ORIGINAL ARTICLE

AEROBIC BACTERIAL CONTAMINATION IN PLATELET CONCENTRATES AT THE REGIONAL BLOOD CENTRE, PESHAWAR

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Background: Platelet concentrates play a crucial role in transfusion medicine, aiding in the management of various medical conditions, including haemorrhage, thrombocytopenia, and platelet dysfunction. However, their storage conditions at 22° C present an optimal environment for bacterial growth, making them susceptible to contamination. Of particular concern is the transmission of microorganisms from the skin flora during the phlebotomy process, which can lead to the transfusion of contaminated platelet concentrates. Such contamination poses significant risks to patients, potentially resulting in morbidity and mortality. Determining the frequency and identifying causative organisms of bacterial contamination in platelet concentrates. **Methods:** It was a descriptive cross-sectional study conducted at the Institute of Pathology and Diagnostic Medicine, Khyber Medical University, and the Regional Blood Center in Peshawar from May to October 2021, spanning a duration of six months. The study included 500 participants aged between 18 and 50 years (mean: 28.13±7.67 years. A simple convenient sampling technique was employed. Blood products underwent screening for Hepatitis B, Hepatitis C, HIV, Syphilis, and Malaria. Leaked units were excluded from the study. Platelets were prepared using a Cryofuge and subsequently subjected to culture media. **Results**: The mean age of the participants included in the study was 28.13±7.67 years, with an age range of 18 to 50 years. Out of the total sample size of 500, there were 483 (96.6%) male participants and 17 (3.4%) female participants. Among the collected samples, bacterial growth was observed in only 11 (2.2%) platelet concentrates. The isolated organisms were Staphylococcus epidermidis, found in 7 (1.4%) platelet concentrates, and Staphylococcus aureus, found in 4 (0.8%) platelet concentrates. **Conclusion:** Bacterial contamination of platelet bags is higher compared to developed countries. Therefore, implementing quality control procedures is necessary to reduce the risk of bacterial contamination in platelet concentrates. Additionally, employing enhanced skin disinfection techniques at the phlebotomy site can significantly minimize bacterial contamination.

Keywords: Aerobic; Bacterial contamination; Platelet concentrates; Transfusion transmissible infections

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INTRODUCTION

Transfusion of blood and blood components is a common source of infections by a wide variety of infectious agents. Among these infections, bacterial and viral infections are more common.[1] However, bacterial infection remains the potential transfusion-associated infectious risk, causing high morbidity and mortality, especially in the recipients of blood products.[2]

Among the blood components (red blood cells, plasma, and platelets), platelet concentrates (PCs) carry more risk of bacterial contamination because they are stored at 22 °C with continuous gentle shaking that promotes rapid bacterial growth and proliferation.[3] Screening of PCs for bacterial contamination is routinely performed in all developed countries. To detect contamination of bacteria in PCs, different methods are used and implemented worldwide. However, this is not the case for the majority of developing countries.

It is estimated that bacterial contamination occurs at an incidence rate of 1:1,000–1: 3,000 in PCs, with the occurrence of fatal sepsis in 1 of 500,000 recipients.[4] The estimated rate of bacterial contamination of platelet is 1 out of 2,000–3,000 units (complete blood and apheresis platelets) and 1 out of every 6 contaminated units of platelets is associated with severe sepsis.[5,6] In Pakistan, screening of PCs for bacterial contamination is not routinely performed and screening is limited to five markers namely Hepatitis B, Hepatitis C, HIV, Syphilis, and Malaria.

Severe sepsis associated with blood transfusion is

frequently misdiagnosed, and also the true clinical and lethal incidence is likely understated.[7] In the United States, the second utmost widespread reason for death associated with blood transfusion is bacterial contamination after transfusion mistakes.[8] The risk of death is projected to be 1 in 500,000 platelet concentrates in the United States.[8]

The contamination by the skin flora near the puncture spot is the most common among all infections.[9] Essentially most of the pathogens are gram-positive bacteria including Viridans group Streptococci, Staphylococcus aureus, Bacillus spp., Corynebacterium, and coagulase-negative Staphylococci, also some other gram-positive anaerobic bacteria like Propionibacterium acne.[6]

No study has been conducted thus far to determine the frequency of aerobic bacterial contamination in platelet concentrates within our region. Therefore, the purpose of this study is to investigate the frequency of bacterial contamination in platelet concentrates and identify the common causative pathogens involved.

MATERIAL AND METHODS

This descriptive cross-sectional study was conducted at the Institute of Pathology and Diagnostic Medicine (IPDM), Khyber Medical University (KMU) in Peshawar, along with the Regional Blood Centre (RBC) in Peshawar. The study was carried out over a period of six months, from May to October 2021. The sample size was determined using the WHO sample size calculator, with an expected frequency of 50%, a confidence level of 95%, and a margin of error set at 5%. Based on these parameters, the calculated sample size was 385. Non-probability simple convenient sampling technique was employed to enroll donors in the study.

