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Origin of Catheter-Related Bloodstream Infections Caused by *Staphylococcus epidermidis* in Critical Neonates

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Abstract

Bloodstream infection (BSI) remains the most frequent adverse event among premature infants worldwide, associated with increased hospital stay length and costs, poor outcomes, and even death. The aim of this study was to investigate the origin of catheter-related bloodstream infections (CR-BSIs) by *Staphylococcus epidermidis* in critical neonates, using molecular biology techniques. This was an observational study comprising neonates presenting BSI using central venous catheters (CVCs) for over 24 hours. Skin cultures obtained from the catheter insertion site, CVC hub, and neonate nasal and intestinal mucosa, were performed weekly from 48 hours from the beginning of the invasive procedure, as well as from the tip of the CVC after its withdrawal. Quantitative cultures were also performed of the tip with modifications and the "roll-plate" technique. Isolates identification were obtained using a VITEK II automated system. Molecular subtyping by pulsed-field gel electrophoresis (PFGE) was used to determine the origin of all BSIs. Among 19 primary BSI caused by *S. epidermidis*, BSI origin was defined in only 21.1% of the cases, where one was characterized as definite intraluminal, two as definite extraluminal, and one as translocation. Origin was indeterminate in 78.9% of the cases analyzed by PFGE. A total of 27 different genotype profiles were obtained. The spread of a prevalent clone in the unit (clone A) was detected in 28.6% of the samples. Most BSIs related to catheter caused by *S. epidermidis* were not able to prove the origin of most microorganisms present in neonate blood, demonstrating the difficulty in determining it in this population of patients. The lack of a better explanation of the origin of CR-BSI in neonates limits the direction of specific intervention measures.

Keywords
- bloodstream infection
- neonates
- pathogenesis
- central venous catheter
- *S. epidermidis*
- pulsed-field gel electrophoresis

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Introduction

Bloodstream infection (BSI) remains the most frequent adverse event among premature infants worldwide, associated with increased hospital stay length and costs, poor outcomes, and even death.[1,2]

The association of these infections with the use of central venous catheters (CVCs) in newborns ranges from ~17.3/1,000 CVC-day in neonates weighing from 1,501 g to 2,500 g to 34.9/1,000 CVC-day in neonates <1,000 g.[3] Although the pathogenesis of catheter-related bloodstream infections (CR-BSIs) is multifactorial and complex, references from studies in adults indicate two main potential routes of contamination of the catheter tip: (1) extraluminal, that is, organisms present in patient skin at the insertion site can migrate into the catheter tract, resulting in colonization of the catheter tip—regarding short-term catheters, this is the most common source of infection;[4,5] and, (2) intraluminal, that is, after the first week of placement, intraluminal contamination and colonization after hub manipulation occurs, which is responsible for most CR-BSI.[6,7]

In premature neonates, microbial translocation through the colonized mucosa is an underestimated source of infection that requires investigation. Currently, few comparable information concerning pathogenesis in high-risk neonates, mainly caused by *Staphylococcus. epidermidis*, is available in Brazil.[8,9] This microorganism is considered the major bacteria species responsible for CR-BSI, mainly due its ability to colonize the catheter tip and establish a biofilm, which accounts for its greater resistance to antibiotics, particularly methicillin.[2,10] Therefore, this study aimed to investigate the origin of CR-BSI by *S. epidermidis* in critical neonates using the pulsed-field gel electrophoresis (PFGE) method. In addition, we propose a new definition for probable intraluminal/extraluminal BSI and translocation.

Materials and Methods

Design, Setting, and Study Population

This study was performed from a prospective trial to assess the origin of CR-BSI in critical neonates. Those eligible for participation in the trial included 19 neonates with BSI admitted to the Neonatal unit of the Uberlândia Federal University (UFU) hospital in Uberlândia, Minas Gerais, Brazil, from January 2011 to August 2012, who required at least one CVC placed for longer than 24 hours. Those excluded were patients who were not in use of CVC or those for whom it was not possible to follow from admission to discharge. Ethical approval was obtained from the UFU Ethics Committee according to the Brazilian Ministry of Health requirements, number 033/11 and CEP/UFU 464/10 registration protocol.

