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A Molecular Biology Approach for the Rescue of Central Venous Catheters during Bloodstream Infections

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Abstract

Background: Infants who are preterm are at a high-risk of infections. A central venous catheter-related bloodstream infection (CRBSI) is a type of infection that happens when a tube is put into a baby's vein in the hospital. This tube is called a central venous catheter (CVC). It is the most common infection that happens in the hospital for babies who need extra care. Coagulase-negative *staphylococci* (CoNS) are a type of bacteria that can make babies very sick when they are born early. The main goal of the study was to use methods to tell the difference between bloodstream infection (BSI) that does not have a known cause and CRBSI, and to compare two ways of finding out what kind of CoNS causes CRBSI: phenotypic methods and genotypic methods.

Methods: We looked at the medical records of 28 babies who were born too early and needed special care in the hospital from 2020 to 2022. We found that at least one of them had a serious infection caused by CoNS BSI. The germs were found in the blood and the CVC tip cultures. The Wilcoxon-Mann-Whitney and Fisher's exact test were used to look at variables. We chose the best model from two criteria: Akaike information criterion and Bayesian information criterion. We looked at 28 different phenotypes of CoNS isolates by biotyping and antibiotyping and 24 of these isolates were genotypically studied by Pulsed-Field gel electrophoresis (PFGE).

Results: The most common types of CoNS were *Staphylococcus haemolyticus* (32.5%), *Staphylococcus epidermidis* (30%), and *Staphylococcus saprophyticus* (12.5%). Most of the bacteria were very resistance to penicillin and cefoxitin (100%). The PFGE revealed five patterns (A to E).

Conclusion: The risk factors for CoNS CRBSI included the birth weight, age at onset of infection, plasma lactate, platelet count, catheter lenght of stay, lipide emulsion, antenatal corticosteroid, antenatal antibiotic therapy, neonatal antibiotic therapy, patent ductus arteriosus, proton pump inhibitor, and the presence of a CVC.

Keywords: Coagulase-negative staphylococci, Central venous catheter, Catheter-related bloodstream infection, Risk factors, Methicillin-resistant CoNS, Neonatal intensive care unit.

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Abbreviations: AIC: Akaike information criterion; APUH: Amiens Picardie University Hospital; AUC: Area Under the Curve; BCA: blood Columbia agar; BIC: Bayesian information criterion; bpm: beats per min; BSI: bloodstream infection; BW: birth weight; CFU: colony-forming Units; CoNS: coagulase-negative *staphylococci*; Cpm: cycle per min; CRBSI: catheter-related bloodstream infection; CRI: CVC-related infection; CRP: C-reactive protein; CVAD: central venous access device; CVC: Central vein catheter; ELBW: extremely low birth; GA: gestational age; HAI: healthcare-associated infection; LBW: low birth weight; LOS: late onset sepsis; MSA: mannitol salt agar; NICU: Neonatal intensive care unit; PFGE: Pulsed Field Gel Electrophoresis; PI: preterm infant; PLAT: platelet; PPI: proton pump inhibitor; RFs: risk factors; SD: standard deviation; ROC: Receiver Operating Characteristic; VLBW: very low birth weight; WBC: white blood cell.

Introduction

Central venous catheters (CVCs) play a crucial role in the management of critically ill patients, providing reliable vascular access for the administration of medications, fluids, and blood products, parenteral feeding, as well as for hemodynamic monitoring [1,2]. Despite their clinical utility, CVCs are associated with significant risks, including hospital-acquired infections (HAIs) [3], mechanical complications during insertion, and CRBSIs [4,5]. CRBSIs, in particular, are a major concern in healthcare settings, contributing to increased morbidity, mortality, and healthcare costs.

A central venous access device (CVAD) is an infusion device inserted through different parts to make the tip of the catheter reach the vena cava. In the clinic, CVAD is mainly divided into the following four categories: tunneled CVC, nontunneled CVC, peripherally inserted central catheter, and totally implantable venous access port [6].

When bacteria develop on or close to the CVC and cause illness in the patient, this is known as a CVC infection [7]. Neonatal intensive care unit (NICU) patients require specialized treatment and care. They carry food that enters their veins, a liquid that helps them stay hydrated, and a tube in their heart [8]. CRBSI are the most prevalent complications that patients experience during their hospitalization, which is also known as HAIS [9-11]. Some hospital-acquired infections may be explained by the fact that patients receive nutrition via a tube in their vein. These infections happen more often in babies who need extra care in the NICU [12].

Other than in patients with weakened immune systems, colonization from an infectious cognizance isn't unusual, even after intestinal translocation. Despite some progress in preventing infections caused by CVCs, 10-20% of all HAIs still occur as a result of their use. Due to its elevated morbidity and mortality rates, the CRBSI is regarded as the most severe. It's something to do with staying alive longer in the NICU. According to studies, an additional fee of between US $3,700 and $29,000 per event is expected [11-14].

The following factors are associated with the host: chronic illness, history of malignant hematological tumors from bone marrow transplants, immunodeficiency, neutropenia, malnourishment, CRBSI records, extremes in age, the duration of time the catheter was in, the type of CVC, and conditions of CVC insertion. Three risk factors (RFs) for CRBSI on CVC are repeated catheterizations, catheter thrombosis, and the neighborhood care provided [15-20]. Most of the bacteria that live in a preterm baby's gut are Gram-positive especially CoNS. There have been cases of CoNS BSI with or without a catheter, and the CVC taking over from the umbilical twine is often linked to these cases. Preterm newborns weighing more than 1200 g require this for nutritional support [21].

The study's objective was to differentiate BSI with an unknown starting point, and CVC was the point of the study. Moreover, the characterization of CoNS CRBSI can be investigated through the use of phenotypic techniques (biotyping and anti-biotyping) and the genotyping method (PFGE).

Materials and Methods

The purpose of this prospective and retrospective study changed into having a look at newborns with signs of sepsis hospitalized in the NICU at the Amiens Picardie University Hospital (APUH) between 2018 and 2022. The affected person's digital scientific records were the source of all of their statistics. This study included all preterm infants born before 37 weeks of gestation and who had at least one episode of CoNS BSI. Additionally, they were admitted to the NICU after a positive blood culture for CoNS. They also had a catheter that was either positive or negative for CoNS in the vein. In this study, duplicates and full-term infants (gestation period of 37 weeks or more) were not included.

The demographic traits, underlying situations, and medical and laboratory findings had been gathered. Their strains were isolated from critical blood culture and catheter tip subculture. A blood subculture was carried out on 28 (17.8%) of the 157 PNs. The last 128 (82.2%) preterm babies had bad blood cultures and were taken out of the test.

