

ORIGINAL RESEARCH

Evaluation of bacterial contamination of the blood and blood components at a Tertiary Care Blood Centre in North India

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ABSTRACT

Background and Aim: Blood should be free from any kind of pathogen or contamination for safe and effective blood transfusion services. Bacterial contamination of blood products may cause sepsis or transfusion reactions. Transfusion associated sepsis is a major health problem which has been overlooked. So this study has been carried out to evaluate the potential contaminants in blood and blood components. **Materials & Methods:** This retrospective study was done in Department of Immunohaematology and Blood Transfusion, Government Medical College, Jammu, India for five years from Jan 2018- Dec 2022 to evaluate the frequency of bacterial contamination of blood and blood components. 5ml of blood was collected from tubing of Whole blood, packed red blood cells, platelet units using a needle and syringe under a laminar air flow taking care of all aseptic precautions and dispensed in nutrient liquid broth media and then sent to Microbiology Department for culture sterility testing. Subcultures were done in blood culture media and growth was identified using standard microbiological methods. **Results:** A total of 533 samples were taken 71 whole blood units; 279 packed RBC units and 183 platelet units and sent to Microbiology Department for sterility testing. Out of 533 units 23 (4.3%) were found to be positive for bacterial contamination. In the present study, whole blood had (5.6%) had highest prevalence of bacterial contamination followed by random donor platelets (4.3%) and Packed red blood cells (3.9%). Main bacteria found were Coagulase negative Staphylococcus, Klebsiella spp., Aerobic Spore Bearers etc. **Conclusion:** This study concludes that bacterial contamination of blood and blood components is common in developing countries including India. There is an urgent need for continuous monitoring, strict quality control program and strategies like proper donor selection, good phlebotomy practices and good manufacturing practices in blood centres to limit bacterial contamination in order to maintain and enhance blood safety.

Keywords: bacterial contamination, blood components, packed red blood cells, platelets, whole blood, etc..

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INTRODUCTION

BACKGROUND & AIM

Blood and blood components are a potential source of infection by various organisms. With the emergence of 3rd and 4th generation enzyme linked immunosorbent assay (ELISA) testing and introduction of Nucleic acid testing for transfusion transmitted infections, the rate of viral transmission through blood and blood products has reduced drastically (1). However, there is definite risk of bacterial contamination through blood and blood products and it has emerged as the residual but major threat of transfusion transmitted disease. (2) Bacterial

contamination of blood components may cause transfusion associated sepsis and is the most frequently reported cause of mortality after haemolytic reaction, which accounts for 10% of transfusion fatalities. This is because contaminated units may contain large numbers of virulent bacteria as well as endotoxins that are considered to be fatal to the recipient (3). The rate of bacterial contamination of RBC unit ranges from 0-0.2% while for platelets, it varies from 0-10%. It is estimated that 1 in 1000-3000 platelet units are bacterially contaminated of which 1 in 25000 transfusions resulted in clinical sepsis. (4) Approximately 57% of all transfusion-transmitted

infections and 16% of transfusion-related deaths have been associated with bacterial contamination (5). In the United States, bacterial contamination of blood accounts for as many as 500 to 750 deaths annually (6), and between 1986 and 1991, bacterial contamination accounted for 15.9% of all transfusion-related fatalities (3). In France, the 'Haemovigilance' surveillance system of the French Blood Agency attributed 18 deaths between 1996 and 1999 to bacterially contaminated blood components (7). The main bacteria involved most often are Gram-positive skin commensals such as *Staphylococcus epidermidis* and *Bacillus cereus*. Asymptomatic donors with transient bacteraemia are presumed to be responsible for most cases of gram negative bacterial contamination like *Klebsiella* spp., *Serratia* spp., *Escherichia coli*, *Acinetobacter* spp., *Enterobacter* spp., *Providencia* spp., *Yersinia enterocolitica*, etc. (8) For effective blood transfusion services, blood should be collected and processed following aseptic precautions. (9) Possible sources of bacterial contamination are well known. The contamination may occur during phlebotomy as a result of incomplete disinfection or skin core removal by the collection needle. Collected blood may also be endogenously contaminated as a result of an asymptomatic bacteraemia in the donor. It can also occur due to improper processing or during storage. (10) It has been observed that bacterial contamination in developed countries has been reduced due to systematic and comprehensive donor selection, improved skin disinfection and good storage conditions. However maintaining proper disinfection, clean environment, comprehensive screening of donors is still a challenge in developing countries like India.