The enrolled blood donors underwent a donor selection questionnaire based on the WHO donor selection guidelines. Phlebotomy procedures were performed using aseptic techniques, and a volume of 500 mL of blood was collected into blood collection bags, specifically Triple blood bags manufactured by JMS Healthcare PHL INC. These bags contained the anticoagulant citrate, phosphate, dextrose, and adenine (CPDA-1). Subsequently, the blood bags were centrifuged using the Thermo Fisher Scientific™ Cryofuge at the Regional Blood Centre in Peshawar, and the platelets were separated into a satellite bag. The platelet concentrates were then stored at a temperature of 24 °C in a Helmer platelet incubator with an agitator.

For inclusion in the study, platelet concentrates needed to exhibit a positive swirling effect and test negative for HBsAg, Anti-HCV, Anti-HIV, Syphilis, and Malaria. Any leaked units were excluded from the study to ensure the integrity of the data.

The sample collection procedure for platelet concentrates (PCs) involved securing the tube of the bag by clamping it at two distinct sites. One of the clamped sites was disinfected using 70% isopropyl alcohol. A sterile disposable syringe was then used to puncture the tube between the clamped segments, allowing for the collection of 4 ml of PCs. The collected sample was dispensed into culture bottles containing nutrient broth. Samples of PCs were cultured in bottles containing nutrient broth in a laminar flow hood and incubated for 24–72 hours at 37 °C. Strict aseptic techniques were followed throughout the process. During this incubation period, careful observation was made for any signs of bacterial growth, such as turbidity and colour changes in the culture bottles. In cases where bacterial growth was observed, the samples were sub-cultured onto Blood agar and MacConkey agar plates. These agar plates were then incubated at 37°C and inspected for bacterial growth after 24 to 48 hours. If no growth was observed, the result was recorded as negative. However, if bacterial growth was detected (indicating positive cultures), further steps were taken to identify the specific bacterial species involved.

The data collected for this study was recorded and analyzed using SPSS version 26. Descriptive statistics were utilized to calculate the mean, standard deviation, and other relevant statistical measures for continuous variables. Categorical variables were summarized using percentages and frequencies. Analytical statistics were performed using the Chi-square test to determine the association between variables. A significance level of 0.05 was set, and any p-value below this threshold was considered statistically significant. The findings were presented in the form of graphs, tables, and figures to provide a clear representation of the results and facilitate a better understanding and interpretation of the data.

RESULTS

The study participants had a mean age of 28.13±7.67 years. Figure 1 illustrates the gender distribution of the sample. Table 1 provides an overview of the age groups of the participants.

Figure-2 presents the prevalence of bacterial contamination in platelet concentrates. Table 2 provides details on the organisms that were isolated during the study. Furthermore, Table 3 demonstrates the stratification of positive organisms in relation to demographic data, allowing for a better understanding of the association between the isolated organisms and various demographic factors.

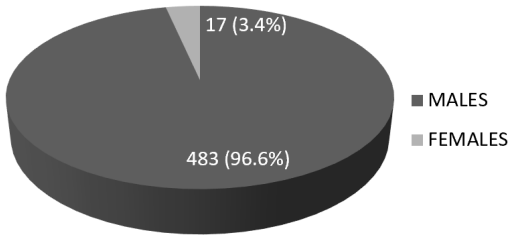

Figure-1: Gender distribution in the study sample (n=500)

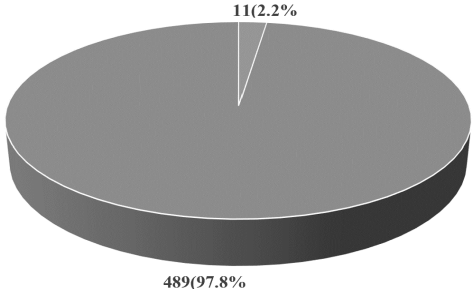

Figure 2: Culture results of the platelet concentrate (n=500)

Table 1: Age group of the participants (n=500)

Age Group	Frequency	Percent
18-20 years	93	18.6
21-30 years	215	43.0
31-40 years	152	30.4
41-50 years	30	6.0
51-60 years	10	2.0

Table 2 : Organisms isolated from platelet concentrates (n=11)

Organism Isolated	Frequency	Percent
Staphylococcus Aureus	4	0.8
Staphylococcus Epidermidis	7	1.4

Table 3: Stratification of the positive organisms with demographic data.

Variables	Growth positive	Growth negative	*p*-value
Gender			
Male	11	472	0.52
Female	0	17	
Age group			
18-20 years	1	92	0.350
21-30 years	6	209	
31-40 years	3	149	
641-50 years	0	30	
51-60 years	1	9	
Blood group			
A negative	0	17	0.860
A positive	2	107	
AB negative	0	10	
AB positive	1	53	
B negative	0	18	
B positive	5	123	
O negative	0	18	
O positive	3	143	

DISCUSSION

In transfusion medicine, bacterial contamination of platelet concentrates (PCs) has long been a problem. The function of transfused platelets is greatly influenced by the ex-vivo storing abrasion along with the in-vivo surrounding of the transfused individuals.[10] PCs are transfused to patients within 1 to 2 days subsequent to blood donation and have a greater chance of reclamation rate, also better endurance and function.

