Incidence of bacterial contamination among platelet concentrate units in selected blood service facilities in the Province of Batangas, Philippines

Student Researchers: Harold Marasigan, Mary Ann Cueto, Ray Carlo de la Cruz, Amy Guico,Sherwin Regondola

Faculty Researcher: Oliver Shane R. Dumaoal, RMT, MSMT

ABSTRACT

Bacterial contamination of platelet units represents the largest infectious disease risk in transfusion medicine. This study determined the incidence of bacterial contamination of platelet units from three major blood service facilities (BSFs) in the Province of Batangas, Philippines. Furthermore, this study also identified the bacterial organisms present in the contaminated platelet units and formulated good laboratory practices to improve the bacteriological safety of platelet units. Laboratory practices and blood collection procedures of the staff from the three blood service facilities were also evaluated through direct observation with a checklist. A total of 106 platelet samples representing 35.57% of the total platelet production of selected blood service facilities were screened for bacterial contamination from July to September 2010. Of the total units screened, 10 units tested positive for bacterial contamination with a detection rate of 9.43% resulting to a bacterial contamination incidence rate of 3.36%. Six samples tested positive for *Bacillus subtilis,* two samples were positive for coagulase-negative *Staphylococcus species* and two other samples had inconclusive identification results. Assessment of the working practices of participating BSFs revealed that all blood centers always adhere to good manufacturing practices in the production of platelets. Lowest performance scores noted during the observation period were on the monitoring of the quality of platelet units during storage. It is recommended that risk reduction strategies for post-transfusion sepsis be implemented as well detection of bacteria in samples of blood components prior to transfusion. Implementation of patient safety through patient monitoring and investigation of transfusion–related-reactions should also be employed.

Keywords: Platelets, Bacterial Contamination, Transfusion, GMP

INTRODUCTION

With contemporary advances in the field of transfusion medicine, risks of contracting viral and protozoal diseases via blood transfusion have decreased significantly over recent years. In the Philippines, the government has mandated the routine screening of

donated blood products for HIV, Hepatitis B and C, malaria, and syphilis.

Transfusion medicine has particularly evolved from a mostly laboratory-centered service focusing only on the serological aspects of blood, into a clinically oriented discipline that highlighted patient care. This change brought with it the necessary emphasis on blood safety, appropriate use of blood components, screening of blood products, and informed consent for transfusion resulting to substantial advances in the reduction of potential risks and complications associated with blood transfusion (Goodnough et al., 2003).

Extraction and concentration of platelets from whole blood is a distinctive development in the field of transfusion. Platelets are the components of the blood that are primarily involved in hemostasis through clotting. Through the process of apheresis, platelets can be separated from whole blood and will be available for transfusion to a patient in need. It is especially useful in the treatment of thrombocytopenic bleeding, hematologic malignancies, solid tumors, major surgical bleeding, trauma, and platelet dysfunction syndromes (Heal and Blumberg, 2004). However, bacterial contamination of platelets is still a pressing issue (McDonald et al., 2002, Cazenave, 2007). Platelet transfusion–associated sepsis is now recognized as the most frequent infectious complication of transfusion therapy (Yomtovian et al, 2006). In the US, bacterial contamination is considered as the second most common cause of death overall from transfusion, after clerical errors, with mortality rates for platelet-related sepsis ranging from 1:20,000 to 1:85,000 donor exposures (Hillyer et al., 2003).

The incidence of platelet bacterial contamination is approximately 1 per 2,000 to 3,000 units, more common than the occurrence of viral transfusion-related transmissions by up to 2 orders of magnitude (Dreier et al., 2004, Yomtovian et al., 2006). This clearly presents dangers to the community, with over 17 million patients receiving platelet component transfusions annually, amounting to nearly 8,000 patients at risk of fatally developing septicemia from contaminated platelet units.

The major sources of bacterial contamination of platelets are either endogenous or exogenous. The endogenous causes are related to the donor due to an asymptomatic bacteremia or a preexisting infection and these can involve all types of bacteria present in the blood stream. The exogenous causes are predominantly related to the introduction of skin organisms into the units of whole blood or apheresis units during venipuncture (Vasconcelos et al., 2004). As such, the possible mechanisms of blood product contamination can include donor bacteraemia, the collection procedure, the collection pack, and blood processing procedures (Goldman, 2004).