Clinical and biological information of sepsis

Within the first three days of neonate lifestyles, scientific-related episodes of clinical sepsis are symptoms that encompass: fever, cold extremities, refusal to feed, vomiting, belly distension, signs of respiratory distress (grunting, apnea, cyanosis), jaundice, pallor, lethargy, excessive crying, convulsions, diarrhea, tachycardia (>180 bpm), bradycardia (<100 bpm), polypnea (respiratory rate > 60 cpm) with desaturation, hemodynamic or awareness alterations. It is frequently sought for the following biological symptoms of infection: C-reactive protein (CRP) (everyday, <10 mg/L), plasma lactates (everyday, 0.88-2.2 mmol/L), white blood cell (WBC) count (regular, 6, 000-18, 000/mm^3), platelet (PLAT) count number (ordinary,150, 000-400, 000/mm^3).

Blood samples and CVC tip cultures

Much less than 0.5 mL of blood from the CVC and peripheral vein was installed in a Bactec Peds Plus F bottle and incubated in a Bactec™ Becton Dickinson device (BD Diagnostic Systems, Spark, MD, USA). Within the initial blood subculture broth, subcultures have been seeded on sheep

blood (5%) Columbia agar (BCA) (Oxoid, Dardilly, France) and mannitol salt agar (MSA) (Biorad, Marnes-la-Coquette, France) plates.

After being taken out, CVC segments were analyzed for usage using the quantitative Brun-Buisson technique [22]. The distal (5 cm) section for long CVCs or the whole indwelling segment for short CVC is vortexed vigorously in 1 mL of sterile 0.9% saline solution for 1 minute. There were 100 µL aliquots of each kind seeded on BCA and MSA plates. Agar plates had been incubated aerobically for 24 hours at 35±2°C. The number of colony-forming units (CFU) turned into a decision for catheter tip culture.

A couple of blood cultures with a relevant quantitative blood culture/peripheral quantitative ratio of ≥5 and a differential put-off time of positivities between the central and peripheral blood cultures of ≥2 hours were used to verify BSIs. This indicates the life of a BSI episode [23]. The colonization of catheter tip cultures changed into positive while the CFU/mL became $\geq 10_3$

Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) (Brucker Daltonics, Bremen, Germany) has been used to analyze all strains of *Staphylococcus spp.* in accordance with a previously defined procedure [24]. The serum was separated using colorimetric (450 nm) analysis after two mL of blood samples were collected. The Human CRP package was used in accordance with the manufacturer's instructions (Thermo Fisher Scientific, Invitrogen, France). Following the manufacturer's instructions, lactate was determined using an L-lactate assay kit (Abcam, France).

Antibiotic susceptibility testing

The antimicrobial susceptibility of all CoNS isolates (with penicillin, cefoxitin, kanamycin, gentamicin, tobramycin, erythromycin, pristinamycin, rifampin, ofloxacin, vancomycin, fusidic acid, trimethoprim-sulfamethoxazole, and fosfomycin) was determined using the disk diffusion technique in step with the guidelines issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [25].

Pulsed-field gel electrophoresis (PFGE) typing

Chromosomal DNA macrorestriction evaluation was accomplished in step with the manufacturer's recommendations (Bio-Rad, France) using SmaI (5U/µL) as the limit restriction enzyme and turned into an analysis with a contour-clamped homogeneous electric field system (CHEF-DRII device; GenePath, Bio-Rad, France) at 14°C and 200V for 19.7 hours. Patterns had been interpreted as previously defined [26].

Maternal and preterm toddler's risk factors for CoNS infection

Maternal risk factors (RFs) that increase the likelihood of infection include the mode of delivery, antenatal corticosteroid use, and antenatal antibiotic treatment. The subsequent RFs were identified for preterm infants (PIs): GA (gestational age) (weeks), birth weight (BW) (grams), age at onset of sepsis (days), hemodynamic disorders, lipid emulsion, proton pump inhibitor (PPI), and patent ductus arteriosus. History of jaundice, neonatal antibiotic therapy, catheter length of stay, CRP, plasma lactate, WBC, and PLAT should all be considered as RFs.

Based on threat factors, the patients were divided into two groups: Group 1 consisted of protected patients whose catheter tip lifestyle was excellent and whose bloodstream subculture changed to a nice state (CVC+). Patients in Group 2 had a positive bloodstream subculture and a catheter tip tradition that was suboptimal (CVC-).

Statistical analysis

The RFs for infection among PIs with BSI with and without catheter tip infection have been looked at. Facts are expressed as the means ± standard deviation (SD) for quantitative variables and the frequencies for qualitative variables. As suitable, proportions were compared for categorical variables using of Fisher's exact test. A Wilcoxon-Mann-Whitney rank sum test for two-group comparisons was used to research the continuous variables. Multivariable evaluation included clarification of covariables with a p-value ≤ 0.05 for univariable analysis. The Akaike information criterion (AIC) and the Bayesian information criterion (BIC) had been used to pick for fashions [27].

Results

Neonates'characteristics

Out of 28 preterm neonates, 13 (46.5%) were men and 15 (53.5%) were females, for a intercourse ratio of 0.86. Twenty newborns (71.4%) had been delivered vaginally, and 8 (28.6%) have been brought via cesarean section. On the prmise of GA (weeks) at birth, there have been 10 (35.7%) extremely preterm newborns, 12 (42.8%) very preterm newborns, and 6 (21.5%) moderately preterm newborns. The BW (gram) was extremely low birth (ELBW) in 13 (46.4%) newborns, range: 592-995; very low birth weight (VLBW) in 9 (32.2%) newborns, range: 1060-1370; and low birth weight (LBW) in 4 (14.2%) newborns, range: 1720-2400. The other traits are maternal age on the delivery (years): 29.7±6.2, median, 29.5, range: 19-45; GA: 29.2±3.3, median, 29, range: 25-36; BW (g): 1287.1 ± 606.7, median, 1075, range: 592-2800; age on the onset of infection (days): 20.8 ± 21.1, median,13, range: 5-104; catheter period of stay (days): 12.5 ± 4.5, median, 12.4, range, 4.5-20.5. The non-unique marker effects have been CRP (mg/L): 38.2 ± 43.5, median, 23.3, range, 0-178; abnormality charges had been determined in 18 (67.8%), range: 10-178; serum lactate (mmol/L): 5.2 ± 1.6, median, 5.1, range: 2.9-7.9; WBC/mm3: 26,418 ± 48,694, median, 15,400 range: 5,000-271,000). Leukocytosis became observed in 13 (46.4%), range: 18,200-271,000/mm^3; and PLAT/mm^3,162, 643 ± 98,181, median,154,500, range: 12,000-447,000. Many of the 28 preterm neonates, 14 (50%) developed thrombocytopenia with a PLAT matter of < 150,000/mm^3. Of those 14 instances, there's one (7.2%) of very severe thrombocytopenia (12,000/mm^3), 9 (64.3%) of intense thrombocytopenia (60,000-98,000/mm^3), and 4 (28.5%) of mild thrombocytopenia (103,000-136,000/mm^3). Preterm infant nutrition changed into mixed with a lipid emulsion by infusion in 12 (42.8%) of preterm newborns, 16 (57.1%)

