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Original Article

Assessment of Bacterial Contamination in Stored Platelet Concentrates at Standard Temperature in a Tertiary Care Hospital

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Abstract:

Background: *Over the last two decades, contamination of blood product with pathogenic bacteria has become a major concern in blood transfusion practice, being recognized as the second leading cause of transfusion-related fatalities after ABO incompatibility. Platelet transfusions pose a higher risk of bacterial sepsis than red cell concentrates due to their storage conditions at standard temperature, suspension in plasma, and storage bags allowing oxygen diffusion. To detect incidence and type of micro bacterial contamination of platelet concentrates (PCs) stored at standard temperature.*

Materials & Methods: *This cross-sectional study was implemented at a tertiary care university hospital in Bangladesh involving 44 healthy blood donors. With all available aseptic precautions, each donor provided 450 ml of blood into a CPDA-1 anticoagulant-containing blood bag system. Collected whole blood was promptly cooled to 20–24°C within two hours and then processed for separation into components. Platelet concentrates were obtained through centrifugation. Each Platelet concentrate was maintained at standard temperature which is 20°C to 24°C with consistent, mild agitation throughout the 5-day storage period. Five ml samples were collected from each platelet concentrate bag on day 0, 3, 5 and culture sensitivity was done in the Dept of Microbiology. Statistical analysis was conducted using SPSS-22 software.*

Results: *Most (63.6%) of the blood donor participants' age belonged to 21-30 years and the male female proportion was almost 3.9:1. The most common blood group (38.6%) among blood donors was B (+ve). Samples collected from each platelet conc. bag on day 0, 3 and 5 showed no change in physical appearance or color of the component, and no bacteremia was detected in any of the samples.*

Conclusion: *No bacterial contamination was observed in this study, and it reflects the institute's best efforts to adhere to the required practice guideline for a satisfactory storage of PC at 20°C to 24°C.*

Keywords: Stored blood, Bacterial contamination, Platelet concentrate, Blood culture.

Introduction:

Platelets are small, disc-shaped, anucleate blood cells measuring about 3–5 μm in diameter. They originate from bone marrow megakaryocytes and carry ABO antigens on their surface.[1] Playing a crucial role in hemostasis, platelets form the initial plug that supports fibrin clot development and release various cytokines and growth factors.[2] Platelets express some antigens which are important in transfusion medicine like A and

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B red blood cell antigens, class I human leukocyte antigen (HLA), and platelet-specific antigens like human platelet antigen (HPA) on their surface.[3]

The therapeutic benefit of platelet transfusion was first noted in 1910. A great physician William W. Duke documented three thrombocytopenic patients who experienced reduced bleeding times after receiving fresh whole blood containing platelets.[4] Therapeutic platelet transfusion has a vital role in the support of surgical, hematological, cancer and transplant patients.[5,6] Platelet transfusion has undergone many changes since its inception over 40 years ago. Until the late 1960s, platelets were transfused only in whole blood.[7] Today, platelets are separated from the whole blood as concentrate for specific component transfusion therapy. The demand for platelet concentrates has risen constantly over recent years, mainly because of an increasing number of hematological and oncological patients requiring repeated platelet transfusions.[8]

Common viruses which transmit through transfusion are hepatitis C virus (HCV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV). By the execution of standard procedures for donor selection and maintaining screening procedure strictly transmission of these viruses has been reduced.[9] Bacterial infection due to transfusion through platelet concentrate is a major source of bacteremia. It is associated with significant morbidity and mortality rates.[10] A definitive relationship between contaminated platelet transfusion and the occurrence of fetal sepsis has not yet been established. Although probable death resulting from transfusion of platelet contaminated with bacteria occurs at a rate of 1 per 500,000.[11] Besides, the contamination rate for platelets is estimated at 16.6 per 100,000 donations and a current estimate suggests that one death occurs in 7,81,936 transfusions.[12]

Bacteria can proliferate in blood due to their ubiquitous nature. As platelets are stored in standard temperature, they are prone to contamination by bacteria.[10] This optimal storage condition provides a conducive environment for bacterial growth.[12] Numerous studies have reported that platelet concentrates are predominantly contaminated with bacteria.[11]