The bacterial contamination of whole blood or blood components can occur at several points: production of the blood bag, donor venipuncture, blood donor bacteremia, during the blood component preparation, or at the transfusion time. Contamination at the

time of blood collection is the major cause of bacterial contamination of platelet units (Cunha et al., 2008).

The most prevalent bacteria causing platelet transfusion-related infections are *Bacillus cereus,* coagulase-negative staphylococci, *Streptococcus* spp., *Staphylococcus aureus,* and *Propionibacterium acnes* for gram-positive bacteria, and *Klebsiella* spp., *Serratia* spp., *Escherichia coli*, *Acinetobacter* spp., *Enterobacter* spp., *Providencia rettgeri*, and *Yersinia enterocolitica* for gram-negative bacteria (Brecher and Hay, 2005). It thus becomes apparent that skin flora is a major source of bacteria contaminating platelet units (Nussbaumer et al., 2007; Schrezenmeier et al., 2007). Listeriosis caused by transfusion has not yet been reported, at least in the literature (Guevara et al., 2006).

Platelets are stored at room temperature to maximize their in vivo survival and hemostatic efficacy post-transfusion. For this reason, the contamination of platelets is easier and more common than that of red cells or any other blood component because the storage temperature of platelets, at 20-24^{o}C favors the proliferation of bacteria, unlike the storage temperature of red cells which is 2-6^{o}C (Liumbruno et al., 2009). Although microbial pathogens can grow in red blood cell fractions stored at 48°C, the room temperature storage of platelets permits the survival and rapid proliferation of a broader spectrum of bacteria (Burns and Werch, 2004). Transfusion reactions caused by contaminated platelets have also been shown to be correlated to storage time of platelets (Munksgaard et al., 2004).

There have been many studies concerning the prevention and detection of the presence of bacteria in platelet units. They detailed some strategies to reduce bacterial infection, including augmented skin cleansing method, predonation sampling by diversion of initial 20 ml of blood into a sample pouch and bacterial screening of blood components (Macauley et al., 2002). Other studies have also suggested measurement of pH and glucose changes for indirect measurement (Boekhorst et al., 2005).

It has been demonstrated that there is partial effectiveness in diverting the first 10 to 30 ml of blood from the initial collection, the first 10 ml of donor blood was diverted from the primary bag, showed that 16 of the first 5-ml aliquots were bacterially contaminated while only 2 of the second 5-ml aliquots were positive after culture (Brecher et al., 2005).

In 2002, the College of American Pathologists had recommended that a method be introduced to detect bacterial contamination of platelets aiming to reduce and detect the presence of bacterial platelet contamination (Yomtovian et al., 2007). Since then, a number of automated detection systems have become available to test for contaminated platelet components, but their utility to some extent is restricted by the time they take to indicate the presence of bacteria and/or their lack of sensitivity to detect initially low bacterial loads (Blajchman et al., 2004). Thus, despite improved methods for detecting

bacterial contamination of blood products, bacterial sepsis remains a significant risk in blood transfusion (Lee et al., 2002).

Knowledge of the potential for bacterial proliferation in blood components, which are stored at a range of temperatures, is essential before considering implementation of a detection strategy (Stormer et al., 2006). Also, the number of bacteria in contaminated platelet products increases over time of storage, so low sensitivity nonculture methods have been proposed to screen platelets before transfusion, including visual inspection of the blood products, direct staining of bacteria, or indirect observation of bacterial metabolism by measuring pH, glucose concentration, oxygen consumption, or carbon dioxide production (Trampuz et al., 2007).Several screening methods were suggested for this purpose including measurement of pH reduction; microscopic examination with Gram's, Wright's, and acridine orange staining; or culture of an aliquot of the platelet (Dunne Jr. et al., 2005).

In Canada, screening for bacterial contamination in apheresis platelets with the BacT/ALERT system has been successfully implemented. Results of the validation assays showed 100 percent specificity and 100 percent sensitivity for the bacterial species, concentrations, and inoculation volumes tested. However, the study did not find differences between Gram-positive and Gram-negative bacteria (Arcos et al., 2007).

For this study, we employed microbial culture growth media to detect the presence of bacteria from platelet samples collected from blood service facilities in the province of Batangas, Philippines. The study also advocates the implementation of good laboratory practices to improve the bacteriological safety of platelet units.

