

## Bacterial contamination of random-donor platelets in a university hospital in the midwestern region of Brazil

Geraldo Cunha, Lara Leão, and Fabiana Pimenta

**BACKGROUND:** Bacterial blood contamination was probably the first recognized transfusion-transmitted disease. Although the transfusion-associated bacterial sepsis has been known for a long time, it remains an important health problem. At present it is the most frequently reported cause of infectious transfusion-related fatalities. The aims of the study were to determine the prevalence of microbiologic contamination in random-donor platelets (RDPs) and to identify the isolated microorganism obtained from a Brazilian university hemotherapy service.

**STUDY DESIGN AND METHODS:** A total of 2000 RDPs were analyzed from November 2004 to June 2005. The time of storage of the platelet (PLT) concentrates studied ranged from the day of donation (Day 0) to the fifth day of storage (Day 5). The RDP cultures were initially performed in pools with bottles containing brain heart infusion (BHI) growth medium, and cultures were incubated aerobically at 37°C for up to 7 days and subcultured onto chocolate agar at 37°C for 48 hours under conditions of microaerophilia. In the cases of positivity of one pool, the culture was individually performed for all the samples of that pool.

**RESULTS:** Eight units (0.4%; 95% confidence interval, 0.31-0.49) were found to be contaminated. Isolated microorganisms were three *Acinetobacter lwoffii*, one *Enterobacter intermedium*, one *Serratia phymuthica*, one *Staphylococcus haemolyticus*, one *Staphylococcus hominis*, and one *Bacillus* sp.

**CONCLUSION:** PLT concentrates were contaminated with bacteria in 0.4 percent of tested units, which represents a potential risk to patients and a public health problem. Regarding the contaminant microorganisms, a predominance of Gram-negative agents was observed (62.5%).

Bacterial sepsis was probably the first recognized transfusion-transmitted disease.<sup>1</sup> Strumia and McGraw<sup>2</sup> reported a case of transfusion reaction associated with bacterial blood component contamination in 1941, the same year in which the first case of transfusion death due to bacterial contamination was reported in the United States.<sup>3,4</sup> Transfusion-associated bacterial sepsis is the second most frequently reported cause of transfusion-related fatalities in the United States, after ABO mismatch, accounting for 46 (17%) of 277 reported transfusion deaths in the period 1990 to 1998.<sup>5</sup> In the United Kingdom, 26 transfusion-transmitted infections deaths were reported from 1995 to 2000 in the Serious Hazards of Transfusion (SHOT) system, among which 15 were caused by bacteria.<sup>6</sup>

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 (opening systems to wash and/or filter red cells), or at the transfusion time.<sup>7</sup> Contamination at the time of blood collection is the major cause of bacterial contamination of platelet (PLT) units. Most organisms present in culture studies and case reports of PLT-associated sepsis are usually Gram-positive skin microbiota.<sup>8</sup> Investigators have speculated that skin plugs, which might form from a needle, occurring during phlebotomy, is responsible for the introduction of skin microorganisms into whole blood.<sup>9</sup>

---

**ABBREVIATION:** RDP(s) = random-donor platelet(s).

---

From the Laboratory of Clinical Microbiology, Institute of Tropical Pathology and Public Health of Federal University of Goiás, Goiás, Brazil.

Address reprint requests to: Geraldo Sant'ana da Cunha Júnior, Dr Francisco Augusto César Street, Number 770, Apt. 51, Jardim Irajá quarter, 14.020-530, Ribeirão Preto, São Paulo, Brazil; e-mail: cunhags@terra.com.br.

Received for publication June 5, 2007; revision received July 26, 2007, and accepted July 29, 2007.

doi: 10.1111/j.1537-2995.2007.01520.x.

TRANSFUSION 2008;48:282-285.

Because PLTs are stored in plasma at room temperature, they provide an appropriate growth medium and conditions for a large variety of Gram-negative and Gram-positive organisms.<sup>10</sup> The risk of receiving bacterial contaminated PLTs is estimated to be 50 to 250 times higher than the combined risk per unit of transfusion-related infection with human immunodeficiency virus I and II, hepatitis C virus, hepatitis B virus, and human T-lymphotropic virus-I and -II.<sup>11</sup> The exact prevalence of bacterial contamination of blood products is unknown. The hemovigilance system is not present in all countries; the bacterial contamination screening is not universally applied due to the use of varied methods for bacterial detection in different studies and because of the usage of different definitions of positivity.<sup>3,12</sup>