Definitions

Primary CR-BSI: It was identified when a positive blood culture was obtained for the same microorganism present at the tip of the catheter and the clinical and microbiological absence of another source of infection was observed, with symptomatology[6,11] (i.e., temperature >38°C and with clinical signs of sepsis, including apnea, temperature instability, lethargy, feeding intolerance, worsening respiratory distress, or hemodynamic instability).[12]

Translocation: Microbial translocation was diagnosed if the microorganisms isolated from the blood culture were indistinguishable from those carried in the rectum or nostril within 2 weeks preceding the BSI episode. When the same microorganisms in the catheter hub or insertion site were detected, isolation dates were considered.

Origin determination: CR-BSI was classified as extraluminally acquired when similarities between isolates recovered from blood samples and recovered from insertion-site samples were observed. CR-BSI was classified as intraluminally acquired if similarity was demonstrated solely between isolates recovered from hub samples and blood. If results suggested more than one route of acquisition, the BSI origin was classified as indeterminate.[5]

Microbiological Methods

Hemoculture

Blood cultures were obtained by Neonatal Intensive Care Unit (NICU) health care professionals at the discretion of the attending neonatologist, taken from neonates presenting signs and symptoms compatible with primary BSI. Cultures were processed at the hospital laboratory using the BACT/Alert® system (bioMérieux, Inc.; Durham, USA).

CVC Tip Cultures

Catheters were removed when no longer required for patient care, when the patient experienced an adverse event, or when catheter exchange was deemed necessary. Catheters were removed under aseptic conditions, and their tips were cut off with sterile scissors and transferred into sterile tubes and transported to the Microbiology Laboratory. Quantitative cultures were performed according to Brun-Buisson et al[13] and considered positive when $\geq 10^3$ colony forming units (CFU)/mL were found. The "roll-plate" technique was performed according to Maki et al[14] and considered positive when ≥15 CFU/mL were found.

CVC Insertion-Site Skin

Skin material was obtained 48 hours, 7 days, and 14 days after catheter insertion, or until hemocultures were positive, and collected in a sterile saline prewet swab over a 20 cm^2 area. The swabs were placed into tubes containing 1 mL sterile saline and stirred using a vortex mixer. Subsequently, 0.1 mL of the liquid was inoculated onto blood agar plates, which were then incubated at 35°C for 24 hours. Cultures were considered positive when a growth of ≥200 CFU/20 cm^2 was observed.

Intestinal/Nasal Mucosa and Catheter Hub

Qualitative cultures of material collected from the nostrils, perianal surface, and catheter hub were performed 48 hours, 7 days, and 14 days after catheter insertion or until blood cultures were positive. The swabs were placed into tubes

containing 1 mL sterile saline and stirred using a vortex mixer. Subsequently, 0.1 mL of the liquid was inoculated onto blood agar plates, which were then incubated at 35°C for 24 hours.

Microorganism Identification

Isolate identification and resistance profile of blood isolates were obtained using the VITEK II automated system (bioMérieux) at the Microbiology Laboratory of the Clinical Hospital of the UFU.

Molecular Tests

Analysis of the Macro-Restriction Profiles of Chromosomal Deoxyribonucleic Acid after Cleavage with the Restriction Enzyme *Sma*I and Pulsed-Field Gel Electrophoresis

S. epidermidis strains (14 from blood, 9 from intestinal mucosa, 6 from nostrils, 9 from catheter hubs, 5 from CVC tips, and 6 from skin from insertion sites) related to pathogenesis were typed by PFGE, formulated based on the proposed methodologies by Goering (2010)[15] and McDougal et al.[16] Following this, digestion of impact genomic deoxyribonucleic acid (DNA) was performed with the SmaI restriction enzyme (Promega, Brazil). *S. epidermidis* DNA fragments were separated on 1% (W/V) agarose gel in 0.5% Tris-Borat-ethylene diamine tetra-acetic acid buffer using a CHEF DRIII apparatus (Bio-Rad) at 6 V/cm, applying pulses from 5 to 40 seconds for 21 hours and at 12°C. Gel were stained with ethidium bromide and subsequently photographed under ultraviolet light. Computer-assisted analyses were performed using the BioNumerics 7.5 software (Applied Maths, Bélgica). Comparison of the banding patterns was performed applying the unweighted pair-group method with arithmetic averages (UPGMA), using the Dice similarity coefficient.

