



Original Research Article

Bacterial Contamination of Platelet at University Hospital: "A Prospective Surveillance Study"

Subhashish Das^{1*}, Mamatha Kale², P.M.Benna² and Harendra Kumar¹

¹Department of Pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar, India

²Department of Microbiology, SDUMC, India

*Corresponding author

A B S T R A C T

The incidence of blood-borne viral diseases has decreased over the last several years, but the risk of blood-borne bacterial diseases has remained the same. Bacterial contamination of platelets occurs because platelets are stored at 20-24°C temperature which facilitates bacterial growth. Approximately 1 in 1,000 to 3,000 platelet units may be contaminated with bacteria with occurrence of fatal sepsis in 1 of 500,000 recipients. However, the actual risk of transfusion-associated sepsis has not yet been accurately estimated because infections resulting from contaminated platelets are under reported. The aim of our study was to (a) detect incidence and type of bacterial contamination of platelets; (b) optimum sampling time for PC culture, (c) to explore the feasibility of shelf life of platelets till 7 days. 410 Random donor platelets were prepared by platelet rich plasma method. 5ml platelet concentrate (PC) was inoculated in blood culture bottle and incubated at 37°C in BacT /ALERT (BioMerieux, USA) for 7 days. The PC was sampled on 0, 3, 5, and 7 days of preparation. Only repeat isolation of the same organism on subsequent days was considered as confirmed positive. A total of two (0.48%) PCs were bacterially contaminated. The isolates were skin commensals coagulase negative staphylococci (CONS) and Diphtheroides. CONS was isolated on day 3 of collection and Diphtheroides on day 1. There were no isolations from PCs sampled on day 5 and 7. The implementation of automatic bacteriological culture allows result to be obtained more quickly. Bacterial contamination of platelets is mainly due to skin flora which can be reduced by better skin disinfection, collection of blood with a diversion pouch set. Third day of collection is a better sampling time which allows detection of most of the organisms. Prolongation of PC storage for more than 5 days had no incidence of bacterial contamination.

Keywords

Bacteria,
Platelet
contamination,
BacT/Alert

Introduction

The incidence of blood-borne viral diseases has decreased over the last several years, but the risk of blood-borne bacterial diseases has remained the same. Bacterial contamination

of platelet concentrates (PCs) is a longstanding problem in transfusion medicine. The reported incidence of clinically relevant reactions to contaminated

platelet products much lower. Approximately 1 in 1,000 to 3,000 platelet units may be contaminated with bacteria with occurrence of fatal sepsis in 1 of 500,000 recipients. It is estimated that 10% of blood transfusion-associated deaths are caused by bacterial contamination of the transfused product (Sazama, 1990).

The major cause of bacterial contamination of PCs is during venepuncture when skin bacteria can gain access to the unit (Gibson and Norris, 1958). Investigations have speculated that a skin-plug is punched out by the needle during phlebotomy. Others possible mechanisms include asymptomatic bacteremia of the donors, contamination of the collection bags, and contamination during the blood processing procedures.

In the last decade, increased attention has been given to the prevention of possible systematic contamination during the collection by following aseptic control measures and by the use of sterile-docking devices, etc., during preparation of blood components (De Jorte *et al.* 2001). The bacterial contamination of whole-blood collections by skin flora can also be reduced by diversion of the first volume of whole blood collected (Blajchman, 1998). Other strategy of reducing contamination is by strict donor selection to exclude donors with risk of bacteremia. Some authors propose the use apheresis platelet concentrates (APC) instead of whole blood (WB) derived pooled concentrates as pooled platelets have higher chance of being contaminated (Wagner, 2000).

In 2002, an open letter calling for immediate action to reduce the risk of platelet bacterial contamination led to the proposal of All American Blood Banks (AABB) standard 5.1.5.1. The standard AABB 5.1.5.1 states that “The blood bank or transfusion service

shall have methods to limit and detect bacterial contamination in all platelet components. Standard 5.6.2 applies for [skin disinfection]” (Fridey, 2003). As of now, three culture-based bacterial detection systems are licensed in the US for quality control use: BacT/Alert (BioMerieux), Ebds (Pall) and Scansystem (Hemosystem).

The aim of our study was to (a) detect incidence and type of bacterial contamination of platelets; (b) optimum sampling time for PC culture, (c) to explore the feasibility of shelf life of platelets till 7 days.

Materials and Methods

The study was conducted in the in the Blood bank and department of Microbiology, RL Jalappa Hospital and Research Centre, Kolar. Blood was collected from eligible donors as per Drugs and Cosmetic Act, 1945, by Ministry of Health, Govt. of India along with written consent. Donors who gave a history of analgesics and antibiotics usage for last 7-10 days were excluded from the study.

