Blood Culture Contamination in a Neonatal Intensive Care Unit in Shiraz, Southwest-Central Iran

Zahra Hashemizadeh a Abdollah Bazargani a Mohammad Ali Davarpanah b

a Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, and
b HIV/AIDS Research Center, Internal Medicine Ward, Namazi Hospital, Shiraz, Iran

Key Words
Blood culture · Contamination · Bacteremia

Abstract
Objective: To measure bacterial contamination rates in blood culture specimens and distinguish sepsis from blood culture contamination in newborn hospitalized patients in a neonatal intensive care unit and to recognize the most commonly isolated bacteria. Materials and Methods: Blood samples of 578 neonates were collected and cultured throughout the year of study (March 2006 to February 2007). Isolated bacteria were identified by traditional biochemical tests. Clinical criteria combined with laboratory data were used to differentiate the contaminated cultures from clinically significant cultures. Results: Of the 578 neonatal blood culture samples, 78 (13.49%) were positive for bacteria, and 49 isolates (8.47%) were classified as contaminants. Pseudomonas aeruginosa and Staphylococcus aureus were the most common isolates from true bacteremia, and Staphylococcus epidermidis and diphtheroids were the most common contaminants. Conclusion: The blood culture contamination rate in our studied neonatal intensive care unit was high. A variety of measures are recommended for reducing the rate of blood culture contamination to avoid undesirable outcomes associated with blood culture contamination.

Introduction
Blood culture is a valuable tool for the diagnosis of septicemia in patients, especially in pediatric wards. Blood culture contamination is a major confusing problem that may happen at various stages of collecting blood and culture [1]. In most instances, the source of the contaminants probably is the existing normal flora on the patient’s skin [2]. On the other hand, some of the leading causes of bacteremia are the frequent contaminants of blood cultures in hospitals, such as coagulase-negative staphylococci and Bacillus spp. [3]. Thus it is sometimes a difficult task to differentiate true septicemia from pseudobacteremia. As a solution to the problem, some clinical and laboratory criteria were described for the diagnosis of true septicemia [4, 5]. Blood culture contamination is also a major concern in medical care centers, especially in teaching hospitals in developing countries, where overpopulation and limited resources are common. Blood culture contamination may lead to a prolonged hospital stay, the administration of unnecessary antibiotics and ordering of additional clinical or laboratory tests that consequently may be responsible for a more than 50% increase in total hospital charges [6, 7].

According to the American Society for Microbiology standards, the blood culture contamination rate should be under 3% [8]. Determining the contamination rate of
Table 1. Bacteria isolated from the blood cultures and classified as true pathogens

<table>
<thead>
<tr>
<th>Number of isolates</th>
<th></th>
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</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>8 (27.6)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>6 (20.7)</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>2 (6.9)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>2 (6.9)</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>2 (6.9)</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>2 (6.9)</td>
</tr>
<tr>
<td><em>Enterobacter agglomerans</em></td>
<td>2 (6.9)</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>2 (6.9)</td>
</tr>
<tr>
<td><em>Salmonella Typhi</em></td>
<td>2 (6.9)</td>
</tr>
<tr>
<td>Viridans streptococci</td>
<td>1 (3.5)</td>
</tr>
<tr>
<td>Total</td>
<td>29 (100)</td>
</tr>
</tbody>
</table>

Values in parentheses denote percentages.

Materials and Methods

From March 2006 to February 2007, at the NICU of Namazi Hospital affiliated with the Shiraz University of Medical Sciences, 578 neonatal blood samples were collected. The neonates were 2–52 days old; their average age was 27 days, 301 were males and 277 females. Blood specimens were requested by physicians for neonates with major septicemic signs such as fever (rectal temperature ≥37°C), leukocytosis (white blood cell count >12,000/mm³) or leukopenia (<4,000/mm³) and tachypnea (>24 breaths/min). The specimens were obtained by nurses or physicians and sent to the laboratory. Following skin preparation with alcohol and then 10% povidone-iodine solution, the skin was allowed to dry for 1 min prior to venipuncture [9]. A blood sample of 2–5 ml was collected with a syringe and then transferred immediately to a blood culture bottle (Darvash Company, Iran). The bottles were incubated aerobically for 1–10 days at 37°C. After 24 h of incubation, the blood cultures were inoculated onto blood agar, eosin methylene blue agar and chocolate agar, and these cultures were incubated for 24 h. Gram staining was performed for every isolate. All isolates were identified by standard biochemical tests [10].

