Blood Bank

Bacterial Contamination Testing of Platelets in the Blood Bank of a Tertiary Care Hospital

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Abstract: Platelet Concentrates (PC) are routinely stored in a blood bank at 20-24 ^{0} C in order to maintain their functional activity and viability. However this room temp storage also renders them more liable to bacterial growth. Prevalence of bacterial contamination of blood products worldwide is varied, attributed to lack of universal bacterial contamination screening practices. As a result septic reactions, also leading to occasional fatalities, continue to occur which are hard to overlook? This study was conducted on Random Donor Platelets (RDP) with an aim to evaluate and quantify the significance of bacterial contamination of RDP units.RDP units from 4000 consenting healthy voluntary blood donors formed our study pool. RDP samples were initially incubated aerobically at 37^0 C for upto 7 days in Bactec 9120 fully automated blood culture system. In case of positive growth, further sub-cultures were done on blood agar. The growth characteristic pattern & identification of the isolated organisms was evaluated. Of the 4000 random donors platelet samples tested, 03 samples were positive. Amongst the positive samples, two samples showed growth on day 2. The isolated organisms were *Micrococci sp.* & *Diptheroid sp*. The prevalence of confirmed bacterial contamination was low. Prevention of transfusion of contaminated products with routinely used bacterial screening by culture methods is questionable due to delay in detection & false negative results.
Key word: Random Donor Platelet, Bacterial Contamination, Automated Culture System.

INTRODUCTION

Over the past three decades, introduction of sensitive & sophisticated technologies have significantly reduced the risk of viral Transfusion-transmissible infections (TTI), still the risk for bacterial contamination (BC) has remained fairly stable. At present, BC of blood components represents the most common infectious risk from transfusion, and is currently ranked the third most common cause of transfusion related fatalities after Transfusion related lung injury and hemolytic transfusion[1,2].

BC represents an ongoing challenge in transfusion medicine. The risk of BC is more with platelet concentrates (PC) which are particularly susceptible to sustainable microbial growth and are currently the most implicated blood component for septic transfusion reactions.[3]The exact prevalence of bacterial contamination is varied and most of the times understated due to lack of hemovigilance system and universal bacterial contamination screening practices.

BC rates of PC have been estimated to be approximately 1:1,000 - 3,000 PC units[4]. Transfusion-transmitted sepsis has been recognized and culture-confirmed in at least 1 of 100,000 recipients, with resultant fatality rate of 1:500,000 among recipients. [5] Fatality rate of 1:17,000 with pooled, Random donor platelets concentrates (RDP) and 1:61,000 with apheresis platelets concentrates(APC) have been reported[6]. These high BC rates and their resultant sequelae have been ascribed to storage conditions of PC, wherein storage is done at room temperature (22 ± 2^0 C) under aerobic conditions to maintain their functional activity and viability.

There are two main issues associated with the BC of platelet concentrates. First is patient's perspective, wherein BC of PC can lead to catastrophic complications for patients receiving such PC? Second is from Transfusion Services perspective, wherein because of the increased risk of BC at currently stipulated room

temperature storage, the shelf life of PC is limited to maximum five days

The exact source of BC cannot be determined in most of cases and has been ascribed to factors such as donor bacteraemia, improper donor arm disinfection, contaminated component collection/processing/storage equipments or infusion sets used during actual blood component transfusion[7]. It is worth mentioning the fact that a large proportion of platelet concentrates are administered to patients who are immuno-compromised, primarily hemato-oncology and transplant patients, many of whom are on chemotherapy or other immunosuppressant regimen. Even seemingly harmless donor skinfloracancauseseriousillnessordeathinasusceptiblerecipientwhen transfused in large numbers, due to both Gram-positive and Gram-negative bacteria present in transfused contaminated PC.

Additionally, fever, which is a prime symptomatology of infection, may go unnoticed because of the inherent immunological dysfunction associated with the disease state in these hemato-oncological patients, thereby leading to catastrophic complications.

Keeping all these factors in mind we conducted a study to evaluate the BC rates of RDPs so as to find out its significance in our transfusion services setup. In our study we focused on BC of RDP units as unlike our western counterparts, the penetration of apheresis platform in our country is still low and majority of PCs transfusion still occur in form of RDPs.

