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Research Article

Coagulase-Negative Staphylococcal Bacteremia by Bacterial Translocation of Gastrointestinal Origin in Preterm Infants: Role of Molecular Analysis

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Abstract

Background: One hundred fifty-seven preterm infants were enrolled in the study. Only 28 (17.8%) were included in this work. The aim of this study was to assess the proportion of intestinal bacterial translocation associated with coagulase-negative staphylococcal bacteremia.

Methods: Blood cultures, stool cultures, antibiotic susceptibility, and genotyping were performed. All Staphylococcus isolates were identified by MALDI-TOF MS.

Results: Sixteen resistance patterns were identified from blood and stool based on antibiotic susceptibility testing. Ten of the coagulase-negative staphylococcus strains isolated from blood samples exhibited R pattern e (35.7%) and 11 of the coagulase-negative staphylococcus strains isolated from stool samples exhibited R pattern e (39.2%). Fifteen isolates exhibited: three ERIC-2 patterns, A (S. epidermidis), B (S. haemolyticus), and C (unidentified coagulase-negative staphylococcus), and three RAPD patterns, D (unidentified coagulase-negative staphylococcus), E (S. haemolyticus), and F (S. epidermidis).

Conclusion: Molecular typing confirmed an intestinal bacterial translocation in the preterm infants with coagulase-negative staphylococcal bacteremia.

Key words: bacterial translocation; coagulase-negative staphylococcus; blood culture; stool culture; preterm infants; molecular typing

Abbreviations

AC-FMS: Antibiogram Committee of the French Microbiology Society; APUH: Amiens-Picardie University Hospital; BT: bacterial translocation; CI: confidence interval; CoNS: coagulase-negative staphylococcus; CRP: C-reactive protein; ERIC: enterobacterial repetitive intergenic Consensus; EUCAST; European Committee on Antimicrobial Susceptibility Testing; GIT: gastrointestinal tract; LBP: LPS binding protein; LPS: lipopolysaccharide; MALDI-TOF MS: matrix-assisted laser desorption ionization time flight mass spectrometry; MODS: multiple organ dysfunction syndrome; MLN: mesenteric lymph modes; MSA: mannitol salt agar: nICU: neonatal intensive care unit; OR: odds ratio; pICU: pediatric intensive care unit; RAPD: random amplification polymorphic DNA; RF: risk factor; RR: relative risk; SBCA: sheep blood Columbia agar; SD: standard deviation; uCoNS: unidentified coagulase-negative Staphylococcus; WG: week of gestation

Introduction

Bacterial translocation (BT) is defined as the passage of live bacteria, bacterial DNA, or bacterial degradation products [lipopolysaccharide

(LPS), bacterial lipoprotein, peptidoglycan, flagellin, unmethylated CpG DNA, tri-acyl lipopeptide, and di-acyl lipopeptide)] or both, across the lamina propia to local mesenteric lymph nodes (MLN) and from there to extranodal sites [1-3]. The intestinal tract has multiple functions in the body apart from its primary function of absorption of nutrients. It represents a real barrier protecting the body from living microorganisms, and antigens of the intestinal lumen.

Many physiological and pathological conditions, such as immaturity, fasting, mesenteric ischemia-reperfusion, shock states, can alter intestinal functioning. Alteration of the intestinal barrier results in a systemic inflammatory process and, more rarely, potentially fatal multiple organ dysfunction syndrome (MODS). Gastrointestinal tract (GIT) epithelial and immune cells play an essential role in initiation and resolution of the inflammatory process. Any adverse alteration of the intestinal barrier leads to disruption of this process, resulting in increased permeability of the intestinal barrier, promoting the passage of live bacteria, bacterial DNA or bacterial degradation products from the intestines to extraintestinal sites [4, 5]. The incidence of BT in humans undergoing emergency laparotomy has been reported to be 15.4%, and 14 to 21% [3, 6]. Numerous molecules have been evaluated as potential biomarkers for BT, including bacterial DNA, soluble CD14, l Lipopolysaccharide (LPS)/endotoxin. LPS binding protein (LBP), calprotectin, and d-lactate [7]. In humans, BT occurs commonly in clinical situations such as laparotomy, hematological malignancies, intestinal obstruction, trauma, liver resection, hemorrhagic shock, Crohn' s disease, colorectal cancer, and aortic aneurysm repair [8, 9, 10, 11, 12]. The incidence of BT and its relationship to sepsis and MODS in preterm newborns remain unclear. Coagulase-negative Staphylococcal (CoNS) bacteremia is common in neonates and is often associated with the presence of a catheter. The mechanism responsible for intestinal BT remains poorly elucidated. It would therefore be interesting to study the proportion of BT responsible for CoNS bacteremia in a population of preterm infants. The objectives of this study were to assess the proportion of intestinal BT and its role in causing sepsis in clinical conditions, and to evaluate the correlation between CoNS isolated from blood cultures and stool cultures using molecular typing.

Materials and Methods

Ethical approval and consent to participate

All procedure performed in studies involving human participants were undertaken in accordance with the guidelines laid down in the Declaration of Helsinki and/or national research committee (Ethical Committee of Amiens-Picardie University Hospital (APUH), n° 139. Clinical and Laboratory data 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. Study participants were all breastfeeding mothers considered to be autonomous and independent to decide on the participation of their baby in the study.

Study design and study population

During the study period, a total of 157 neonates (less than 28 days of life) born prematurely (<37 weeks of gestation-WG) were immediately hospitalized in the neonatal intensive care units (nICUs) and in the pediatric intensive care units (pICUs) of the APUH, Amiens (France) between 2016 and 2018. Twenty-eight (17.8%) of these infants had experienced at least one episode of CoNS bacteremia with a concomitant positive stool culture were included in the study. However, 129 (82.2%) children including full term infants (≥ 37 WG), and duplicate cases were all excluded in this study.