The current study aimed at evaluating the bacterial contamination of the blood and blood components at a tertiary care blood centre in North India.

MATERIAL AND METHODS

STUDY PLACE

This retrospective study was done in the Department of Immunohematology and Blood Transfusion, Government Medical College Jammu for five years from Jan 2018 - Dec 2022 to evaluate the frequency of bacterial contamination of blood and blood components.

NO. OF SAMPLES

A total of 533 stored blood and blood component units comprising of 71 Whole Blood, 279 Packed Red Blood Cell and 183 Platelet Concentrate units were

sent for sterility culture testing and were included for the study.

SAMPLING METHOD

A 5ml of blood sample was collected from tubing of each blood component viz. Whole Blood, Packed Red Blood Cells and Platelet Concentrate units. The sampling was under a laminar air flow following all aseptic precautions, the tubing of blood component unit bag was first stripped using a stripper and the tubing was disinfected using methylated spirit/alcohol followed by collection of 5ml blood component sample by puncturing of the blood bag tubing with a sterile needle and syringe. The 5ml sample so collected was then dispensed in nutrient liquid broth media and sent to Microbiology Department for sterility culture testing.

CULTURE TESTING

Samples were incubated at 37°C in the nutrient liquid broth media for initial 24 hours followed by subcultures in Blood agar and MacConkey agar culture plates and growth was evaluated after incubation at 37°C for 24-48 hours. Positive bacterial growths were identified by various methods like Gram staining, biochemical testing and other appropriate microbiological techniques. Repeat sampling and culture sterility testing was done for the blood component units found to be positive for bacterial growth. Culture positive blood unit and their components units were discarded as per hospital biomedical waste management protocols.

STATISTICAL ANALYSIS

The bacterial contamination of blood and blood components were calculated as rates and were expressed as percentages and proportions.

ETHICAL CLEARANCE

Ethical Clearance was taken from the Institutional Ethical Committee for conducting the study.

RESULTS

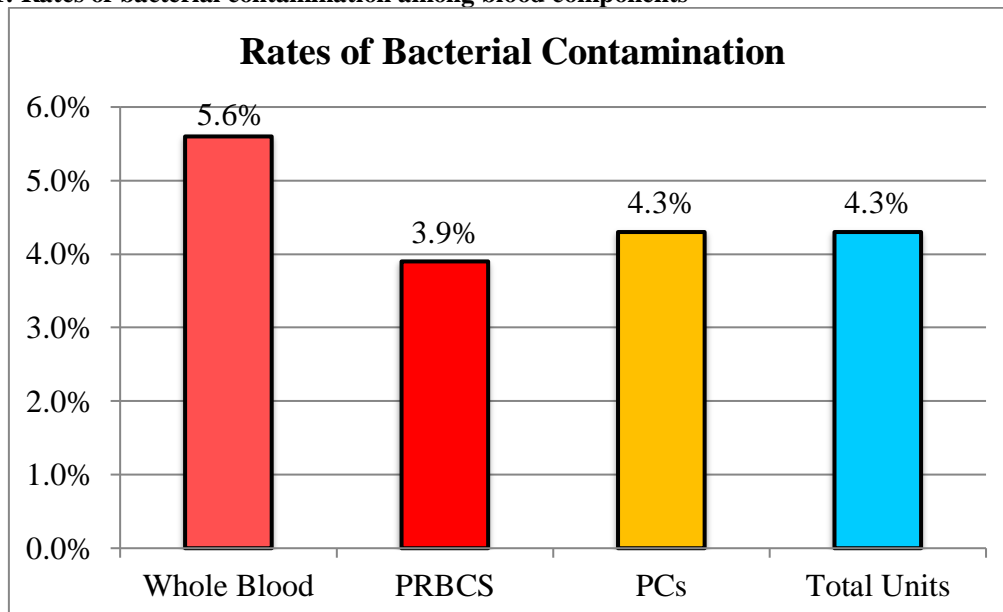
A total of 533 samples were taken from 71 (13.4%) whole blood units; 279 (52.3%) packed RBC units and 183 (34.3%) platelet units being sent to Microbiology Department for culture sterility testing as part of routine quality control of blood & its components. Out of total 533 blood units sent for testing, 23 (4.3%) were found to be positive for bacterial growth with rates of bacterial contamination in Whole Blood, Packed Red Blood Cells and Platelet Concentrate Units being 5.6%, 3.9% and 4.3%, respectively as shown in Table 1 & Figure 1.

