ABSTRACT

This prospective study evaluated semiquantitative and qualitative catheter-culture methods for diagnosis of catheter-related infection (CRI) in newborns. Catheter tips from newborns admitted to the Neonatal Unit of the University Hospital of the Botucatu Medical School, UNESP were included in the study. Catheter cultures were performed with both semiquantitative and qualitative techniques. For CRI diagnosis, microorganisms isolated from catheter cultures and from peripheral blood cultures were identified and submitted to agent susceptibility test. The gold standard was the certain CRI diagnosis when same microorganism (specie and profile of susceptibility to agents) was isolated from both catheter tips and peripheral blood culture. A total of 85 catheters from 63 newborns were included in the study. The semiquantitative culture method, despite presenting lower sensitivity (90%), showed higher specificity (71%) when compared to 100% of sensitivity and 60% of specificity in the qualitative method. The identification of the microorganisms obtained from the catheter cultures showed a prevalence of coagulase-negative staphylococci (CNS) species. The specie Staphylococcus epidermidis (77.5%) was the prevalent in the catheters with positive semiquantitative cultures. Among 11 episodes with CRI diagnosis, 8 (72.7%) were associated with CNS species, of which 6 were S. epidermidis. Two episodes of CRI by S. aureus and one by Candida parapsilosis were also detected. The semiquantitative catheter-culture method showed advantages for CRI diagnosis in newborns when compared to the conservative qualitative method.

Key-words: Catheter-related infection, catheter culture, semiquantitative culture, newborns, coagulase-negative staphylococci.

INTRODUCTION

The medical advances achieved in neonatal intensive care units (Neonatal ICU) in the last few decades have enabled a significant increase in preterm newborns’ survival rates, particularly in those with low birth weight (17,19). Numerous resources have been routinely used in these units, such as mechanical ventilation, parenteral nutrition and the insertion of umbilical catheters as well as the extensive use of antibiotics and long periods in neonatal ICU. These factors, despite contributing to lives of newborns (NB) preservation, act as predisposing factors to the development of nosocomial neonatal infections (14,19,25,32,34,35).

Among all the used resources, intravascular catheters are distinguished. They are frequently used in numerous procedures in neonatal ICU, since they allow rapid intravenous access for the medication administration, parenteral nutrition and others (31). Although the use of catheters is a fundamental...
procedure for NB’s survival in neonatal ICU, it is also considered an important risk factor for infection acquisition. Additionally, it contributes to increase the incidence of bloodstream infections and, therefore, increases morbidity rates and hospitalization periods (3).

Similarly to what occurs in adults, nearly 60% of the catheter-related bloodstream infections in NB are caused by gram-positive bacteria, among which coagulase-negative staphylococci (CNS) species are distinguished as they are associated with 37.7% of such infections (5). There are some important catheter contamination sources, however the most frequent is the NB’s flora on the catheter insertion site (31). This explains why CNS are the most frequent microorganisms associated with such infections. Since they are the major component of NB skin and mucosal flora, they are recognized as the most frequent etiologic agents in NB infections, mainly in those with low birth weight (<1,500g) (9,16,20).

Catheter-related infections (CRI) are diagnosed when identical microorganisms are isolated from catheter and blood cultures in absence of other apparent source of infection, except the catheter (4). The culture’s reliability depends on the adopted technique. The major catheter-culture methods utilized for CRI diagnosis are the qualitative and semiquantitative methods. The qualitative or conservative broth method is the simplest and the most commonly used. However, the semiquantitative method proposed by Maki et al. (24) is recommended for CRI diagnosis by the Centers for Disease Control and Prevention (4). According to Maki’s proposal, semiquantitative catheter-tip culture is considered positive in the presence of 15 or more Colony-Forming Units (CFU) growth. Hence, this study aimed to compare two methods for CRI diagnosis in NB.

MATERIAL AND METHODS

Sampling
Eighty-five catheter tips from 63 newborns admitted to the Neonatal Unit of the University Hospital of the Botucatu Medical School, UNESP, between September 2001 and June 2003, were included in this prospective study.

Catheter tips from patients who had presented one or more blood cultures collected close to the date of catheter removal were included. Catheters from NB whose clinical data and laboratory records referring to a one-week period prior to the device’s removal date were not available were excluded of the study. The procedures were approved by Medical School Research Ethics Committee.

Catheter culture
The catheter-tip cultures were performed by Maki’s semiquantitative method (24). The catheters were aseptically removed by the medical staff and the approximately 5 cm distal tips were collected, placed in dry sterile vials and immediately transported to the laboratory for processing. The segments were rolled on the surface of Blood Agar plates and incubated at 37°C for 72 hours. The plates were examined daily and counted as soon as growth was detected, the result was expressed in CFU. The catheter tips were also cultured with Qualitative or Conservative method that consisted in immersing the catheter tips in Brain Heart Infusion (BHI) with subsequent incubation at 37°C for 72 hours. The broths were examined daily and when cloudy, a subculture was performed in Blood Agar.

Blood culture
The blood cultures were collected and cultivated by the Bactec Automated System, according to Koneman et al. guidelines (23).