Antibiotics used in patient treatment

Sixteen (57.1%) of the 28 preterm infants included in this study had received the following antibiotics during neonatal life: amoxicillin (9 cases), vancomycin (6 cases), amoxicillin + gentamicin (5 cases), fluoconazole (4 cases), piperacillin- tazobactam+ gentamicin (2 cases), vancomycin + amikacin (2 case), cefotaxime (2 cases), cilastine-imipenem + gentamicin (1 case), teicoplanin + amoxicillin (1 case), meropenem (1case), ceftazidime (1case), trimethoprim-sulfamethoxazole (1 case), metronidazole (1 case), and josamycin (1 case). These antibiotics were used individually or in combination in 15 suspected cases of maternal-fetal infections, and piperacillin-tazobactam+ gentamicin, vancomycin and metronidazole were used in one case of septic shock. Sixteen (57.1%) of the 28 mothers who delivered preterm had received the following antibiotics prior to delivery: amoxicillin (9 cases), ceftriaxone (2 cases), amoxycillin-clavulanic acid, piperacillin-tazobactam (1case), cefotaxime (1 case), ceftriaxone, cefixime and erythromycin (1 case), clindamycin and trimethoprim-sulfamethoxazole (1 case). These antibiotics were used individually or in combination to treat urinary tract infections, vaginal infections, and premature rupture of membranes.

Data collection

All data were obtained from the patient's electronic medical records. Patient demographic characteristics, underlying conditions, and clinical and laboratory findings were collected.

Diagnostic criteria for bacteremia

One positive blood culture in the presence of suggestive clinical signs: bradycardia, oxygen desaturation, increased respiratory requirements, total leukocyte count > 18,000/mm^3, C-reactive protein (CRP) > 10 mg/L, serum lactate> 1.65 mmol/L, temperature > 38.3°C or < 36°C.

Blood and stool samples

Blood was obtained for routine hematological, biochemical and bacteriological tests, including polymorphonuclear leukocyte, platelet counts and CRP. Less than 0.5 mL of blood was inoculated into a Bactec Peds Plus F bottle and incubated in Bactec^{TM} Becton Dickinson instrument (BD Diagnostic Systems, Spark, MD, USA). Subcultures of initial blood culture broth were seeded on sheep blood (5%) Columbia agar (SBCA) and mannitol salt agar (MSA) (Oxoid, France) were incubated aerobically at 37°C for 24 hours. Anal or rectal samples using sterile swabs were seeded on SBCA and MSA and were incubated aerobically at 37°C for 48 hours.

Identification

Staphylococcus spp. were identified after examining all colonies. All isolates that were negative for mannitol and for bound coagulase (Pastorex Staph Plus-Bio-Rad, France) and positive for catalase and for Gram staining were classified as CoNS. All these *Staphylococcus* strains were examined by Matrix-Assisted-Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) (Brucker Daltonics, Bremen, Germany) according to a previously described procedure [13,14] when the child's blood cultures were negative, the corresponding stool cultures and blood cultures were discarded.

Antibiotic susceptibility testing

Isolates were tested by the disk diffusion method on Mueller-Hinton (MH) agar according to the zone size criteria as recommended by the Antibiogram Committee of the French Microbiology Society (AC-FMS) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) – 2015 [15]. The antibiotics used were kanamycin (K), gentamicin (G), tobramycin (T), erythromycin (E), lincomycin (L), pristinamycin (PT), rifampin (RIF), ofloxacin (OFX), vancomycin (VAN), fusidic acid (FA), trimethoprim-sulfamethoxazole (SXT), and

fosfomycin (FOS). Susceptibilities to benzylpenicillin (P) (disk loaded with 6 µg) and cefoxitin (FOX) (disk loaded with 30 µg) were determined by the disk diffusion method on salted MH at 37°C for 24 hours.

DNA isolation and molecular typing

Total nucleic acid extraction was performed using the bioMérieux NucliSENS easy MAG platform (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. ERIC-PCR (Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction) was performed as previously described [16]. Extracted DNA (100 ng from each isolate) was amplified in a final volume of 50 µL of the ERIC-2 primer (5'-AAG-TAA-GTG-ACT-GGG-GTG-AGC-G-3') and 5 µL of CoralLoad PCR buffer. PCR cycling consisted of 94°C for 7 min followed by 45 cycles of 94°C for 1 min, 45°C for 1 min and 72°C for 2 min, and 72°C for 7 min. RAPD-PCR (Random Amplification Polymorphic DNA) was performed as previously described [17]. The primer used was, 5'-GGT-TGG-GTG-AGA-ATT-GCA-CG-3'. Amplification reactions were carried out with a final volume of 50 µL containing 25 µL of Top Taq Master MIX (QIAGEN, Les Ulis, France), 1 µL of RAPD1 primer, 2 µL of DNA as template, 5 µL of CoralLoad PCR buffer. The cycling conditions were 95°C for 3 min, followed by 35 cycles of 94°C for 1 min, 40°C for 1 min, and 72°C for 2 min, and 72°C for 10 min. After amplifications, PCR products were resolved by electrophoresis on 1.2% agarose gels at 90 V for 6 h, followed by ethidium bromide staining and were visualized under UV light. A photograph was also taken.

Risk Factors (RFs) for gastrointestinal BT in preterm infants with CoNS bacteremia

The following clinical, laboratory and treatment parameters were analyzed as potential RFs for BT in this study. These RFs were categorized in two groups according to molecular typing: group1 included patients in whom BT was indicated; group 2, comprised patients in whom BT was not indicated.

Statistical analysis

We calculated the relative risk (RR) of BT among preterm infants with and without documented BT. Data are expressed as means ± standard deviation (SD) for quantitative variables and frequencies for qualitative variables. We calculated the RR of BT with associated 95% confidence intervals (CIs) using Fisher's Exact test. Comparisons between documented BT and undocumented BT groups were performed with Wilcoxon-Mann-Whitney test for quantitative data and Fisher's Exact test for qualitative data. All tests of significance were two-sided and a *p* value of < 0.05 was considered to indicate statistical significance.