Phlebotomy site was prepared with a povidine-iodine scrub followed by povidine-iodine application. For donors who were allergic to iodine, a chlorhexidine scrub was substituted and kept for 30 seconds. The first portion (20 ml) of WB collected was diverted in a diversion pouch.

Whole blood derived platelets was prepared using platelet rich plasma (PRP) method. In the PRP method whole blood was collected into an anticoagulant solution and then subjected to a soft spin separate the PRP. The PRP was centrifuged again to prepare the platelet concentrate (PC) which contains 60-75% of the platelets present in the whole blood unit from which it is derived.

The present study has used the FDA approved BacT/ALERT 3D (BioMerieux, USA) which is an automated colorimetric blood culture method, based on the detection of carbon dioxide produced by proliferating microorganism, which allows the detection of both aerobic and anaerobic bacteria as well as yeasts and fungus. The bottom of the bottles contains a pH sensitive liquid sensor which changes its color according to the amounts of CO₂ released.

One blood culture bottle was aseptically inoculated with 5ml of PC in a laminar air flow hood after the bottle tops were cleaned with alcohol. After inoculation the bottles were incubated in BacT/ALERT classic 3D for 7 days with continuous monitoring of signals. If the blood culture bottle beeped a positive signal, a subculture from the bottle was done onto blood agar, chocolate agar and MacConkeys agar for bacterial growth. The organism was further identified by biochemical reactions. Each platelet concentrate was sampled on day 1, 3, 5 and 7 of preparation. Repeat isolation of the same organism on subsequent days of sampling was considered as confirmed positive.

A positive signal triggered immediate actions: the concerned PC units were blocked for issue or called back or if already transfused the responsible physician was informed about the initial positive result.

Result and Discussion

A total of 410 whole blood derived platelet concentrate were included in the study.

The results obtained were categorized into:

Confirmed positive: PCs with first positive culture along with identification of the microorganism and with a positive repeat

culture with identification of identical microbial species on both the occasions.

Probable contamination

- i. PCs with a first positive culture along with detection of microorganism but with a negative observation in repeat culture from the sample bag or
- ii. BacT/Alert instrument error: a positive signal beep but no growth on subculture from the blood culture bottle

Negative: The PCs that did not exhibit a positive signal over the 7 day incubation period in BacT/ALERT were described as negative.

A total of two (0.48%) PCs were bacterially contaminated. Coagulase negative Staphylococci (CONS) and Diphtheroids both of which are skin commensals were isolated from PCs. Nine (2.19%) were considered probable contamination due to instrument error 3 (0.73%) and 6 (1.46%) were inoculation contamination as repeat culture did not yield any growth. Three hundred and ninety nine (97.31%) PCs were negative for bacterial culture.

CONS was isolated on day 3 of collection and Diphtheroids on day 1. There were no isolations from PCs sampled on day 5 and 7.

Over the years, with improvement in donor screening transfusion-transmitted viral infections have dropped with bacterial contamination of platelets incidence assuming a new prominence as the most frequent infectious risk of transfusion. While room temperature storage allows transfused platelets to circulate *in vivo*, it also has the risk of promoting bacterial contamination with a limited shelf life of 5 days and thereby making platelet inventory

management extremely challenging (Ness, *et al.* 2001).

In the 1990s, numerous studies demonstrated that contaminating bacteria, usually representing skin flora from the donor, could be cultured in approximately 1/3000 platelet units. Clinically apparent septic transfusion reactions were thought to occur following 1/25,000 platelet transfusions (Mitchell, *et al.* 1999). The most commonly reported agents are Gram-positive bacteria such as coagulase negative *Staphylococcus*, viridans group *Streptococci*, *Staphylococcus aureus*, and Diptheroids (Leiby, *et al.* 1997).

Olthuis et al. were the first to show that for apheresis collections, the first 10 ml had a higher rate of bacterial contamination than the next two aliquots of 10 ml (Wagner, 2000). Wagner et al. showed the effectiveness of the diversion approaches in an in vitro model system (Wagner, *et al.* 2000). Bruneau et al. showed that the first aliquot (15-ml) of whole blood collected has a higher contamination rate than the second aliquot, without testing the final whole-blood unit collected (Bruneau, *et al.* 2001).

Various approaches have been suggested to reduce the chance of bacterial contamination. Augmented skin cleansing preparations and diversion of the first milliliters of blood may reduce the risk of skin commensal bacterial entering the unit by 75 to 90 % (De Korte, D *et al.* 2006).

