemophilus influenzae,* and *Enterococcus* spp. [5,6] whereas the bacteria in the environment commonly associated with LOS include CoNS*, S. aureus, Klebsiella pneumoniae, E.coli, Enterobacter* spp*, Pseudomonas aeruginosa* and *Acinetobacter* spp. [5,6].

In the first week of life, newborns become rapidly colonized by microorganisms originating from the environment. During this period, the risk of CoNS infection is substantially increased (i) the use of central venous catheters (CVCs), mechanical ventilation, and parenteral nutrition, and (ii) exposure to other invasive skin-or mucosa-breaching procedures [3]. Coagulase-negative staphylococci are common inhabitants of the skin and mucous membranes. Although a small proportion of newborns acquire CoNS by vertical transmission, most acquisitions horizontally [3]. Consequently, infants admitted to hospital obtain most of their microorganisms from the hospital environment, familly members, and the hospital staff [7]. Transmission from the hospital staff's hands can lead to the circulation of endemic strains for long periods of time. Given that CoNS is a ubiquitous skin commensal, some researchers have suggested that colonization's on the skin and/or of on indwelling catheters are major sources of sepsis [8]. Selective perinatal antibiotic exposure, also significantly influences antibiotic resistance patterns among microorganisms isolated from newborns [3]. The objectives of the present study were to determine the frequency of CoNS strains in blood cultures and in other sample cultures (stools, CVCs, tracheobronchial fluids (TBF), and nasopharyngeal fluids (NPF), and antibiotic susceptibility patterns in the neonatal Intensive Care Unit (nICU) at Amiens Picardie University Hospital (APUH), France.

Material and Methods

This prospective study was performed in the nICU, between January 20th, 2017 and January 20th, 2019. A total of 157 newborns (aged from 0 to 28 days on admission) with clinically suspected NS were enrolled. Blood cultures were performed in all cases. Only, newborns with a positive blood culture for CoNS and who had not received any antibiotics prior to testing were included in the study. Newborns who had received antibiotics prior to admission to the nICUs, and newborns who died before the blood culture results were excluded from the study.

The demographic and clinical variables recorded for CoNS-positive newborns with suspected sepsis included sex, GA, birth weight, age at onset, maternal age at the time of delivery, and mode of delivery. Sepsis was suspected when one or more of the following symptoms were present: body temperature instability, lethargy, abdominal distension, refusal to feed, respiratory distress, hemodynamic instability, neonatal seizures, and neonatal jaundice.

In all clinically suspected cases of NS, the newborns' blood samples were assayed for nonspecific markers of sepsis, included C-reactive protein (CRP), serum lactate, the total leukocyte count (TLC), to diagnose leukocytosis (>26000/mm^3) or leukopenia (<10000/mm^3), and the platelet count/mm^3 to diagnose thrombocytopenia (<150000/mm^3).

The etiological diagnosis of sepsis was defined as a positive-blood culture. Samples from other sites (stools, CVCs, TBFs, and NPFs were also screened for pathogens.

Bacteriological analysis of different collected samples

Peripheral vein blood samples were performed for diagnostic purposes. Stool, CVC, TBF, and NPF samples were cultured as part of routine. Less than 0.5 mL of blood was inoculated into a Bactec Peds Plus F bottle, and incubated in Bactec™ Becton Dickinson instrument (BD Diagnostic System, Spark, MD, USA). Subcultures of the initial blood culture broth were seeded on blood (5%) Columbia agar (BCA), (Oxoid, Dardilly, France), and mannitol salt agar (MSA) (Biorad, Marnes-la-Coquette, France) plates. Stool samples were collected with sterile swabs were seeded on BCA and MSA plates. After removal, CVCs were analyzed using the quantitative Brun-Buisson method [9] as follows, the distal (5 cm) for long CVCs or the whole indwelling segment for short CVCs was vortexed vigorously in 1 mL of sterile saline solution 0.9% for 1 minute, and 100 µL aliquots were then seeded on BCA and MSA plates. TBF and NPF samples were collected through the intubation probe, placed in a sterile tube containing 1 mL of sterile saline solution 0.9%, and vortexed vigorously for 1 minute. Lastly,

100 µL aliquots of the homogenate were seeded on BCA and MSA plates. All agar plates were incubated aerobically at 35±2°C for 24 hours. The number of CFU/mL of CoNS was determined. Colonization was defined as CFU/mL ≥ 10^3/mL [10].