MATERIALS AND METHODS

Sampling of Platelet Units

Selected platelet samples were collected weekly from the three major blood service facilities in the province of Batangas, Philippines from July 7 to September 8, 2010. Both whole blood-derived and apheresis-collected platelets were included in the study through random collection twice a week for the entire testing period.

Microbiologic procedures

The platelet units were mixed and two aliquots of approximately 0.1 mL were obtained from the sealed off sample tubing. One of these aliquots was used for culture and the other was frozen at -20°C for confirmatory testing. Samples were cultured in 4.0 to 5.0 mL of brain heart infusion and thioglycollate nutrient growth medium (BD Diagnostic Systems, Franklin Lakes, NJ). With aseptic technique in a laminar airflow cabinet, the samples were inoculated in the culture broth and incubated at 37°C for up to 7 days. The cultures were examined

daily for signs of microbiologic growth. When the presence of bacterial multiplication in the form of turbidity had been observed, the cultures were then subcultured in blood agar plate, nutrient agar plate, or chocolate agar and incubated aerobically at 37°C for 48 hours (Cunha et al., 2008).

The laboratory practices of the staff from the three blood service facilities were evaluated, through direct observation with a checklist (Asanza et al., 2008). Laboratory staffs were observed during blood extraction and were assessed if they are observing laboratory precautions regarding the collection of donor blood, component preparation and storage of platelet units.

Sample Collection and Transport

Samples were collected from the platelet-concentrated satellite bag of the blood bag. A segment of the platelet bag tubing 4-6 inches long, was created by sealing the end with a heat sealer. The segment was then cut from the remainder of the tubing with a sterile cutting instrument.

RESULTS

A total of 106 platelet samples were collected from July to September 2010 from the three participating BSFs (Table 1). From Hospital A, we were able to collect 64 sample units, 32 for the month of July, 22 for the month of August and 10 for the month of September. The total number of platelet units processed by Hospital A was 145, so we were able to process 44.14% of their platelet units. 15.62% of those processed platelets tested positive and 84.38% tested negative. From Hospital B, we were able to collect 38 sample units, 3 for the month of July, 28 for the month of August and 7 for the month of September. The total number of platelet units processed by Hospital B was 135, so we were able to process 28.14% of their platelet units. 100% of the processed platelets were tested negative. From Hospital C, we were able to collect 4 sample units for the month of August only. The total number of platelet units processed by Hospital C was 18, so we were able to process 22.22% of their platelet units. 100% of the processed platelets were tested negative. Over-all, 35.57% of the total platelet production was assessed for bacterial contamination for the entire surveillance period.

Table 1. *Number of platelet concentrate units collected from each corresponding blood service facilities of hospitals A, B, and C.*

	July	**August**	**September**
A	32	22	10
B	3	28	7
C	0	4	0

Of the 106 platelet samples tested, 10 of them tested positive. This represents a detection rate 9.43% of all the units tested. Of the positive samples, 60% were positive for *Bacillus subtilis* and 20% of them were coagulase-negative *Staphylococcus* and two non-bacterial species (Table 2).

Table 2.*Results of microbial identification tests on platelet units that tested positive for bacterial contamination.*

Bacteria Isolated	**No. of Isolates**	**Percentage**
B. subtilis	6	60%
Coagulase-negative *Staphylococcus*	2	20%
Non-bacterial species	2	20%

In the evaluation of the working practices, all participating BSFs were found to be implementing high adherence to good manufacturing practices in the production of platelets. In the over-all tally, Hospital A has a weighted average of 4.29, Hospital B has a weighted average of 4.29 and Hospital C has a weighted average of 4.14. During the observation of blood collection for donors, all BSFs ranked low in the proper washing of the forearms of donors prior to phlebotomy. This is significant because published studies have reported a significant impact of proper asepsis of the phlebotomy site of donors on contamination rates of blood and blood products (Spiess et al., 2010). During component collection, BSFs ranked low in the decontamination of equipment and in the proper aseptic techniques during component preparation. Lastly, the participating BSFs also ranked low in ensuring the monitoring of platelet units for bacterial contamination during storage. The equipment used in particular has no cover increasing the risk of contamination as well as the visual inspection of units for contamination prior to the issuance of units. In general, the BSFs ranked lowest in the good manufacturing practices during storage of platelet concentrates despite the high over-all adherence to GMP standards.

