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Brief Report

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Detection of Bacterial Contamination of Platelet Concentrates Produced at Selected Blood Processing Centres

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ABSTRACT

Introduction: The most common microbiological cause of transfusion related morbidity and mortality is bacterial contamination of blood and its cellular components. Out of those cellular components, bacterial contamination of platelet concentrates is more common. Therefore, time-to-time detection of bacterial contamination of platelet concentrates produced at selected blood processing centers is crucial. **Objective:** To detect the pathogenic bacteria associated with bacterial contamination of platelet concentrates (PCs) produced at selected blood processing centers. **Methodology:** Single donor platelet units, prepared from different donations were used for this cross-sectional study. PCs which were injected into universal culture bottles, which contained Brain Heart Infusion (BHI) broth were observed for any evidence of growth. When there was any evidence of growth, those were inoculated on blood agar and chocolate agar, and a direct smear was prepared to perform a Gram stain. Particular biochemical tests were performed to identify the organism as far as possible. When there was no evidence of growth, only sub-culturing on blood agar and chocolate agar were done. All the bottles those showed negatives were re-incubated, and they were inspected daily. The above procedure was followed for up to 7 days. **Results:** Only three samples (3%) showed true positivity for contamination of both culture bottles. Therefore, the percentage of bacterial contamination of platelet concentrates at Day -7 was 3%. The isolated organism in all positive platelet concentrate units was a Gram-positive bacillus of the Bacillus species. **Conclusion:** Storing platelets at 22 °C up to 6 days and not extending the storage period are necessary to protect the sterility of platelets and to prevent platelet transfusion-associated adverse effects.

Keywords: Platelet concentrates, Blood transfusion, Bacterial contamination, Transfusion reactions

INTRODUCTION

Platelets are small, anucleated fragments which are transfused to the patients who have thrombocytopenia, platelet dysfunction, active bleeding, or a high risk of bleeding (1). As any other blood product, platelet transfusion also has its adverse events out of which, the most common microbiological cause of morbidity and mortality associated with transfusion is bacterial contamination of platelets. That is because,

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platelet concentrates are stored at room temperature in gas-permeable containers with continuous agitation, which supports bacterial proliferation easily (2). Platelets are stored at 22 ℃ (range 20–24 ℃) up to 6 days, to ensure the functionality and viability of the platelets .Over the course of the PC storage period, bacteria can multiply to potentially dangerous proportions in this temperature, despite the initial inoculate being tiny (1). The additives in the storage solution may work as an additional energy source for some bacteria (3). The platelet preparation itself exhibits a high glucose content (about 500 mg/dl) and a perfect physiological pH which is partly provided by an anticoagulant that contains dextrose (4). Extending the period that PCs are stored could expose patients to potential declines in the effectiveness of platelet transfusions as well as potential increases in adverse events in addition to transfusion-associated sepsis. One of the causes for limiting PCs storage to 6 days is that there is a direct correlation between bacterial growth and the duration of storage (2).

There are Gram-positive, Gram-negative and anaerobic contaminants that cause the contamination of PCs. Examples of Gram-positive contaminants are coagulase-negative Staphylococci mostly Staphylococcus epidermidis, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Corynebacterium spp. (non diphtheriae), and unidentified Gram positive rods. Examples of Gram-negative contaminants are Enterobacterales including Escherichia coli, Serratia spp., Klebsiella spp., Enterobacter spp., Yersinia enterocolitica, Acinetobacter spp., Pseudomonas aeruginosa, Pasteurella multocida, and unidentified Gram-negative rods. Clostridium perfringens, Propionibacterium acnes and etc. are examples of anaerobic contaminants (4).The reason for 60% of fatalities are Gram-negative bacilli (GNB), while 40% are due to Gram-positive organisms, including Staphylococcus aureus, Staphylococcus epidermidis, Group B/C/G Streptococci, Enterococci and unidentified Gram-positive rods (4).

Bacterial contamination of donated blood can be occurred due to endogenous (from the donor) or exogenous (during collection and processing) routes (5). The primary source of bacterial contamination of PCs is the skin flora which gains access during collection processes due to inadequate sterilization of the puncture site (6). S. epidermidis strains have the ability to strongly adhere to donors' skin in spite of cleansing procedures (4). Spore-forming organisms in PCs are a challenge in transfusion as they are highly resistant to physical and chemical agents. The members of genera Bacillus and Clostridium are the most important spore formers. Bacterial endospores may become vegetative during storage (7). Although the donors are healthy, bacteria can still enter the body through a skin plug that is punctured by the needle during the donation, even after adequate cleaning. Regular donors may have fibrous scar tissue present in phlebotomy areas that is resistant for cleaning (8). Bacterial contamination of platelets is mainly related to the donor phlebotomy arm. A small portion of skin containing viable bacteria that enters the collection bag may occur when a phlebotomy needle passes through the skin. Contaminated collection equipment, donor bacteremia, contamination occurred during processing and storage, the phlebotomist and the surrounding environment, including air are the other ways of bacterial contamination of PCs (7).

Therefore, this study was carried out to detect the bacterial growth in PCs and to identify the organisms responsible for contamination.