Results

Patient characteristics

The characteristics of 28 preterm infants included in this study were as follows: gestational age (weeks): mean±SD:29.2±3.3, median: 29, range:25-36; birth weight (gram): mean± SD: 1,288±606.2, median: 1,075, range: 592-2,800; age at onset of infection (days): mean± SD: 19.8±21.5, median: 11, range: 3-104; Lactate (µmol/L): mean± SD: 38.2±43.5, median: 23.3, range: 3.0-43.5; CRP (mg/L): mean± SD: 3.28±1.62, median: 5.15, range: 0-178; Leukocytes (/mm^3): mean± SD: 26,775±48,733.7, median: 15,400, range: 5,000-271,000; Platelets (/mm^3): mean± SD : 162,642.8±98,180.5, median: 54,500, range: 60,000-286,000 (**Table 1**); maternal age at birth (years): mean± SD: 29.7±6.2, median: 29.5, range: 19-45.

Patient No.	Gestational age*(WG)	Birth weight (g)	Age at onset of infection (days)	Gastrointestinal disorders	Lactate mmol/L	*** CRP mg/L	Leukocytes /mm^3	Platelets /mm^3
1	26	710	48	yes	7.2	0	9900	70000
2	25+4d**	950	47	yes	7.3	5	9800	214000
3	36	2800	8	yes	5.5	53	9900	230000
4	26+6d	700	8	yes	7.0	5,7	34200	173000
5	28+3d	1200	30	yes	6.9	40	19900	65000
6	29	1370	9	yes	6.8	23	18400	200000
7	27	975	11	yes	7.5	62	25100	85000
8	28+5d	960	13	yes	7.7	17	13400	103000
9	30	1060	7	No	3.5	0	12000	213000
10	31+2d	1720	10	No	4.5	178	20500	70000
11	30+5d	1730	10	yes	5.8	84	19000	60000
12	27+2d	986	19	No	4.6	3,4	11800	316000
13	25+5d	700	28	yes	6.5	0	11400	194000
14	35	2371	14	yes	6.8	99	10500	136000
15	26	592	15	yes	7.9	103	16100	72000
16	27	990	8	yes	2.9	89	271000	12000
17	29+4d	1330	3	yes	3.4	58	37000	123000
18	29+2d	755	6	yes	4.2	0	27600	98000
19	32	1090	53	yes	4.3	23,7	9500	298000
20	35	2400	6	yes	5.0	41,5	20400	73000
21	36	2730	104	yes	5.9	15	9100	199000
22	29+4d	949	5	yes	5.3	31	10700	232000
23	34	1340	24	yes	3.5	45	14700	185000
24	30	1250	13	yes	3.7	10	18200	447000
25	29	1320	5	No	4.1	0	5000	120000
26	28+4d	1192	11	yes	3.0	0	12000	286000
27	26	995	33	yes	3.3	84	36900	75000
28	25+4d	900	9	No	4.0	0	35700	205000
Mean	29.2	1288	19.8	Y: 82.2% N: 17.8%	38.2	3.28	26775	162642.8
SD	3.38	606.2	21.5		43.5	1.62	48733.7	98180.5
Median	29	1075	11		23.3	5.15	15400	54500

**WG: weeks of gestation; **d: days; ***CRP: C-reactive protein. The mean term of birth of the 28 preterm infants was 29 WA and 4 days with a mean birth weight of 1288g. The CoNS infectious episode occurred at a mean age of 19.8 days. Mean CRP was 38.2mg/L at the time of diagnosis.*

Table 1: *Characteristics of the 28 children with coagulase-negative Staphylococcus spp. included in the studyBlood culture and stool culture results*

Blood culture and stool culture

S. h*aemolyticus*, unidentified coagulase-negative Staphylococcus spp. (uCoNS), and *S. epidermidis* were isolated from 39.3%, 35.7% and 17.8% of blood cultures respectively. *S. haemolyticus*, uCoNS, and *S.epidermidis* were isolated from 39.3%, 35.7% and 17.8% of stool cultures, respectively. (**Table 2**).

Coagulase-negative *Staphylococcus* species	**Blood culture n (%)**	**Stool culture n (%)**	**Total n (%)**
**S. epidermidis*	5 (17.8)	5 (17.8)	10 (17.8)
S. haemolyticus	11 (39.3)	12 (42.8)	23 (41.0)
S. warneri	1 (3.6)	0	1 (1.8)
S. capitis	1 (3.6)	0	1 (1.8)
**uCoNS	10 (35.7)	11 (39.4)	21 (37.6)
Total	**28 (100.0)**	**28 (100.0)**	**56 (100.0)**

S. haemolyticus was the most widely represented species in these 28 cases of CoNS bacteremia (39.3% of blood cultures and 42.8% of stool cultures) followed by uCoNS (35.7% of blood cultures and 39.4% of stool cultures).

**S.: Staphylococcus; **uCoNS: Unidentified Coagulase-negative Staphylococcus*

Table 2*: Distribution of coagulase-negative Staphylococcus strains isolated from blood culture and stool culture*

Antibiotics susceptibility of CoNS strains

One hundred percent of the 28 CoNS strains isolated from blood cultures were resistant to penicillin, cefoxitin and kanamycin, and 96.4% of isolates were resistant to gentamicin, tobramycin and netilmicin. The resistance of these strains to other antibiotics tested are shown in **Table 3**.