Microorganism identification
The microorganisms were submitted to Gram staining for purity assessment and morphology and specific stain examination. After confirmation of these characteristics, identification tests were performed as recommended by Koneman et al. (23).

Identification of coagulase-negative Staphylococci
Identification was performed according Kloos and Bannerman (21), Kloos and Schleifer (22) and Cunha et al. (10) with a simplified scheme of biochemical tests which includes the performance of catalase and coagulase tests, as well as of sugar utilization tests: xylose, arabinose, saccharose, trehalose, mannitol, maltose, lactose, xylitol, ribose and fructose, in addition to the characterization of hemolysins, nitrate reduction, urease production, ornithine descarboxylase and Novobiocin resistance (5 μg).

Susceptibility test
The susceptibility test was performed using the technique of agent diffusion from impregnated discs on agar, according National Committee for Clinical Laboratory Standards-NCCLS guidelines (27). For inoculum preparing, BHI cultures of the microorganisms obtained from catheter tips and blood cultures were previously incubated for 4 to 6 hours and adjusted to 0,5 McFarland density scale before plating. The following discs were utilized: Penicillin G (10U), Oxacillin (1μg), Tetracycline (30 μg), Chloramphenicol (30 μg), Erythromycin (15 μg), Cephalothin (30 μg), Gentamicin (30 μg), Cloxacillin (5 μg), Cefotaxime (30 μg), Cefaclor (30 μg), Levofloxacin (5 μg), Ofloxacin (5 μg), Rifampin (5 μg), Vancomycin (30 μg) and Teicoplanin (30 μg). Following the incubation at 37°C for 24 hours, the halos were measured (mm), and the results obtained were compared between the microorganisms isolated from the same NB (catheter and blood cultures) in order to observe the similarity between samples.
Catheter-related infection (CRI) diagnosis

CRI was diagnosed according to CDC guidelines (4) by the presence of two or more of the following signs or symptoms: fever (≥38°C), hypothermia (<36°C), apnea, bradycardia or shock signs, in addition to the presence of one or more positive blood cultures in patients whose catheter semiquantitative culture was positive, if the same microorganism (specie and agent susceptibility) had been isolated from the catheter and the peripheral blood culture without another apparent source of infection focus except the catheter.

Statistics

Culture accuracy was obtained by determination of sensitivity (S) and specificity (SP), and CRI was calculated from the positive predictive value (PPV) and the negative predictive value (NPV).

RESULTS

Eighty-five catheters from 63 NB admitted to the Neonatal Unit of the University Hospital of the Botucatu Medical School, UNESP were studied. The mean time of catheter use was 10 days, ranging from 1 to 33 days. Among the catheters, 36.6% were the umbilical vascular type, 34.1% umbilical arterial, 17.1% central vascular and 12.2% peripheral.

Of all the catheters included in the study, 54 (63.5%) had negative cultures by Maki’s semiquantitative method, of which 13 (15.3%) showed growth with less than 15 CFU, whereas the remaining 41 (48.2%) catheters did not present growth of any colony. The semiquantitative culture was positive, by showing growth equal or superior to 15 CFU, in 31 (36.5%) catheter tips (Table 1).

In relation to the qualitative method, it was observed that 45 (52.9%) catheter tips had negative cultures, whereas the remaining 40 (47.1%) had positive culture by this method, by presenting culture medium clouding (Table 1).

The catheter-related infection diagnosis was observed in 11 episodes. In six (54.5%) of them, it was observed a confluent growth on Blood Agar plates (Fig. 1); other 4 (36.4%) CRI episodes presented culture growth ≥100 CFU, and, in the remaining episode it was verified an 8 CFU growth. Besides this last episode did not present a culture growth superior to 15 CFU, it consisted in a certain CRI diagnosis since it presented infection by the same specie, Staphylococcus aureus, with similar agent susceptibility in both catheter and blood culture in this patient.

The results regarding the identification of the microorganisms obtained from the catheter semiquantitative cultures revealed a predominance of CNS species (Table 2) either in cultures with growth <15 CFU (76.9%) or in those presenting growth ≥15 CFU (83.9%).

Table 1. Results from the catheter cultures by the semiquantitative and qualitative methods.

| Catheter culture | Semiquantitative | Qualitative |
|------------------|------------------|-------------|
| No growth        | <15 CFU          | ≥15 CFU     |
| N                | 41               | 13          | 31          |
| %                | 48.2             | 15.3        | 36.5        |
|                  | 45               | 40          |
|                  | 52.9             | 47.1        |

N= Total number of catheters. CFU= Colony-Forming Units.

