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Original article

Genotypic study documents divergence in the pathogenesis of bloodstream infection related central venous catheters in neonates

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ABSTRACT

Objective: To investigate the pathogenesis of bloodstream infection by *Staphylococcus epidermidis*, using the molecular epidemiology, in high-risk neonates.
Methods: We conducted a prospective study of a cohort of neonates with bloodstream infection using central venous catheters for more than 24 h. "National Healthcare Safety Network" surveillance was conducted. Genotyping was performed by DNA fingerprinting and *mec*A genes and *ica*AD were detected by multiplex-PCR.
Results: From April 2006 to April 2008, the incidence of bloodstream infection and central venous catheter-associated bloodstream infection was 15.1 and 13.0/1000 catheter days, respectively, with *S. epidermidis* accounting for 42.9% of episodes. Molecular analysis was used to document the similarity among six isolates of bloodstream infection by *S. epidermidis* from cases with positive blood and central venous catheter tip cultures. Fifty percent of neonates had bloodstream infection not identified as definite or probable central venous catheter-related bloodstream infection. Only one case was considered as definite central venous catheter-related bloodstream infection and was extraluminally acquired; the remaining were considered probable central venous catheter-related bloodstream infections, with one probable extraluminally and another probable intraluminally acquired bloodstream infection. Additionally, among *mec*A+ and *ica*AD+ samples, one clone (A) was predominant (80%). A polyclonal profile was found among sensitive samples that were not carriers of the *ica*AD gene.
Conclusions: The majority of infections caused by *S. epidermidis* in neonates had an unknown origin, although 33.3% appeared to have been acquired intraluminally and extraluminally.

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We observed a polyclonal profile between sensitive samples and a prevalent clone (A) between resistant samples.

Introduction

Central venous catheters (CVCs) are widely used in adults and newborns and are the leading cause of bloodstream infection (BSI) in hospitals.[1] Infections associated with the use of CVCs represent 10–20% of hospital infections (IHs) in adult patients[2] and approximately half of those observed in neonates.[3] Infections related to the use of CVC (CR-BSI), i.e. those in which the microorganism detected in the bloodstream is also present in the catheter tip, represent up to 29% of these infections in neonatal intensive care units (NICUs) with an incidence varying from 2 to 49/1000 days CVC.[4]

The pathogenesis of CR-BSI is multifactorial and complex. Data from studies in adults showed two main potential routes of contamination of the catheter tip with BSI: dissemination of intraluminal microorganisms through contamination of the hub in long-term CVCs, and extraluminally due to migration of microorganisms to the skin at the site of insertion in those short duration CVC.[5,6]

Although evaluation of samples by molecular techniques is essential, due to their discriminatory power, their use in investigating the pathogenesis of BSIs associated/related to CVC has been scarce; moreover, there are few studies on the pathogenesis of CVC-related BSI in critical newborns.[7,8]

Gram-negative rods are the main pathogens of neonatal sepsis in developing countries.[9] In NICUs, following the adoption of sophisticated tertiary neonatal care with a high rate of invasive device use, coagulase-negative staphylococci (CoNS) stand out as the main agents of neonatal nosocomial sepsis, with *Staphylococcus epidermidis* being the most frequently isolated from late onset sepsis, accounting for 70% of cases.[10,11] Neonatal infection by CoNS is less severe but causes significant morbidity, especially among infants of very low birth weight.[12]

The main factor in the pathogenicity of *S. epidermidis* is its ability to form biofilms mediated by polysaccharide intercellular adhesin (PIA), encoded by the ica operon.[13] This bacterium adheres to the surface of invasive devices such as CVCs and forms biofilms, which account for its greater resistance to antibiotics, particularly to meticillin, increasing the length of hospital stay and costing around $ 2 billion US dollars just in the United States.[14] The bacteria that form biofilms are difficult to eradicate and it is estimated that this mechanism is associated with 65.0% of infections in hospitals.[15]

Objective

To investigate the pathogenesis of CR-BSI by *S. epidermidis* in high-risk neonates through a prospective nested cohort study in a randomized trial using molecular epidemiology to determine the origin of microorganisms responsible for BSI.

Materials and methods

Patients, study design and setting

This was a prospective cohort study of patients with a CVC with laboratory confirmed primary BSI by *S. epidermidis* conducted in the NICU with 10 level-3 beds within a 500-bed teaching hospital from April/2006 to April/2008.

The population analyzed was followed five times/week from birth until discharge or death. Ethical approval was obtained from the Ethics Committee of Uberlândia Federal University according to the requirements of the Ministry of Health.

The study sample consisted of neonates who had at least one CVC placed for longer than 24 h, followed-up through epidemiologic vigilance as part of the "National Healthcare Safety Network" (NHSN).

Definitions

Laboratory-confirmed primary BSI
Isolation of recognized pathogens from two blood cultures that were not related to infection at another site, with temperature >38 °C and with clinical signs of sepsis, including apnea, temperature instability, lethargy, feeding intolerance, worsening respiratory distress, or hemodynamic instability.[16]

Catheter tip colonization
Absence of signs of infection at the catheter insertion site and microorganism growth $\geq 10^3$ CFU/mL at the catheter tips (by quantitative culture) or ≥15 UFC/mL (by the "roll- plate" technique).[17]

CVC-associated BSI
Bacteraemia (isolation of the same organism with identical antibiogram from blood drawn from peripheral veins and CVC), clinical manifestations of sepsis, defervescence after removal of the implicated catheter, but without laboratory confirmation of CVC colonization.[18]

Definite catheter-related BSI
For neonates with BSI that did not have an identified source, definite catheter-related BSI was defined by a culture of a peripheral, percutaneously obtained blood sample that was positive for the same organism found to be colonizing the catheter hub or tip (i.e., concordant colonization of the catheter hub or tip).[19]

Probable catheter-related BSI
Probable catheter-related BSI was defined as BSI without an identified source that met one of the following criteria: (1) a blood sample obtained through a peripherally inserted