Isolates were identified as CoNS on the basis of a positive catalase assay and negative mannitol and bound coagulase assays (Pastorex™ Staph Plus, Bio-Rad, Marne-La-Coquette, France). Species were identified using Matrix-Assisted-Laser Desorption Ionization Time Flight Mass Spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany) according to manufacturer's instructions. The antimicrobial susceptibility of all CoNS isolates (with penicillin, cefoxitin, kanamycin, gentamicin, tobramycin, erythromycin, pristinamycin, rifampin, ofloxacin, vancomycin, fusidic acid, trimethprim-sulfamethoxazole, and fosfomycin) was determined using the disk diffusion method according to the guidelines issued by the French Society of Microbiology (FSM) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [11].

Two mL blood samples were collected, and serum was separated for colorimetric (450 nm). CRP was performed using the Human CRP ELISA Kit, according to manufacturer's instructions (Thermo Fisher Scientific, Invitrogen, France). Lactate was performed by L-lactate assay Kit according to manufacturer's recommendations (abcam, France). Normal values are <10 mg/mL for CRP, and ≤1.65 mmol/L for lactate in the newborns.

Statistical analysis

Quantitative variables (such as maternal age at childbirth, GA, the newborn's birth weight, age at onset of sepsis, CRP, lactate, TLC, and the platelet counts) were described as the mean ±, standard deviation (SD), or the median (range). Fisher's Exact test, and Spearman's rank correlation coefficient (*rs*) were used to compare blood culture results with the results for other sample culture, and to compare EOS results with LOS results. The mortality rates in EOS *vs* LOS were compared using a Wilcoxon-Mann Whitney. The threshold for statistical significance was set to *p*-value ≤ 0.05.

Results

The blood cultures were positive in 28 (17.8%) cases and negative in 129 (82.2%). The 28 positive cases included, 15 (53.5%) females and 13 (46.5%) males (female: male ratio 1.1: 1 (Table 1). Twenty newborns (71.4%) had been delivered vaginally and 8 (28.6%) had been delivered by emergency cesarean section (Table 1). Eighteen of the 28 cases (64.2%) presented with EOS and 10 (35.8%) presented with LOS (Tables 2,3). Based on the GA at birth,there were 10 (35.7%) extremely preterm newborns, 12 (42.8%) very preterm newborns, and 6 (21.5%) moderately preterm newborns (Table 2). The birth weight was ELBW in 13 (46.4%) newborns, very low birth weight (VLBW) in 9 (32.2%), l ow birth weight (LBW) in 4 (14.2%) and normal in 2 (7.2%) (Table 3).

The most common clinical findings observed on admission of the 28 preterm infants were respiratory distress (32.2%) followed by refusal to feed (25%), abdominal distension (14.3%), and neonatal seizures (14.3%). Neonatal jaundice, hypothermia, and lethargy were the other presenting symptoms (Table 1). The mean± SD GA, birth weight and age at onset sepsis and the laboratory results for the 28 newborns with blood culture-positive sepsis are summarized in table 4. The mean maternal age was 29.7 ± 6.2 (range: 19-45) (Table 4).

The stool culture was positive in all 28 newborns (100%) with a positive blood cultures. The CVC, TBF, and NPF samples were positive in 46.4%, 42.8%, and 32.1% of newborns respectively (Table 5). The most common CoNS isolated by blood culture was *S. haemolyticus* (n=11, 39.3% of the newborns), followed by *S. epidermidis* (n=8, 28.6%). Most of the infections in EOS were due to *S. haemolyticus*

Neonatal variables	Number (%)
Sex	
Male	15 (53.5)
Female	13 (46.5)
Male-to Female ratio of	1. 1: 1
Maternal age (years)	
≤ 19	4 (14.2)
20-29	15 (53.6)
30-39	6 (21.5)
≥40	3 (10.7)
Mode of delivery	
Vaginal	20 (71.4)
Cesarean section	8 (28.6)
Clinical features	
Respiratory distress	9 (32.2)
Refusal of feeds	7 (25.0)
Abdominal distension	4 (14.3)
Neonatal seizures	4 (14.3)
Neonatal jaundice	2 (7.2)
Hypothermia	1 (3.5)
Lethargy	1 (3.5)

Table 1: General characteristics of the 28 suspected cases of neonatal sepsis.

