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Bacterial Contamination of Whole Blood-Derived Platelet Concentrates: Results of a Prospective Multicentre Study from Pakistan

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Author`s Contribution

[1,2,3]Substantial contribution to the conception or design of the work; or the acquisition; [2,4]Active participation in methodology and literature review; [2,5]Analysis, or interpretation of data for the work, [1,5]Drafting the work and revising it critically for important intellectual content

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ABSTRACT

Objective: To assess the prevalence of bacterial contamination and the characterization of the bacterial isolates in whole blood-derived platelet concentrates.

Methods: This prospective study included 1,254 samples of 72 hours' post-donation PCs (whole blood-derived) studied from January to December 2023. PCs from three different blood centres were studied, including Peshawar Regional Blood Centre, Peshawar (n = 766), Mirpur Regional Blood Centre, Mirpur (n = 425), and Dr. Akbar Niazi Teaching Hospital Blood Bank, Islamabad (n = 63). Using aseptic technique, a 10 ml blood sample was obtained from the PC bag and inoculated into BD BACTEC™ aerobic/anaerobic platelet quality control testing culture bottles. The bottles were incubated in the BD BACTEC™ blood culture system for seven days at 37°C. Culture bottles indicating bacterial growth were sub-cultured, and microbiological tests were used to identify and classify bacterial strains.

Results: The study revealed that seven PCs were found to be contaminated, showing a contamination rate of 1 in 179 (0.55%). None of the platelet units with positive screening tests were ultimately transfused. The bacteria detected were consistent with skin microbial flora that are connected to non-fatal septic blood transfusion reactions. The majority (71.42%; n = 5) were coagulase-negative *Staphylococcus aureus*, with two cases of gram-positive *Propionibacterium acnes* (28.58%).

Conclusion: The study underscores the critical importance of implementing screening measures for bacterial contamination of blood products.

Key words: Platelets, Contamination, Pakistan

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Introduction

Bacterial contamination of platelet concentrates (PCs) remains a major challenge in transfusion medicine, particularly due to the susceptibility of PCs to bacterial growth under storage conditions.[1] Platelet concentrates are typically stored at room temperature to preserve platelet function, creating an environment conducive to bacterial proliferation if contamination occurs.

Unlike other blood components, which are stored at lower temperatures, PCs are therefore at an inherently higher risk of bacterial contamination.[2] This contamination poses a significant risk to recipients, with potential outcomes ranging from mild febrile reactions to severe and sometimes fatal septic reactions. It is estimated that in the USA more than two million platelet transfusions are administered every year while in the UK, 300,000 are administrated every year.[3]

In Pakistan, around 2.7 to 3.0 million blood units are collected annually but 35% of these collected units are not separated into components.[4] A study from Karachi revealed that 25% whole blood and 80-85% other blood components were inappropriately transfused to the patients.[5] The utility of platelets has increased over the last 15 years due to regular Dengue outbreaks in the country. Pakistan's blood transfusion practices are evolving, yet there is limited data on the bacterial contamination rates of blood products, particularly PCs, in the country.

There are concerns about the safety of platelet transfusions due to risks of acquired bacterial contamination that can result in serious consequences like sepsis, shock, and death.[6] The risk of the bacterial contamination in the whole blood-derived platelet concentrates increases due to contamination of bacterial flora of the skin which grows during platelet incubation at room temperatures.[7] With the advancement in the screening and testing technologies, the chances of transfusing an infected platelet unit have reduced significantly but still carries a challenge in transfusion medicine. There is a reported rate of 1 in 15,000 platelets units having bacterial contamination in the United States.[8] Contaminated platelets are a source of wastage in developing countries with limited resources.[9-11]

In a recent study published in the year 2023, the wastage of the bacterial contaminated platelet units resulted in a loss of $12 million apart to additional cost related to restock the necessities.[12] The majority of bacterially contaminated PCs are due to inadequate skin preparation and testing/screening procedures.[13] Inappropriate storage temperatures leads to bacterial growth.[14] Additionally, storage conditions like gas-permeable blood bags kept at room temperature (20-24 °C) and continuous agitation support bacterial growth.[15] Adaptation to the automated system for the platelet processing and storage has demonstrated to control the risks of acquiring bacterial contamination during the aforementioned activities.[16]The most common pathogens are gram-positive bacteria including Viridans group; Streptococci, *Staphylococcus aureus, Bacillus spp., Corynebacterium,* and coagulase-negative *Staphylococci*. The gram-positive *Propionibacterium acne* bacteria are reported in some studies involved in the contamination of the PCs.[17]

The current study aimed to evaluate the presence of bacterial contamination in whole blood platelet concentrates and characterization of bacterial isolates. This research is particularly relevant as Pakistan continues to develop its blood transfusion infrastructure and work toward aligning its practices with international safety standards. The frequency and types of bacterial contaminants in PCs within Pakistan have not been extensively studied, and available data are often limited to single-center studies, making it difficult to form a national picture.