Patient No.	**Antibiotics [Inhibition diameter (mm)]**																	
	***Staphylococcus* Isolates**	**P**	**FOX**	**K**	**G**	**T**	**N**	**E**	**L**	**PT**	**RIF**	**OFX**	***VAN**	**FA**	**SXT**	**FOS**	**DOX**	**Resistance phenotype**
1	*S. epidermidis*	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	a
2	*S. epidermidis*	R	R	R	R	R	S	R	R	S	R	R	S	R	S	S	S	b
3	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	c
4	*S. epidermidis*	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S	d
5	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
6	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
7	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
8	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
9	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
10	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
11	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
12	CoNS	R	R	R	R	R	R	R	R	S	R	R	S	S	S	R	S	f
13	CoNS	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
14	CoNS	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
15	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
16	*S. capitis*	R	R	R	R	R	R	S	S	S	R	S	S	S	S	R	S	g
17	CoNS	R	R	R	R	R	R	R	S	S	R	R	S	R	S	S	R	h
18	*S. epidermidis*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	R	i
19	*S. epidermidis*	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	j
20	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	R	R	k
21	CoNS	R	R	R	R	R	R	R	S	S	S	R	S	R	S	S	S	l
22	CoNS	R	R	R	R	R	R	S	S	S	S	R	S	R	S	R	S	m
23	CoNS	R	R	R	S	S	S	R	S	S	S	S	S	R	S	S	S	n
24	*S. haemolyticus*	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S	d
25	CoNS	R	R	R	S	S	S	S	S	S	S	R	S	S	S	S	S	o

26	CoNS	R	R	R	R	R	R	S	S	S	R	S	S	S	S	R	S	g
27	*S. warneri*	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	S	p
28	CoNS	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	S	p

One hundred percent of the 28 CoNS strains isolated from blood samples were resistant to penicillin, cefoxitin and kanamycin, and 96.4% of isolates were resistant to gentamicin, tobramycin, and netilmicin.

*R: resistant; S: susceptible; CoNS: Coagulase-negative Staphylococcus; P: benzylpenicillin (R < 26 mm); FOX: cefoxitin (R < 24 mm); K: kanamycin (R < 14 mm); G: gentamicin (S ≥ 22 mm, R < 22 mm); T: tobramycin (S ≥ 22 mm, R < 22 mm); N: netilmicin (S ≥ 22 mm, R < 22 mm); E: erythromycin (S ≥ 21 mm, R < 18 mm); L: lincomycin (S ≥ 22 mm, R < 19 mm); PT: pristinamycin (S ≥ 21 mm, R < 18 mm); RIF: rifampin (S ≥ 26 mm, R< 23 mm); OFX: ofloxacin (S ≥ 20 mm, R < 20 mm); Van: vancomycin (S ≤ 2 mg/L); FA: fusidic acid (S ≥ 24 mm, R < 24mm); SXT: trimethoprim-sulfamethoxazole (S ≥ 17 mm); FOS: fosfomycin (S ≥ 6mm, R < 6 mm); DOX: doxycycline (S ≥ 22 mm; R < 19 mm). *MIC: minimum inhibitory concentrations of VAN were determined using E-test strips (BioMérieux, Marcy l'Etoile, France)*

Table 3: *Susceptibility of coagulase-negative Staphylococcus strains isolated from blood samples*

The distribution of resistance patterns of these isolates showed sixteen antimicrobial resistance patterns (R patterns) a-p, and 10 of these strains exhibited R pattern e [(35.7%) (isolates 5-11,13-15)] (**Table 4**).

R patterns	Antimicrobial resistance	Isolate numbers
a	P^R FOX^R K^R G^R T^R N^R E^R OFX^R	1
b	P^R FOX^R K^R T^R E^R L^R RIF^R OFX^R FA^R	2
c	P^R FOX^R K^R G^R T^R N^R E^R OFX^R	3
d	P^R FOX^R K^R G^R T^R N^R E^R L^R RIF^R OFX^R	4, 24
e	P^R FOX^R K^R G^R T^R N^R E^R RIF^R OFX^R	5, 6, 7, 8, 9, 10, 11, 13, 14, 15,
f	P^R FOX^R K^R G^R T^R N^R E^R L^R RIF^R OFX^R FOS^R	12
g	P^R FOX^R K^R G^R T^R N^R RIF^R FOS^R	16, 26
h	P^R FOX^R K^R G^R T^R N^R E^R RIF^R OFX^R FA^R DOX^R	17
i	P^R FOX^R K^R G^R T^R N^R E^R RIF^R OFX^R DOX^R	18
j	P^R FOX^R K^R G^R T^R N^R RIF^R DOX^R	19
k	P^R FOX^R K^R G^R T^R N^R E^R RIF^R OFX^R FOS^R DOX^R	20
l	P^R FOX^R K^R G^R T^R N^R E^R OFX^R	21
m	P^R FOX^R K^R G^R T^R N^R OFX^R FA^R FOS^R	22
n	P^R FOX^R K^R E^R FA^R	23
o	P^R FOX^R K^R OFX^R	25
p	P^R FOX^R K^R G^R T^R N^R RIF^R	27, 28

Sixteen antimicrobial resistance patterns were observed, 10 of which exhibited R pattern e [(35.7%) (Isolates 5-11, 13- 15)].

Table 4: *Antimicrobial resistance patterns (R patterns) of the 28 strains isolated from blood samples*

One hundred percent of the 28 CoNS strains isolated from stool samples were resistant to penicillin, cefoxitin, and kanamycin, and 96.4% of isolates were resistant to gentamicin, tobramycin and netilmicin (**Table 5**).

Patient No.	Antibiotics [Inhibition diameter (mm)]																	
	***Staphylococcus* Isolates**	**P**	**FOX**	**K**	**G**	**T**	**N**	**E**	**L**	**PT**	**RIF**	**OFX**	**VAN**	**FA**	**SXT**	**FOS**	**DOX**	**Resistance phenotype**
1	**S. epidermidis*	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	a
2	*S. epidermidis*	R	R	R	R	R	R	R	R	S	R	R	S	R	S	S	S	b
3	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	c