There are many sources of bacterial contamination which is difficult to identify. Presence of bacteria in circulation of blood donor, bacterial infiltration during collection and processing of blood are the possible cause of bacterial contamination. In case of platelet associated sepsis most of the cultural report shows that majority of organism are *Staphylococcus aureus and Staphylococcus epidermidis*. These bacteria are normal flora which might be contaminate platelet during donation process. Despite skin cleansing, contamination rates are observed within 2 to 6%. So, it may be virtually not possible to disinfected human skin completely.[12] In Bangladesh, the demand for PC has consistently increased due to the growing number of hematological and oncological patients requiring repeated transfusions of such product.

Ideal storage condition for PC can be maintained in a Helmer shaker incubator with gentle, continuous agitation at 20°C to 24°C for 5 to 7 days. To reduce the risk of bacterial contamination, AABB standards advise using aseptic techniques during blood collection and an enclosed system for preparing platelet concentrates, ensuring they are free from bacterial contamination. This approach is critical for maintaining the safety and efficacy of blood transfusions. Bacterial sepsis, though rare, can result from the transfusion of contaminated platelets, leading to serious complications.[13] This study was aims to detect any bacterial contamination in stored PC, which can pave the way for the establishment of standard operating procedures which can ensure safe platelet transfusions

Materials and Methods:

This cross-sectional study was conducted involving platelet concentrate prepared from healthy donors' blood in the Department of Transfusion Medicine, Bangladesh Medical University (BMU) Dhaka, Bangladesh from May 2022 to July 2023. A total of 44 healthy donors who fulfilled the eligibility criteria[14] were selected for whole blood donation from where PCs were prepared. Apheretic platelet donations were excluded from the study.

Healthy donors were identified by fulfilling the following criteria which are selected and authorized by World Health Organization and institutional standard operating procedure.[14,15] Donor should be fit mentally and physically and should not have any history of having multiple sex partners, drug-addiction or a jail inmate. Weight should not less than 45 kg and age limit should be between 18 to 60 years. Pulse rate of Donor should be in normal range which is 60 to 100 per minute with regular rhythm. Blood pressure should be within normal range. In case of systolic blood pressure normal range is 100–140 and diastolic blood pressure is 60–90 mm of Hg. The interval between successive whole blood donations should be at least 12 weeks for males and 16 weeks for females. A minimum deferral period of 4

weeks is also required before whole blood donation following an apheresis platelet procedure. For male, the minimum accepted haemoglobin level is 12.5 g/dl and for female, haemoglobin level is 11.5 gm/dl. Any skin disease especially at the site of phlebotomy should not be considered for donor. Donor should not have any acute respiratory distress, any puncture site or scar mark on arms or forearms and screening test should be negative for HIV, HBV, HCV, malaria, and syphilis.

The study began by thoroughly explaining its aims and objectives to donors and obtaining written informed consent from each of them for utilizing PCs for the study purpose, prepared from their whole blood donations. Donors were interviewed face-to-face, and comprehensive data including age, sex, blood group, history of previous blood donations, time since last meal, and any drug usage were meticulously recorded based on national blood transfusion guidelines. All information was carefully documented on a data collection sheet. Donor eligibility was confirmed through physical examination measuring weight, checking different parameters like anemia, clinical vital sign comprising pulse, blood pressure, body temperature, respiratory rate etc. Besides, lab parameters such as, hemoglobin percentage and screening tests for HBV, HCV, HIV, VDRL, and syphilis were done to ensure overall donation as well as transfusion safety. The triple pack system was utilized for collecting and storing different blood components. Platelet concentrate bags were made of UPX80 (JMS, Japan) 1-L platelet storage PVC. Each healthy donor donated 450 ml of blood into a primary CPDA-1 anticoagulant-containing primary bag of triple blood bag system. Phlebotomy involved a two-step disinfection process following World Health Organization protocols: swabbing the antecubital fossa site with 7% povidone iodine for 30 seconds followed by 70% isopropyl alcohol for another 30 seconds. After collection, whole blood units were promptly cooled to 20-24°C within 2 hours and processed for component separation. Platelet concentrates were prepared using the platelet-rich plasma (PRP) method. The bags were balanced and centrifuged at 2000 x g for 3 minutes at 22°C, then the PRP was expressed into satellite bags, followed by a second centrifugation at 5000 x g for 5minutes at 22°C to separate platelet-poor plasma, thus obtaining PCA portion of the PC (5 ml) was sampled for culture each day for three consecutive days; day 0, day 3, and day 5 of storage, bacterial culture performed using conventional methods in the Microbiology Department of BMU. PC were maintained in a Helmer shaker incubator with gentle, continuous agitation at 20°Cto 24°Cthroughout the study duration which is 5 days storage period for each bag.

