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# Significance of Coagulase Negative Staphylococcus Species in Blood Culture

Microbiology Section

SAPNA MALIK, K. RAVISHEKHAR

## ABSTRACT

A retrospective study was carried out from August 2009 to July 2010 to evaluate the rate of contamination in the blood cultures in a tertiary care hospital. A total number of 755 samples were tested for blood culture, 18% of these samples were labeled as contaminants; however, on further scrutiny, this was reduced to 16%.

Determining whether a growth in the blood culture is a pathogen or a contaminant is a critical issue and multiple parameters have to be considered before arriving at a conclusion. Without a gold standard for truly distinguishing the contaminants from the true pathogens, it becomes inherently difficult to interpret the results and to institute preventive measures.

**Key Words:** CONS, Blood Culture pathogens, contaminants

## INTRODUCTION

Septicaemia is a clinical syndrome which is characterized by fever, chills, malaise, tachycardia, hyperventilation and toxicity or prostration, which results when the circulating bacteria multiply at a rate that exceeds their removal by phagocytosis [1]. During septicaemia, organisms are released into the blood stream at a fairly constant rate and also during the early stages of certain specific infections, bacteria continuously present in the blood stream. In patients with undrained abscesses, bacteria are found intermittently in the blood stream. In cases of the transient seeding of blood from a sequestered focus, bacteria are released into the blood approximately 45 minutes before a febrile episode [2].

The mortality rate from septicaemia may be 40% or higher and hence, the timely recovery of bacteria from the patients' blood can have a great diagnostic and prognostic importance. Hence, it becomes mandatory that every precaution must be taken to minimize the percentage of contaminated blood cultures. The critical factors which must be decided by the laboratory include the type of collection, the number and timing of the blood cultures, the volume of blood, the amount and the composition of the culture medium, when and how frequently to subculture and the interpretation of the results [1].

The bacteraemia may be transient, continuous or intermittent and the blood cultures which are taken during this period may give false positive results.

Coagulase Negative Staphylococcus (CONS) is frequently isolated from clinical samples including blood cultures, but their significance is difficult to interpret. CONS, which are often previously dismissed as culture contaminants, are assuming greater importance as true pathogens. The infections which are caused by these organisms involve indwelling foreign bodies and these are increasing as the number of catheters and artificial devices which are being inserted through the skin becomes higher. These infections are characterized by their indolence, but they may necessitate the removal of the catheter or the foreign device.

The resistance of the infecting isolates to multiple antibiotics may further complicate the therapy. The importance of CONS as nosocomial pathogens has prompted more interest in their detailed characterization. A working knowledge of the biology and the antimicrobial susceptibility of these organisms may be necessary to distinguish the infecting from the contaminating isolates and to devise the appropriate therapy. The various infections which are caused by CONS are urinary tract infections, osteomyelitis, native valve endocarditis, bacteraemia in immunosuppressed patients, endophthalmitis after ocular surgery and the infections which are caused by indwelling foreign devices [3].

The blood culture contamination represents an ongoing source of frustration for the microbiologists and the clinicians alike [4]. The ambiguous culture results often lead to a diagnostic uncertainty in the clinical management and these are associated with increased health care costs due to the unnecessary treatment and testing [4]. The contaminated cultures have been recognized as a troublesome issue for decades. The increase in the use of central venous catheters (CVC) and other indwelling vascular devices have complicated the issue even more. The interpretation of the culture results for patients with CVC is challenging, because these individuals are at an increased risk for bacteraemia as well as for culture contamination or colonization of the line [4].

## MATERIALS AND METHODS

Castenedas culture bottles from Himedia (dual performance medium) and the Hi Safe blood culturing system having a solid phase – 20 ml and a liquid phase – 40 ml for adults and 7 ml(solid phase) and liquid phase 20 ml for the paediatric age group were used for doing the blood culture testing. These bottles were incubated at 37ºC for 7 to 10 days and subcultures were performed after 24 hours of incubation (day 1), 72 hours of incubation (day 3), 120 hours of incubation (day 5) and 240 hours of incubation (day 10) on chocolate agar, blood agar and Mac Conkey's agar. These plates were incubated for 18 to 24 hours at 37ºC and the growth, if any, was identified by the standard CLSI procedures [1].

Two samples of blood cultures were obtained from all the groups, except neonates, from which only one sample was obtained.

## RESULTS

A retrospective study was done from August 2009 to July 2010, in which a total number of 755 samples were received. This included 263 adult, 352 paediatric and 140 neonatal samples. 136 of the total number of 755 blood cultures which were done, grew organisms such as Micrococcus, coagulase negative Staphylococcus spp. (CONS) and diphtheroids. Among the 263 adult samples, 28 grew Micrococcus spp., 11 samples grew CONS and 3 grew diphtheroids.