4	*S. epidermidis*	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S	d
5	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
6	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
7	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
8	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
9	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
10	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
11	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
12	**CoNS	R	R	R	R	R	R	R	R	S	R	R	S	S	S	R	S	f
13	CoNS	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
14	CoNS	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
15	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	e
16	CoNS	R	R	R	R	R	R	R	S	S	R	R	S	S	S	R	S	e
17	CoNS	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	R	g
18	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	R	h
19	CoNS	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	R	g
20	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	R	h
21	CoNS	R	R	R	R	R	R	R	S	S	R	S	S	S	S	S	S	l
22	CoNS	R	R	R	R	R	R	S	S	S	S	R	S	R	S	R	S	j
23	CoNS	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	R	k
24	*S. epidermidis*	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	S	l
25	CoNS	R	R	R	S	S	S	R	S	S	S	R	S	S	S	S	S	m
26	*S. haemolyticus*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	R	S	n
27	*S. epidermidis*	R	R	R	R	R	R	R	R	S	R	S	S	S	R	S	S	o
28	CoNS	R	R	R	R	R	R	S	S	S	R	R	S	S	S	S	S	p

One hundred percent of the 28 CoNS strains isolated from stool samples were resistant to penicillin cefoxitin, and kanamycin, and 96.4% of isolates were resistant to gentamicin, tobramycin, and netilmicin.

Table 5. *Susceptibility of coagulase-negative Staphylococcus strains isolated from stool samples*

The resistance of these isolates to other antibiotics tested are shown in Table 5. All these isolates were classified into sixteen R patterns a-p, and 11 of these strains exhibited R pattern e [(39.2%), (isolates 5-11,13-16)] (**Table 6**).

R patterns	Antimicrobial resistance	Isolate numbers
a	P^R FOX^R K^R G^R T^R N^R E^R OFX^R	1
b	P^R FOX^R K^R G^R T^R N^R E^R L^R RIF^R OFX^R FA^R	2
c	P^R FOX^R K^R G^R T^R N^R E^R OFX^R	3
d	P^R FOX^R K^R G^R T^R N^R E^R L^R RIF^R OFX^R	4
e	P^R FOX^R K^R G^R T^R N^R E^R RIF^R OFX^R	5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16
f	P^R FOX^R K^R G^R T^R N^R E^R L^R RIF^R OFX^R FOS^R	12
g	P^R FOX^R K^R G^R T^R N^R E^R OFX^R DOX^R	17, 19
h	P^R FOX^R K^R G^R T^R N^R E^R RIF^R OFX^R DOX^R	18, 20
i	P^R FOX^R K^R G^R T^R N^R E^R RIF^R	21
j	P^R FOX^R K^R G^R T^R N^R OFX^R FA^R FOS^R	22

k	P^R FOX^R K^R G^R T^R N^R DOX^R	23
l	P^R FOX^R K^R G^R T^R N^R RIF^R	24
m	P^R FOX^R E^R OFX^R	25
n	P^R FOX^R K^R G^R T^R N^R E^R RIF^R OFX^R FOS^R	26
o	P^R FOX^R K^R G^R T^R N^R E^R L^R RIF^R SXT^R	27
p	P^R FOX^R K^R G^R T^R N^R RIF^R OFX^R	28

All isolates were classified into 16 antimicrobial resistance patterns (designated R patterns (a to p), 11 of which exhibited R pattern e (39.2%).

Table 6: *Antimicrobial resistance patterns (R patterns) of the 28 strains isolated from stool samples*

Clinical/laboratory/ treatment parameters	**Documented translocation (n=15)**	**Undocumented translocation (n=13)**	**Wilcoxon-Mann -Whitney test (*p*-value)**	**Fisher's exact test (*p*-value)**
Gestational age (weeks) mean±SD median (range)	 28.6±3.35 28 (25-36)	 29.9±3.40 29 (25-36)	*P*=0.27	
Birth weight (g) mean±SD median (range)	 1254.9±644.3 986 (592-2800)	 1326.2±582.8 1192 (755-2730)	*P*=0.0098	
Delivery mode Vaginal Cesarean	 46.6% (7/15) 53.4% (8/15)	 53.8% (7/13) 46.2% (6/13)		*P*=1 OR: 0.7878 95%CI [0.1323; 4.2017]
Gastrointestinal Disorders	80.0% (12/15)	84.6% (11/13)		*P*=1 OR: 0.7355 95%CI [0.0521; 7.7488]
Age at onset of Infection (days) mean±SD median (range)	 18.4±13.65 13(7-48)	 1.5±28.61 9 (3-104)	*P*=0.015	
CRP (mg/L) mean±SD median (range)	 44.8±52.15 23(0-178)	 30.5±31.35 23.7 (0-89)	*P*=0.59	
Leukocytes (/mm^3) mean±SD median (range)	 15120±7799.0 12000 (1900-34200)	 39061.5±70588 18200 (5000-271000)	*P*=0.042	
Platelets (/mm^3) mean±SD median (range)	 142733±8385 136000(25000-316000)	 181000±11705 185000 (12000-447000)	*P*=0.33	
Lactate (mmol/L) mean±SD median (range)	 6.36±1.30 6.8 (3.5-7.9)	 4.04±0.90 4.0 (2.9-5.9)	*P*=0.0002	
Lipid emulsion	12 (80%)	4 (30.7%)		*P*=0.02 OR: 8.1821 95%CI [1.2555; 73.4536]
Antenatal corticosteroid	14 (93.4%)	11 (84.6%)		*P*=0.58 OR: 2.4627 95% CI [0.1141; 160.529]
Proton pump inhibitor	13 (86.6%)	10 (76.9%)		*P*=1 OR: 0.6605 95% CI [0.01; 14.4325]
Patent ductus arteriosus	7 (46.6%)	10 (76.9%)		*P*=0.03 OR: 0.0961 95% CI [0.0018; 0.9895]
Hemodynamic disorders	10 (66.6%)	2 (15.3%)		*P*=0.009 OR: 9.9418 95% CI [1.3872; 127.034]
History of jaundice	14 (93.3%)	7 (53.8%)		*P*=0.02 OR: 10.9341 5% CI

				[1.0273; 587.9893]
Antenatal antibiotic therapy	9 (60%)	7 (53.8%)		*P*=1 OR: 1.2742 95% CI [0.2241; 7.3857]
Neonatal antibiotic therapy	12 (80%)	4 (30.7%)		*P*=0.02 OR: 8.1821 95% CI [1.2555; 73.4236]