Table 3.*Overall scores of hospitals A, B and C, based on their laboratory practices during specific stages of platelet concentrate processing*

Hospital	Blood Collection	Platelet Extraction	Component Storage
A	4.13	4.75	4.00
B	4.25	4.75	3.83
C	4.38	4.71	3.67

CONCLUSION

This study established the incidence of bacterial contamination of platelet units from selected BSFs in the province of Batangas, Philippines. Up to 9.43% of processed samples tested positive for the presence of bacteria representing a bacterial contamination rate of 3.53%. This is significant because it is much higher than reported incidence rates of platelet bacterial contamination at 0.05% or 1 in 2000 (Hillyer, et al., 2003) thus representing a greater risk of transfusion-related sepsis from platelet transfusion in the local setting.

The primary contaminant in the processed samples is *B. subtilis.* This affirms results of published studies (Nussbaumer et al., 2007; Schrezenmeier et al., 2007; Brecher and Hay, 2005) where *Bacillus* speciesare the predominant contaminant. The said gram-positive microorganism is primarily implicated as an environmental contaminant thus suggesting that majority of platelet contamination in the locale is caused by poor environmental control during the collection, processing and storage of platelet units.

All platelet units that tested positive for bacterial contamination came from Hospital A which uses plateletpheresis as collection procedure. It is therefore concluded that plateletpheresis poses a greater risk of bacterial contamination for platelet components. This may be due to the larger volume of blood collected from the donor or by the prolonged collection period as opposed to a single-unit random donation.

RECOMMENDATIONS

It is recommended that similar researches should be conducted in other medical institutions. Other hospitals should be monitored for platelet contamination so that if similar problems are found, concerted efforts may be exerted to improve the safety of transfusion. Hospitals should also perform tests for the detection of platelet contamination prior transfusion to avoid any platelet-related reactions. The use of rapid tests in the detection of platelet contamination is recommended for immediate detection of contamination prior to transfusion. Proper aseptic techniques and good laboratory practices should always be observed in the laboratory to

minimize the risk of contamination as well the implementation of risk reduction strategies such as the use of diversion pouches and other similar methods.

REFERENCES

1. Arcos, S., C. Jenkins, J. Dion, F. Bernier, G. Delage and M. Goldman. Canadian experience with detection of bacterial contamination in apheresis platelets. Transfusion, Vol.47, 2007.
2. Asanza, R., V. De Chavez, C. De Rosales, C. Dinglasan, R. Manacap, G, Navarro. Incidence of Bacterial Contamination of Platelet Concentrates in the Blood Bank Centers of Batangas Regional Hospital and Mary Mediatriz Medical Center. 2008
3. Blajchman M., E. Beckers, E. Dick meiss, L. Lin, G. Moore and L. Muylle. Bacterial detection of platelets: current problems and possible resolution. Transfusion Medicine Reviews, Vol.19, No.4, 2005.
4. Blajchman, M., M. Goldman and F. Baeza. Improving the bacteriological safety of platelet transfusions. Transfusion Medicine Review, Vol.18, 2004.
5. Boekhorst, P., E. Beckers, M. Vos, H. Vermiej and D. Rhenen. Clinical significance of bacteriologic screening in platelet concentrates. Transfusion Vol.45, 2005.
6. Brecher, M. and S. Hay. Bacterial contamination of blood components. Clinical Microbiology Reviews, 2005.
7. Burns K., J. Werch. Bacterial contamination of platelet units.Arch Pathol Lab Med., 2004.
8. Cazenave, J.P. Bacterial contamination: Should it be detected or inactivated? Publié par Elsevier Masson SAS, 2007.
9. Cunha, G., L. Leão and F. Pimenta. Bacterial contamination of random-donor platelets in a university hospital in the midwestern region of Brazil. Transfusion, Vol.48, 2008.
10. Dreier, J., M. Störmer and K. Kleesiek. Two novel real- time reverse transcriptase PCR assays for rapid detection of bacterial contamination in platelet concentrates. Journal of Clinical Microbiology, 2004.
11. Dunne, Jr. M., L. Case, L. Isgriggs and D. Lublin. In-house validation of the BACTEC 9240 blood culture system for detection for bacterial contamination in platelet concentrates. Transfusion, Vol. 45, 2005.
12. Goldman, M. Bacterial contamination of platelet concentrates. Vox Sanguinis, 2004.
13. Goodnough, L. T., A. Shander and M. Brecher. Transfusion medicine: looking to the future. The Lancet, Volume 361, 2003.
14. Guevara, R., M. Tormey, D. Nguyen and L. Mascola. Listeria monocytogenes in platelets: a case report. Transfusion, Vol.46, 2006.

