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Bacteremia Detected by Lysis Direct Plating in a Neonatal Intensive Care Unit

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The density of bacteremia was determined in 787 neonatal blood specimens by using the 1.5-ml Isolator microbial tube. Coagulase-negative staphylococci were the organisms isolated most frequently from both true-positive cultures (25 of 50) and contaminated cultures (57 of 131). Based on the first positive culture in an episode of sepsis, there were no cases of coagulase-negative staphylococcal sepsis associated with counts of ≤5 CFU/ml. Indwelling intravascular lines were associated with the majority of the episodes of sepsis. The distribution of pathogens causing sepsis in this neonatal population was similar to the distribution of microorganisms associated with cannula-related sepsis in other hospitalized patients.

Neonatal sepsis is one possible cause of instability in infants hospitalized in a neonatal intensive care unit setting. Because it is difficult to exclude infection as the cause of instability, cultures from many sites, including blood, are obtained and broad-spectrum antibiotic therapy is started. A positive blood culture does not necessarily confirm infection as the cause of instability, since contamination of blood can occur. Consequently, physicians and microbiologists have developed guidelines to distinguish contaminated from true-positive cultures (5).

The identification of the microbe itself remains the most important piece of information that is used to interpret the significance of a positive blood culture. The recovery of organisms traditionally regarded as pathogens, such as *Streptococcus agalactiae*, *Escherichia coli*, and *Listeria monocytogenes*, pose no problem of interpretation even when recovered at densities of ≤1 CFU/ml. On the other hand, the recovery of organisms which colonize the skin, such as coagulase-negative staphylococci, diphtheroids, and *Candida* spp., is often difficult to interpret, especially when these organisms are present in low numbers. Thus, additional information, i.e., density of bacteremia, number of positive cultures, number of days to a positive culture, presence of risk factor(s), and underlying disease, is required in order to determine whether infection is truly present. While multiple blood cultures are rarely obtained from low-birth-weight neonates, the density of bacteremia can easily be determined. Here we report our experience with lysis direct plating as an aid in differentiating true-positive blood cultures from those that can be considered falsely positive.

MATERIALS AND METHODS

The Neonatal Service at Emanuel Hospital comprises a 44-bed neonatal intensive care unit and a 20-bed normal newborn nursery. When sepsis is suspected in a neonate, blood is drawn by the nursing staff after preparation of the skin or catheter port of an indwelling intravascular line. The nursing staff is instructed to follow the directions accompanying the 1.5-ml Isolator (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) when collecting blood. In the laboratory, equal aliquots of blood (usually 0.4 ml per plate) are inoculated onto a single chocolate agar plate, which is incubated in an atmosphere of 5% CO_2, and onto a single brucella agar plate, which is incubated in a GasPak jar (BBL Microbiology Systems, Cockeysville, Md.) under anaerobic conditions. These plates are read daily for 7 days. Isolates are identified by standard techniques (4).

The medical records of each infant with any organisms isolated from a blood culture were reviewed by one of us (J.S.B.) for evidence of infection. A judgment, based on clinical signs and laboratory data, was made as to whether the organism(s) isolated from the blood was associated with sepsis or represented contamination of the culture. The clinical signs included fever, leukocytosis, recovery of the same microorganism from other concurrently obtained specimens, apneic and bradycardic episodes, feeding intolerance, and increased oxygen requirements. In addition, if the neonate responded to antibiotic therapy appropriate for eradication of the microorganism(s) recovered from the blood, the diagnosis of sepsis was considered confirmed. If empiric therapy was discontinued within 72 h because another cause for the instability in vital signs was discovered and if the infant remained stable after therapy was stopped, the diagnosis of sepsis was considered excluded.

Sepsis was defined as the condition characterized by clinical signs and laboratory data consistent with infection associated with the presence of microorganisms in the blood. Catheter sepsis was defined as sepsis occurring when an indwelling catheter was the only apparent focus of infection. A contaminant was defined as an organism that was recovered from a culture of the blood but was not deemed responsible for the clinical signs and symptoms that prompted the blood culture.