Clinical criteria combined with laboratory data were used to differentiate the contaminated cultures from clinically significant cultures [11]. In addition to the above mentioned major clinical signs of septicemia, the following data were also considered for each patient: platelet count (<150,000/mm³); positive C-reactive protein; elevated erythrocyte sedimentation rate (ESR); the presence or absence of arterial lines or central venous catheter at the time of blood sampling; the results of other concurrent microbiology tests (e.g., cultures of other specimens); the presence of infection in other systems (e.g., urinary tract infection, central nervous system); predisposing factors such as dialysis, cancer or neoplasm and urinary tract anomaly; the number of positive blood cultures for each patient, and the identity of the organism that is the most important predictor in a predictive model of differentiating contamination from bacteremia.

True blood culture was defined as the growth of any bacteria other than coagulase-negative staphylococci, *Propionibacterium* spp., *Micrococcus* spp., *Corynebacterium* spp., *Bacillus* spp., non-hemolytic *Streptococcus* spp. and *Clostridium* spp., which were classified as contaminants in previous studies [5], and the patient continuing to have at least 2 major clinical signs of bacteremia. In cases involving the above microorganisms of doubtful significance, blood sampling was repeated. Clinically relevant cases were only episodes in which there was evidence of clinical manifestations of bacteremia and the same bacteria were isolated in the second or more different blood cultures [12].

SPSS software (version 13; SPSS Inc., Chicago, Ill., USA) was used for statistical analysis. The χ² test was used to compare the groups. p < 0.05 was considered as statistically significant. The study was approved by the institutional ethics committee.

Results

Of 578 samples, 78 (13.49%) were positive for bacteria and 49 isolates (8.47%) were classified as contaminants. The species of bacteria recovered from the blood cultures classified as true pathogens are given in table 1. *P. aeruginosa* and *S. aureus* were the most frequently isolated true pathogens, with frequencies of 27.6 and 20.7%, respectively. Nine *P. aeruginosa* were isolated from blood specimens, and all of them were classified as pathogens except 1 isolate. This isolate belonged to a patient with the signs and symptoms of sepsis and meningitis. In addition to blood culturing, cerebrospinal fluid (CSF) analysis and CSF culture were done for this patient. After 48 h and based on the results of the CSF analysis (polymorphonuclear prominent pleocytosis, increase in protein concentration, decrease in sugar concentration) and CSF culture positive for *E. coli*, we labeled the patient as having meningitis caused by *E. coli*; thus the patient received specific treatment for meningitis and *Pseudomonas* isolate considered as a contaminant. *Staphylococcus epidermidis* and diphtheroids were the most common contaminants, with frequencies of 79.6 and 8.2%, respectively (table 2).

A comparison between those cultures with contaminants and those with pathogens is shown in table 3. There was no statistically significant difference in mean
The blood culture contamination still remains a persistent problem. In recent years, it has been documented that contaminated blood cultures are common [13, 14], highly costly [7, 15] and confusing for clinicians in their selection and administration of antibiotics [16–19]. Most studies on this subject have been reported from developed countries. The dimensions of the problem in developing countries, where the resources are limited and the hospitals are more likely to be crowded, are more essential. The blood culture contamination rate in peripheral blood cultures taken from neonates was 8.9% at our hospital compared to rates of 2–10% reported in the literature [7, 20–23]. Based on reports by the American Society for Microbiology, the rate of blood culture contamination should not exceed 3% [8]. Based on the findings of previous studies, we suggest the following methods for reducing the blood culture contamination rate in NICU: (1) Strict adherence to using dedicated phlebotomists or medical technologists to collect cultures [24, 25]; trained phlebotomy or blood culture teams can decrease blood culture contamination rates [26–28]. (2) Adherence to a sterile venipuncture technique [11]; skin of the patient at the site where the cultures are obtained is the most common source of contamination, so adequate skin preparation is highly recommended before percutaneous collection of the blood specimen [23, 29]. (3) Culture bottle preparation according to the standard practice; the top of culture bottle before inoculating with blood must be disinfected [2, 9, 29]. (4) Obtaining the blood specimen for culture percutaneously instead of by vascular catheters; despite of some good reasons for obtaining cultures from vascular catheters – such as preventing pain, reduction in difficulty of venous access in the neonate population –, there are many undesirable consequences of this practice, including the probability of bacterial colonization in vascular catheters that can be pulled into blood specimens drawn from those sites, which may lead to the ordering of unnecessary diagnostic studies and unnecessary use of antibiotics.

**Conclusion**

The blood culture contamination in our studied intensive care unit was high. We recommend proper equipment, correct techniques and a designated team of phlebotomists as the main strategies for reducing blood culture contamination rates especially in NICU.

**Acknowledgment**

This study was financially supported by grant No. 86-3912 from the Shiraz University of Medical Sciences.
References