Results

A total of 19 neonates with primary BSI by *S. epidermidis* were included in this CR-BSI study. Five neonates were excluded of molecular analyses, as their samples did not grow in culture or did not form clear PFGE bands. All isolated microorganisms are detailed in ►**Table 1**.

In addition to blood from these 19 infants, *S. epidermidis* isolates were also identified totaling 9 (47.4%) on catheter tips, 14 (73.7%) on catheter hubs, 11 (57.9%) on catheter insertion sites, 14 (73.7%) in intestinal mucosa, and 14 (73.7%) in the nostril samples. Of the total, 8 samples were not cultured as they were lost during experiments, 13 did not grow in culture, and 11 comprised other microorganisms, and not *S. epidermidis.*

Table 1 Microorganism isolates from neonates included in the origin of bloodstream infection caused by *Staphylococcus epidermidis* in neonates with central venous catheter

Patient	Isolation site				
	CVC tip	Hub	Insertion site	Gut	Nostrils
1	NEG	*Staphylococcus epidermidis*	NEG	*S. epidermidis*	*S. epidermidis*
13	NEG	*S. epidermidis*	*Staphylococcus capitis*	*S. epidermidis*	*Staphylococcus warneri*
15	NEG	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*	NEG
20	*S. epidermidis*	NEG	*S. epidermidis*	*Staphylococcus lugdunensis*	*S. epidermidis*
22	*S. epidermidis*	*S. capitis*	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*
26	*S. epidermidis*	*S. epidermidis*	NP	*S. epidermidis*	*S. epidermidis*
29	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*	NEG	*S. epidermidis*
37	NEG	*S. epidermidis*	*S. epidermidis*	*Staphylococcus haemolyticus*	*S. epidermidis*
45	NP	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*
47	NEG	*S. epidermidis*	*S. epidermidis*	NP	NP
49	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*	*S. capitis*
51	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*
52	NEG	*S. haemolyticus*	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*
57	*S. epidermidis*	*Staphylococcus aureus*	*S. capitis*	*S. epidermidis*	*S. epidermidis*
62	NP	*S. epidermidis*	NP	*S. epidermidis*	*S. epidermidis*
69	NEG	NP	NP	*S. epidermidis*	*S. epidermidis*
75	NEG	*S. epidermidis*	*Enterococcus faecium*	NEG	*Enterococcus faecalis*
82	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*
86	*S. epidermidis*	*S. epidermidis*	NEG	*S. epidermidis*	*S. epidermidis*

Abbreviations: CVC, central venous catheter; NEG, negative culture; NP, not performed.

Table 2 Origin of bloodstream infection caused by *Staphylococcus epidermidis* in neonates with central venous catheter

Infected patient	CVC type	Duration of parenteral nutrition BSI onset, days	Sites with concordant isolates[a]	Route of acquisition[b]
1	Umbilical	09	Hub, gut, blood	Indeterminate
13	Phlebotomy	22	Gut, blood	Translocation
15	PICC	11	No concordance	Indeterminate
20	PICC	17	Tip, nostril, blood	Definite extraluminal
22	PICC	42	Hub, gut, blood	Indeterminate
26	PICC	46	NP	Indeterminate
29	PICC	17	Hub, blood	Definite intraluminal
37	PICC	13	No concordance	Indeterminate
45	PICC	05	Hub, gut, nostril, blood	Indeterminate
47	PICC	37	NP	Indeterminate
49	PICC	04	No concordance	Indeterminate
51	PICC	31	NP	Indeterminate
52	PICC	06	Skin, nostril, blood	Definite extraluminal
57	PICC	10	Tip, blood	Indeterminate
62	PICC	0	No concordance	Indeterminate
69	PICC	09	NP	Indeterminate
75	PICC	0	No concordance	Indeterminate
82	PICC	07	NP	Indeterminate
86	Umbilical	07	No concordance	Indeterminate

Abbreviations: CVC, central venous catheter; BSI, bloodstream infection; PICC, peripherally inserted catheter; NP, not performed.
[a]Molecular subtyping by pulsed-field gel electrophoresis was used to determine the concordance of all *S. epidermidis* isolates.
[b]For definitions, see Methods section.