In the paediatric age group of 352 samples, 54 grew Micrococcus spp. 17 grew CONS and 6 samples grew diphtheroids. Among the 140 neonatal samples, 8 samples grew micrococcus and 9 grew CONS. No diphtheroids were isolated from this group of patients.

| Age Group | No. of samples | No. of positive isolates |
|---|---|---|
| Adults | 263 | 42 |
| Pediatric | 352 | 77 |
| Neonates (NICU) | 140 | 17 |
| Total | 755 | 136 |

**[Table/Fig-1]:**Total number of samples received according to age distribution & number of positive isolates.

[Table/Fig 1] – Total number of samples received according to age distribution & number of positive isolates.

| Organisms | Adults > 14 Years | Pediatric < 14Years | Neonates 0-28 Days | Total Number of Isolates |
|---|---|---|---|---|
| Micrococcus | 28 | 54 | 08 | 90 |
| Cons | 11 | 17 | 09 | 37 |
| Diphtheroids | 03 | 06 | 00 | 09 |

**[Table/Fig-2]:** Various organisms isolated from various age groups.

[Table/Fig 2] – Various organisms isolated from various age groups.

| Day of Culture | Number of Isolates |
|---|---|
| Day 1 | 00 |
| Day 2 | 97 |
| Day 3 | 16 |
| Day 4 | 09 |
| Day 5 | 06 |
| Day 6 | 08 |
| Day 7 | 01 |

**[Table/Fig-3]:** Number of positive blood cultures according to days of sub -culture.

[Table/Fig 3] – Number of positive blood cultures according to days ofsub-culture.

| Organisms | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|
| Micrococci | 00 | 64 | 09 | 06 | 06 | 05 | 01 |
| Cons | 00 | 28 | 06 | 01 | 00 | 02 | 00 |
| Diphtheroids | 00 | 05 | 01 | 02 | 00 | 01 | 00 |

**[Table/Fig-4]:** Contaminant organisms according to day wise break up.

[Table/Fig 4]-Contaminant organisms according to day wise break up.

## DISCUSSION

As per our study, the contamination rate of the blood culture was 18 %, while the target rates for the contamination should ideally not exceed 2%-3% [5,6]. The actual rates for the contamination vary widely from institution to institution, ranging from 0.6% to over 6% [4].

Certain organisms which are found to represent the contamination included, coagulase negative Staphylococcus spp. (CONS), Corynebacterium spp., Bacillus spp. other than Bacillus anthracis, Propionibacterium acnes, Micrococcus spp., viridans group Streptococci, Enterococci and Clostridium perfringens [7,8]. However, each of these organisms could represent true bacteraemia with devastating consequences, particularly if they were left untreated due to their misinterpretation as contaminants and hence the determinants of the contamination in the blood cultures is very essential.

Studies which were done in Virginia (USA) and south India also showed that these organisms, particularly CONS, were an increasing source of true bacteraemia, especially in patients with prosthetic devices and central venous catheters [4,9]. Therefore, it is extremely important that certain criteriae are set for determining the contamination in the blood cultures like

1. **Source of the culture** – Percutaneous vs Catheter drawn When the blood cultures which are drawn from catheters are positive, it could indicate three possibilities.

   a. True Bacteraemia
   b. Catheter Colonization
   c. Culture Contamination

   The catheter colonization may or may not progress to cause symptoms of infection or true bacteraemia. 15%–25% of the short term central venous catheters are colonized by CONS and most of them have no evidence of infection and hence a substantial number of patients who have CVC are expected to be positive due to colonization [10]. Sterilizing the catheters prior to the blood collection is more difficult than sterilizing the skin.
2. **Time to positivity (Time to growth)** – Cultures that are positive for more than 3–5 days after incubation are more likely to represent contamination, as the continuous monitoring of the blood cultures to detect the growth advances the time to growth and the sensitivity for detecting the growth can be expected to change. However, some experts say that this should not be relied upon to distinguish the contaminants from the pathogens in the blood cultures [11].
3. **Quantity of growth per culture bottle** – Similar to the quantitation of urine, the quantitation of sputum and catheter related blood stream infections can be done for routine blood cultures. However, a low colony count should not be dismissed as a contamination in a high risk population [11,12].
4. **Number of blood culture sets** – Usually one set of blood cultures involves one aerobic and one unaerobic bottle. A minimum of two blood culture sets per episode should be drawn. However, these two sets are not obtained always. The results of multiple positive cultures may be helpful, but they are still imperfect with respect to the discernment the contamination.
5. **Clinical condition of the patient** – The clinical criteria to detect true bacteraemia from the contamination should also be used. In our study, we found that 6 out of 17 CONS isolates in the paediatric age group were MRCONS and that they all grew