METHOD

Ethical clearance was obtained from the Ethics Review Committee (ERC) of Faculty of Allied Health sciences, University of Peradeniya and the permission was granted from the National Blood Transfusion Services Sri Lanka.

This was a cross sectional study. The bags of platelet concentrates which were prepared from different donations received to the selected blood processing centers were collected on 7th day after the preparation of PCs. The calculated sample size of this study was 196. As I have completed this study during my undergraduate period, there was a limited time duration. Availability of media and laboratory facilities were somewhat limited. There were some practical issues when collecting samples. I have used platelet concentrates (PCs) which were on 7th day after the preparation, which were about to discard from blood processing

centers. Therefore I had to collect samples without interfering the process of blood bank. As a result of all about limitations, finalized sample size was 100. When collecting samples, more donations were tried to cover according to the availability, to improve the accuracy of the laboratory based work. Samples were transported accordingly, by public transport services. . Because of the limitation of samples, I needed to improve the specificity of the laboratory based results of these 100 samples. Therefore two samples were taken from a single donor platelet unit to duplicate the process. Environmental contamination was monitored during the procedure. All the aseptic techniques were followed from the beginning to the end of the procedure to avoid contamination as much as possible. All media and reagents used for laboratory-based work were quality-controlled.

The culture bottles which were used in this study were aerobic. Brain heart infusion (BHI) was a good growth medium to facilitate the growth of aerobic bacteria in the presence of oxygen. Anaerobic culture methods require specialized culture media, chambers, anaerobic jars and techniques. They are expensive and not affordable to use in this study. All the universal culture bottles into which platelets were injected, were observed for presence or absence of growth. When there was any evidence of growth, those were sub-cultured on blood agar and chocolate agar and a direct smear was prepared to perform Gram stain. Particular biochemical tests were performed to identify the organism as far as possible. When there was no any evidence of growth, only sub-culturing on blood agar and chocolate agar were done. All the bottles those showed negatives were re-incubated, and they were inspected daily. The above procedure was followed up to 7 days.

RESULTS

The percentage of the total positive number of cultures were taken as the final result. Out of the tested PC units 97 (97 %) were negative for bacterial growth and 3 (3 %) PC units showed bacterial growth.

Therefore, the percentage of bacterial contamination of platelet concentrates at Day -7 was 3%. The isolated organism in all positive platelet concentrate units was a Gram-positive bacillus of the Bacillus species.

DISCUSSION

Bacterial contamination of PC is a serious adverse event and prompt actions are needed to prevent it. Storage conditions of PC are in favor of bacterial growth. Hence, time to time preventive measures should be taken to minimize the occurrence. The general objective of conducting this research is to detect the pathogenic bacteria associated with bacterial contamination of platelet concentrates (PCs).

In this study, the isolated organism was a Gram-positive bacillus which belongs to the Bacillus species. As a result of spore formation, Bacillus species are difficult to eliminate because of the resistance to heat, disinfectants and etc. Bacillus species are a frequent cause of laboratory environmental contamination and they are frequently isolated as a culture contaminant. According to the previous literature, examples of Gram- positive contaminants are, coagulase-negative Staphylococci mostly Staphylococcus epidermidis, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, and unidentified Gram-positive rods and etc. Forty percent (40%) of fatalities are due to Gram positive organisms including Staphylococcus aureus, Staphylococcus epidermidis, Group B/C/G Streptococci, Enterococci, and unidentified Gram-positive rods (4).

Two samples were taken from a single donor platelet unit to duplicate the process to improve the specificity of the laboratory-based work. That means, if only one culture bottle is positive for contamination, out of the two culture bottles of a particular single donor platelet unit, it can be excluded as negative or it can be considered as a false positive result. But if both bottles of a particular sample are positive, and if there is no growth on the culture plate which is used to monitor environmental contamination, we can consider that result as true positive. As a result of duplicating the process, false positives can be excluded and only the true positives can be included. As a recommendation, it is better to consider a prevalence of bacterial contamination of

PCs by increasing the sample size covering other blood processing centers in Sri Lanka.

Because of the initial concentration of the contaminant is tiny, it may be inoculated into only one culture bottle. It may be the reason for the 6% false positivity of single donor platelet units, which exhibited positive for contamination of one culture bottle. 91% PC units showed negative for bacterial growth in both culture bottles. In this study, the percentage of bacterial contamination of platelet concentrates was 3%, and the isolated organism was a Gram-positive bacilli of Bacillus species. The previous literature has indicated that, contaminated collection equipment, during processing and storage, surrounding environment including air are some of the ways of bacterial contamination of PCs (7). As PCs are stored at room temperature in gas-permeable containers with continuous agitation, which supports bacterial proliferation easily (2). The bags that are used to keep PC are composed of gas-permeable plastic to allow sufficient oxygen for platelets to maintain aerobic respiration. The aim of platelet agitation is to ensure gas exchange between the storage medium and the atmosphere. Over the course of the PC storage period, bacteria can multiply to potentially dangerous proportions, despite the initial inoculate being tiny (1). Therefore, screening for bacterial growth of platelet concentrate is a crucial action to prevent the transfusion of highly contaminated PCs at the end of their shelf lives, and to ensure the product safety (9).