Results from the CR-BSI pathogenesis caused by *S. epidermidis* isolates, undergoing PFGE or not, are summarized in ►**Table 2**. Among 19 primary BSI cases, BSI origin was identified in only 21.1% of the cases, where one was characterized as definite intraluminal, two definite extraluminal, and one as translocation case. The BSI origin was not possible to determine in most of the cases; 78.9% of the total cases were analyzed by PFGE (15 patients).

PFGE was used to document concordance among *S. epidermidis* isolates for 14 of the 19 CR-BSI cases (►**Fig. 1**). A total of 27 different genotype profiles were obtained. The spread of a prevalent clone in the unit (clone A) was detected in 28.6% of the samples. The other clones were detected in fewer samples.

Discussion

Understanding the pathogenesis of CR-BSIs is essential to better define the adoption of more effective strategies for the prevention and control of these infections. However, few studies applying molecular techniques regarding identifying the origin and potential spread route of microorganisms to the tip of the catheter and blood are available.[5]

Like the study conducted by Garland et al,[5] where the route of acquisition of the microorganism present in blood was determined in only 40% of the cases, in the present study performed on critical neonates, most BSIs were indeterminate (78.9%) and only 21.1% were from a confirmed source. This difficulty in confirming CVC contamination routes strongly demonstrates that CR-BSI pathogenesis in neonates cannot be easily defined, in contrast with adult cases, in which the two main routes of contamination of the catheter tip with potential to infect blood are intraluminal (10–50%) and extraluminal (75–90%).[5]

In premature infants, the combination of an immature immune system and an atrophied intestinal mucosa, due to the frequent use of parenteral nutrition in addition to the presence of potentially pathogenic microorganisms in the intestinal and nostril microbiota, represents increased risk of microorganism translocation to the blood.[17] Thus, microbial translocation from the mucosa can be considered a substated origin of microorganisms in blood.[18] Although translocation was determined in only one case herein, most neonates with BSI caused by *S. epidermidis* presented this microorganism in both the intestinal mucosa and nostril, and were in frequent use of early parenteral nutrition for a long period of time.

The detection of identical clones in different patients during the same period confirms cross-transmission in the assessed NICU, reinforcing problems with hand hygiene in the unit. The importance of hand hygiene among health professionals concerning the transmission of health care-related infection is known worldwide and represents the