Table 2. Microorganisms isolated from catheter tips by the semiquantitative culture.

| Microorganisms isolated from catheter tips | <15 CFU | ≥15 CFU | CRI |
|-------------------------------------------|---------|---------|-----|
| S. epidermidis                             | 9 (69.2)| 24 (77.5)| 6 (54.5) |
| S. warneri                                 | -       | 1 (3.2)  | 1 (9.1)  |
| S. simulans                                | -       | 1 (3.2)  | 1 (9.1)  |
| S. haemolyticus                            | 1 (7.7) | -        | -        |
| Total CNS                                  | 10 (76.9)| 26 (83.9)| 8 (72.7) |
| S. aureus                                  | 1 (7.7) | 1 (3.2)  | 2 (18.2) |
| Acinetobacter baumannii                    | -       | 1 (3.2)  | -        |
| Candida parapsilosis                        | 1 (7.7) | 3 (9.7)  | 1 (9.1)  |
| Gram Positive Rods                         | 1 (7.7) | -        | -        |
| Total                                      | 13      | 31       | 11       |

Figure 1. Catheter-tip semiquantitative culture with confluent growth.
The advances achieved in neonatology in the last few decades have enabled significant increase in the survival of preterm newborns as well as in that of newborns with low weight at birth; nevertheless, a progressive increase in the diagnosis of nosocomial infection in neonatal ICU (17) have also been verified as a result. The use of invasive procedures in such units is an important risk factor for the development of nosocomial infections (33), during catheter insertion or from the health care staff’s hands. The two former agents mainly originate from the skin representatives of this gender in catheter-related infections (12.6%), whereas Candida spp. represented 8% to 9% of the findings. Various preceding studies on adult patients also showed that the three major agents causing CRI are coagulase-negative staphylococci, S. aureus and Candida spp. (2,12,15). The two former agents mainly originate from the skin during catheter insertion or from the health care staff’s hands. Candida albicans, followed by C. parapsilosis are the main representatives of this gender in catheter-related infections (6,13,15).

Among the 8 CRI cases associated with CNS species, Staphylococcus epidermidis was present in 6 (54.5%) cases. Such higher frequency of S. epidermidis in this disease is expected, since, according to D’Angio (11), this species is predominant in NB’s flora around the fourth day of life. Such predominance in the colonization of individuals and the greater pathogenicity of some strains can explain the fact that S. epidermidis is the species most commonly associated with infectious processes in NB as reported in a study performed by
Cunha et al. (9). CNS were also the prevalent organisms in catheter colonization, that is, catheters with growth ≥ 15 CFU.

The comparison between the qualitative and semiquantitative catheter-culture techniques showed agreement with data in the literature. In a metaanalysis by Sañdar et al. (30), the sensitivity mean found for the qualitative culture was of 90%, while specificity was 72%. Nevertheless, although the semiquantitative culture showed lower sensitivity (85%), it exhibited higher specificity (82%). In this study, although the sensitivity found in the qualitative-culture method was 100% in contrast to 90% shown by the semiquantitative method, specificity was lower for the qualitative method, where 60% was found in contrast to 71%. The semiquantitative culture also showed larger positive predictive value (PPV), in addition to being a more rapid technique, since it is capable of detecting positive results in up to 24 hours.

It was concluded that the semiquantitative culture is a rapid and efficient technique for diagnosing catheter-related infection in NB. However, it requires careful interpretation, and its result must be part of a set of factors that can indicate diagnosis and a specific treatment.

RESUMO

Comparação entre culturas qualitativa e semiquantitativa de ponta de cateter: Diagnóstico laboratorial de infecção relacionada a cateter em recém-nascidos

Este estudo prospectivo avaliou os métodos semiquantitativo e qualitativo de cultura de cateter para o diagnóstico de infecção relacionada a cateter (IRC) em recém-nascidos (RN). Foram incluídas pontas de cateteres provenientes de recém-nascidos internados na Unidade Neonatal do Hospital das Clínicas da Faculdade de Medicina de Botucatu, UNESP. Foram utilizadas as técnicas semiquantitativa e qualitativa de cultura de cateter. Para o diagnóstico de IRC, os micorganismos isolados das culturas de cateteres e de hemoculturas periféricas foram identificados e submetidos ao teste de sensibilidade a antimicrobianos. O padrão ouro correspondeu ao isolamento do mesmo micorganismo (espécie e perfil de sensibilidade a antimicrobianos) isolado em hemocultura periférica. Foram estudados 85 cateteres provenientes de 63 RN. A cultura semiquantitativa, embora tenha apresentado menor sensibilidade (90%), apresentou uma maior especificidade (71%) em comparação à sensibilidade de 100% e especificidade de 60% encontradas na cultura qualitativa. Através da identificação dos micorganismos obtidos nas culturas de cateteres, observou-se uma predominância de espécies de Estafilococos coagulase-negativa (ECN). A espécie Staphylococcus epidermidis foi a prevalente (77,5%) nos cateteres com culturas semiquantitativas positivas. Dos 11 episódios de IRC diagnosticados, 8 (72,7%) foram associados a espécies de ECN, dos quais 6 eram da espécie S. epidermidis. Também foram detectados dois casos de IRC por S. aureus e um caso por Candida parapsilosis. O método de cultura semiquantitativa cateter apresentou vantagens para o diagnóstico de IRC em RN quando comparado com o método qualitativo tradicional.

Palavras-chave: Infecção relacionada a cateter, cultura de cateter, cultura semi-quantitativa, recém-nascidos, Estafilococos coagulase-negativa.

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