Except the manual culture-based methods, there are several other methods like molecular methods and automated culture-based methods to detect the bacterial contamination of PCs (4). The manual culture-based methods appear to be time-consuming and slow-growing organisms can go undetected. But it was difficult to perform these costly molecular or automated culture-based methods due to a lack of funding as this is self-funded research. Therefore, this study was done using manual blood culture-based identification. As a recommendation, it is better to use a molecular or automated culture-based method to get results with high sensitivity and specificity.

The sterility of the platelet concentrates to be transfused must be 100%. However, it is important to note that shelf life of PC is limited to 6 days in Sri Lanka, but in this study the sample was taken on day 7. Hence, the findings do not prove any clinical matter. It is crucial to regularly check for bacterial contamination of the platelet concentrates prepared at various blood processing centers. Developing and following standard safety practices, regular monitoring of the storage environment of PCs, proper disinfection of the working surfaces regularly, enhancing the quality of air and etc. are some of the recommendations to prevent the contamination of PCs by environmental bacterial contaminants. It will be highly valued to conduct studies to identify the sources of bacterial contamination of platelet concentrates and to improve the awareness of healthcare personnel regarding the fact that bacterial contamination of platelet concentrates is a significant source of morbidity and mortality related to blood transfusion.

CONCLUSION

Bacterial contamination is possible in PCs and in this study, the isolated organism is a Bacillus species. Steps must be taken to avoid contamination further and time to time testing is recommended. Furthermore, limiting the shelf life of PC to 6 days is appropriate until further evaluation is achieved.

Author declaration

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Authors' contributions:
Conceptualisation and Design: EMSKS, EPPAJ; Sample Collection and Laboratory Work: EPPAJ; Supervision – Laboratory Work: WKHD; Writing – Original Draft: EPPAJ; Supervision – Manuscript Writing: EMSKS; Writing – Review & Editing: All authors.

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Data are available with the first author to be submitted on request.

REFERENCES

1. Cloutier M, Korte D De. Infectious IT. Residual risks of bacterial contamination for pathogen-reduced platelet components. 2022;(February):879–86. https://doi.org/10.1111/vox.13272
2. Strand S, Hervig T, Seghatchian J, et al. Transfusion and Apheresis Science Bacterial contamination of blood components : Norwegian strategies in identifying donors with higher risk of inducing septic transfusion reactions in recipients. 2014;51(2):97–102. https://doi.org/10.1016/j.transci.2014.08.007
3. Prax M, Oleg IB. Microbiological Screening of Platelet Concentrates in Europe. 2019;76–86. https://doi.org/10.1159/000499349
4. Savini V, Catavitello C, Astolfi D,et al. Bacterial Contamination of Platelets and Septic Transfusions : Review of the Literature and Discussion on Recent Patents About Biofilm Treatment. 2010;168–76.
5. Agzie M, Niguse S, Tsegay E, et al. Bacterial contaminants of stored blood and blood components ready for transfusion at blood banks in Mekelle , Northern Ethiopia. 2019;1–6. https://doi.org/10.1186/s13104-019-4217-0
6. Resolutions P. T RANSFUSION M EDICINE. 2005;19(4):259–72. https://doi.org/10.1016/j.tmrv.2005.05.002
7. Mathai J. Transfusion and Apheresis Science.2009;41(2):139–44. https://doi.org/10.1016/j.transci.2009.07.012
8. Ketter PM, Kamucheka R, Arulanandam B, et al. Platelet enhancement of bacterial growth during room temperature storage: mitigation through refrigeration. Transfusion. 2019;59(S2):1479–89. https://doi.org/10.1111/trf.15255
9. Vollmer T, Hinse D, Diekmann J, et al. Extension of the Storage Period of Platelet Concentrates to 5 Days by Bacterial Testing : Is it Worth the Effort ? 2019;111–3. https://doi.org/10.1159/000499543
10. White SK, Schmidt RL, Walker BS, et al. Original research. 2020; https://doi.org/10.1111/trf.15762
11. Blajchman MA, Goldman M. Bacterial contamination of platelet concentrates: Incidence, significance, and prevention. Vol. 38, Seminars in Hematology. 2001. p. 20–6. https://doi.org/10.1016/s0037-1963(01)90120-9
12. Farrugia C, Baron B, Zammit V. Platelet Storage – Current Limitations and Future Solutions. 2022;5(2):278–83.
13. Greco C, Martincic I, Gusinjac A, et al. Blood components. https://doi.org/10.1111/j.1537-2995.2007.01249.x
14. Gong J, Hogman CF, Lundholm M, et al. Novel automated microbial screening of platelet concentrates. 1994;(9):72–8.
15. Corash L. Bacterial contamination of platelet components : potential solutions to prevent transfusion-related sepsis. 2011;509–25.
16. Vos MC, Vermeij H, Rhenen DJ Van. T r a n s f u s i o n c o m p l i c at i o n s. 2005;45(April):514–9.