*Gestational age (weeks)	Range	**EOS N (%)	**LOS N (%)	Total N (%)
Extremely Preterm (<28 W)	(25-27 W)	10 (55.5)	0	10 (35.7)
Very Preterm (28-< 32 W)	(28-31 W)	8 (44.5)	4 (40)	12 (42.8)
Moderate Preterm (32-<37 W)	(32-36 W)	0	6 (60)	6 (21.5)
Total		18 (64.2)	10 (35.8)	28 (100)

*Preterm birth categorization according to the World Health Organization [29] and Quinn et al. [30]
**Neonatal sepsis is categorized into Early Onset Sepsis (EOS) which presents within the first 3 days or below 3 days of life and Late Onset Sepsis (LOS) presenting after 3 days of life.

Table 2: Distribution of blood culture positive neonatal sepsis according to gestational age and onset of sepsis (n=28).

*Neonatal birth Weight (gram)	Range	EOS N (%)	LOS N (%)	Total N (%)
Extremely low birth weight (<1000 g)	(592-995)	12 (66. 7)	1 (10)	13 (46. 4)
Very low birth weight (<1500 g)	(1060-1370)	4 (22. 2)	5 (50)	9 (32. 2)
Low birth weight (<2500 g)	(1720-2400)	2 (11. 1)	2 (20)	4 (14. 2)
Normal birth weight (> 2500 g)	(2370-2800)	0	2 (20)	2 (7. 2)
Total		18 (64. 2)	10 (35. 8)	28 (100)

*Birth weigth category according to newborn infants classification by weight [30,31]

Table 3: Distribution of positive-blood -culture neonatal sepsis according to birth weight and onset of sepsis (n=28).

Variables	Distribution of values		
	Mean ± SD*	Median	Range
Mother's age at childbirth (years)	29. 7 ± 6.2	29.5	19-45
Gestational age (weeks)	29. 2 ± 3. 3	29.3	25-36
Birth weight of neonate (gram)	1288 ± 606. 2	1075	592-2800
Age at onset of sepsis (days)	9. 8 ± 21. 2	3	1-104
Lactate (mmol/L	38. 2 ± 43. 5	23.3	2. 9-7. 9
CRP (mg/L)	38. 2 ± 41. 3	30	0-178
Leukocytes/mm3	26775 ± 4833. 7	15400	5000-271000
Platelets/mm3	162642. 8 ± 98180. 5	54500	12000-447000

*SD: standard deviation

Table 4: Characteristics of neonates with suspected sepsis on admission at Neonatal Intensive Care Unit at Amiens Picardie University Hospital.

CoNS isolates	Blood culture (n=28)			Stool culture (n=28)			* CVC (n=28)			**TBF (n=28)			***NPF (n=28)		
	EOS N (%)	LOS N (%)	Total N (%)	EOS N (%)	LOS N (%)	Total N (%)	EOS N (%)	LOS N (%)	Total N (%)	EOS N (%)	LOS N (%)	Total N (%)	EOS N (%)	LOS N (%)	Total N (%)
S.haemolyticus	8 (44. 5)	3 (30)	11 (39.3)	9 (50)	3 (30)	12 (42. 8)	2 (25)	0	2 (15.3)	4	0	4 (33. 4)	2	0	2 (22.2)
S.epidermidis	5 (27. 8)	3 (30)	8 (28. 6)	6 (33.3)	3 (30)	9 (32. 2)	6 (75)	2 (40)	8 (61.6)	6	0	6 (50. 0)	5	0	5 (55.6)
S.sprophyticus	1 (5. 5)	3 (30)	4 (14. 2)	1 (5.5)	3 (30)	4 (14. 2)	0	1 (20)	1 (7.7)	0	0	0	0	0	0
S.hominis	2 (11. 2)	1 (10)	3 (10. 8)	2 (11.2)	1 (10)	3 (10. 8)	0	1 (20)	1 (7.7)	2	0	2 (16.6)	2	0	2 (22.2)
S.warneri	1 (5. 5)	0	1 (3. 5)	0	0	0	0	0	0	0	0	0	0	0	0
S.capitis	1 (5. 5)	0	1 (3. 5)	0	0	0	0	0	0	0	0	0	0	0	0
S.lugdinensis	0	0	0	0	0	0	0	1 (20)	1(7.7)	0	0	0	0	0	0
Total	18 (64.2)	10 (35.8)	28 (100)	18 (64.2)	10 (35.8)	28(100)	8 (44.4)	5 (50)	13 (46.4)	12	0	12 (66.6)	9 (50)	0	9 (32.1)