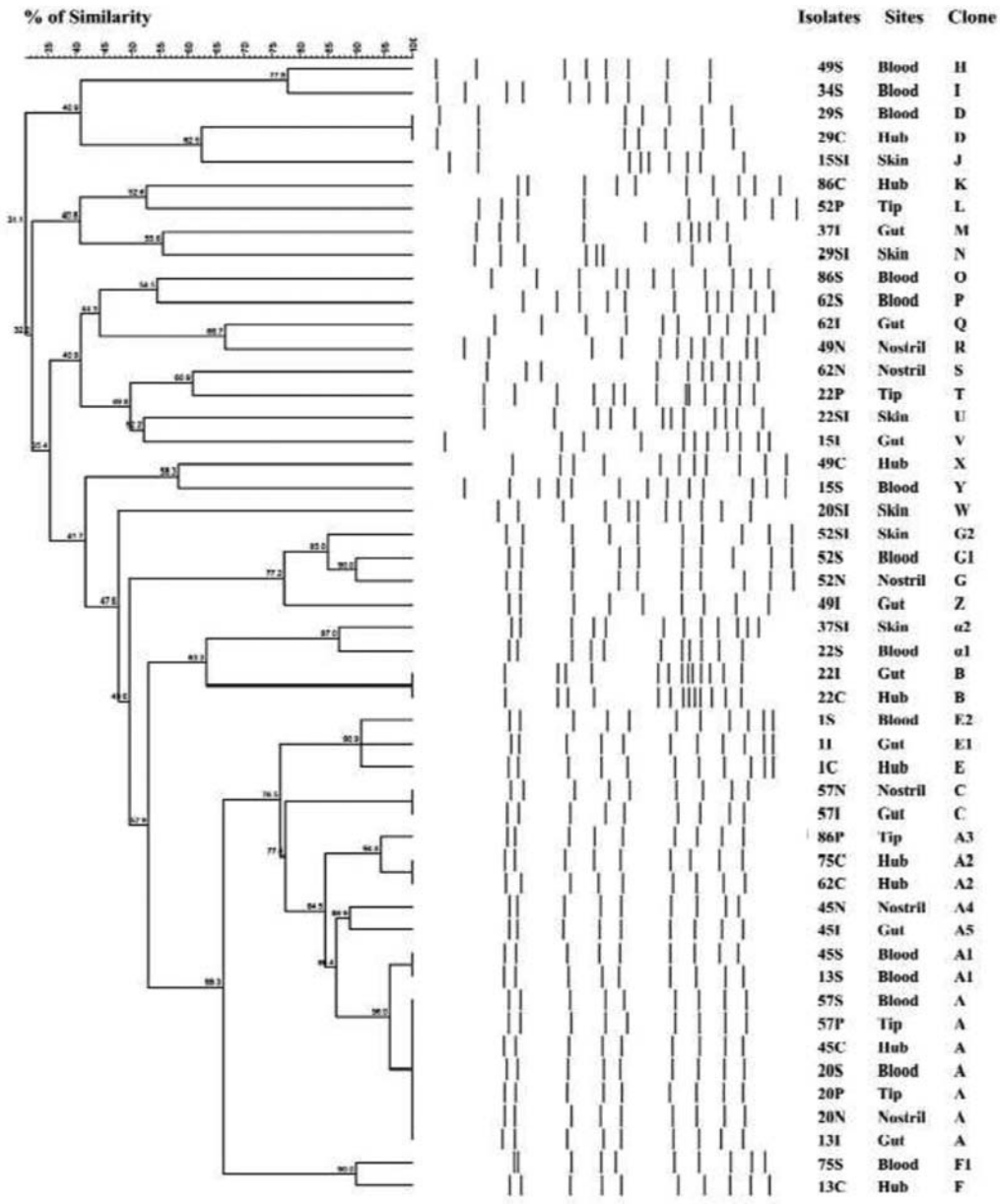

Fig. 1 Clonal deoxyribonucleic acid profile of *Staphylococcus epidermidis* isolated from catheter-related bloodstream infections and sites included in the pulsed-field gel electrophoresis-based origin analyses.

most common way of disseminating microorganisms in the hospital environment.[19,20]

Generally, in low- and middle-income countries, such as Brazil, several factors can lead to the emergence and spread of a resistant clone, especially in NICUs. These include a high quantity of antibiotic use, which results in selective pressure responsible for the emergence of resistant and multiresistant isolates, adding to the existence of dominant clones and failures in basic infection prevention and control practices, which may justify the dissemination of this microorganisms in the hospital environment.[21]

Although the origin of the microorganism was confirmed in some cases, strategies designed to prevent extraluminally acquired BSI should be combined with infection prevention strategies to avoid infections that originate from intraluminal contaminants. Despite efforts to elucidate the pathogenesis of CR-BSI in critical neonates using molecular techniques, studies have not been sufficient in this regard and this process remains unclear, requiring more thorough studies.

Our study has some strengths and weaknesses. Although we used a gold standard molecular technique, the number of *S. epidermidis* samples was low, so it was not possible to prove the origin of most microorganisms present in neonate blood, demonstrating the difficulty in determining it in this population of patients. In addition, this study demonstrates that, despite the high clonal diversity displayed by isolates, cross-transmission still occurred. Even so, our study shows mucosal translocation in one case and provides indirect data suggesting mucosal translocation as a way transmission in remaining cases.

The lack of a better explanation of the pathogenesis of CR-BSI in neonates limits the direction of specific intervention measures. At the time, the use of comprehensive interventions

that can control all possible pathways that can lead the microorganism to the bloodstream is required.

Conflict of Interest
None declared.

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