Reports of random-donor platelet (RDP) concentrates contamination range from 0.03 to 2.5 percent.<sup>7,12-20</sup> Because random-donor units are pooled, the risk of contamination with RDP should increase by the factor of the number of units in the pool.<sup>21</sup>

Ten microorganisms comprise more than 98 percent of the fatalities attributable to transfusion of bacteria-contaminated PLTs reported by the Food and Drug Administration between 1976 and 1988.<sup>22</sup> In Brazil, all the regulations for hemotherapy are under the responsibility of the National Agency of Sanitary Monitoring (Agência Nacional de Vigilância Sanitária [ANVISA]), a governmental health department that requires bacterial screening of 1 percent of PLT units for quality control. There is no recommendation for this screening before transfusion.

Very few studies on this field have been conducted in Brazil. The goals of the present study were to determine the prevalence of microbiologic contamination in RDP, which is a PLT component most commonly used in Brazil, and to identify the isolated microorganisms.

## MATERIALS AND METHODS

### Study design and sample collection

This study was developed in the hemotherapy service of a university hospital in Goiânia, the capital city of the State of Goiás, located in the Midwestern Region of Brazil, after approval by the local institutional ethical committee. The microbiologic procedures were performed in the Public Health and Tropical Disease Institute (Instituto de Patologia Tropical e Saúde Pública [IPTSP]), at the Federal University of Goiás (Universidade Federal de Goiás [UFG]). All subjects were volunteers and provided informed consent before participation. They were selected among eligible blood donors, who had fulfilled the donor selection criteria according to the standard operating procedures currently used in Brazil.<sup>23</sup>

Preparation of the phlebotomy site followed a two-stage procedure comprising an initial scrubbing followed

by application of an antiseptic solution. Axillary temperature was measured in all donors. Phlebotomy diversion and leukoreduction were not used. All components were prepared in closed systems. All PLT concentrates were stored under ideal conditions, according to the ANVISA recommendations. Time of PLT concentrates storage before sampling for microbiologic testing ranged from the day of donation (Day 0) to the fifth day of storage (Day 5), from November 2004 to May 2005.

### Microbiologic procedures

The PLT 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  $-20^{\circ}\text{C}$  for confirmatory tests. The cultures were initially done in pools. The criterion used for the pool composition was the number of days the PLT concentrates were under storage. These pools could contain either 1 to 5 or 6 to 10 samples, cultured in 4.5 or 9.0 mL of brain heart infusion growth medium (Bacto, BD Diagnostic Systems, Franklin Lakes, NJ), respectively. With aseptic technique in a laminar airflow cabinet, the samples were inoculated in brain heart infusion broth and incubated at  $37^{\circ}\text{C}$  for up to 7 days. The cultures were examined daily for signs of microbiologic growth. Whether the presence of bacterial multiplication was evidenced or not, the cultures were subcultured in chocolate agar and incubated aerobically at  $37^{\circ}\text{C}$  for 48 hours under conditions of microaerophilia.

In the cases where bacterial growth was observed, the samples were considered true positive when a microorganism was identified and the respective frozen samples were used for new individualized cultures for all the samples of the pool. The isolated microorganisms were identified through standard microbiologic procedures, identification by morphologic and biochemical methods and automated methods (MicroScan system, with Pos-Combo 21 and Neg-Combo 32 kits and autoSCAN 4 reader, Dade Behring, West Sacramento, CA).

## RESULTS

A total of 2000 RDPs were included in the analysis. The distribution of samples according to the time of storage was: Day 0, 411 samples (20.55%); Day 1, 364 samples (18.2%); Day 2, 374 samples (18.7%); Day 3, 329 samples (16.45%); Day 4, 304 samples (15.2%); and Day 5, 249 samples (10.9%). Thirty-six cultures (1.8%) were positive and eight (0.4%; 95% confidence interval of rate, 0.31-0.49) were found to be true positive. The distribution of the microorganisms detected in the positive cultures was as follows: Gram-negative rods were detected in five (62.5%) positive cultures—three *Acinetobacter lwoffii*, one

**TABLE 1. Isolated microorganisms and time of storage**

Microorganism	Time of storage
<i>A. lwoffii</i>	Day 0
<i>A. lwoffii</i>	Day 4
<i>A. lwoffii</i>	Day 4
<i>E. intermedium</i>	Day 2
<i>S. phymuthica</i>	Day 3
<i>S. haemolyticus</i>	Day 0
<i>S. hominis</i>	Day 5
<i>Bacillus</i> sp.	Day 2

*Enterobacter intermedium*, and one *Serratia phymuthica*. Two (25.0%) were represented by Gram-positive coccus—one *Staphylococcus haemolyticus* and one *Staphylococcus hominis*; one (12.5%) was represented by Gram-positive bacilli—*Bacillus* sp.