had obtained postnatal antibiotic remedy, 12 (42.8%) had evolved premature jaundice, and 14 (50%) had supplied a hemodynamic disease, 15 (53.5%) and 10 (35.7%) of their mothers were given antenatal corticosteroid and antenatal antibiotic remedy respectively. The alternative parameters tested had been, patent ductus arteriosus, 16 (57.1%), PPI, 17 (60.7%), and mode of delivery: cesarean section, 12 (42.8%) and vaginal delivery, 16 (57.2%).

Blood culture and CVC culture results

CRBSIs contain bacteria that are part of the normal skin microbiota. Table 1 shows the types of CoNS that can be found in the blood culture or the catheter tip culture. The most common types of CoNS were *S. haemolyticus*, *S. epidermidis*, and *S. saprophyticus*. They were found in 39.3%, 28.6%, and 14.2% of blood cultures, respectively. About two-thirds of the catheter tips that were tested had *S. epidermidis* on them (61.6%), and about one-fifth had *S. haemolyticus* on them (15.3%). *S. epidermidis* was the most common one found in the blood of patients with infections. It was found in 28.5% of the samples. Another type of bacteria called *S. haemolyticus* (25.0%) and *S. saprophyticus* (14.1%) were also found in blood samples. *S. haemolyticus* is the CoNS sp. that is more common in CVC (50.0%), followed by *S. epidermidis* (33.4%).

Antibiotic susceptibility of CoNS isolates results

The outcomes of antibiotic susceptibility checking out are shown in tables 2 and 3. In summary, most of the CoNS spp. isolated from blood subculture and catheter tip culture became resistant to penicillin and cefoxitin (methicillin-resistant strains). A heterogeneous resistance was determined to aminoglycosides (kanamycin, gentamicin, tobramycin, and netilmicin), to macrolides (erythromycin), to quinolones (ofloxacin), and to rifampin. They remain susceptible to glycopeptide (vancomycin), fusidic acid, trimethoprim-sulfamethoxazole, fosfomycin, and doxycyclin. These types of isolates had been classified into six R-patterns (a, b, c, d, e, f) for blood culture isolates and into three R-patterns (a, g, f) for catheter tip way of life isolates.

Comparison of the phenotypic outcomes of strains from blood and catheter tip cultures.

Twenty-eight venous blood samples and 28 catheter tips removed had been analyzed in this examination of those samples: 28 blood cultures had been positive (100%), and catheter cultures were positive in 12 (42.8%) preterm toddlers. The ultimate 16 (57.2%) have been negative (Table 4). In this series of 28 CoNS isolates, blood subculture outcomes were similar to catheter tip culture results in 12 (42.8%) of cases. Twelve concordant strains exhibited R-patterns (a) and corresponded to six *S. haemolyticus* isolates and four *S. epidermidis* isolates respectively. The R-pattern (b) changed into what was observed in a single strain and corresponded to the *S. saprophyticus* isolate. The R-pattern (c) was observed in one strain and corresponded to *S. hominis* isolates. Eight CoNS strains isolated from blood cultures, such as one *S. haemolyticus* strain (phenotype a), four *S. epidermidis* strains (phenotype a), and three *S. saprophyticus* strains (phenotype b), were no longer removed from catheter tip cultures. In catheter tip cultures, three *S. capitis* strains (phenotype d) and three *S. warneri* (phenotype e) have been now not discovered.

PFGE results

To detect CRBSI, only the *Staphylococcus spp.* that were isolated from each CVC tip subculture and peripheral blood lifestyle and showed the same antibiotic resistance pattern were genotyped using PFGE. A total of 24 strains of Staphylococcus spp. that fulfilled the specified conditions were genotyped.

The *SmaI* of chromosomal DNA from 24 *Staphylococcus spp.* isolates studied produced seven exclusive patterns of 13-17 fragments ranging in size from 48.5 to 533.5 Kbp arbitrarily designated PFGE patterns (PFGEp). Five PFGEps (A, B, C, D, and E) were identified. PFGEp A became essential 12 (50%). With the help of PFGEp B, 8 (33.4%), PFGEp D, 2 (8.4%), PFGEp C, 1 (4.1%), and PFGEp E, 1 (4.1%). The PFGEp A sample had *S. haemolyticus* from blood cultures (isolates 1-3, 16-18) and a CVC tip culture (isolates 19-24). *S. epidermidis* (isolates 4, 5, 8, and 9) from blood cultures and (isolates 11 and 13-15) from the CVC tip cultures were safeguarded by PFGEp B. PFGEp D included *S. hominis* (isolate 7) from the blood way of life and (isolate 10) from CVC tip subculture. Those isolates were strongly linked to each other within the same genotype. PFGEp C contained *S. saprophyticus* (isolate 6) from blood way of life, while PFGEp E safeguarded *S. saprophyticus* (isolate 12) from CVC tip subculture. The genotypes of these last few isolates have no genetic link to each other (Figure 1).

The evaluation of phenotypic strategies with the genotypic technique

We were able to confirm CRBSI by comparing phenotypic and genotypic methods. The phenotype (a) became identical to *S. haemolyticus* genotype A and *S. epidermidis* genotype B.

CoNS isolates	Blood culture	Central venous catheter (CVC) Tip culture	Total (%)
	N=28 (%)	N=28 (%)	
S.haemolyticus	7 (25.0)	6 (50.0)	13 (32.5)
S.epidermidis	8 (28.5)	4 (33.4)	12 (30.0)
S.saprophyticus	4 (14.1)	1 (8.3)	5 (12.5)
S.hominis	3 (10.8)	1 (8.3)	4 (10.0)
S.warneri	3 (10.8)	0	3 (7.5)
S.capitis	3 (10.8)	0	3 (7.5)
Total	28 (100.0)	12 (42.8)	40 (100.0)

Table 1: Neonatal sepsis distribution of CoNS isolates according to the kind of sampling.