*CVC,Central Venous Catheter; **TBF, tracheobronchial fluid; ***NPC, nasopharyngeal fluid

Table 5: Neonatal sepsis distribution of Coagulase-negative Staphylococcus isolates according to the kind of sampling.

(n=8, 44.5%) and *S. epidermidis* (n=5, 27.8%) whereas most of the infections in LOS were due to *S. haemolyticus* (n=3, 30%), *S.epidermidis* (n=3, 30%), and *S. saprophyticus* (n=3, 30%) (Table 5). The predominant CoNS isolates in other sample cultures and their proportions for cases of EOS, and LOS are listed in table 5.

The CoNS strains' susceptibility to individual antibiotics is summarized in tables 6-10. All the CoNS strains isolated in the present study were resistant to cefoxitin (100%) (methicillin-resistant strains). Furthermore, all the strains showed high resistance to aminoglycosides, and ofloxacin (100%). However, all the strains demonstrated high susceptibility to vancomycin, pristinamycin, trimethoprim-sulfamethoxazole, and fusidic acid (100%).

When comparing the blood culture susceptibility profile with the stool culture profile, 78.5% (22/28) of cases were concordant and 21.5% (6/28) of cases were discordant. Similarly, 8 (28.5%) of the blood culture vs CVC profiles were concordant and 20 (71.5%) of cases were discordant, along with 9 (42.8%) of the blood culture vs TBF fluid were concordant and 19 (57.8%) of cases were discordant, and 7 (25%) of the blood culture vs NPF were concordant and 21(75%) of cases were discordant.

Fisher's Exact Test was used to compare the blood culture results with the results for the other samples. There was a significant difference between the blood culture results and the stool culture results. Odds ratio (OR): 3.6113, 95% confidence interval (CI) [1.186 to 12.5998], *p*-value: 0.017 and between the blood culture results and NPF results, OR: 0.3377, 95% CI [0.1039 to 0.9913], *p*: 0. 03. There was no

Antibiotic tested	*S.haemolyticus* N=11		*S.epidermidis* N =8		*S.saprophyticus*		*S.hominis*		*S.capitis*		*S.warneri*	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Penicillin	11 (100)		8 (100)		4 (100)		3 (100)		1	0	1	0
Cefoxitin	11 (100)		8 (100)		4 (100)		3 (100)		1	0	1	0
Kanamycin	11 (100)		8 (100)		4 (100)		3 (100)		1	0	1	0
Gentamicin	11 (100)		8 (100)		4 (100)		3 (100)		1	0	1	0
Tobramycin	11 (100)		8 (100)		4 (100)		3 (100)		1	0	1	0
Netilmicin	11 (100)		8 (100)		4 (100)		3 (100)		0	1	1	0
Erythromycin	11 (100)		7 (87.5)	1(12.5)	3 (75)	1(25)		3 (100)	0	1	0	1
Lincomycin	1 (9. 1)	10 (90.9)	2 (25)	6 (75)		4 (100)	1 (33.4)	2(66.4)	0	1	0	1
Pristinamycin		11 (100)		8 (100)		4 (100)	0	3 (100)	0	1	0	1
Rifampin	10 (90.9)	1 (9. 1)	7 (87.5)	1 (12.5)		4 (100)	1 (33.4)	2(66.4)	1	0	1	0
Ofloxacin	11 (100)	0	8 (100)	0	4 (100)	0	3 (100)	0	0	1	0	1
Vancomycin		11 (100)		8 (100)		4 (100)		3 (100)	0	1	0	1
Fusidic acid		11 (100)		8 (100)		4 (100)		3 (100)	0	1	0	1
Trimethoprim Sulfamethoxazole		11 (100)		8 (100)		4 (100)		3 (100)	0		0	1
Fosfomycin		11 (100)		8 (100)		4 (100)		3 (100)	1	0	0	1
Doxycyclin		11 (100)		8 (100)		4 (100)		3 (100)	0	1	0	1