RESULTS

During 1986 and 1987, 787 neonatal blood specimens were submitted to the Microbiology Laboratory of Emanuel Hospital for blood culture. A positive blood culture was found in 243 of these 787 (31%) specimens that were cultured. These specimens were obtained from 441 neonates. Positive blood cultures represented 50 episodes of sepsis from 43 patients. Contaminated blood cultures were found in 131 of the 787 (16.6%) blood samples submitted.

As shown in Table 1, the microorganisms causing sepsis in the low-birth-weight neonates were primarily gram-positive bacteria and yeasts. The coagulase-negative staphylococci were the organisms isolated most frequently from neonates

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TABLE 1. Microorganisms isolated from 43 patients with sepsis

Organism	No. of isolates	No. of sepsis episodes	No. of cathether sepsis episodes	Median CFU/ml[a]
Gram-positive bacteria				
Staphylococcus aureus	9	4	2	>100
Coagulase-negative staphylococci	70	25	18	—[b]
Streptococcus pneumoniae	1	1	0	1
Group B streptococci	2	2	2	22
Enterococcus faecalis	4	3	1	5
Listeria monocytogenes	1	1	0	1
Gram-negative bacteria				
Haemophilus influenzae type b	1	1	0	8
Fusobacterium nucleatum	1	1	0	6
Pseudomonas luteola	1	1	0	3
Yeasts				
Candida albicans	3	2	1	26
Candida parapsilosis	6	2	2	18
Malassezia species	1	1	1	96
Mixed cultures				
Mixed coagulase-negative staphylococci	1	1	1	>100
Mixed *Candida* spp.	2	1	1	>100
Mixed *Candida* and coagulase-negative staphylococci	7	33	3	66
Mixed *Candida* and *Enterococcus faecalis*	2	1	1	>100

[a] For each episode, if paired or sequential isolation occurred, the median was based on the first specimen drawn from direct percutaneous sampling from a vein in an extremity.
[b] Percutaneous extremity specimens, 39.5 CFU/ml; catheter specimens, >100 CFU/ml.

with sepsis (25 of 50 episodes of sepsis) and from contaminated cultures (57 of 131 cultures). The single episodes of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b were recovered from infants who were discharged from the normal newborn nursery and subsequently admitted to the neonatal intensive care unit from the Emergency Department of Emanuel Hospital. The identification of organisms from blood cultures judged to be contaminated are shown in Table 2. The majority of these cultures consisted of normal skin or oropharyngeal flora. Mixed cultures were obtained in 20 of the 131 (15%) contaminated cultures. *Staphylococcus aureus* was judged to be a contaminant on two occasions; one culture had 2 CFU/ml and the other had 3 CFU/ml. In both of these cases, respiratory distress syndrome appeared to be the cause of instability; when empiric antibiotic therapy was discontinued, the infants remained stable. In those cultures in which *Staphylococcus aureus* was considered to be responsible for sepsis (Table 1), densities of greater than 100 CFU/ml were recovered. In neonates with sepsis, the median counts of coagulase-negative staphylococci were 39.5 CFU/ml for the first peripheral specimens that were drawn and >100 CFU/ml for the first line specimens that were drawn. These counts differed significantly from a median of 1 CFU/ml (range, 1 to 12 CFU/ml) when coagulase-negative staphylococci were recovered from cultures judged to be contaminated. There were no cases of coagulase-negative staphylococcal sepsis associated with counts of ≤5 CFU/ml (Fig. 1). Only 3 of 57 coagulase-negative staphylococcal contaminants had counts of >5 CFU/ml.

Candida spp., alone or as part of a mixed culture, were never recovered as contaminants. There was a wide range in densities of fungemia; the median count for *Candida albicans* was 26 CFU/ml in two episodes and 18 CFU/ml for *Candida parapsilosis* in two episodes. All nine of the infants with sepsis in which *Candida* spp. were recovered alone or as part of mixed gram-positive flora had indwelling intravascular catheters and were receiving antibiotics.