Table 1: Rates of bacterial contamination among blood components

Blood component type	No. of blood units tested	Bacterial contamination blood units n, (%)
Whole Blood	71	4 (5.6%)
Packed Red Blood Cells	279	11 (3.9%)

Platelets Concentrate	183	8(4.3%)
Total blood units	533	23 (4.3%)

Figure 1: Rates of bacterial contamination among blood components

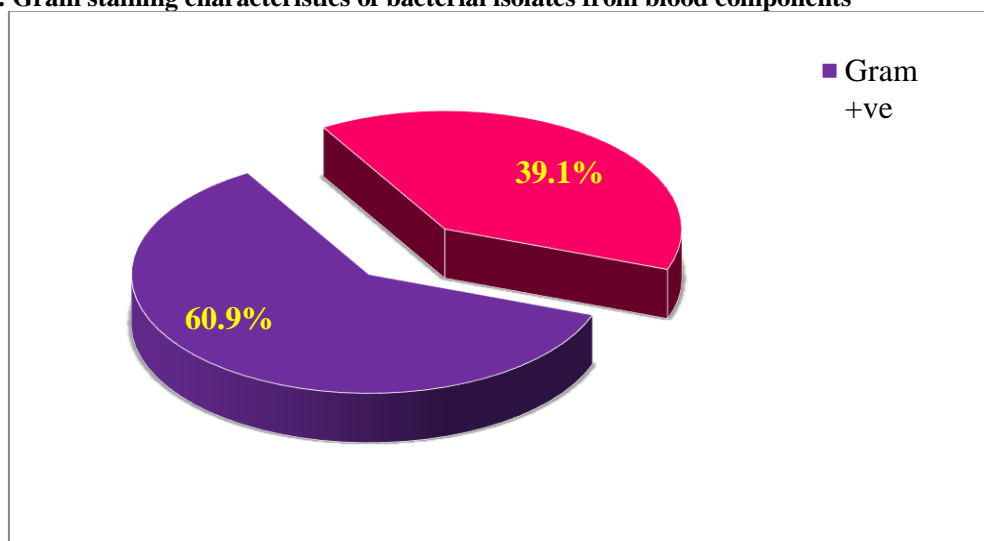


Out of total 23 bacterial isolates, 60.9% (14) and 39.1% (9) were gram positive and gram negative bacteria, respectively as shown in Table 2 & Figure 2.

Table 2: Gram staining characteristics of bacterial isolates from blood components

Gram Positive (n=14; 60.9%)			Gram Negative (n=9; 39.1%)		
Bacteria	Frequency	%	Bacteria	Frequency	%
Coagulase Negative Staphylococci (CONS)	4	28.6	Klebsiella spp.	4	44.4
Aerobic spore bearers	4	28.6	Citrobacterfreundii	2	22.2
Enterococci spp.	3	21.4	Pseudomonas spp.	2	22.2
Micrococci spp.	2	14.2	E. coli	1	11.2
Staphylococcus aureus	1	7.2			

Figure 2: Gram staining characteristics of bacterial isolates from blood components



The type of bacterial contamination with respect to day of storage 0 to 9 days is shown in Table 3. Gram positive bacteria were mainly isolated early phase of storage and gram negative weremainly isolated in later phase of storage.