According to the storage time, the contamination range was: Day 0, two positive samples (one *S. haemolyticus* and one *A. lwoffii*); Day 1, no positive sample; Day 2, two positive samples (one *A. lwoffii* and one *Bacillus* sp.); Day 3, one positive sample (*S. phymuthica*); Day 4, two positive samples (two *A. lwoffii*); and Day 5, one positive sample (*S. hominis*; Table 1).

*Acinetobacter lwoffii* was the most common microorganism identified in this study: two positive cases on Day 4 and one on Day 0. *Staphylococcus* sp. was the second most common agent, with one positive case of *S. haemolyticus* on Day 0 and one positive case of *S. hominis* on Day 5. The other agents were present in one case each: one *E. intermedium* on Day 2, one *S. phymuthica* on Day 3, and one *Bacillus* sp. on Day 2.

## DISCUSSION

This study showed 0.4 percent bacterial contamination prevalence in RDPs in a Brazilian university hospital hemotherapy service. The prevalence found in this research is in agreement with the literature reports (Table 1) and was similar to the results reported by de Korte and colleagues,<sup>18</sup> Munksgaard and colleagues,<sup>17</sup> and Vasconcelos.<sup>19</sup> A predominance of Gram-negative rods as RDP contaminants was observed and *A. lwoffii* was the most common microorganism identified in the samples. These microorganisms are part of the natural human skin microbiota and can also be commensals in the oropharynx and vagina.<sup>24</sup>

This result is not in accordance with the literature reports in which it is reported that Gram-positive species predominate as RDP contaminants.<sup>25</sup> A possible reason for the predominance of Gram-negative contamination in the present study could be due to the small number of RDP samples analyzed.

This difference becomes more important due the fact that although the prevalence of Gram-negative

agents is usually smaller, they represent a majority of fatalities from sepsis due to PLT transfusion and are usually not observed until the third day of storage. In contrast, although more prevalent, Gram-positive agents are associated with nonfatal reactions to RDPs stored for up to 5 days.<sup>26</sup> According to the FDA, among the 51 deaths between the years 1976 and 1988 associated with bacteria-contaminated PLTs, 59.7 percent were caused by Gram-negative agents.<sup>27</sup>

The positive RDP samples represent a risk to transfusion recipients. It should be pointed out that RDPs in Brazil are commonly used as pools. The usual dosage of RDPs consists of 1 unit per each 10 kg of the patient's weight, resulting in 7 units for a typical adult. In this case, the risk of receiving contaminated PLTs should increase to 2.8 percent for each transfusion.

The current Brazilian regulation in hemotherapy approaches the problem of bacterial contamination in a superficial way, not establishing the realization of pre-transfusion screening to detect bacterial contamination. To minimize the impact of bacterial contamination in public health, it is vital that the Brazilian vigilance system implements the same Standards for Blood Banks and Transfusion Services adopted by the AABB in 2004, which requires measures to detect and limit bacterial contamination in all PLT components.<sup>5</sup>

## REFERENCES

- Blajchman MA. Bacterial contamination of blood products and the value of pre-transfusion testing. *Immunol Invest* 1995;24:163-70.
- Strumia MM, McGraw JJ. Frozen and dried plasma for civil and military use. *JAMA* 1941;116:2378-82.
- Yomtonovian R. Bacterial contamination of blood: lessons from the past and road map for the future. *Transfusion* 2004;44:450-60.
- Sazama K. Bacteria in blood for transfusion. A review. *Arch Pathol Lab Med* 1994;118:350-65.
- Centers for Disease Control and Prevention (CDC). Fatal bacterial infections associated with platelet transfusions—United States, 2004. *MMWR Morb Mortal Wkly Rep* 2005; 54:168-70.
- Love EM, Williamson LM, Cohen H, Jones H, Todd A, Soldan K, Revill J, Norfolk DR, Barbara J, Atterbury C, Asher D. Serious hazards of transfusion annual report 1999-2000. Manchester (UK): SHOT Office; 2001.
- Illert WE, Sanger W, Weise W. Bacterial contamination of single-donor blood components. *Transfus Med* 1995;5:57-61.
- Blajchman MA. Bacterial contamination of cellular blood components: risks, sources and control. *Vox Sang* 2004;87 Suppl 1:98-103.
- Wagner SJ, Robinette D, Friedman LI, Miripol J. Diversion of initial blood flow to prevent whole-blood contamination