Antibiotic tested	*S.haemolyticus*		*S.epidermidis*		*S.saprophyticus*		*S.hominis*		*S.capitis*		*S.warneri*	
	N=7		N=8		N=4		N=3		N=3		N=3	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R	S	R	S
Penicillin	7 (100)		8(100)	0	4(100)		3(100)	0	3(100)	0	3(100)	0
Cefoxitin	7 (100)		8(100)	0	4(100)		3(100)	0	3(100)	0	3(100)	0
Kanamycin	7 (100)		8(100)	0	4(100)		3(100)	0	3(100)	0	3(100)	0
Gentamicin	7 (100)		8(100)	0	4(100)		3(100)	0	3(100)	0	3(100)	0
Tobramycin	7 (100)		8(100)	0	4(100)		3(100)	0	3(100)	0	3(100)	0
Netilmicin	7 (100)		8(100)	0	4(100)		3(100)	0	3(100)	0	3(100)	0
Erythromycin	7 (100)		8(100)	0	4(100)		3(100)	0	0	3(100)	0	3(100)
Lincomycin	1 (14.3)	6 (85.7)	2(25)	6 (75)	3(75)	1(12.5	0	3(100)	0	3(100)	0	3(100)
Pristinamycin	0	7 (100)	0	8 (100)	0	4(100)	1(33.4)	2(66.4)	0	3(100)	0	3(100)
Rifampin	6 (85.7)	1 (14.3)	7(87.5)	1(12.5)	0	4(100)	0	3(100)	0	3(100)	3(100)	0
Ofloxacin	7 (100)	0	8(100)	0	0	4(100)	1(33.4)	2(66.4)	3(100)	0	0	3(100)
Vancomycin	0	7 (100)	0	8(100)	4(100)	0	3(100)	0	3(100)	0	0	3(100)
Fusidic acid	0	7 (100)	0	8(100)	0	4(100)	0	3(100)	0	3(100)	0	3(100)
***SXT**	0	7 (100)	0	8(100)	0	4(100)	0	3(100)	0	3(100)	0	3(100)
Fosfomycin	0	7 (100)	0	8(100)	0	4(100)	0	3(100)	0	3(100)	0	3(100)
Doxycyclin	0	7 (100)	0	8(100)	0	4(100)	0	3(100)	0	3(100)	0	3(100)
R patterns	a		a		b		c		d		e	

*SXT: Trimethoprim-sulfamethoxazole
High resistance: 90.9-100%; Moderate resistance: 75-87.5%
High susceptibility: 90.9-100%; moderate susceptibility: 66.4-75%
R patterns: resistance patterns(a, a, b, c, d, e)

Table 2: Antibiotic susceptibility of CoNS strains isolated from blood samples.

Antibiotic tested	*S.haemolyticus*		*S.epidermidis*		*S.hominis*		*S.saprophyticus*	
	N=6		N=44		N=1		N=1	
	R	S	R	S	R	S	R	S
Penicillin	6 (100)	0	4 (100)	0	1	0	1	0
Cefoxitin	6 (100)	0	4 (100)	0	1	0	1	0
Kanamycin	6 (100)	0	4 (100)	0	1	0	1	0
Gentamicin	6 (100)	0	4 (100)	0	1	0	1	0
Tobramycin	6 (100)	0	4 (100)	0	1	0	1	0
Netilmicin	6 (100)	0	4 (100)	0	1	0	1	0
Erythromycin	6 (100)	0	4 (100)	0	0	1	1	0
Lincomycin	1(16.6)	5 (83.4)	0	4 (100)	0	1	0	1
Pristinamycin	0	6 (100)	0	4 (100)	0	1	0	1
Rifampin	6 (100)	0	4 (100)	0	0	1	0	1
Ofloxacin	6 (100)	0	4 (100)	0	1	0	1	0
Vancomycin	0	6 (100)	0	4 (100)	0	1	0	1
Fusidic acid	0	6 (100)	0	4 (100)	0	1	0	1
***SXT**	0	6 (100)	0	4 (100)	0	1	0	1
Fosfomycin	0	6 (100)	0	4 (100)	0	1	0	1
Doxycyclin	0	6 (100)	0	4 (100)	0	1	0	1
R patterns	a		a		c		b	

*SXT: Trimethoprim-sulfamethoxazole
High resistance: 100%; moderate resistance: 87.5%
High susceptibility: 100%; moderate susceptibility: 75%
R patterns: Resistance patterns (a, a, c, b)

Table 3: Antibiotic susceptibility of CoNS strains isolated from Central Venous Catheter (CVC) tip culture.

Phenotype (c) matched *S. hominis* genotype D. The phenotype (b) was identified as genotype C of *S. saprophyticus* from blood culture and genotype E of *S. saprophyticus* from catheter tip culture.

Role of the genotypic in the analysis of CRBSI episodes

We evaluated the episodes of presumptive CRBSI that were observed in this study. In both blood and catheter tip cultures, they exhibited signs and symptoms that were comparable to those of CRBSI and contained one or more additional isolates of CoNS. A total of 74 episodes and 28 positive blood cultures (37.8%) were observed during those episodes. Sixteen episodes had 16 positive blood cultures (100%), eight episodes had 4 positive blood cultures (50%), three episodes had one positive blood culture (33.3%), twelve episodes had 3 positive blood cultures (25%), twenty-one episodes had 3 positive blood cultures (14.2%), and fourteen episodes had one positive blood culture (7.1%).

Patient N°	Positive blood culture	Positive catheter tip culture ≥10^3	Patient N°	Positive blood culture	Negative catheter culture
N= 28	Group 1	Group 1	N=28	Group 2	Group 2
2	+	10^6	1	+	0
5	+	10^3	3	+	0
8	+	10^4	4	+	0
10	+	10^6	6	+	0
11	+	10^5	7	+	0
14	+	10^3	9	+	0
17	+	10^4	12	+	0
18	+	10^6	13	+	0
20	+	10^5	15	+	0
21	+	10^4	16	+	0
23	+	10^4	19	+	0
27	+	10^5	22	+	0
			24	+	0
			25	+	0
			26	+	0
			28	+	0
12 (42.8%)			**N=16 (57.2%)**		

Table 4: Distribution of 28 preterm infants based on blood cultures and intravenous catheter cultures.