APUH: Amiens Picardie University Hospital; SD: Standard Deviation; CRP: C-reactive protein; OR: Odds Ratio, CI: confidence interval; RR: relative risk

Table 7*: Rates and relative risk factors for digestive bacterial translocation in preterm infants hospitalized in APUH, classified according to two groups: documented translocation and undocumented translocation*

Comparison of blood culture and stool culture results according to phenotype resistance pattern

In this series of 28 *Staphylococcus* isolates, blood culture results were concordant with stool culture results in 53.5% (15/28) of cases and discordant in 46.5% (13/28) of cases. Ten of the fifteen concordant strains exhibited R pattern e and corresponded to eight *S. haemolyticus* and two *uCoNS* isolates; five strains exhibited R patterns a, b, d, e, and f, and corresponded to three *S. epidermidis*, one *S. haemolyticus* and one *uCoNS* isolates, respectively. The following resistance patterns were detected on blood cultures from the 12 discordant cases: g (*S. capitis* isolate), h (*uCoNS* isolate), j (*S. epidermidis* isolate), k (*S. haemolyticus* isolate), l (*uCoNS* isolate), m (*uCoNS* isolate), n (*uCoNS* isolate), d (*S. haemolyticus* isolate), o (*uCoNS* isolate), g (*uCoNS* isolate), p (*S. warneri* and *uCoNS* isolates). Similarly, the following R patterns were detected on stool cultures from the 12 discordant cases: e (*uCoNS* isolate), g (*uCoNS* isolate), h (*S. haemolyticus* isolate), i (*uCoNS* isolate), j (*uCoNS* isolate), k (*uCoNS* isolate), l (*S. epidermidis* isolate), m (*uCoNS* isolate), n (*S. haemolyticus* isolate), o (*S. epidermidis* isolate), and p (*uCoNS* isolate). The *S. epidermidis* (R pattern i) and *S. haemolyticus* (R pattern h) strains were isolated from blood culture and stool culture of patient 18, respectively.

Molecular typing results

Phenotyping results suggested BT from the GIT to the circulatory system in 15 preterm infants. When the same *Staphylococcus* spp. were isolated from both stool and peripheral blood, and exhibited the same resistance pattern, they were further genotyped by ERIC-PCR and RAPD-PCR to confirm BT. Fifteen isolates were selected to obtain a diverse sample of patients (blood and stool samples), and R patterns. These 15 selected *Staphylococcus* strains were compared by ERIC-PCR and RAPD-PCR.

Three different ERIC-2 patterns (A, B, C) (**Figure 1**) and three different RAPD patterns (D, E, F) (**Figure 2**) were identified in the 15 selected isolates. ERIC-2 patterns comprised A [*S. epidermidis* (isolates 1, 2 and 4)]; B [*S. haemolyticus* (isolates 3,5-11, and 15)], and C [*uCoNS* (isolates 12-14)].

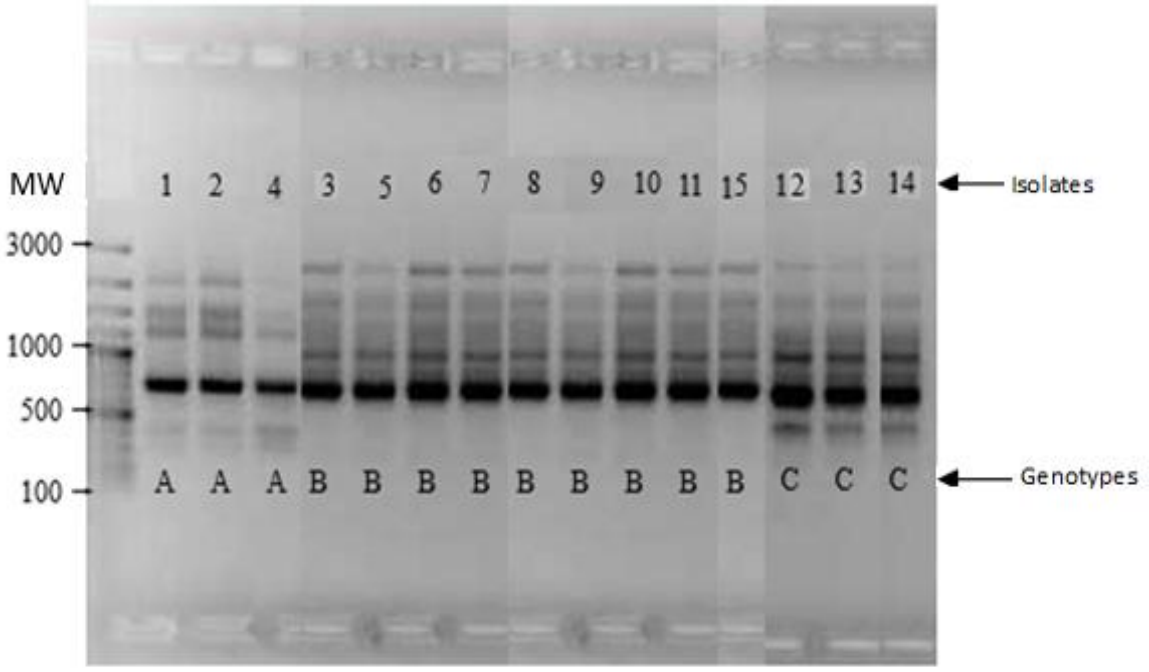

Figure 1: Representative ERIC- PCR types of 15 CoNS spp.isolated from blood cultures. Isolate numbers are Indicated below [*S.epidermidis* (1, 2, 4); *S.haemolyticus* (3, 5-11, 15); CoNS (12-14)]. Pattern types are indicated below (A, B, C). Molecular weights (MW) are expressed in base pairs (bp). The 15 isolates were differentiated into three distinct ERIC-PCR types called A, B, and C.