15. Heal, J. and N. Blumberg. Optimizing platelet transfusion therapy. Elsevier, 2003.
16. Kerrigan, S. and D. Cox. Platelet-bacterial interactions. Cellular and Molecular Life Sciences, 2009.
17. Lee, C. K., P. Ho, N. Chan, A. Mak, Jong and C. K. Lin. Impact of donor arm skin disinfection on the bacterial contamination rate of platelet concentrates. Blackwell Science, 2002.
18. Liumbruno, G., L. Catalano, V. Piccinini, S. Pupella and G. Grazzini. Reduction of the risk of bacterial contamination of blood components through diversion of the first part of the donation of blood and blood components. Immunoematologia e Medicina Transfusionale e di Patologia Clinica, Roma, Italy, 2008.
19. Macauley, A., A. Chandrasekar, G. Geddis, K. G. Morris and W. McClelland. Operational feasibility of routine bacterial monitoring of platelet. Transfusion Medicine, 2003.
20. McDonald, C. P., A. Rogers, M. Cox, R. Smith, A. Roy, S. Robbins, S. Hartley, J.A.J. Barbara, S. Rothenberg, L. Stutzman and G. Widders. Evaluation of the 3D BacT/ALERT automated culture system for the detection of microbial contamination of platelet concentrates. Transfusion Medicine, 2002.
21. Munksgaard, L., L. Albjerg, S. Lillevang, B. Hansen and J. Georgsen. Detection of bacterial contamination of platelet components : six years' experience with the BacT/ALERT system. Transfusion, 2004.
22. Murphy, W., M. Foley, C. Doherty, G. Tierney, A. Kinsella, A. Salami, E. Cadden and P. Coakley. Screening platelets concentrates for bacterial contamination: low numbers of bacteria and slow growth in contaminated units mandate an alternative approach to product safety. Vox Sanguinis, 2008.
23. Nussbaumer, W., D. Allesdorfer, C. Grabmer, M. Rheinshmidth, L. Lin, D. Schonitzer and C. Lass-Florl.Prevention of transfusion of platelet components contaminated with low levels of bacteria:a comparison of bacteria culture and pathogen inactivation methods. Transfusion, Vol.47, 2007.
24. Schrezenmeier, H., G. Walther-Wenke, T. muller, F. Weinuaer, A. Younis, T. Holland-letz, G. Geis, J. Asmus, U. Bauerfeind, J. Burkhart, R. Deitenbeck, E. Forstemann, W. Gebauer, B. Hochsmann, A. Karakassopuolos, U. Leibscher, W. Sanger, M. Schmidth, F. Schunter, W. Sereis and E. Seifried.Bacterial contamination of platelet concentrates: results of a prospective multicenter study comparing pooled whole blood- derived platelets and apheresis platelets.Tranfusion Vol.47, 2007.
25. Seo, H., S. Michalek and M. Nahm. Lipoteichoic acid is important in innate immune responses to gram-positive bacteria. Infection and Immunity, Vol.76, No. 1, 2008.

26. Trampuz, A., S. Salzmann, J. Antheaum and A. Daniels. Microcalorimetry: a novel method for detection of microbial contamination in platelet products. Transfusion, Vol.47, 2007.
27. Vasconcelos, E. and J. Seghatchian. Bacterial contamination in blood components and preventative strategies: an overview. Elsevier, 2004.
28. Verax BioMedical Incorporated. Platelet PGD Test. Fenwal Inc., 2009
29. Wenke, G., R. Doerner, Th. Montag, O. Greiss, B. Hornei, R. Knels, J. Strobel, P. Volkers and W. Daubener. Bacterial contamination of platelet concentrates prepared by different methods: results of standardized sterility testing in Germany. Vox Sanguinis, 2006.
30. Yomtovian, R., E. Palavecino, A. Dysktra, K. Downes, A. Morrissey, S. Bajaksouzian, M. Pokorny, H. Lazarus and M. Jacobs. Evolution of surveillance methods for detection of bacterial contamination of platelets in a university hospital, 1991 through 2004. Transfusion, Vol.46, 2006.
31. Yomtovian, R., P. Tomasulo and M. Jacobs. Platelet bacterial contamination: and assessing progress and identifying guandaries in a rapidly evolving field. Transfusion, Vol.47, 2007.