In this study, random blood group donors who are directed and non-remunerated were selected as study participants using the inclusion and exclusion criteria. Detailed information regarding the study was provided to all participants. Then, before enrollment written informed consent was obtained from each participant for utilizing PCs for this study purpose prepared from their whole blood donation. The study was not involving any additional investigation that may cause any financial burden or added risks to the participants.

“Institutional Review Board” of BMU approved this study. All the data which were collected from the donors and reports of the laboratory tests were exclusively available to investigators, regulatory authority and Institutional Review Board (IRB) to ensure confidentiality. The identities of the participants were kept anonymous during data analysis and publication of the results.

All relevant data was compiled on a master chart first and then statistical analysis of the result was done by using the Statistical Package for Social Science (SPSS) version 22.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results:

The average age of the participating blood donors is 27.8 ±6.7 years varied from 19 to 51 years and the distribution of age is shown in table 1.

Table 1: Distribution of the study participants by demographic characteristics (N=44)

Demographic characteristics Age (years)	Number of participants (Percentage)
<20	4 (9.1)
21-30	28 (63.6)
31-40	10 (22.7)
41-50	1 (2.3)
>50	1 (2.3)

The majority of participants in the sample were male, accounting for 79.5%. Sex distribution among the participants is shown in figure 1.

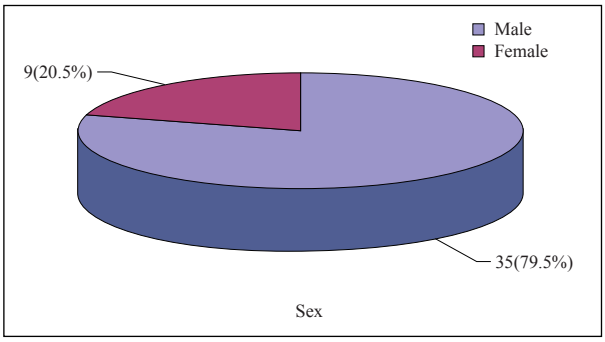

Figure 1: Distribution of the study participants by sex

The most common blood group among the study samples was B (+ve) which is 38.6% and O (-ve) blood group is the least represented. The distribution of blood groups is shown in table 2.

Table 2: Distribution of the study participants by blood group (N=44)

Blood group	Number of participants (Percentage)
B (+ve)	17 (38.6)
O (+ve)	14 (31.8)
A (+ve)	10 (22.7)
AB(+ve)	2 (4.5)
O (-ve)	1 (2.3)

Table 3 presents data related to physical appearance of the samples collected (5 ml each day). No physical alternation or change of color was observed in any of the samples.

Table 3: Distribution of stored platelet concentrates by sample color on Day 0, 3 and 5 (N=44)

Day	Sample amount	Sample Color	Number of Sample (Percentage)
Day 0	5 ml	Yellow	44 (100)
Day 3	5 ml	Yellow	44 (100)
Day 5	5 ml	Yellow	44 (100)

Table 4 showing the data on the presence of bacteria over the storage period of PC which indicates there was no bacteremia in any of the samples.