High resistance: 90.9-100% ;Moderate resistance: 75-87. 5%
High susceptibility :90.9-100%; Moderate susceptibility: 66.4-75%; Low susceptibility: 12.5-25%

Table 6: Antibiotic susceptibility of Coagulase-negative Staphylococcus strains isolated from blood samples.

Antibiotics tested	*S.haemolyticus N=12*		*S.epidermidis N=9*		*S.saprophyticus N=4*		*S.saprophyticus N=3*	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Penicillin	12 (100)	0	9 (100)	0	4 (100)	0	3(100)	0
Cefoxitin	12 (100)	0	9 (100)	0	4 (100)	0	3(100)	0
Kanamycn	12 (100)	0	9 (100)	0	4 (100)	0	3(100)	0
Gentamicin	12 (100)	0	9 (100)	0	4 (100)	0	3(100)	0
Tobramycin	12 (100)	0	9 (100)	0	4 (100)	0	3(100)	0
Netilmicin	12 (100)	0	9 (100)	0	4 (100)	0	3(100)	0
Erythromycin	12 (100)		8 (88.8)	1 (11.2)	4 (100)	0	3(100)	0
Lincomycin	0	12 (100)	7 (77.8)	2 (22.2)		4 (100)	0	3(100)
Pristinamycin	0	12 (100)	0	0	0	4 (100)	0	3(100)
Rifampin	11(91.6)	1(8.4)	8 (88.8)	9 (100)	0	4 (100)	0	3(100)
Ofloxacin	12 (100)	0	9 (100)	1 (11.2)	4 (100)		3(100)	0
Vancomycin	0	12 (100)	0	9 (100)	0	4 (100)	0	3(100)
*SXT	0	12 (100)	0	9 (100)	0	4 (100)	0	3(100)
Fosfomycin	1(8.4)	11(81.6)	0	9 (100)	0	4 (100)	0	3(100)
Doxycyclin	2(16.6)	10(83.4)	0	9 (100)	0	4 (100)	0	3(100)
Fusidic acid	0	12 (100)	0	9 (100)	0	4 (100)	0	3(100)

*SXT: Trimethoprim-sulfamethoxazole
High resistance: 91.6-100%; Moderate resistance: 77.8%-88.8; Low resistance: 8.4%-16.6%
High susceptibility: 100%; Moderate susceptibility: 81.6-88.8%; Low susceptibility: ≤ 22.2%

Table 7: Antibiotic susceptibility of Coagulase-negative Staphylococcus strains isolated from stool.

Antibiotics tested	*S.haemolyticus N=2*		*S.epidermidis N=8*		*S.hominis N=1*		*S.saprophyticus N=1*		*S.lugdinensis N=1*	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Penicillin	2	0	8(100)	0	1	0	1	0	1	0
Cefoxitin	2	0	8(100)	0	1	0	1	0	1	0
Kanamycn	2	0	8(100)	0	1	0	1	0	1	0
Gentamicin	2	0	8(100)	0	1	0	1	0	1	0
Tobramycin	2	0	8(100)	0	1	0	1	0	1	0
Netilmicin	2	0	8(100)	0	0	1	1	0	1	0
Erythromycin	2	0	7(87.5)	1(12.5)	0	1	0	1	1	0
Lincomycin	0	2	2(25)	6 (75)	0	1	0	1	0	1
Pristinamycin	0	2	0	8(100)	0	1	0	1	0	1
Rifampin	2	2	7(87.5)	1(12.5)	1	0	1	0	0	1
Ofloxacin	2	0	8(100)	0	0	1	0	1	1	0
Vancomycin	0	2	0	8(100)	0	1	0	1	0	1
Fusidic acid	0	2	0	8(100)	0	1	0	1	0	1
*SXT	0	2	0	8(100)	0	1	0	1	0	1
Fosfomycin	0	2	0	8(100)	0	1	0	1	0	1
Doxycyclin	0	2		6 (75)	0	1	0	1	0	1

*SXT: trimethoprime-sulfamethoxazole
Haigh resistance: 100%; Moderate resistance: 87. 5%; Low resistance: 25%
High susceptibility: 100%; Moderate susceptibility: 75%; Low susceptibility: 12. 5%

Table 8: Antibiotic susceptibility of Coagulase-negative Staphylococcus strains isolated from Central Venous Catheter (CVC).