In this study, 64% of the episodes of sepsis occurred in neonates in which an indwelling catheter was the only apparent focus of infection (Table 1). Attempts were often

TABLE 2. Microorganisms which were judged to be contaminants cultured from blood of 116 patients

Organism	No. of isolates	Median CFU/ml
Gram-positive bacteria		
Micrococcus sp.	1	1
Staphylococcus aureus	2	2.5
Coagulase-negative staphylococci	57	1
Viridans group streptococci	4	1
Peptostreptococcus sp.	1	1
Aerobic diphtheroids	14	1
Anaerobic diphtheroids	20	1
Bacillus sp.	6	1
Gram-negative rods		
Enterobacter sp.	1	1
Acinetobacter lwoffii	1	1
Flavobacterium sp.	1	>100
Hemophilus parahaemolyticus	1	1
Mixed cultures		
Mixed gram-negative and -positive cocci	2	2[a]
Mixed oropharyngeal flora[b]	5	4[a]
Mixed skin flora[c]	13	2[a]
Fungi, mold	2	1

[a] Total colonies.
[b] Viridans group streptococci, *Neisseria* sp., and coagulase-negative staphylococci.
[c] Diphtheroids, coagulase-negative staphylococci, and *Bacillus* sp.

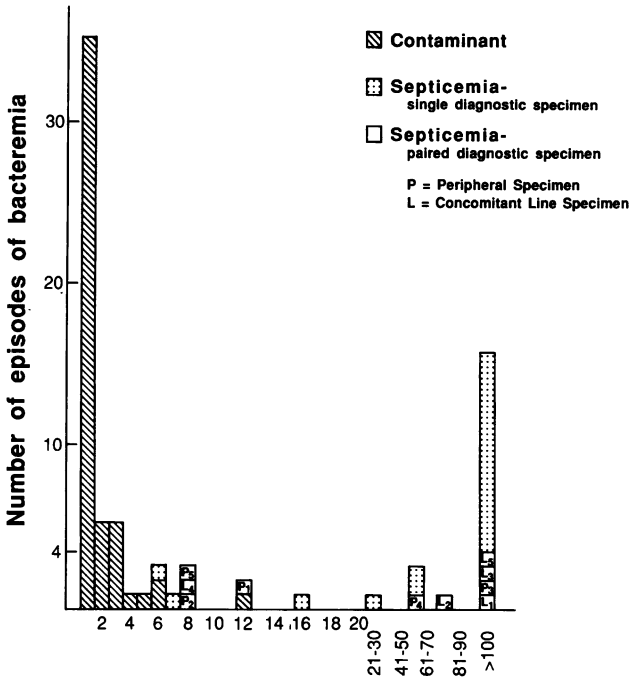

FIG. 1. Distribution of coagulase-negative staphylococci.

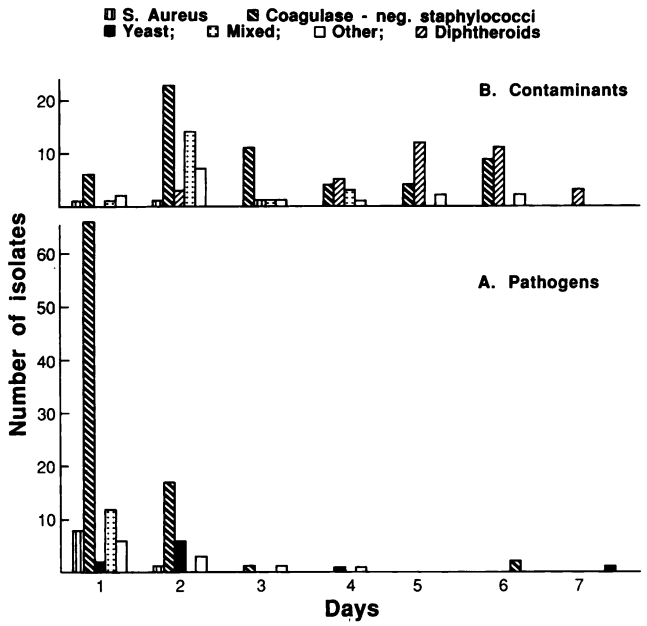

FIG. 2. Comparison of the times to positivity between pathogens (A) and contaminants (B).

made to identify the indwelling line as the source of infection by concomitantly obtaining blood through the line and from a peripheral venous site (9). When paired peripheral and line specimens were drawn for diagnostic purposes (data not shown), line counts were greater than or equal to peripheral counts 93% of the time. The microorganisms recovered in venipuncture and catheter cultures were identical. For contaminated paired blood cultures, peripheral cultures were usually higher (median, 2 CFU/ml) than line cultures, which were sterile in 85% of the paired cultures. In two instances different organisms were recovered from paired line and venipuncture samples.