Table 4: Distribution of stored platelet concentrates by presence of bacteria (N=44)

Presence of bacteria	Day 0	Day 3	Day 5
	N (%)	N (%)	N (%)
Yes	0 (0.0)	0 (0.0)	0 (0.0)
No	44 (100.0)	44 (100.0)	44 (100.0)

Discussion:

In this current study, it was observed that the study participants primarily consist of young adults. A previous study[16] found maximum (58.5%) donors' age belonged to 20-29 years and the age of the blood donors ranged from 19 to 45 years with mean age was 28.2±6.3 years, which is in close resemblance with the present study. Other studies[17,18] similarly found that the majority of donors were aged between 20 and 29 years.

This study revealed that the majority (79.5%) of participants were male, mirroring the findings of[16] who reported 83.0% male and 17.0% female donors. The dominance of male donors aligns with the observations of previous studies.[17,18]

In the recent study, it was noted that the most prevalent blood group among the donor is B positive, accounting for 38.6% of participants, followed by O positive at 31.8%; A positive at 22.7%, AB positive at 4.5%, and O negative at 2.3%, representing the least frequency. Likewise, a study by Quader observed a higher frequency of blood group B, at 35.07%, followed by 33.02% for blood group O, 23.86% for blood group A, and 8.05% for blood group AB, Rh-D positivity was observed in 97.04%, while Rh-D negativity accounted for 2.96%.[19] Another study also found B positive to be the most common blood group, at 34.3%.[17]

In this study, the culture sensitivity indicated presence of no bacteria in the samples collected on Day 0, Day 3, and Day 5 which is different from a similar study conducted in Bangladesh where they reported bacteremia of 5.7%.[17] In other studies, conducted in Ghana, Egypt and Zimbabwe, rate of bacterial contamination in PCs were respectively 9.09% (20), 17.9%[21] and 10.3%[22] which is relatively higher than our present study finding. It may be due to not maintaining proper donor selection and inappropriate storage conditions. On the contrary, in Pakistan 1%[23] and in Iran

0.02%[24] bacterial contaminations was found in stored platelet concentrates which is relatively low as they maintained proper donor selection, standard aseptic precaution and ideal storage condition.

The study revealed that the color of samples collected from the stored PC son day 0, 3 and 5 was yellow and the physical properties remained same. Presence of bacteria may alter the color of sample due to metabolic activities and lack of color change is a physical indicator of absence of bacterial contamination in stored PC.

Factors contributing to contamination include inadequate disinfection, repeated needle insertions during donation presence of donor infections and poor storage of blood product. Sub-Saharan Africa reports higher rates of contamination than the developed countries.[21,25-27] Variations in healthcare systems may affect these rates. Understanding these factors and regional differences is vital for creating prevention strategies. Continued research and developing proper infection control strategies are key to safe, high-quality blood supplies. The global rate of infiltration of bacteria in blood component is not precisely known, but studies offer some insight. For example, contamination rates in the USA, UK, and France are 0.2%, 0.15%, and 0.1% respectively.[28,29]

The variation in prevalence observed in different studies may be attributed to inadequate infrastructure and adherence to infection prevention standards. Furthermore, recent advancements in screening tests of donor blood for virus may have overshadowed the necessity for screening for bacteria.[30]

Though variations in sample size, culture methods, and platelet concentrate preparation and storage procedures may impact the identification of the prevalence of bacterial contamination, this study highlights successful prevention of bacterial contamination in stored PC at 20°C to 24°C. The institute's extensive testing, likely including regular microbiological checks, allows for early contamination detection and prompt resolution, safeguarding patient health. Astringent sanitation protocols, aseptic techniques, and equipment disinfection contribute to a sterile environment, preventing bacterial contamination in platelets. This is crucial, as contamination poses serious risks to transfusion recipients. The institute's dedication to preventing bacterial contamination in platelet stored at 20°C to 24°Cthrough careful storage, comprehensive testing, and rigorous cleanliness supports required practice compliance, thus maintaining overall transfusion safety and better patient care.

Conclusion:

No evidence of bacterial contamination observed in this current study indicates it is crucial to maintain a satisfactory storage condition of platelets at 20°C to 24°C with gentle agitation. This success reflects the institute's strict adherence to the required collection and storage guidelines as per the SOP for maintaining the overall product quality which may impact the blood and transfusion safety positively.

Conflict of interest: There is no conflict of interest.

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