MATERIALS & METHODS

This study was conducted at a Premier transfusion services setup of Northern India catering to two large tertiary health care centers. It was a cross sectional descriptive study conducted on 4000 platelet concentrate samples (random donor platelets only). Study period was from Jan 2016 to July 2016. Blood units were collected from eligible voluntary healthy blood donors that qualified the pre-donation criteria as mandated by our standard operating procedures based on guidelines for Blood Banks issued by various Government agencies.

Phlebotomy site was specifically examined & adequately disinfected at the time of blood donation. Blood was collected in a sterile closed 450 ml CPD-SAGM bag (Mfg Terumo Penpol) following all aseptic precautions and after diversion of minimum 20 ml of venous blood into diversion pouch. The whole blood collected was subjected to preparation of components within 6-8 hrs of collection. For the preparation of RDPs, initially the bags were centrifuged at 2000 rpm for 8 minutes at 20°C (low spin). Subsequently, packed cells were separated & labeled appropriately. The Platelet Rich Plasma (PRP) was further centrifuged at

3200rpm for 9 minutes at 20°C (High spin).The final products obtained were RDP, which were stored at 22+/- 2°C in a platelet agitator & FFP (Fresh Frozen Plasma) were stored at -80^0 c after appropriate labeling.

Using proper asepsis, 8 ml sample of RDP was collected from the platelet bag on the day of blood collection under laminar air flow and incubated at 37^0C for 7 days in the aerobic culture vial in BacT/ALERT 9120 Fully Automated Microbiology Detection System (bio Merieux, Inc). All samples were monitored daily for growth characteristics for a period of 7 days. In case of cultures, showing growth after incubation at 37^0C for 48 hrs (True Positive), were further sub-cultured on Mc Conkey's Medium & Blood Agar. Colonies were analyzed for growth morphology on plates & subsequently identified microbiologically.

RESULTS

A total of 6, 285 voluntary allogeneic donations took place during the study period, from which 4000 RDP units were prepared. As routine release of RDP units was not obstructed, the storage time of PC evaluated varied from Day 0 to Day 5 of storage.

Out of the 4000 RDP samples tested, 03 samples were culture positive with 02 samples showing growth on day 2 i.e., 48 hrs after incubation at 37^0C and 1 sample showed growth on Day 1. The pattern of growth remained same on all the subsequent days of culture. Out of the 03 positive growths in cultures, it was possible to isolate bacteria only in 02 samples (66.67%). These two RDP contaminants were identified as *Micrococcus* & *Dithered* species (Gram positive microorganisms). Remaining one was contaminant, not of any clinical significance (False positive) ie, growth seen on day 01.

None of the transfused patients, including the two patients of Acute Myeloid Leukemia and Mitral valve replacement (post op) to whom the culture positive PC had been transfused before the availability of culture reports, showed sepsis or any systemic manifestation.

DISCUSSION

Bacterialcontaminationoftransfusionproducts,especiallyPC,isasignificantclinicalproblemwit multiple causes. In a study by Hillyer *et al*. upto33.9per100,000units of whole blood derived RPD were found to be bacterially contaminated [7]. Bhat *et al*. in their study reported 1.16 % of RDP units as failed. [8] In our study, the rate of bacterial contamination is 0.08% which falls somewhat in between the wide range reported in the available literature (range 0.05% to 1.06%) [9,10].

A study done by Morel P et al in France revealed that the residual risk of transfusion reaction

due to bacterial contamination of PC has been decreasing slowly since 1994[11]. Various strategies are being considered for implementation so as to reduce BC risk, namely stringent donor selection, adequate donor arm cleansing, diversion of first 10-30 mL blood and visual inspection of swirl[7]. Rather an impressive reduction in BC risk of upto 50% has been demonstrated by some researchers by improving donor arm disinfection & diversion of the initial 20 mL of the donation[4,12].

In our results, the isolated organisms were Gram positive skin commensals. This compares well in relation to the skin contaminants seen in few reports.[13–16] The organisms isolated are responsible for fatal complications in immunocompromised patients & in patients undergoing cardio-vascular surgeries. Surprisingly, none of the transfused patients with the contaminated units showed any systemic manifestation. This is of importance because these patients were already on prophylactic broad spectrum antibiotics. Additionally, majority of the fatalities reported in literature are caused by Gram negative bacteria[10,17].