Antibiotics tested	*S.haemolyticus N=4*		*S.epidermidis N=6*		*S.hominis N=2*	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Penicillin	4 (100)	0	6 (100)	0	2	0
Cefoxitin	4 (100)	0	6 (100)	0	2	0
Kanamycin	4 (100)	0	6 (100)	0	2	0
Gentamicin	4 (100)	0	6 (100)	0	2	0
Tobramycin	4 (100)	0	6 (100)	0	2	0
Netelmicin	4 (100)	0	6 (100)	0	2	0
Erythromycin	4 (100)	0	6 (100)	0	0	2
Lincomycin	0	4 (100)	0	6 (100)	0	2
Pristinamycin	0	4 (100)	0	6 (100)	0	2
Rifampin	4 (100)		6 (100)	0	0	2
Ofloxacin	4 (100)		6 (100)	0	2	0
Vancomycin	0	4 (100)	0	6 (100)	0	2
Fusidic acid	0	4 (100)	0	6 (100)	0	2
*SXT		4 (100)	0	6 (100)	0	2
Fosfomycin	0	4 (100)	0	6 (100)	0	2
Doxycyclin	0	4 (100)	0	6 (100)	0	2

*SXT: Trimethoprime-sulfamethoxazole
High resistance: 100%
High susceptibility; 100%

Table 9: Antibiotic susceptibility of Coagulase-negative Staphylococcus strains isolated from Tracheobronchial fluid (TBF).

significant difference between the blood culture and CVC, or the blood culture and TBF results (p-> 0.05).

Spearman's rank correlation coefficient (*rs*) was used to compare EOS and LOS with regard to the blood, stool, and CVC culture results. The correlation was strong, positive and statistically significant for blood cultures, (Qobs 15.647 ; *rs*: 0.7206, *p*: 0.05), and stool cultures; (Qobs 6.680; *rs*: 0.8807, *p*: 0.008), but not CVC culture, (*rs*: 0.2887, *p*>0.05). For TBF, and NPF samples, the *p*-values could not be calculated.

The CRP and lactate assay results were available for all 28 clinically confirmed cases of NS. The CRP assay was positive in all 18 (64. 2%) cases of EOS and negative in all 10 (35.8%) cases of LOS. The lactate assay was positive in a 28 (100%) newborns (i.e. in all cases EOS and LOS). According to the TLC, leukocytosis was diagnosed in 6 (21.4%) of the 28 cases (range: 27, 600-271, 000/mm^3), and leukopenia was diagnosed in 5 (17.8%) (range: 5, 500-9,900/mm^3). Thrombopenia was observed in 14 (50%) of the 28 newborns (range: 12,000-136,000/mm^3). In our study, the mortality rate among newborns with blood- culture positive sepsis was 25%, overall, 27.7% in cases of EOS, and 20% in cases of LOS.

Discussion

Neonatal sepsis remains a major clinical problem in neonatology and is associated with high morbidity and mortality rates. In the present study, EOS (18 out of 28 blood -culture- positive cases, 64.2%) was more common than LOS (10 cases, 35.8%). This agrees with the reports from Lakhey et al. (75% for EOS and 25% for LOS) [12], Sathyamurthy et al. (62%, and 38% LOS respectively) [13], and Jyothi et al. (74.8% and 25.2% respectively) [1]. In the present study, 17.8% of the newborns had bacteriologically