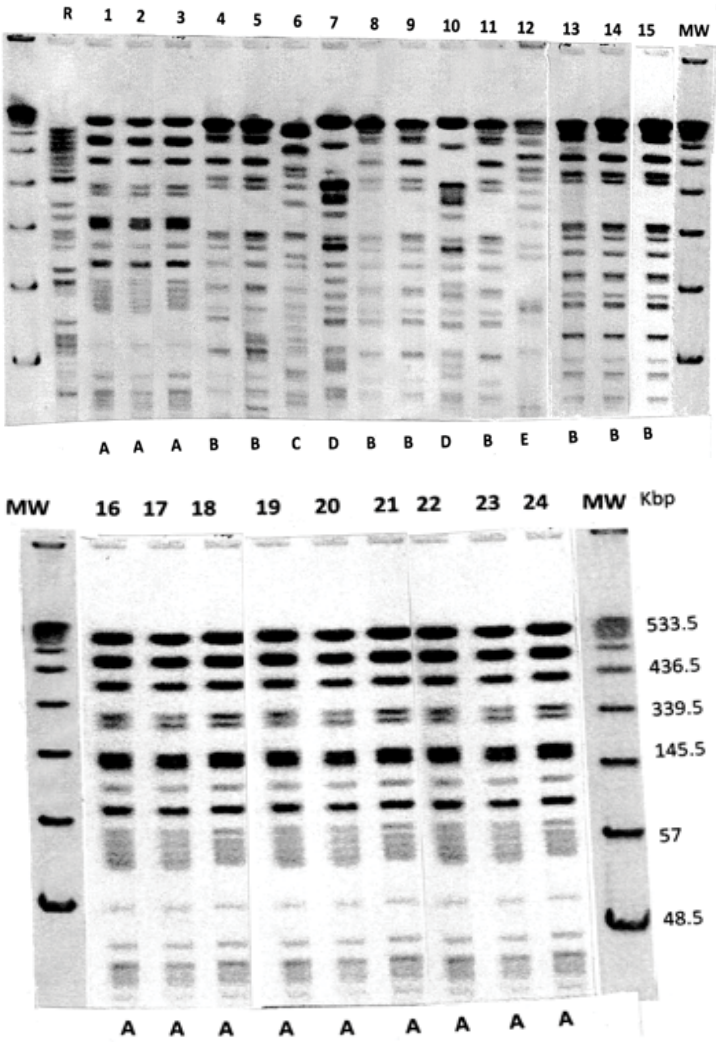

Figure 1: The molecular size of these PFGEps of 16-24 fragments ranged from 48.5 to 533.5 Kb. PFGEp A become observed to be related to 12 *S. haemolyticus* isolates (R-pattern a); eight isolates of *S. epidermidis* (R-pattern a) were a part of PFGEp B; PFGEp C was one *S. saprophyticus* strain isolated from catheter tip (R-pattern b); Two *S. hominis* isolates (R-pattern c) have been blanketed in PFGEp D. R=reference strain.

We have examined 26 CoNS isolates following 66 episodes of suspected CRBSI based solely on PFGE outcomes. Five distinct PFGEps (A-E) were identified, which helped us find 64 real cases of CRBSI (96.9%). Eight positive blood cultures were observed in 17 episodes (47%), twelve positive blood cultures were observed in 45 episodes (26.6%), and two positive blood cultures were observed in 2 episodes. In two episodes, we found 2 positive blood cultures. One episode's positive blood culture was associated with PFGEp C, while another episode's positive blood culture was associated with PFGEp E. We failed to find a comparable PFGEp between the catheter tip culture and the blood culture in these last two cases. Because of this, they were not thought to have CRBSI.

Baseline traits

There were 28 preterm babies with BSIs and their corresponding consequences. Of these, 12 (42.8%) were classified as CVC+, while,16 (57.2%) were classified as CVC-. When these two groups, CVC+ and CVC-, were used together, the following results were seen: GA (weeks): 30.5 ± 3.7, 29.5, range 25-36 versus 28.2 ± 2.8, 27.5, range 25-36; BW (g): 1360 ± 693, 1265, range 240-2730 versus 1098 ± 509, 988, range 592-2800; age at onset of infection (days): 33 ± 25.8, 26, range 10-104 versus 11.6 ± 10.1, 9, range 5-48; CRP >10 mg/L: 66.1 ± 48.3, 51.5, range 15-178 versus 54.9 ± 31.8, 53, range 23-103; lactate < 2.2 mmol/L: 6.1 ± 1.3, 6.8, range 3.5-7.7 versus 4.6 ± 1.4,4.1, range 2.9-7.9; WBC/mm^3: 25900 ± 8064, 20500, range 19000-37000 versus 67100 ± 100170, 29650, range 18200-271000; PLAT/mm^3: 89222 ± 27092, 75000, range 60000-136000; catheter period of stay (days): 15 ± 3.4, 14.9, range 10-20.5 versus 9.3 ± 3.8, 8.7, range 4.5-17.8.

Univariate analysis of factors associated with CRBSI

Some clinical and biological factors that have been essential for clinical and biological diagnosis of CRBSI were found in our study. Table 5 shows that there were significant differences in BW (p=0.03), age at onset of infection (p=0.003), catheter period of stay (p=0.001), lactate (p=0.008), and PLAT remember (p=0.02) between groups CVC+ and CVC-.

Furthermore, table 5 indicates that there were no statistically significant differences in GA (p=0.11), WBC count (p=0.39), and CRP (p=0.06) between groups CVC+ and CVC-.

Additionally, qualitative parameters were investigated concurrently. Lipid emulsion (p=0.0003), antenatal corticosteroid (p=0.003), antenatal antibiotic therapy (p=0.04), neonatal antibiotic therapy (p=0.02), patent ductus arteriosus (p=0.02), and PPI (p=0.005) are all significantly

Variables	Group 1 (CVC+)	Group 2 (CVC-)	p-value
Gestational age (weeks)			
Mean±SD	30.5±3.7	28.2±2.8	0.11
Median	29.5	27.5	
Range	25-36	25-36	
Birthweight (g)			
Mean±SD	1360±693	1097±509	**0.03**
Median	1265	988	
Range	240-2730	592-2800	
Age at onset of infection (days)			
Mean±SD	33±26	12±10	**0.0003**
Median	26	9	
Range	10-104	5-48	
CRP mg/L			
Mean±SD	66±48	55±32	0.06
Median	52.5	53	
Range	15-178	23-103	
WBC count/mm^3			
Mean±SD	25900±8063	67100±100170	0.39
Median	20500	29650	
Range	19000-37000	18200-271000	
Platelet count/mm3			
Mean±SD	89222±27091	71800±38977	**0.02**
Median	75000	72000	
Range	60000-136000	12000-120000	
Catheter length of stay (days)			
Mean±SD	15±3.4	9.3±3.8	**0.001**
Median	8.7	15	
Range	4.5-17.8	10-20.5	
Serum lactate mmol/L			
Mean±SD	6±1.3	4.6±1.4	**0.008**
Median	6.8	4	
Range	3.5-7.7	2.9-7.9	
p-values in bold are statistically significant.			