In our study, most of the participants were males (96.6%) with a mean age of 28 years. While female participants in this study were only 3.4% (n=17), that is because the blood donation trend among female participants is low in our society.

In this study, platelet concentrates were prepared from donated blood by platelet- rich plasma (PRP) technique/differential centrifugation and PCs were held for a minimum of 24–36 hours after donation before collecting the sample, allowing bacteria to proliferate in enough numbers for their detection. This is in line with similar research conducted by Noor Raihan *et al* in 2014 in the United States.[11]

In our study culture-based method was used for bacterial screening in the PCs. Samples from platelet concentrates were cultured in bottles containing nutrient broth and incubated for 24–72 hours at 37 °C. Culture bottles indicating bacterial growth were sub-cultured onto Blood agar and MacConkey agar. Agar plates were inspected for bacterial growth after 24–48 hours. The positive growth of bacteria was further processed to identify different types of bacterial species by gram staining and microscopy. Numerous conventional tests were applied for the identification step like catalase, coagulase, indole, etc. Although these are some old techniques used for the identification of bacterial species but some modern techniques can also be applied to further investigate the types of bacteria in depth.

Our study demonstrates that after screening the platelets for aerobic organisms it was positive in 2.2% and negative in the rest 97.8%. In comparison to other research studies, our reported results are on the lower side as reported from developing countries like Ethiopia (12.5%), Ghana (9.0%), Nigeria (8.8%), Zimbabwe (3.1%) and Tanzania (2.8%).[12–16] However, the prevalence rate is higher than in developed countries like Belgium, Australia, Germany, China, Norway, USA and Canada with the prevalence rate of 0.74%, 0.67%, 0.18%, 0.06%, 0.05%, 0.04%, 0.03%, 0.02% and 0.01% respectively.[17–24]

In our study, the implicated organisms were

gram-positive facultative anaerobes. Staphylococcus Aureus and Staphylococcus Epidermidis were the two organisms isolated from platelets bags with a prevalence of 0.8% and 1.4% respectively. A similar study conducted by Noorulamin *et al* in 2018 shows that the implicated organism was Staphylococcus Aureus with a prevalence of 1% in platelet concentrates.[4] Another study by H Schrezenmeier et al reported *Staphylococcus epidermidis* and Propionibacterium acnes to be the most prevalent contaminants in platelet concentrates.[25] This can be attributed to the fact that Staph. Epidermidis is a predominant colonizer of the skin and can be found specifically in the antecubital area from where it is supposed to be entered into PCs by venipuncture during phlebotomy.[26,27]

Limitations of the study:

Samples were taken from a single centre, i.e., Regional Blood Centre, Peshawar. Secondly, a conventional method (Culturing) of screening was used which takes a lot of time to get results. Thirdly, screening was done. Future research could address these limitations by incorporating multiple centers, utilizing advanced screening methods, considering a broader range of microorganisms, and investigating the potential sources of contamination to enhance the understanding and management of bacterial contamination in platelet concentrates. Only for aerobic organisms. Lastly, the source of bacterial contamination was not identified.

CONCLUSION

The findings of this study support the notion that bacterial contamination in platelet concentrates in Pakistan is higher compared to developed countries. To enhance blood safety in the country, it is crucial to implement standardized protocols for screening and quality control measures. Interestingly, the majority of the bacterial species identified in our study, as well as in previous studies, originate from the skin microflora. This highlights the importance of improving skin disinfection techniques at the phlebotomy site and emphasizing personal hygiene practices among the staff involved in blood collection. Additionally, raising awareness within the blood bank community about the potential sources of contamination can significantly contribute to limiting bacterial contamination.

By implementing these measures, the overall quality and safety of platelet concentrates can be improved, reducing the risk of transfusion-associated infections and ensuring better patient outcomes in Pakistan.

Recommendations:

To enhance the safety and quality of platelet concentrates, it is essential for blood banks to be equipped with advanced bacterial detection technologies. These technologies can provide more accurate and timely identification of bacterial contaminants, enabling prompt interventions and minimizing the risk of transfusion-associated infections. By combining advanced bacterial detection technologies, stringent quality control procedures, and improved skin disinfection techniques, blood banks can effectively limit bacterial contamination in platelet concentrates, thereby improving the safety and reliability of transfusion therapy.

AUTHORS' CONTRIBUTION

MI: Literature review, write-up, results compilation. YMY: Main idea, supervision, data analysis. MNK: Data collection, data interpretation. SAA: Literature search, results compilation. MA: Proofreading. NES: Article review.

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