Table 3: Storage time and type of bacterial contamination of stored blood and blood components

Day of storage	Isolated bacteria (n=23)	Frequency (n)	Percentage (%)
2-5 days	Coagulase negative Staphylococci	4	17.4%
2-8 days	Klebsiella spp.	4	17.4%
2-8 days	Aerobic spore bearers	4	17.4%
3-6 days	Enterococci spp.	3	13.0%
5-6 days	Citrobacterfreundii	2	8.7%
8-9 days	Escherichia coli	1	4.35%
1-3 days	Micrococci spp.	2	8.7%
6-8 days	Pseudomonas spp.	2	8.7%
2-5 days	Staphylococcus aureus	1	4.35%
	Total	23	100%

DISCUSSION

Though blood banks have standard operating procedures to minimize bacterial contamination of blood and blood components, there are reports of bacterial contamination from various blood centres with varying rates. This retrospective study was done at Blood Centre, Government Medical College Jammu, India in which five year data from Jan 2018- Dec 2022 was evaluated to determine the frequency and type of bacterial contamination of blood and blood components.

In the present study, overall prevalence of bacterial contamination among all blood components was found to be 4.3% which was lower in comparison to bacterial contamination prevalence of 9-12.5% reported in similar studies from India & other developing countries.(10,11,12). In contrast, prevalence of bacterial contamination of blood components reported in studies from developed countries ranged from 0.19%-0.2% which was quite low as compared to present study.(13,14,15,16) Low prevalence in developed countries may be attributed to strict blood donor screening procedures and good phlebotomy practices during blood collection and

efficient infection control protocols and good manufacturing practices during blood component preparation and processing.

In the present study, highest rate of bacterial contamination was seen in Whole Blood (5.6%) followed by Platelet Concentrates (4.3%) and Packed Red Blood Cells (3.9%). Similar study done in India has reported platelet concentrates (5.38%) as major component to be contaminated than whole blood (1.9%), PRBC units were found to be sterile.(2) Another study from India has reported Packed Red Blood Cells (21.21%) as major component to be contaminated by bacteria followed by Platelet Concentrates (10.41%) and Whole Blood (9.09%).(12) Studies done in Ethiopia and Ghana have similar results with Whole Blood as major component to be contaminated by bacteria.(10,18) As opposed to the current study, a study done in Zimbabwe showed the highest contamination rate in Platelet units (10.3%) followed by Packed Red Blood Cells (1.3%) while no bacterial contamination was seen in Whole Blood.(5) The prevalence of bacterial contamination in the present study is compared with various studies in Table 4.

Table 4: Comparison of bacterial contamination of present study with various studies

Study	Country	Bacterial Contamination (%)
Present Study	India	4.3%
Sehgal S <i>et al</i> ² , 2022	India	2.54%
Gupta Set <i>al</i> ¹² , 2018	India	21.50%
Esmael Aet <i>al</i> ¹¹ , 2014	Ethopia	12%
Adjei AAet <i>al</i> ¹⁰ , 2009	Ghana	9%
Makuni Net <i>al</i> ⁵ , 2015	Zimbabwe	3.1%
Love E Met <i>al</i> ¹³ , 2002	United Kingdom	0.19%
Kuehnert M Jet <i>al</i> ¹⁴ , 2001	United States of America	0.2%
Perez Pet <i>al</i> ¹⁶ , 2001	France	0.1%
Dickson Met <i>al</i> ¹⁵ , 2013	New Zealand	0.04%

Studies done in Japan, United States and France have reported Platelets units as the major component with bacterial contamination.(14, 16, 17)