Table 5: Univariate analysis of factors associated with CRBSI.

Analyzed parameters	Group 1 (CVC+)	Group 2 (CVC-)	Wilcoxon-Mann Whitney test (p-value)	Fisher's exact test (p-value)
Gestational age (weeks)				
Mean±SD	30.5±3.7	28.2±2.86	Qobs: 130	
Median (Range)	29.5 (25-36)	27.5 (25-36)	P=0.11	
Birthweight (g)				
Mean±SD	1360±692.4	1097±508.8	Qobs: 141.5	
Median (Range)	1265 (240-2,730)	988 (592-2,800)	**P=0.03**	
Age at onset of infection (days)				
Mean±SD	33±25.8	11.6±10.1	Qobs: 173.5	
Median (Range)	26 (10-104)	9 (5-48)	**P=0.0003**	
CRP≥10/mm³				
Mean±SD	66±48.3	54.9±31.8	Qobs: 135	
Median (Range)	51.5 (15-178)	53 (23-103)	P=0.6	
Serum lactate mmol/L				
Mean±SD	6±1.3	4.6±1.4	Qobs: 153	
Median (Range)	6.8 (3.5-7.7)	4 (2.9-7.9)	**P=0.008**	
WBC count/mm³				
Mean±SD	25,900±8,063.4	67,100±100.170.3	Qobs: 113.5	
Median (Range)	20,500 (19,000-37,000)	29,650 (18,200-271,000)	P=0.39	
Platelet count/mm³				
Mean±SD	89,222.2±27,091.4	71,000±38,976.9	Qobs: 143	
Median (Range)	75,000 (60,000-136,000)	72,000 (12,000-120,000)	**P=0.02**	
Catheter length of stay (days)				
Mean±SD	9.3±3.8	15.0±3.4	Qobs: 26	
Median (Range)	8.7 (4.5-17.8)	14.9 (10-20.5)	**P=0.001**	
Lipid emulsion	10 (83.4%)	2 (12.5%)		**P=0.0003** OR: 28.428 95% CI[3.2095; 476.2275]
Antenatal corticosteroid	11 (91.6%)	4 (25%)		**P=0.003** OR: 27.2597 95% CI [2.7094;1517.2448]
Hemodynamic disorders	3 (25%)	5 (31.2%)		P=0.06 OR: 6.1128 95% CI [0.9744 ;51.6683]
History jaundice	8 (66.6%)	4 (25%)		P=0.06 OR: 5.5744 95% [0.9054;42.9234]
Antenatal antibiotic therapy	7 (58.3%)	3 (18.7%)		**P=0.04** OR: 5.5744 95% [0.8634;48.4832]
Neonatal antibiotic therapy	10 (83.4%)	6 (37.5%)		**P=0.02** OR: 7.6616 95% [1.0876;95.7421]
Patent ductus arteriosus	10 (83.4%)	6 (37.5%)		**P=0.02** OR: 7.6616 95% CI [1.0876; 95.7421]
Mode of delivery				
Cesarean section	8 (66.6%)	4 (25%)		P=0.06 OR: 5.5744 95% CI [0.9054; 42.9234]
Vaginal	4 (33.4)	12 (75%)		
Proton pump inhibitor	11 (91.6%)	6 (37.5%)		**P=0.005** OR: 16.3688 95% CI [1.6493; 860.9408]
p-values in bold are statistically significant				

Table 6: Rates and relative RFs for catheter-related bloodstream infection as a result of CoNS.

For each of the risk factors related to the infection studied, significant differences were observed both by the Wilcoxon-Mann Whitney test and by Fisher's exact test (<0.05) between the two groups. On the other hand, the following parameters: WBC count, hemodynamic disorders and history jaundice are not statistically significant (p>0.05) and therefore do not constitute likely risk factors related to infection in this study.

0	Parameter Number in the model	ONSET	GA	BW	CRP	PLAT	WBC	AIC	BIC
1	6	V	V	V	V	V	V	30.70215	40.02768
2	5	V	V		V	V	V	28.71205	36.70527
3	5	V	V	V		V	V	29.17177	37.16499
4	5	V	V	V	V		V	28.70611	36.69933
5	5	V	V	V	V	V		33.23517	41.62284
6	5	V	V	V	V	V	V	30.28223	38.27323
7	4	V				V	V	27.41080	34.07183
8	4	V	V		V		V	26.72015	33.38117
9	4	V	V	V			V	27.42134	34.08236
10	4	V	V		V	V		31.27269	37.93371
11	4	V	V	V		V		32.17604	38.83706
12	4	V		V		V	V	28.67476	35.33578
13	3	V		V	V			29.75714	35.08596
14	4	V		V	V		V	28.2936	34.95462
15	4	V	V	V	V			31.44426	38.10528
16	4	V		V	V	V		31.68335	38.34437
17	4	V			V	V	V	30.65144	37.31247
18	3	V	V				V	25.69952	31.02578
19	3	V	V			V		30.39824	35.72706
20	3	V	V		V			29.48198	34.8108
21	3	V		V		V		30.70391	36.03272
22	3	V			V		V	29.25369	34.58251
23	3	V		V			V	26.84445	32.17327
24	3	V			V	V		31.10912	36.43794
25	3	V				V	V	32.40407	36.975
26	3	V	V	V				32.04537	37.37419
27	2	V			V			29.15658	33.15319
28	2	V				V		32.38367	36.35028
29	2	V	V					30.54763	34.54424
30	2	V		V				30.17781	34.17443
31	2	V					V	30.57355	34.57017
32	1	V						31.35667	34.02107

Table 7: Selection of fashions with Akaike information criterion (AIC) and Bayesian information criterion (BIC).

more expensive. These factors were examined using the Wilcoxon-Mann-Whitney and Fisher's Exact tests (Table 6). In order to investigate the substantial variables included in the analysis, a logistic regression model was implemented. The remarkable factors located in the evaluation had been entered right into a logistic regression version in which the simplest age at the ONSET of infection remained an independent RF related to CRBSI.