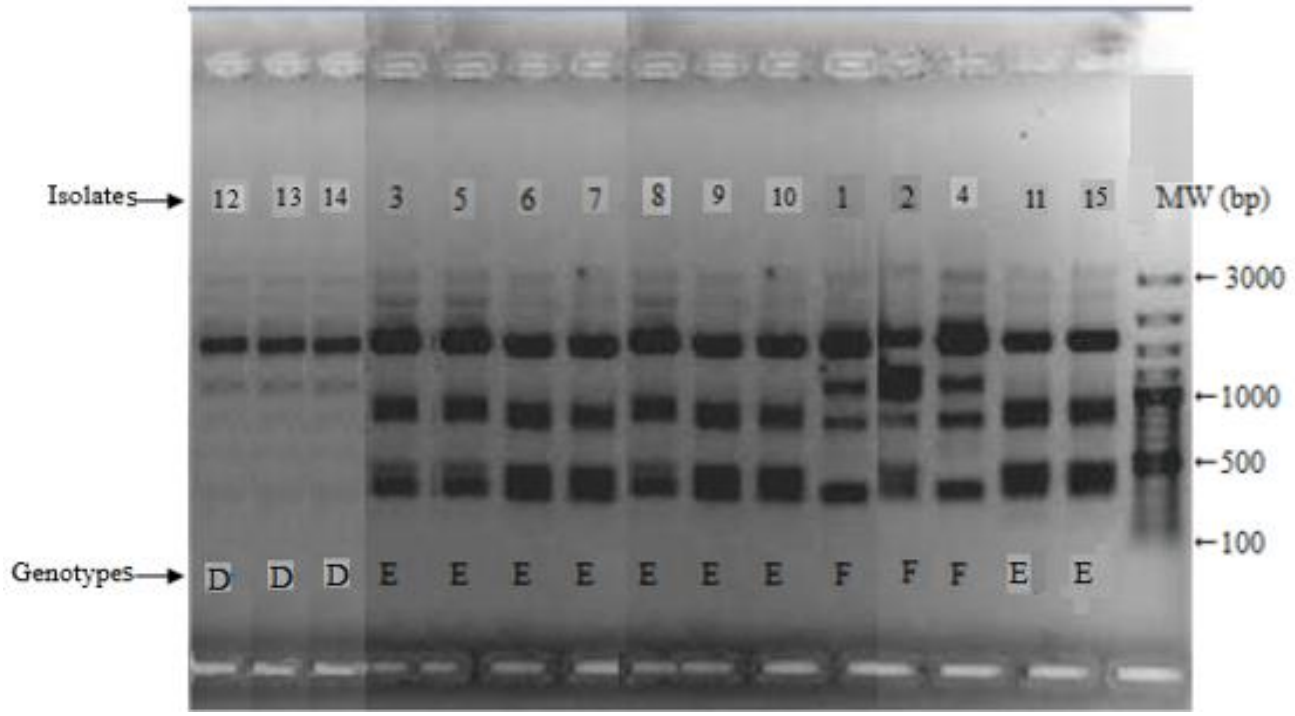

Figure 2: Representative RAPD-PCR types of 15 CoNS spp. isolated from stool cultures. Isolate numbers are indicated below [CoNS (Isolates 12-14); *S. haemolyticus* (isolates 3, 5-11, 15); *S. epidermidis* (1, 2, 4)]. Pattern types are indicated below (D, E, F). Molecular weights (MW) are expressed in base pairs (bp). The 15 CoNS spp. isolated were differentiated into three distinct RAPD-PCR types, called D, E and F.

RAPD patterns consisted of D [*uCoNS* (isolates 12-14)], E [*S. haemolyticus* (isolates 3,5-11, and 15)], and F [*S. epidermidis* (isolates 1, 2, and 4)]. The three *S. epidermidis* R patterns a, b, and d exhibited the AF genotype; The three other *uCoNS* strains with resistance patterns e and f exhibited the CD genotype. Finally, nine *S. haemolyticus* phenotype e strains exhibited the BE genotype. This major epidemic BE profile included 60% of *S. haemolyticus* strains (9/15) isolated in both blood culture and stool culture, and was identified in both units participating in this study. The remaining strains (three *S. epidermidis* and three *uCoNS*) exhibiting AF and CD genotypes, respectively, were considered to be sporadic cases.

Combined analysis of ERIC-2 and RAPD results identified three different genomic groups (gg): I to III. The strains isolated from blood culture and stool culture in each group were more similar to each other than to the other strains.

Bacterial Translocation results

Translocation from the GIT to the circulatory system was documented in 53.5% (15/28) of preterm infants. The same *Staphylococcus* spp. was not found in blood and stool in 46.5% (13/28) of preterm infants, strongly suggesting the absence of BT in these preterm infants. In patient 18, blood culture was positive for *S. epidermidis*, and stool culture was positive for *S. haemolyticus*, although culture of a nasopharyngeal sample taken prior to the onset of bacteremia isolated *S. epidermidis*, suggesting that the respiratory tract was the probable source of bacteremia in this child (this source was not included in this study).

Risk factors for the occurrence of BT in preterm infants with CoNS bacteremia

Comparison of documented (group1) and undocumented (group2) gastrointestinal BT is shown in Table 7. Two tests, Wilcoxon-Mann-Whitney test and Fisher's Exact test, identified the presence of BT RFs, such as: birth weight (p=0.0098); age at onset of infection (p=0.01); leucocytes/mm^3 (p=0.042); lactate/mmol/L (p=0.0002) (Wilcoxon-Mann-Whitney); intravenous perfusion lipid emulsion (OR:8.1821; 95% CI [1.2555; 73.4536]), p=0.02; treated patient ductus arteriosus (OR: 0.0961; 95% CI [0.0018; 0.9895]), p=0.03; hemodynamic disorders (OR: 9.9418; 95% CI [1.3972; 127.034]), p=0.009; history of jaundice (OR: 10.9341; 95% CI [1.0273; 587.9893], p=0.02; neonatal antibiotic therapy (OR: 8.1821; 95% CI [1.2555; 73.4236]), p=0.02 (Fisher's Exact test) were direct independent RFs for the occurrence of gastrointestinal BT. None of the other RFs tested were significant.