Antibiotics tested	*S.haemolyticus N=2*		*S.epidermidis N=5*		*S.hominis N=2*	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Penicillin	2	0	5 100)	0	2	0
Cefoxitin	2	0	5 100)	0	2	0
Kanamycin	2	0	5 100)	0	2	0
Gentamicin	2	0	5 100)	0	2	0
Tobramycin	2	0	5 100)	0	2	0
Netilmicin	2	0	5 100)	0	2	0
Erythromycin	2	0	5 100)	0	0	2
Lincomycin	0	2	0	5 100)	0	2
Pristinamycin	0	2	0	5 100)	0	2
Rifampin	2	0	4 (80)	1 (20)	0	2
Ofloxacin	2	0	5 100)	0	2	0
Vancomycin	0	2	0	5 100)	0	2
Fusidic acid	0	2	0	5 100)	0	2
*SXT	0	2	0	5 100)	0	2
Fosfomycin	0	2	0	5 100)	0	2
Doxycyclin	0	2	0	5 100)	0	2

*SXT: trimethoprime-sulfamethoxazole
High resistance: 100%; Moderate resistance: 80%
High susceptibility: 100%; low susceptibility: 20%

Table 10: Antibiotic susceptibility of Coagulase-negative Staphylococcus strains isolated from Nasopharyngeal fluid (NPF).

confirmed sepsis. This proportion is in agreement with the values found in several studies in the literature, e.g.,16.9% |5],17.3% [14], and 20.5% [15]. However, the proportion of blood-culture positive cases of NS differs greatly from one study to another. Khante et al. reported value of 36.22% [16], and Islam et al. found a value of 31% [17]. These disparities might be due to interstudy differences in culture-techniques, designs, predisposing factors, infection control practices, and prior antibiotic administration.The most common clinical manifestation of NS in our study was respiratory distress (32.2%). Similar findings were reported by Pokhrel et al. [15].

Coagulase-negative *staphylococci* are major pathogens in NS [1-3]. According to the literature data, the mean infection-related mortality rate in VLBW infants is 10% [3] but the value for some pathogens is as high as 40% [18]. Preterm newborns have a high risk of developing neonatal infections, which result in high mortality rates and serious long-term morbidity [3]. In the present study, the mortality rates for EOS (27.7%) and LOS (20%) did not differ significantly (p=0.33). Our results agree with the mortality rates reported by Khante et al. [16] 25.4% for EOS and 19.11% for LOS, (p=0.306). The greater incidence of mortality in EOS may be due to weaker resistance by, under weight newborns, associated birth trauma, anoxia, and early circulatory problems (intraventricular hemorrhage and periventricular leukomalacia).

A CRP level ≥ 10 mg/mL was considered to be a positive result for sepsis. In the present study, 18 (64.2%) of the 28 newborns with blood-culture-positive sepsis were positive for CRP (range: 17-178 mg/mL). The negative correlation was moderately strong (*rs*: 0.5269) and statistically significant (p: 0.024), which consistent with the results of another study [16]. Elevated CRP levels are more difficult to interpret, (especially for the dignosis of EOS) because they may also be associated with factors such as prolonged rupture of membranes, maternal fever, pregnancy-induced hypertension, prenatal steroid use, and fetal distress. Furthermore, some studies have suggested that variations in the CRP during the first few days are normal [16].

In the presente study, the lactic acid level in the capillary blood was greater than >1.65 mmol/L (range: 2.9-7.9) in all cases of NS (i.e. both EOS and LOS). This elevation might have been due to either to NS-related variations poor-tissue oxygenation,i.e. (hypoxia and metabolic acidosis) or physiological variations related to age, digestion, and muscle activity.

Six (21.4%) of the 28 newborns with blood-culture-positive sepsispresented leukocytosis, whereas 5 (17.8%) presented leukopenia. Misra et al. [19] reported that leukopenia was a better predictor of septicemia than leukocytosis (specificity, 87.5% *vs* 25% respectivity). Severe bacterial sepsis may lead to an elevated TLC in general and a greater number of mature and immature neutrophils in particular. This might be caused by release of various growth factors and cytokines. (such as granulocyte colony-stimulating factor [G-CSF], granulocyte-macrophage colony-stimulating factor [GM-CSF], interleukin [IL-3, and IL-6] that stimulate the bone marrow [19]. In the first few days after delivery, the TLC varies physiologically as a function of age and sex. Furthermore, very severe cases of bacterial sepsis are often characterized by leukopenia [20].