Marker choice by using Akaike information criterion (AIC) and Bayesian information criterion (BIC)

The findings of the analyses between the likelihood of developing CRBSI and the following variables are compiled in table 7. Using easy logistic regression, we did a discriminant analysis. The significance of comparing models with varying numbers of parameters is demonstrated by this. Version comparisons are acceptable when implemented with either the AIC [27-29] or the BIC [28-30]. When the AIC and BIC variable choices were implemented in ONSET, a set of markers was chosen that optimized the statistics. The only variable that is stastically associated with the risk of CRBSI according to a multivariable approach, is age at infection onset. To find a good multivariate model, we use the time period ONSET to fit the other variables (GA, PLAT, and WBC). The parsimony criterion is satisfied by version n°18, which features the simplest three terms (ONSET, GA, and WBC) and leaves the lowest residual information (AIC=25.7; BIC=31.02) among all candidates. Weaker merit fashions were the result of other factors, such as CRP, BW, and PLAT (Table 7).

Discussion

Management of catheter-related bloodstream infections caused by CoNS

Catheter infections happen most often, and staphylococci are CoNS. When the insertion site is contaminated, a feverish state is typically evident. Too much sepsis doesn't happen very often. This issue is resolved by the catheter that is associated with antibiotic treatment for a period of 10-14 days. If there aren't problems, we will talk about keeping the catheter in place for 7 days and using an antibiotic lock for 10 to 14 days.

CoNS catheter-related bloodstream infection (CRBSI)

CoNS is the most commonly encountered organism in CRBSIs, causing between 11% and 45% of infections with an incidence of 15.8 per 10,000 hospital admissions. These microorganisms are part of the normal skin microbiota. They have been most often discovered at the catheter tip. In this study, CoNS was found in 42.8% of CRBSI cases. *S. haemolyticus*, *S. epidermidis*, and *S. saprophyticus* make up 32. 5%, 30%, and 12.5% of CoNS. In a previous observe, we validated that *S. haemolyticus* and *S. epidermidis* had been the most isolated CoNS spp., with prevalences of 41.0% and 17. 8% respectively [31]. Talebi et al. discovered that the main CoNS strains have been *S. epidermidis* (45%), *S. hominis* (14%), and *S. haemolyticus* (12.7%) [32]. Durkin et al. and de Oliveira et al. suggested similar findings in 30% and 86.3% of *S. epidermidis*, respectively [11,33].

The use of phenotypic testing to identify and characterize the mechanism of resistance in CoNS

Due to the fact that many CRBSI sellers are resistant to mechanically used antimicrobials, there is also a full size. All of the CoNS strains isolated during this analysis have demonstrated 100% resistance to penicillin and cefoxitin. They confirmed a high resistance to aminoglycoside and ofloxacin (100%) and a high susceptibility to vancomycin, pristinamycin, trimethoprim-sulfamethoxazole, fosfomycin, and doxycyclin. We found the same results in another study we did on the NICU [34]. Additionally, Durkin et al. also proposed comparable findings [11].

Physiopathogenesis of CoNS CRBSIs

The two main ways that CoNS enters the bloodstream through CVC insertion are involved in the pathophysiology of CRBSIs [13]. The CVC tip can get infected in two ways: extraluminal for infections that last less than eight days and endoluminal for infections that last more than eight days. The bloodstream is the most probable source of infection in patients who are very sick [35].

Infection occurs immediately upon the CoNS's arrival at the CVC. The bacteria adhere to the catheter floor, colonize it, and generate biofilm. A biofilm is created when a microorganism sticks to the catheter's outside or inside floor. The duration and location of the CVC biofilm are contingent upon the catheter's duration of use [13].

Our investigation revealed that the presence of CoNS at the CVC tip was an independent RF associated with CVC colonization (p=0.001), illustrating the significance of the skin as a reservoir for this microorganism. After the catheter is put into the vascular system, CoNS spp. usually stick to the bottom of the CVC polymer and form slime or glycocalyx [19]. The disruption of the inflammatory process caused by any unfavorable modification of the intestinal barrier increases its permeability, which facilitates the movement of live bacteria, bacterial DNA, or products of bacterial degradation from the intestines to extraintestinal locations. This is one of the ways that intestinal CoNS bacteremia moves around in PIs population [31].

Risk factors associated with CRBSI

In the NICU, CVCs expose patients to complications, the most common of which is BSI. Studies of the RFs that are associated with CVCs and the identification of the microorganisms can also be beneficial in their prevention. The following RFs were identified as RFs for CRBSI: delivery weight (p=0.03), age at onset of infections (p=0. 003), PLAT count number (p=0. 02), catheter period of stay (p=0. 001), lipid emulsion (p=0.0003), antenatal corticosteroid (p=0. 003), antenatal antibiotic therapy (p=0. 04), neonatal antibiotic therapy (p=0.02), patent ductus arteriosus (p=0.02), and PPI (p=0. 005). Group 1 patients were at a higher risk of developing CRBSI than group 2. The results were comparable among various authors [36-42].

Epidemiological and etiological CoNS CRBSI

Accurate methods must be implemented to identify strain-relatedness in order to investigate the epidemiology and etiology of CoNS CRBSI. Despite our findings, we can't always be sure that all 24 cases of presumptive phenotypically CRBSI CoNS are actually CRBSI. Of these isolates, 22 (91.6%) were genotypically related to CRBSI and were considered the proper cause of CRBSI. The remaining 2 (8.4%) were no longer considered the motive and were no longer associated with CRBSI.

Patients in group 2 were distinguished by the lack of evidence of a BSI supply other than that related to vascular access. While temporary BSI is typically asymptomatic, it may also cause the onset of symptoms that are consistent with many theories. There is a chance of getting a false positive blood culture if pediatric blood culture bottles are overfilled. Additionally, an overfilled blood subculture bottle may contain enough blood cells to produce CO_2 and speed up the special detection algorithms within the incubation module. No microorganisms are cultured due to the possibility that overfilling may be the cause of a false positive result [43].

CoNS are the most frequently isolated bacteria from blood cultures. They are the most frequent source of falsely positive blood cultures and a major contributor to HAIs [44]. These CoNS isolates are classified as contaminants. They may result in the following: a weakened diagnosis of BSI, inappropriate antibiotic therapy, unnecessary catheter removal, an extended length of stay, and increased costs [45].

When to remove CVC from neonates receiving parenteral nutrition

Providing nutrients through a vein facilitates the growth of sick and small babies. Determining how long to administer parenteral nutrition via a vein-based tube presents a dilemma for clinicians. Because using CVC for too long can be bad, but stopping it too soon can impede growth. The risk of infection was found to be lower when parenteral nutrition was discontinued at higher enteral feed volumes by Anne et al.[46]. However, the risk of late-onset sepsis (LOS) was also higher. Their conclusion was that it is still not clear what the best way is to stop parenteral nutrition in newborns. Early removal of CVCs made babies more likely to get sepsis later on, even if their blood did not have bacteria in it.