Methodology

This prospective study was conducted from January to December 2023. A total of 1,254 whole blood-derived platelet concentrate (PC) samples were collected from three different blood banks namely: Peshawar Regional Blood Centre (PRBC) (n = 766) located in the Khyber Pakhtunkhwa province, Mirpur Regional Blood Centre (MRBC) (n = 425) in Azad Jammu and Kashmir, and Dr. Akbar Niazi Teaching Hospital Blood Bank (ANTH BB), Islamabad (n = 63). The three centers were selected in order to evaluate the current practices and any kind of bacterial contamination that can be found in different geographical regions.

Sample Collection

As per recommended practices, 10 mL sample was aseptically collected from each PC bag using a sterile needle and syringe. The samples were then inoculated with aseptic technique into BD BACTEC™ aerobic/anaerobic culture bottles (Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

Inoculation and Incubation

The culture bottles were inoculated and incubated in the BD BACTEC™ blood culture system (Becton, Dickinson and Company, Franklin Lakes®, NJ, USA) for a period of seven days at 37°C. This system utilizes fluorescent sensor technology to detect the presence of microorganisms in the culture bottles.

Bacterial Growth Detection

All culture bottles that showed growth of the bacteria were sub-cultured onto agar plates (Becton, Dickinson and Company®, Franklin Lakes, NJ, USA) and subjected to microbiological tests for identification and classification of bacterial strains. The tests included Gram staining, catalase testing, and biochemical reactions using API 20E strips (bioMérieux, Marcy-l'Étoile, France).

The platelet concentrates that did not show bacterial growth after seven days of incubation period were labeled as negative and were not tested any further.This study was approved by the Institutional Review Board (IRB) of the Peshawar Regional Blood Centre, Peshawar, Pakistan (IRB approval number: PRBC/IRB/2023/01). Informed

consent was obtained from all blood donors prior to participation in the study. SPSS Version 26.0 was used to analyze the data collected during the study. Categorical variables were summarized using percentages and frequencies.

Results

In our study, a total of 1,254 whole blood-derived platelet concentrates (PCs) were tested for bacterial contamination. The mean age of the participants was approximately 27.24 ± 8.12 years as given below in the Table 1.

The participants were grouped into five age categories, ranging from 18-20 years to 51-60 years, to assess potential age-related differences in contamination rates.

Table 1: Age group of study participants (n=1,254)

Age Group	Number	Percentage
18-20 years	270	21.5%
21-30 years	443	35.3%
31-40 years	390	31.1%
41-50 years	65	5.2%
51-60 years	16	1.3%

Out of the 1,254 platelet concentrates, only seven PCs (0.55%) showed positive results for bacterial growth, indicating a contamination rate of 1 in 179. Table 2 given below provides an overview of the total number of PCs tested, the number of contaminated PCs, and the contamination percentage. The contamination rate of 0.55% is comparable to that reported in developing countries but is higher than the rates reported in developed countries. Table 3 shows the distribution of bacterial isolates and in Table 4 the contamination rate by site of sample collection is shown. Coagulase-negative *Staphylococcus aureus* was the most common isolate (71.42%), followed by *Propionibacterium acne* (28.58%).

Table 2: Summary of PC testing results

Age Group	Frequency	Percentage
Contaminated PCs	7	0.55%
Non-contaminated PCs	1,247	99.45%
Total	1,254	100%

Table 3: Distribution of bacterial isolates

Bacterial Isolate	Frequency	Percentage
Coagulase-negative *Staphylococcus aureus*	5	71.42%
Propionibacterium acnes	2	28.58%
Total	7	100%

Coagulase-negative *Staphylococcus aureus* had the highest mean bacterial load (25.6 CFU/mL), followed by *Propionibacterium acnes* (15.8 CFU/mL). These results are given below in Table 5.