on the 2nd day, except one that grew on 3rd day. Similarly, in adults, 4 MRCONS grew on 2nd day and one grew on 3rd day. In the neonatal isolates, 2 MRCONS grew on 2nd day. However, MSCONS across all the age groups also grew on 2nd day or 3rd day of the culture. Thus, it becomes difficult for the laboratory to issue a report of bacteraemia. In these cases, we correlated the antibiotic sensitivity pattern of the isolates and the clinical condition of the patients, as surveillance by antibiotyping with attention to the multiresistant profile and the warning to clinicians is necessary [9,13,14,15]. Studies which were done in Jamaica, West Indies, also stressed the need for a careful evaluation of CONS which were isolated from the blood cultures before instituting the therapy, to avoid the unnecessary use of antibiotics, especially vancomycin, and The consequent increase of antibiotic resistance in hospitals [15]. Based on the above mentioned criteria, 13 more isolates were reclassified as pathogens, thereby bringing down the rate of contamination from 18% to 16%. The specificity of the blood cultures is directly related to the rate of the false positive results which are increasingly caused by the contamination. Reduction of the contamination rates leads to an improved specificity and a better performance of this test.

The factors which are responsible for the contamination would be:

1. **Skin preparation** – Skin antisepsis cannot entirely prevent the contamination of the blood culture because as many as 20 % of the skin associated bacteria have been found to survive disinfection. These bacteria can be located in the deep layers of the skin or in other structures where antiseptics cannot penetrate. Investigators have found that the median contamination rate was significantly lower in settings where tincture iodine was used (2.1%) as against an iodophore (2.6% p = 0.036). A study found that 0.5 % Chlorhexidine and alcohol had significantly lowered the contamination rates than the standard povidone iodine group (p=0.065) The time which is required for the antiseptic to have a maximum effect is an important consideration eg. The Povidone iodine preparation requires 1.5–2.00 mins of contact time to have a maximal antiseptic effect, whereas tincture iodine requires only 30 seconds. This difference in the time may possibly account for the differences in contamination which were seen in many of the studies. Experts have recommended, although it is controversial, that the culture site should be prepared with 70 % isopropyl/ethyl alcohol, allowed to air dry and that a second preparation of 1-2 % iodine or 10 % povidone – iodine should also be applied [16].
2. **Single needle vs double needle** – This effect has been evaluated by several control studies. All the authors admitted to be having inadequate power to detect the level of difference that was actually observed between the two techniques. A CAP survey of 640 institutions in 1997 concluded that the difference in contamination was not statistically significant [17].
3. **Phlebotomy team** – Dedicated and trained phlebotomy teams have been found to decrease the culture contamination rates [7,18,19].
4. **Preparation of the blood culture bottle** – The rubber stopper on each blood culture bottle is not sterile, despite being covered by the lid, that requires its removal prior to the inoculation. It has been found that institution that prepped (preparation of blood culture bottle) the bottle tops had significantly lower contamination rates 2.3 % than those that did not prepped the bottle tops 3.4% [4].

## CONCLUSION

In our study, we found that out of a total of 755 blood cultures, 123 were contaminants, thus bringing the contamination rate to 16 %. However, we did not have any definite criteria to identify the true bacteraemias.

Despite the progress that has been made in distinguishing the contamination from the true bacteraemia, significant barriers remain. Without a gold standard for truly distinguishing the contaminant organisms from the true pathogens, studies that seek to measure the success of the prevention strategies are inherently limited. Additional research on the value of time to the positivity and the quantity of growth for differentiating the culture contamination from the bacteraemia is necessary. Information technology may have a role in facilitating the detection of the contamination, in assisting in the clinical decision making and enabling better systems for tracking the c ontamination rates both within and between institutions. In the paediatric age group, additional studies are needed for interpreting the results of single blood cultures that grow CONS. Blood culture contamination is a complex challenging problem that requires a multidisciplinary approach. More research is needed to refine these models and to test them.

## NOTE

Coagulase negative Staphylococcus

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**AUTHOR(S):**
1. Dr. Sapna Malik
2. Dr. K. Ravishekhar

**PARTICULARS OF CONTRIBUTORS:**
1. Specialist Microbiology, ESIC Hospital Andheri (E).
2. Professor Microbiology, Pad. Dr. D. Y. Patil Medical College, Nerul, Navi Mumbai, India.

**NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:**
Dr. Sapna Malik
C 1201, Jal Vayu Vihar, A.D.S. Marg,
Powai, Mumbai, India 400076.
E-mail: sapnawadhwan@hotmail.com

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