Various techniques have been suggested to screen platelets for bacterial contamination, but none have received general acceptance, because of a lack of sensitivity and specificity, the techno-economic reasons and the lack of legal approval (De Jortete *et al.* 2001). Although rapid bacterial detection systems based on fluorescence, flow

cytometry, or detection of bacterial rDNA are available, in many rural and resource constrained institutions various non-culture methods such as pH and glucose estimation along with Gram staining are performed (Blajchman, 1998). However, the sensitivity and specificity of these non-culture methods is much inferior to the culture-based methods which, in turn, are regarded as the ‘gold standard’ for sterility testing (Blajchman, 1998).

In 1986, the U.S. Food and Drug Administration (FDA) mandated platelet storage be limited to five days. If the platelets are not used within five days, they are considered expired product and must be destroyed (Dodd, 2003). In April 2004, the AABB mandated that a method be performed to limit and detect the presence of bacteria prior to transfusion. As a result, platelets tested by culture methods were not available for use in the first 24 to 48 hours after donation. This mandatory period of quarantine provides time for the bacteria to proliferate and be detected in culture. Hence, the current five-day shelf-life gets further reduced to 3 days for effective and optimal utilization thereby making platelet inventory management extremely challenging (Blajchman, *et al.* 1994).

While the various automated culture systems have increased platelet safety, there are also numerous pitfalls which includes, 1-2% product loss from sampling and 12-30 hours delay in platelet product release, higher cost for platelets, thereby affecting the platelet availability. There might be considerable sampling errors-depending on time of sampling and sample volume (Wagner, 2000). Even if a bacterial contamination is present, it might last up to 7 days or even longer until the culture give a positive result. This can lead to transfusion of PC units that are positive in the culture bottle, however,

too late to prevent issue and transfusion of the contaminated product (Krihsna and Brecher, 1995).

The contamination rates of PC units reported literature show wide variation. This is due to heterogeneity of the various studies with respect to the detection system used, time of sampling, sample volume taken from the PC unit, and also the volume injected into the culture bottle (Mitchell, *et al.* 1999). Most literature is based on culture methods, but not always both anaerobic and aerobic cultures have been inoculated (Yomtovian, *et al.* 1993).

For example, a recent publication on the American Red Cross Experience 2004 on routine bacterial screening of apheresis (APC) unit reports an incidence of confirmed-positive rate of only 0.019 percent or 1 in 5157. A sample volume of only contamination rates can vary greatly depending on the type of culture or bacteria species on which the analysis is based (Fang, *et al.* 2005, Simon, *et al.* 1987).

A Norwegian study reported that on testing of 36896 PC units over a 6-year period, 29 of 88 units (33%) with positive signals had already been transfused whereas a study from Denmark showed 24 of 70 confirmed PC positive units were issued before the bacterial screening system had alarmed. Thus, a negative-to-date issue strategy based on a culture method still bears a considerable risk of issue of contaminated blood products (Mertens and Muylle, 1999).

Our study revealed a high rate of positive culture findings within 24 to 48 hours of incubation. This is because growth characteristic might differ between clinical isolates and laboratory bacterial strains that are used in spiking studies. Hence, performance assessment and decisions on use of bacterial detection systems for blood products should be based only on routine

screening of a large number of products rather than artificial situations produced in spiking studies (Schmidt, *et al.* 2005).

There is also great concern in the possibility of false-negative results. Septic episodes have been reported in the literature despite screening of all PC units. In the Netherlands two cases of life threatening sepsis due to *Bacillus cereus* contamination occurred despite routine bacterial screening with the BacT/LAERT system (Munksgaard, *et al.* 2004). Larsen et al reported that 2 of 1061 outdated PC units that had remained negative in the first culture tested positive in a repeat culture at end of their shelf life and *Bacillus* and *epidermidis*, species respectively, were isolated (Larsen, *et al.* 2005). Cooper and AuBuchon reported their experience in culturing APC on Day 2 with a blood culture system (BacT/Alert). Bacterial growth was detected in 0.6 percent of units after a mean of 26 hours; however, they found that the overall false-positive rate with the BACT/Alert system was 2 of 4794 samplings, rivaling that of the true-positive incidence (Cooper and AuBuchon, 2002).

Three large-scale surveillance studies of septic reactions that were performed prior to the implementation of standard 5.1.5.1: a Johns Hopkins University study, the French BACTHEM study, and the US BaCon study. Bacterial contamination appeared to occur less often with single donor platelets than with pooled RDP. The prevalence of septic reactions was dramatically lower in the BaCon study than in the other two studies (Perez, *et al.* 2001).