Table 4: Contamination rate by site

Site	PCs tested	Contaminated PCs	Contamination rate
Peshawar RBC	766	4	0.52%
Mirpur RBC	425	3	0.70%
ANTH Blood Bank	63	0	0
Total	**1,254**	**7**	**0.55%**

Table 5: Bacterial load of contaminated PCs

Bacterial Isolate	Mean CFU/mL	Range CFU/mL
Coagulase-negative *Staphylococcus aureus*	25.6	10-50
Propionibacterium acnes	15.8	5-30

Discussion

The findings of this study reveal that bacterial contamination in whole blood-derived platelet concentrates (PCs) remains a significant concern in the blood transfusion landscape in Pakistan. The contamination rate of 0.55% aligns with data from other developing countries but is notably higher than the contamination rates reported in more developed healthcare settings and those recommended by WHO and AABB.[18-20] This discrepancy emphasizes the need for improved contamination control practices and bacterial screening procedures for blood products in resource-limited environments. A prominent observation in this study is the predominance of coagulase-negative *Staphylococcus aureus* and *Propionibacterium acnes* as contaminants. Both bacterial species are typically part of skin flora,

suggesting that contamination may occur during the collection or handling process. Coagulase-negative Staphylococci, despite their lower pathogenic potential, have been associated with non-fatal septic transfusion reactions, which can still pose risks to immunocompromised or critically ill patients.[21] This finding underscores the importance of stringent aseptic techniques during the collection, processing, and storage of PCs to mitigate the risk of contamination.

Among the three sampling sites, the highest contamination rate was found at Mirpur Regional Blood Centre (0.70%), followed by Peshawar Regional Blood Centre (0.52%). Despite quality control measures, there are some variabilities in quality monitoring parameters and disinfecting techniques between different blood banks and hospitals.[22] The study also underscores a critical concern in transfusion medicine, i.e. the higher risk of bacterial contamination in PCs compared to other blood products due to the storage conditions that support bacterial proliferation. The seven-day incubation period used for culture detection in this study reflects a reliable approach for identifying bacterial contamination in PCs. However, newer rapid detection technologies could enhance real-time screening and potentially further reduce the risk of bacterial transfusion reactions by identifying contamination prior to transfusion.

Among the contamination causing bacteria, Coagulase-negative *Staphylococcus aureus* was the most common bacterial isolate (71.42%), followed by *Propionibacterium acnes* (28.58%). Earlier studies conducted in other countries also have shown Coagulase-negative *Staphylococcus aureus* to be a common contaminant in platelet concentrates.[23,24]

Coagulase-negative Staphylococcus aureus (CNS) has a noticeably higher average number of bacteria (25.6 CFU/mL) than *Propionibacterium acnes* (15.8 CFU/mL). This indicates, that, Coagulase-negative *Staphylococcus aureus* may be difficult to eliminate during disinfecting technique and might need more steps to be completely removed.[25] Previous studies have revealed that bacterial contamination of platelet concentrates can result to serious concerns, including sepsis and death.[26-28]

The fact that no contaminated PCs were transfused is a positive outcome, indicating effective pre-transfusion screening protocols within the study centers. However, the need for universal and routine bacterial screening of PCs is evident, especially considering the vulnerability of transfusion recipients. This study's findings advocate for regulatory guidelines in Pakistan that mandate bacterial screening of PCs, aligning with practices in developed countries where bacterial screening of PCs is standard.

Conclusion

The rate of confirmed bacterial contamination of PCs (0.55%) was comparable to that reported in developing countries, while it was on the higher side when compared with the developed countries, emphasizing the need to enhance quality control measures and strict adherence to the policy and guidelines to mitigate the risk of bacterial contamination. There is a variation in the contamination rate of the selected sites, suggesting variation in the quality control and disinfecting techniques. There is a potential resilience to disinfecting techniques that is why Coagulase-negative *Staphylococcus aureus* was the predominant bacterial isolate, with a significantly higher mean bacterial load than *Propionibacterium acnes*. Thus, our study highlights the demand for consistent and improved quality control measures to reduce the risk of bacterial contamination in platelet concentrates and emphasizing strict vigilance on the emergence of contamination cases. Bacterial contamination can pose serious risks to transfusion recipients, including sepsis and other life-threatening complications. The blood transfusion authorities (BTAs) can play a pivotal role in ensuring compliance with national standards and guidelines to uphold the safety and quality of blood transfusion services.

Limitations

This study's results must also be interpreted in light of its limitations. The sample size from Dr. Akbar Niazi Teaching Hospital Blood Bank, Islamabad, was relatively small, potentially limiting the generalizability of findings to that region. Future studies could benefit from larger sample sizes across more diverse geographical regions to gain a more comprehensive understanding of contamination rates in the country. Additionally, further research into the cost-effectiveness of implementing universal bacterial screening in Pakistan is warranted.

Disclosure

The authors declare no conflicts of interest. This study was presented as a poster presentation during the 38th International Congress of ISBT (International Society of Blood Transfusion) in Barcelona, June 23-27, 2024. The abstract was published in Vox Sanguinis. 2024;119 (Suppl. 1; doi:10.1111/vox.13650).

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