Discussion

In this study, *S.haemolyticus* and *S.epidermidis* were the CoNS species most isolated, with rates of 41.8% and 17.8% respectively. These pathogens are a major cause of nosocomial bacteremia and catheter infections in nICUs [18], and are the most common cause of late-reported sepsis [19, 20].

The results of this study established that birth weight, age at onset of infection, leucocytes, serum lactates, intravenous lipid emulsion perfusion, treated patient ductus arteriosus, hemodynamic disoders, history jaundice, and neonatal antibiotic therapy were independent RFs for the occurrence of BT in preterm infants with CoNS bacteremia. MacFie et al. [6] showed that the following factors were independently associated with an increased prevalence of BT based on univariate analysis: intestinal obstruction, jaundice, inflammatory bowel disease, malignancies, preoperative total parenteral nutrition (TPN) and emergency surgery. However, multivariate analysis showed that only emergency surgery and preoperative TPN were independently associated with translocation [7]. These results differ from those of our study in that our study population consisted of only a sample of preterm infants due to

our inclusion criteria, whereas the participants in MacFie' study with BT had a median age of 71 years.

Our study also showed that BT in preterm infants with sepsis is responsible of secondary bacteremia and is driven by external factors such as length of stay in the nICUs or prolonged feeding by an enteral feeding tube. Such results have been observed by Jezioski et al. [21].

According to the study conducted by Pappoff et al [2], prematurily appears to play a significant predisposing role by reducing mucosal barrier function. Other factors influencing BT have been reported in the literature [22, 23]. Various publications have identified BT in a wide group of diseases, such as acute pancreatitis, cirrhosis, malignancy, heart failure, aortic aneurysm repair, cardiopulmonary bypass, and bowel transplant [22-24].

In this study, the presence of BT from the gut to the circulatory system in 15 of 28 preterm infants with CoNS bacteremia represents a prevalence of 53.5% according to molecular typing results. O'Boyle et al. observed this phenomenon in 15.4% of cases of patients undergoing laparotomy [3]. Moharem et al. [25] detected BT in 33% of liver transplant patients. Bellot et al. found an incidence of BT of 38% among cirrhotic patients [26], and Jezioski et al. have shown that the BT is associated with significant hemodynamic changes [21].

In this series of 28 CoNS strains, the predominant antibiotic resistance pattern comprised 10 strains, including 8 *S. haemolyticus* strains, mainly presenting a major epidemic profile including the BE genotype that spread rapidly after emergence in the two units participating in this study. The results of this study demonstrate the predominance of this major epidemic profile, corresponding to 28.6% (8/28) of the strains studied. Furthermore, a close correlation was observed between the various genotypes and the associated antibiotic resistance patterns of the CoNS strains isolated. The isolated CoNS strains demonstrated the emergence of multidrug resistance (resistance to β-lactams, aminoglycosides and fluoroquinolones).

The results of this study demonstrate that CoNS species belonging to intestinal microbiota were the most likely source of CoNS bacteremia in hospitalized preterm infants. In the present study, BT was essentially demonstrated by comparison of the ERIC-PCR and RAPD-PCR genomic patterns of the same *Staphylococcus* spp isolated concomitantly from the patient's blood and stool. Genetic analysis of all of these strains showed that gg I, II, and III strains shared genetic patterns AF, BE, and CD, respectively, indicating that the strains of each gg were closely related and were therefore considered to be included in BT process. In this study, the proportion of the 28 preterm infants with CoNS bacteremia caused by BT of an intestinal CoNS undoubtedly underestimates the true prevalence of BT in the study population. It is difficult to demonstrate a correlation between BT and bacteremia, as these forms of bacteremia only appear to have clinical repercussions in the presence of massive BT, when the body's capacity to clear the organism is exceeded, when the organism responsible is particularly virulent [27] or when the host's immune defense mechanism is altered. Under these conditions, micro-organisms from the GIT may be responsible for localized or systemic infections. Molecular biology techniques can now be used to noninvasively study BT in humans [28]. This ERIC-PCR and RAPD-PCR study confirmed the presence of BT in 15 phenotypically selected patients included in the molecular typing study. The CoNS strains isolated from blood and stool presented a high level of phenotypic and genotypic similarity in 53.5% of cases. In this population of patients with CoNS bacteremia stools constituted a definite source of CoNS. These results are in agreement with data in the literature [29]. In the 46.5% of discordant cases with or without bacteremia obtained from blood cultures and stool cultures, BT cannot be formally be excluded because of the existence of other non-intestinal sources that were not analyzed in this study.

Study limitations

This study was performed on a limited sample of 28 preterm infants who had at least bacteremia with concomitant CoNS positive stool culture. This inclusion criterion is thery restrictive. This study only concerned BT from intestinal origin. Other sites, such as respiratory or skin, were not investigated in this study. *Staphylococcus* spp. are the predominant germ within the microbiota of very preterm baby and therefore it can be considered that all preterm infants are carriers of detectable CoNS or not in standard culture. This sample of patients can therefore be considered to be a random sample representative of the population of preterm newborns with CoNS bacteremia.

Conclusions

This study clearly demonstrates that BT from the intestinal tract was the most likely source of CoNS bacteremia in hospitalized preterm infants. BT appears to be an important early step in sepsis in debilitated preterm patients. Reinforcement of the intestinal barrier, regulation of the intestinal microbiota by breast milk and prebiotics or probiotics would be a possible approach to the prevention of intestinal BT.

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Authors' contributions

AL, GA, BED, GM, GK, MB participated in meeting and follow-up discussions that culminated in the preparation of this manuscript. They contributed to the study conception, design, 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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Conflict of interest:

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