A number of recent advances have come up in the field of BC detection of blood components. An entire continuum of tests such as determination of glucose or pH levels, direct Gram's/Acridine orange staining of bacteria, tests with bacteria-specific nucleic acid probes or fluorescent dyes or antibodies have come up [18–20]. But, Culture-based methods are still considered the most useful, popular and economical[10,15,21].

In this study we have tried to investigate the utility of the automated culture method. Traditionally, performance of culture-based tests has been linked with the duration of culture, timing of sampling and sample volume inoculated. First fail scenario occurs in case of delayed true positive culture, wherein by the time a positive growth signal is obtained (usually after the shelf life of PC), the product would have already been transfused. In our study, the median time for the first positive signal was approximately 2.5 days compared to 3.7 days by H Schrezenmeier *et al.*[22] False positive results can affect the positive predictive value of the screening methodology, leading to unnecessarily blockage of platelet concentrates issue.

Second fail scenario is of false-negative culture, wherein sample does not contain viable organisms when it was introduced into the culture bottles depending on inadvertent error in the timing of sampling and sample volume inoculated. At present majority of septic transfusion reactions are ascribed to failed QC tests due to these false-negative tests[18].

Sample volume taken for BC studies can have serious connotations for volume of remaining product available for transfusion. Though a larger sample volume increases sensitivity[21,23], the issue of adequacy of remaining product volume available for transfusion remains. This issue is more so for RDP units which already have only 50-60 ml of PC.

The low rate of prevalence of bacterial contamination in our study can be attributed to rigorous implementation of optimal skin disinfection techniques and sample diversion strategies to reduce contamination, as has been reported worldwide. Conversely, these low rates can also be ascribed to the timing of sample collection in our study which was at the time of component preparation itself. As mentioned above due to low BC levels at the time of collection, sampling at the time of collection can lead to missing of bacteria in the sample taken. Usually 24–48 hours of interval should be allowed between collection and sampling for effective BC detection by allowing bacteria to increase to detectable numbers in the PC[24]. Also in our setup, due to the high demand of the PCs by dependent patients, the time limits of waiting for culture results upto 24-48 hours or end of shelf life could not be enforced.

Inthe United States, 4 million platelet units are transfused annually with annual expected contamination in the range of 2000-4000 units[25]. But no such comprehensive data is available in our country which appears to be a break in the link of transfusion practices. The newer methods for rapid culture of blood products related to bacterial contamination like BacT ALERT, Intercept bacterial detection system, Flowcytometry, Electro-luminiscent detection etc needs to be evaluated and can undoubtedly intercept many contaminated units and should be used for routine study of bacterial contamination given the majority of evidence based studies. Bacterial detection methods to screen for bacterial contamination of platelet concentrates is gaining wide spread importance regarding practice of use of prestoragepooled RDP and extending of shelf life of PC beyond current permissible limit of 5 days.

An additional layer of transfusion safety can also be provided with the implementation of various upcoming Pathogen Reduction Technological platforms, that have shown impressive results in decreasing the septic outcomes of transfusion albeit with some reduction in functional activity of the components transfused[15]. Our result show that prevention of transfusion of contaminated products with routinely used bacterial screening by culture methods is questionable due to delay in detection & false negative results.

However in a financially constrained country like ours, meticulous and ongoing training of medical and paramedical staff regarding the sources of BC, from the collection of blood component to its final transfusion to the intended recipient is the need of the

hour. In this context it may be remembered that already an impressive reduction in BC risk of upto 50% has been demonstrated by some researchers by improving donor arm disinfection & diversion of the initial 20 mL of the donation[4,12]. However new BC detection methods are also coming up in big way which may become economical in future with widespread acceptance, making further reduction in the risk of BC associated transfusion-associated septic reaction surrealistic possibility in the foreseeable future.

Conflict of Interest
None of the authors declare any potential case of conflict of interest.

Ethical approval
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent
Informed consent was obtained from all individual participants included in the study

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