Studies have shown that bacterial contamination of Platelet Concentrate to be more common than Packed Red Blood Cells because of the storage temperature being 20 to 24°C for platelet units which is favourable

for growth of micro-organisms. (19) Food and Drug Administration (FDA) regulations currently limit platelet storage to 5 days at room temperature. (20)

Both gram positive (60.9%) (*Staphylococcus aureus*, *CONS*, Aerobic spore bearers, *Enterococci* spp., *micrococci* spp.) and gram negative (39.1%) (*Pseudomonas* spp., *E. coli*, *Citrobacter freundii*) bacteria were isolated in our study with *CONS* being the most predominant bacterial isolate (17.4%) which is in concordance with the study done by Agzie *Met al*, 2019. (21) Gram positive bacteria were isolated during initial days of storage while gram negative bacterial isolates were detected as the storage period advanced. Findings in this study were in agreement with the studies Gupta *Set al*, 2018, Bolarinwa *RA et al*, 2011, and Sharma *R Ret al*, 2004. (12, 22, 23) This may be due to the fact that gram positive isolates being normal commensals or transient skin flora, contamination is thought to be primarily during phlebotomy due to improper disinfection techniques and isolated during initial days soon after donation while gram negative organisms are detected after a period of proliferation during storage.

The use of sterile disposable blood bags, closed systems and refrigeration has helped decrease, but not eliminate the bacterial contamination. (24) As result of advancements in transfusion transmissible viral marker testing including nucleic acid testing, transfusion-related sepsis is emerging as major cause of concern for blood safety and transfusion recipient morbidity and mortality especially in developed countries while transfusion transmissible infections still poses considerable transfusion risk in developing countries due to lack of advanced viral marker testing facilities. Risk of bacterial contamination is still undermined in developing nations.

Sterility testing of blood and blood components, as part of a quality assurance program, is another essential strategy to further reduce the risk and is being practiced in India. Blood banks and laboratories are employing various methods to detect bacterial contamination of blood components, such as visual inspection (changes in colour and consistency in red cell concentrates, absence of swirling in platelet concentrates) microscope examination of stained samples, evaluation of metabolic parameters during storage (pO₂, pCO₂, pH, glucose levels), endotoxin detection, DNA/RNA studies, immunochromatography, use of the automated liquid media culture systems Bact/ALERT 3D (Organon Teknika, Durham, NC, USA) and BACTECTM 9240 (Becton Dickinson, MD, USA) (25, 26, 27). Pall eBDS is an enhanced bacterial detection method based on the measurement of oxygen consumption by organisms and is highly specific. The Pall eBDS detection system is unable to detect anaerobic bacteria. (28)

The main sources of bacterial contamination are either exogenous or endogenous. The exogenous causes are predominantly due to inadequate disinfection procedures or due to the introduction of skin

commensals from the skin-plug which is punched out by the needle at the time of blood donation. This can be reduced by diversion of few aliquots of blood into diversion pouches. Diversion pouches have made mandatory by FDA for Whole Blood collection bags used for platelet preparation. It has been observed that the rate of contamination by first 10ml is 3%. (29) Available data has shown that diversion of initial blood flow reduces the concentration of bacteria by 90%. (30, 31) The endogenous causes are related to the asymptomatic bacteraemia or a pre-existing infection in the blood donor.

Strategies have been implied to reduce bacterial contamination in blood components like leuco-filtration, gamma irradiation or viral inactivation. Leuco-filtration is a current practice in many countries. It has been documented that the red cell filters have a better efficacy in removing bacteria than filters used for platelets. (32) Pathogen inactivation methods for platelets, based on the combination of the synthetic psoralen amotosalen-HCL with UVA illumination (INTERCEPT) is already in practiced and licensed in Europe. (33)

CONCLUSION

This study concludes that bacterial contamination of blood and blood components is common in developing countries including India. This warrants continuous monitoring, strict quality control program and strategies like proper donor selection, good phlebotomy practices and good manufacturing practices in blood centres to limit bacterial contamination in order to maintain and enhance blood safety.

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