

Prevalence of Bacterial Contamination in Stored Blood, Experience At Tertiary Care Teaching Hospital.

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Abstract

Background : Though the incidence of Transfusion Transmitted Viral diseases have come down, bacterial contamination of Transfusion products is a long standing problem. The main risk of transfusion related infectious diseases is currently that of bacterial sepsis. The clinical consequences of transfusing bacterially contaminated blood range from minimal or no reaction to fatal septic shock and death. The prevalence of bacterial contamination of whole blood in India is not known.

Objectives: This study was conducted to determine the prevalence and type of bacterial contamination of whole blood, at Rajiv Gandhi Institute of Medical Sciences (RIMS) blood bank, Ongole, Prakasam district of Andhra Pradesh.

Methods: Study was conducted on 100 units of randomly selected stored whole blood. Blood was cultured on different media using standard aseptic precautions, isolates were identified by Gram stain, biochemical reactions, and Sugar fermentation tests.

Results: The prevalence rate of bacterial contamination in stored blood was 11%. The Isolates were *S.aureus*, Coagulase Negative Staphylococcus and Micrococci.

Conclusion: Bacterial contamination in blood suggest, there is a risk of developing infection, particularly in Immunocompromised individuals. Precautions should be taken in donor selection, during blood collection, processing and storage.

Keywords: Whole blood, Bacterial contamination, Sepsis, RIMS, Andhra Pradesh, India.

I. Introduction

As the infection risk for other diseases has decreased due to better donor testing, bacterial contamination has come to the forefront and has become a great concern as a transfusion transmitted disease.^[1] Although the incidence of transfusion associated bacterial sepsis is low, the morbidity and mortality rates are high. Common sources of bacterial contamination include donor skin and blood. Less common sources are the environment and disposables.^[2] The mortality rate from sepsis and toxemia due to bacterially contaminated RBC units is higher than 60%.^[2] Platelets have been the most frequent source of septic transfusion reactions, due to the fact that room temperature storage promotes bacterial growth.^[2] Although the number of contaminated platelet units is much larger, the mortality rate is not as high as it is for RBCs. Contaminated rates in US have been estimated to be 0.2% for RBCs and as high as 10% for platelets. It is estimated that a febrile transfusion reaction caused by contaminated blood occurs once for every 10 to 20,000 units and that a death occurs once for every 6 million units.^[3]

The bacteria implicated in the transfusion of blood and its products are gram negative bacilli such as *yersinia enterocolitica*, *pseudomonas fluorescens* and *pseudomonas aeruginosa*. Other species are gram positive including staphylococcus and streptococcus species.^[4,5,6]

According to CDC,^[7] *yersinia enterocolitica* is the most common isolate found in RBC units followed by the *pseudomonas* species. Together, these two accounted for more than 80% of all bacterial infections transmitted by RBCs. *Y. enterocolitica* retain viability and replicate under RBC storage conditions (between 1^o to 6^oC). Because *Y. enterocolitica* lacks siderophores, growth is enhanced in an iron – rich environment such as stored RBCs.

In a study by Kunishima,^[8] *propionibacterium acnes* (Slow growing anaerobic bacteria), a common isolate of human skin, was the most common bacterial contaminant in RBCs. *Staphylococcus epidermidis* and *Bacillus cereus* (both gram positive) are the organisms most frequently recovered from donated blood and contamination of platelets.^[1]

1.1. Common sources of contamination:

Donor Bacteremia.

Blood collection: Majority of organisms isolated from contaminated blood are part of the normal skin flora and are thought to enter into the venesection needle during venipuncture.^[9,10] It has been

postulated that, despite adequate surface disinfection, viable bacteria remain in the deeper layers of skin, especially skin associated with a scarred phlebotomy site.^[9,11] It has also been shown that S.aureus and S.epidermidis can adhere firmly to human hair despite skin disinfection.^[12]

Blood bag and container damage (or) defect.

Blood processing.

II. Materials and Methods

Study was conducted for a period of 6 months from June 2016 to November 2016 in RIMS Medical College Hospital Blood Bank and Department of Microbiology, Ongole located in Prakasam district of Andhra Pradesh. Blood was collected from eligible voluntary donors (both outdoor blood donation camps and those donated in blood bank) as per drugs & cosmetic act 1945, alongwith written consent. Phlebotomy site was prepared with povidine – iodine scrub and 70 % ethanol. Random sampling of 100 bags of screened stored blood were tested for bacterial contamination.