There is some doubt in our minds about this, and they took longer to gain weight after giving birth. Authors say that the chance of LOS goes up when parenteral nutrition is stopped and the CVC is taken out while less food is given by mouth. The babies who were born prematurely required additional time to regain the weight they lost at birth, but when they left the hospital, they were the same size and shape as when they were born.

Eight of the sixteen patients in this study who had a blood culture that was positive but a negative intravascular catheter tip subculture had their catheter removed 5 days before the onset of BSI. This may imply that BSI may be observed in its entirety or over an extended period. Removing the catheter immediately in the event of sepsis may be the optimal healing approach. In order to maintain parenteral nutrition, transfer the umbilical cord in very PIs, and produce an adequate antibiotic, it is essential to set up any additional principal access.

It's no longer always clean to place a new needle in every PI, and it typically requires a significant amount of effort to remove the catheter. Various BSIs can result in the colonization of indwelling invasive systems, dental work, processes on the digestive tract, and genital-urinary catheters. We discovered in another study that 53.5% of the population of PIs had bacterial translocation from the gastrointestinal tract to the circulatory device [31].

Application of information criteria of AIC, BIC, sensitivity and specifity to select model for biological and health research

Numerous simulation studies have been conducted to compare the effectiveness of information criteria such as AIC and BIC. To perform the ONSET analysis, we used CVC culture. In the 95% CI [0.73; 0.98], the AUC is 0.90. This result discriminates at the crucial 14-day threshold. The findings of the two research groups (CVC+ and CVC-) were comparable. Comparing mixture models, the AIC and BIC exhibit a sensitivity of 83.3% and a specificity of 87.5%. Among the models that use both AIC and BIC, Model 18 is the best. Figure 2 illustrates that it contains only three terms (ONSET, GA, WBC) and the lowest residual records (AIC=25.7 ; BIC=31.02).

Study limitations

The study had a small number of people, so it is possible that some things that could make them sick were not in their medical records. Some factors that might have affected the results:(i) the site where the blood was taken was not ready or clean enough; (ii) the hands of the person who took the blood were not clean or disinfected;

(iii) the way the blood was taken was not done well; CoNS are the blood germs that are the farthest away from other germs in newborns' blood. It is both a contaminant and pathogen. Contaminants and a pathogen are hard to tell apart.

Conclusion and Recommendations

For the preterm baby, the CVC may be essential. The deleterious and prolonged maintenance of this item can result in severe repercussions related to secondary infections. The lengh of time the catheter is in place is the main cause of catheter-related infections (CRIs) where we work. But the results make it clear that the CVC isn't always blame for the infection. Molecular biology studies show that it is in 40 to 50% of cases. This gives comfort to the notion that the catheter can be used as a route for medication administration and as optimal nutritional support in cases of well-controlled sepsis, so its removal is not required. Conversely, molecular biology, which is frequently employed in clinical and epidemiological research, may prove advantageous in the event of a bacterial infection recurrence following a properly executed antibiotic treatment, in order

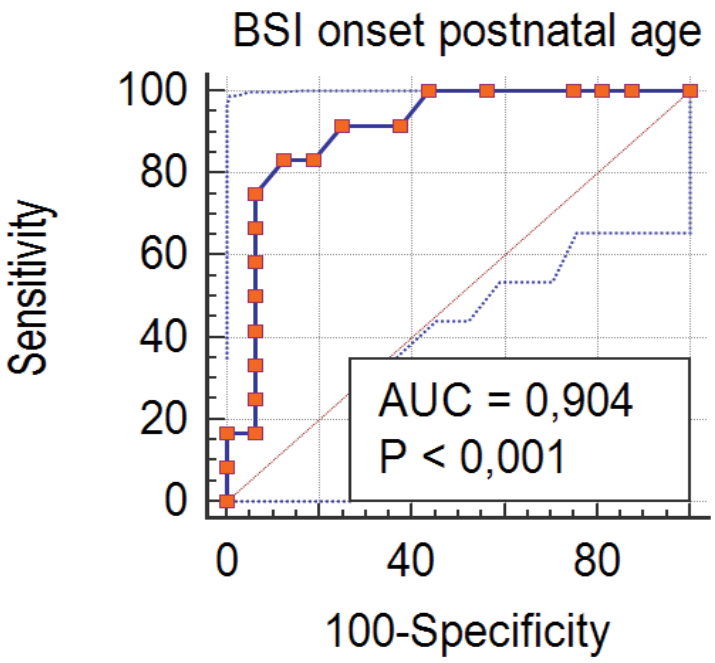

Figure 2: The ONSET analysis was carried out according to the results of CVC culture. The AUC is 0.90 (95% CI [0.73; 0.98]). At the critical threshold of 14 days, this result discriminates well between the two groups (CVC+ and CVC-). There is an 83.3% sensibility and an 87.5% specificity.

to determine whether to retain or eliminate the CVC.

Clinicians are always worried about CRIs in PIs because of their multifactorial etiology and the need to prioritize prevention, especially through asepsis, the use of maximum barrier precautions, and limiting manipulations. Managing intestinal dysbiosis and the possibility of bacterial translocation from digestive tract is a frequently disregarded factor. There are numerous factors that expose PIs to this phenemenon, such as dysbiosis, inflammatory of the immune response, and immaturity of the intestinal barrier. The use of symbiotics, pre-biotics, and enteral feeding, even at low volumes, with fresh human milk are preventive measures against bacterial translocation from the digestive tract.

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Ethical Approval and Consent to Participate

The local Person Protocol Committee that checks and gives permission for research projects in the area of Ile de France 3, N° ID-RCB; 2017-AO3540-53 gave its approval to the study plan. We followed the rules of the Declaration of Helsinki and/or the Ethical Committee of APUH when we did research with human babies who were born too early. We collected and analyzed their medical and laboratory information. The study did not get permission from the parents or other adults who had legal responsibility for the children who took part. The study looks at what happened in the past, without changing anything about the people involved. It uses data that is already available from the patients' medical records, which are kept safe and private. The study involved mothers who were breastfeeding who were thought to be autonomous and independent and who could choose if they wanted their baby to join or no the study. They were also confident and self-reliant.

Author Contributions

AL, GK, GA, and MB worked together on meetings and discussions that led to this paper. They helped make the study idea, plan, and wrote the paper. The authors worked together on all aspects of the research, from collecting and analyzing the data to writing and improving the paper. The final version of the paper was checked and agreed by all the writers.

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Conflict of Interest

The authors say that they have no problem or interest that could affect their work.

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