Figure 2 shows the median time (in hours) to a positive culture. As shown in Fig. 2A, 64% of the pathogens were recovered within 24 h of incubation, and 94.6% of the pathogens were recovered by 48 h. In contrast less than 8% of contaminants were isolated by 24 h, and 15% were isolated by 48 h (Fig. 2B).

DISCUSSION

We found the distribution of microorganisms causing sepsis in a neonatal population to be different from that reported by investigators evaluating the use of the 1.5-ml Isolator in a general pediatric hospital (2, 3, 8). The distribution was similar to those reported for catheter sepsis in a general population (6). At our institution, critically ill newborn infants are managed with vascular catheters; therefore, they are at risk of catheter sepsis. In this study, 64% of the episodes of sepsis occurred in neonates in which an indwelling catheter was the only apparent focus of infection. Of the 32 episodes of sepsis, 18 involved coagulase-negative staphylococci; 4 involved yeasts; 1 involved an *Enterococcus* sp.; and 6 were polymicrobic infections with gram-positive cocci, yeasts, or both. Coagulase-negative staphylococci were the most frequently recovered microorganism from true-positive and contaminated cultures. This finding supports the work of other investigators (1, 7) studying the significance of coagulase-negative staphylococci isolated from infants hospitalized in a neonatal intensive care unit. Baumgart et al. (1) found that growth of coagulase-negative staphylococci from blood cultures was indicative of sepsis in 26% of infants from whom these bacteria were recovered. If coagulase-negative staphylococci recovered from mixed cultures are excluded, recovery of coagulase-negative staphylococci was indicative of sepsis in 30% of our patients.

Some microorganisms recovered from the blood were always associated with sepsis. Such traditionally regarded pathogens of the newborn period as *Streptococcus agalactiae*, *L. monocytogenes*, *Streptococcus pneumoniae*, and *H. influenzae* type b were found in patients with sepsis only, but so were the yeasts, *Candida* spp., and *Malassezia* spp. Other microorganisms were found only as contaminants: viridans group streptococci, *Bacillus* sp., and aerobic and anaerobic diphtheroids. Contamination rates ranging from 8.7% to as high as 14.1% have been reported for the pediatric Isolator (2, 3, 8). In this study, 16.6% of the blood cultures were judged to be contaminated. Mixed skin flora, coagulase-negative staphylococci, *Micrococcus* spp., and diphtheroids (both aerobic and anaerobic) accounted for 80% of the contaminants. Forty-four percent of all contaminants were coagulase-negative staphylococci.

The density of bacteremia correlated with the clinical assessment in patients with staphylococcal sepsis. We found bacterial densities of >5 CFU/ml of blood in every case of sepsis caused by both coagulase-negative staphylococci and *Staphylococcus aureus*, although the numbers of episodes of *Staphylococcus aureus* sepsis were small. The density of bacteremia did not correlate with the clinical assessment for the traditionally regarded pathogens of childhood, namely, *Streptococcus pneumoniae*, group B streptococci, *L. monocytogenes*, and *H. influenzae* type b.

The number of days to a positive culture also correlated well with the clinical evaluation of sepsis. The majority of microorganisms associated with sepsis were recovered

within 72 h, while contaminants were recovered throughout the 7-day incubation period.

In summary, the 1.5-ml Isolator blood culture system differs from other blood culture methods by providing quantification of the density of bacteremia and by providing information on colonial morphology and differential growth requirements at the time an isolate is first detected. In the event of neonatal instability, determination of the density of bacteremia can aid in differentiating true-positive blood cultures from those that are falsely positive. Our data suggest that the following guidelines may be used to interpret culture results. When more than 5 CFU of coagulase-negative staphylococci or *Staphylococcus aureus* per ml are recovered from blood, sepsis should be strongly considered. When 5 or fewer CFU of these same bacteria per ml are isolated, antibiotics may more reasonably be discontinued as contamination of the culture is likely. Any of the traditionally regarded pathogens, however, should be considered the cause of sepsis unless another cause of instability in the vital signs can be documented. A negative blood culture suggests other causes of neonatal instability.

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