The CoNS has been reported in various studies as being the most common cause of NS in the nICUs [21,22]. Our observation of multi-drug resistance (MDR) CoNS (i.e. strains that resist to antibiotics commonly used in the nICUs) is particularly alarming. All the CoNS isolates were resistance to cefoxitin (methicillin-resistance strains) combined with heterogeneous resistance to aminoglycosides, resistance to erythromycin, resistance to rifampin, and resistance to ofloxacin. These MDR CoNS constitute a rapidly emerging and potentially disastrous problem. They are known to colonize hospital personnel and to produce septicemia in

the nICU. Infection with MDR CoNS has been linked to with treatment faillure, higher morbidity and mortality rates, greater health coasts and prolonged hospitalization. During a vaginal delivery (as soon as the membranes rupture), the newborn is colonized by the mother's vaginal flora (mainly GBS, and lactobacilli), fecal flora (mainly enterobacteria, and bifidobacteria), and skin flora (mainly staphylococci). Infants born by cesarean section are colonized by bacteria (mainly enterobacteria, enterococci, and staphylococci) from the environment (i. e. the ambient air, family members, and hospital staff). The risk of CoNS infection is known to be substantially increased by the use of CVCs, mechanical ventilation, parenteral nutrition, and other invasive skin or mucosa-breaching procedures [3]. CoNS isolates are the most prevalent normal flora located on the skin and the nose. Hence, suboptimal hand hygiene by care-staff and the manipulation of peripheral intravenous lines on newborns can contributed to the acquisition of these bacteria. Although a small proportion of newborns acquire CoNS by vertical transmission. Most transmission is horizontally [23,24]. Consequently, infants admitted to hospital can obtain MDR-CoNS from the hospital environment, their familly member (not only the mother), and hospital staff [25]. Newborns are colonized by CoNS living on the mother's skin and on indwelling catheters [26]. Selective pressure caused by perinatal antibiotic exposures is an important additional factor influencing the spectrum and antibiotic resistance pattern of CoNS strains isolated from newborns. Our present results, showed that CoNS were responsible for all cases of EOS (64.2%), and LOS (35.8%). A similar study in a nICU in China, found that Gram-positive organisms were present in 83.3% of cases of EOS, and 70% of cases of LOS [27]. In the present study, the most common isolate was *S. haemolyticus* (in 39.3% of cases overall, 44. 5% of cases of EOS, and 30% of cases of LOS), and *S. epidermidis* (in 28.6% of cases overall, 27.8% of cases of EOS and 30% of cases of LOS). Similar findings were reported by Labi et al. [28]; *S.epidermidis* was the most common isolate in both cases of EOS (59.1%) and LOS (52.8%). Aku et al. [14] also reported that S*. epidermidis* was also the most common isolate in both EOS (in 69.2% of cases) and LOS (in 38.5% of cases).

In summary, in the present study of preterm in the nICU, the prevalence of blood-culture-positive NS was 17.8%. The most frequent CoNS isolate was, *S. haemolyticus*, followed by *S. epidermidis, S.saprophyticus,* and *S. hominis*. CoNS isolates are present in both EOS and LOS. These isolates showed a high prevalence of resistance to cefoxitin associated with heterogeneous resistance to aminoglycosides, erythromycin, rifampin, and ofloxacin. This high degree of antibiotic resistance is typically associated with high morbidity and mortality rates among neonates. To prevent the emergence of drug resistance, a comprehensive approach (with the evaluation of antibiotic use, improvements in laboratory techniques, the rational use of empirical treatments, treatment de-escalation/discontinuation when appropriate, and continuous local epidemiologic monitoring) is needed.

Authors' Contribution

Léké A, Amar G, Elion Dzon B, Kongolo G, Biendo M participated in meeting and follow-up discussions that culminated in the preparation of this manusctipt. They contributed to the study conception, Cdesign, and drafted the manuscript. All authors participated in the acquisition, analysis, and the interpretation of data and also in editing, and final revisions. All authors read and approved the final manuscript.

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Ethical Approval and Consent to Participate

All procedures performed in studies involving human participants were conducted in accordance with the guidelines laid down in the Declaration of Helsinki and/ or National Research Committee (Ref n° 139). Clinical and laboratory concerning the preterm infants were included in this study. Informed consent from legal guardians of the minors included in the study was not specifically requested.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article and the additional information files.

Consent for Publication

Not applicable

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