Blood was mixed before sampling and the tubing was cleaned with 70 % alcohol and cut with sterile scissors. 5 ml of whole blood aseptically inoculated into Castaneda blood culture medium.

After inoculation the bottles were incubated at 37 °C for 7 days with continuous monitoring. If there are any possible signs of bacterial growth, subculture will be done on to chocolate agar, Nutrient agar, MacConkey agar incubated over night at 37 °C for bacterial growth. The organisms are further identified by Gram stain, biochemical reactions and sugar fermentation tests. Repeat isolation of the same organism on subsequent days of sampling was considered as confirmed positive.

Ethical clearance was obtained from Institutional Ethics Committee.

III. Results

Between June 2016 to November 2016, 100 random samples of whole blood were tested for the study. Out of 100 samples tested, 11 were found to be contaminated with various types of bacteria (11 %). Length of storage of the blood ranged from 0 to 5 Weeks. Leading whole blood contaminants isolated were staphylococcus aureus, coagulase negative staphylococcus and Micrococci.

Table1: Level of contamination

No of blood samples tested.	No of bags contaminated (%)
100	11 (11 %)

Table 2: Level of contamination according to type of bacteria

Bacteria Type	Number of blood units contaminated	Percentage
S.Aureus	3	3 %
Coagulase negative Staphylococci	4	4 %
Micrococci	4	4 %

IV. Discussion

Knowledge of the prevalence of bacterial contamination in blood and blood products and their source is important for planning prevention and reduction measures that reduce mortality and morbidity arising from transfusion of contaminated blood and blood products.

This study determined an overall bacterial contamination prevalence of 11 %. Most common organisms isolated were Gram positive bacilli (100 %) i.e S. aureus, coagulase negative staphylococcus and Micrococci. This prevalence is comparable to studies done in Ghana and Ethiopia. The possible explanation for high prevalence in this study could be due to more blood collection from outdoor camps, where proper disinfection is not possible. Moreover the Staphylococcus aureus, Coagulase Negative staphylococcus, Micrococci isolated in this study are part of the normal skin flora and therefore can easily be introduced in the blood, if disinfection is not carried out properly. We also observed that most of the contaminated samples falls into 3rd week of storage.

In a study carried out in Ghana, whole blood accounted for the greatest percentage (13 %) of contamination.^[13] The bacteria isolated were coagulase negative Staphylococcus, S. aureus and Bacillus spp, while Gram negative bacteria included yersinia enterocolitica, citrobacter freundii, E coli, Pseudomonas aeruginosa and klebsiella pneumoniae. These bacterial species have also been implicated in studies carried out

in the United States , Nigeria, Japan and France . This clearly indicating that they are a real threat to blood supplies world wide. ^[3,14,15,16]

High prevalence rate of 12.5 % was also reported in Ethiopia. ^[17] The organisms isolated in this study were Gram positive(S. aureus, Coagulase Negative staphylococcus, S. pneumonia) and gram negative (K. pneumoniae, S.typhi and E coli) accounting for 66.7 % and 33.3 % respectively.

Prevalence rates are very low in US (0.2 %) ^[3] and (0.1 %) in France. ^[16] The possible explanation for this low prevalence in developed countries could be blood transfusion laboratory setup and implementation of more rigorous screening procedures.

Table3: Comparison statement.

Name of the country	Author	Bacterial contamination.
Ethiopia	Esmael A et al	12.5 %
Ghana	Adjei AA et al	13 %
U.S	Kuehnert, MJ, et al	0.2 %
Present study (India)		11 %

V. Conclusion and Recommendation

Bacterial contamination of stored whole blood in our study was 11 %. Staphylococcus aureus, Coagulase Negative Staphylococcus and Micrococci were the major isolates. As they are commensals of normal skin and can adhere firmly to human hair, proper arm preparation for phlebotomy is of paramount importance. Therefore preventive measures like comprehensive donor selection and screening, implementing two stage skin disinfection method, diversion of first 10 to 40 ml of donor blood can significantly reduce the bacterial load in the collection bag. Before issuing one should also inspect for discoloration and clots in the unit which strongly indicate contamination.

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