- by skin surface bacteria: an in vitro model. *Transfusion* 2000;40:335-8.
10. Goldman M. Bacterial contamination of platelet concentrates: where are we today? *Vox Sang* 2004;87 Suppl 2:90-2.
  11. Dreier J, Störmer M, Kleesiek K. Two novel real-time reverse transcriptase PCR assays for rapid detection of bacterial contamination in platelet concentrates. *J Clin Microbiol* 2004;42:4759-64.
  12. Bruneau C, Perez P, Chassaigne M, Allouch P, Audurier A, Gulian C, Janus G, Boulard G, de Micco P, Salmi LR, Noel L. Efficacy of a new collection procedure for preventing bacterial contamination of whole-blood donations. *Transfusion* 2001;41:74-81.
  13. Castro E, Bueno JL. Bacterial contamination of blood components needs to be confirmed. *Transfusion* 2002;42:380.
  14. Goldman M, Sher G, Blajchman M. Bacterial contamination of cellular blood products: the Canadian perspective. *Transfus Sci* 2000;23:17-9.
  15. Kocazeybek B, Arabaci U, Akdur H, Sezgiç M, Erenturk S. The evaluation of microbial contamination in platelet concentrates prepared by two different methods. *Transfus Apher Sci* 2001;25:107-12.
  16. de Korte D, Marcelis JH, Soeterboek AM. Determination of the degree of bacterial contamination of whole-blood collections using an automated microbe-detection system. *Transfusion* 2001;41:815-8.
  17. Munksgaard L, Albjerg L, Lillevang ST, Gahrn-Hansen B, Georgsen J. Detection of bacterial contamination of platelet components: six years' experience with the BacT/ALERT system. *Transfusion* 2004;44:1166-73.
  18. de Korte D, Marcelis JH, Verhoeven AJ, Soeterboek AM. Diversion of first blood volume results in a reduction of bacterial contamination for whole-blood collections. *Vox Sang* 2002;83:13-6.
  19. Vasconcelos E. Leucodepletion, bacterial contamination and viral inactivation: a Portuguese blood centre experience. *Transfus Apher Sci* 2001;25:215-6.
  20. Macauley A, Chandrasekar A, Geddis G, Morris KG, McClelland WM. Operational feasibility of routine bacterial monitoring of platelets. *Transfus Med* 2003;13:189-95.
  21. Engelfriet CP, Reesink HW, Blajchman MA, Muylle L, Kjeldsen-Kragh J, Kekomaki R, Yomtovian R, Hocker P, Stiegler G, Klein HG, Soldan K, Barbara J. Bacterial contamination of blood components. *Vox Sang* 2000;78:59-67.
  22. Ortolano GA, Freundlich LF, Holme S, Russell RL, Cortus MA, Wilkins K, Nomura H, Chong C, Carmen R, Capetandes A, Wenz B. Detection of bacteria in WBC-reduced PLT concentrates using percent oxygen as a marker for bacteria growth. *Transfusion* 2003;43:1276-85.
  23. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. RDC nº 153 de 14 de junho de 2004. Determina o Regulamento Técnico para os procedimentos hemoterápicos, incluindo a coleta, o processamento, a testagem, o armazenamento, o transporte, o controle de qualidade e o uso humano de sangue, e seus componentes, obtidos do sangue venoso, do cordão umbilical, da placenta e da medula óssea. *Diário Oficial da União; Poder Executivo, Brasília, DF, 24 Jun. 2004.*
  24. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, WinnWC Jr. *Diagnóstico microbiológico: texto e atlas colorido*. 5th ed. Rio de Janeiro: Medsi; 2001.
  25. Andreu G, Morel P, Forestier F, Debeir J, Rebibo D, Janvier G, Herve P. Hemovigilance network in France: organization and analysis of immediate transfusion incident reports from 1994 to 1998. *Transfusion* 2002;42:1356-64.
  26. Kuehnert MJ, Roth VR, Haley NR, Gregory KR, Elder KV, Schreiber GB, Arduino MJ, Holt SC, Carson LA, Banerjee SN, Jarvis WR. Transfusion-transmitted bacterial infection in the United States, 1998 through 2000. *Transfusion* 2001;41:1493-9.
  27. Brecher ME, Hay SH. Bacterial contamination of blood components. *Clin Microbiol Rev* 2005;18:195-204. 