The FDA has issued specific guidelines under which the current bacterial detection devices could be validated for released testing. In post-marketing surveillance studies a large sample size of more than 50,000 units needs to be studied to achieve a high degree of statistical confidence with more than 95% confidence level. In this

regard the “Post Approval Surveillance Study of Platelet Outcomes, Release Tested” (PASSPORT) study already been undertaken (Blajchman, *et al.* 2004). Under this study APC were collected using the Spectra or Trima devices and cultured 24 hours after the collection using the BacT/ALERT system for aerobic and anaerobic bacterial growth respectively.

After 24 hours, the products were made available for clinical use with a 7-day shelf life. Any products that outdate were re-cultured on day 8. Final results from the study are still awaited for a definite opinion (Blajchman, *et al.* 2004).

As of now, three cultures based bacterial detection systems are licensed in the US for quality control use: BacT/Alert (BioMerieux), eBDS(Pall) and Scansystem (Hemosystem). “Quality control” in this context means that the test is licensed to verify that the platelet collection process does not introduce contaminating bacteria more frequently than a predefined rate. These tests are not yet licensed as “release tests”, which by definition are used to confirm that a platelet product being issued is not contaminated with bacteria(Benjamin and Mintz, 2005).

Although the aforementioned strategies result in a general reduction of bacterial risk, they do not prevent such events from occurring, as PC with bacteria that evade detection by quality control culture remains a significant residual transfusion risk. The

only approach that is likely to achieve near-absolute bacteriologic safety is the inactivation of bacteria by pathogen reduction technologies (Kieby, *et al.* 2005).

The reported rates of bacterial contamination vary widely (from 0-10%) depending on the surveillance system used. However the true incidence of such contamination is unknown because of similarity with febrile non-hemolytic transfusion reactions (FNHTR): and also due to the frequent premedication's of patients with antipyretics and antibiotics which can mask the signs and symptoms associated with septic transfusion reaction (STR). We detected only 2 units of platelets as confirmed positive which remained in blood bank inventory and hence no (STR) on the recipients were noted. Of the 2 confirmed cases one suspected donor was counseled and referred to medicine department and other donor remained untraceable. This highlights the importance of strict donor screening criteria along with better and cost effective diagnostic methods.

Our study shows that BacT/ALERT automated culture system is an efficient tool for monitoring PCs for bacterial contamination and prevention of transfusion associated septicemia. The system can easily be implemented in the daily routine as shown by us. We did not follow a quarantine period for PC after transferal of the sample to the BacT/ ALERT culture bottle. All PCs that are negative to date can be issued for transfusion as was done in our study.

Table.1 Results of bacterial culture of platelet concentrates: n =410

| Sample Result | Number | Percentage % |
|--------------------------|--------|--------------|
| Confirmed Positive | 2 | 0.48 |
| Probable contamination * | 9 | 2.19 |
| Total positive | 11 | 2.68 |
| Total negative | 399 | 97.31 |

*3 were due to instrument error and 6 PCs yielded no growth on repeat culture.

Keeping the PCs in quarantine can create supply problems because detection of bacterial growth by BacT/ALERT system may occur within 24h or as late as 168h after sampling. In addition, there is substantial under reporting of mild reaction in clinical practice and hence highlights the importance of the physician awareness regarding their responsibilities in the hemovigilance system. In our institute hospital transfusion committee have been constituted to provide necessary guidelines to medical and para medical staff in order to avoid under reporting of STR and promote hemovigilance.

Our study also highlights the need to explore the feasibility of using of rapid tests having a turnaround time of less than a hour by using around 1ml of platelets. Although the existing rapid bacterial test have lower sensitivity as compared to the culture methods, the feasibility of using rapid bacterial tests for quality control or as an adjunct to another test (initial culture, or as a stand-alone release test) needs to be examined. Licensing criteria for rapid bacterial tests needs to be developed by the FDA expeditiously (Wrech, 2002).

Despite the increased concern about and clear awareness of the problem, there has been no consensus as to its real extent or practical solutions for dealing with it. AABB recommends that each institution establish its own measures to prevent or minimize the potential for contamination of blood components and platelet concentrates.

BacT/ALERT is an efficient tool for monitoring PCs for bacterial contamination. We also recommend that every transfusion service review its own procedures for preparing and manipulating platelets in order to detect possible weakness or flaws that may lead to proliferation of bacteria.

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