FOCUS: TRANSFUSION RISKS

Bacterial Contamination of Blood Components

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ABBREVIATIONS: AABB = American Association of Blood Banks; BaCon = bacterial contamination associated with transfusion reactions; CFU = colony forming units; RBC = red blood cell; SHOT = serious hazards of transfusion; UVA = ultraviolet A light.

INDEX TERMS: bacterial contamination; transfusion contamination.

Clin Lab Sci 2003;16(4):230

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As discussed in one of the companion articles, significant progress has been made in reducing the transfusion transmission of viral infections such as hepatitis B, hepatitis C, and HIV. Serologic testing of donors has decreased the risk for acquiring some viral diseases to less than one in a million. Now however, bacterial contamination of blood products has assumed a major role as a cause of morbidity and mortality in recipients of blood products.1-7 Although overall risk for acquiring a transfusion-transmitted bacterial infection is still relatively low, methods to detect infected donors are not available as they are for detection of virally-infected donors. Therefore, detection of bacterial contamination is more problematic and is usually recognized only when the recipient has a severe reaction following administration of the blood component. In fact, after acute hemolytic transfusion reactions, bacterial sepsis is the next most frequent cause of transfusion related fatalities.3,4

In this article we will discuss the major organisms associated with bacterial contamination of blood components, studies of prevalence of bacterial contamination and transfusion associated sepsis, and the methods developed or in development to detect and/or prevent contamination.

BACTERIAL CONTAMINATION

Bacterial contamination of blood units occurs at any one of the following points: collection, processing, pooling of components, or transfusion. Infrequently, it may be due to transient bacteremia in the donor.5-7 High bacterial concentrations are often responsible for a serious septic or fatal reaction in the recipient with most of these reactions occurring in the elderly, neonates, or patients immunocompromised by illness or chemotherapy.5,8 If patients who receive contaminated units are on antimicrobial therapy, there is often decreased severity of the clinical reaction, making recognition more difficult. On the other hand, low levels of bacterial contamination in the transfused component may cause relatively mild symptoms, such as fever and chills, which resemble a febrile, non-hemolytic transfusion reaction.9 It is widely recognized that the similarities in these mild symptoms has led to the underrecognition and underreporting of reactions due to bacterial contamination of blood products.2,7,9,10

Over the years, the literature has described case reports of one or more fatalities due to bacterial contamination of blood components or results from surveillance surveys designed to detect the prevalence of bacterial contamination and/or transfusion-associated bacterial sepsis in a single facility. Comparing these reported data or obtaining an overall incidence rate can be difficult due to different criteria for identification and reporting of a reaction, timing of cultures, and methods used to detect contamination. In the last few years however, there have been concerted efforts in several countries to obtain data about the overall prevalence of bacterial

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contamination in blood products and transfusion-related fatalities in a standardized manner. The English SHOT (Serious Hazards of Transfusion) project, the French Hémovigilance effort, and the U.S. BaC on study (Bacterial Contamination Associated with Transfusion Reactions) are ones that have received wide dissemination and will be discussed later in the article.

Blood is an excellent growth medium but only a few bacterial species such as Yersinia, Serratia, and Pseudomonas grow well at 1 °C to 6 °C, the storage temperature for red blood cells (RBC). Most bacteria, however, grow well at 20 °C to 24 °C, the storage temperature for platelets which is why surveillance studies often show a higher rate of bacterial contamination for platelets than for RBC.

Normal skin flora such as coagulase-negative Staphylococcus, yeast, Streptococcus spp., Bacillus cereus, Propionibacterium acnes, or gram-negative bacilli that are attached to skin cells or colonized in sebaceous glands are introduced into the unit with the initial skin plug when the needle enters the arm during phlebotomy. When the platelet concentrate made from the blood is incubated at room temperature, these bacteria replicate rapidly and can increase from as few as one to five colony forming units (CFU)/mL to greater than 1x10⁸ CFU/mL during the five day shelf-life. Generally bacterial levels greater than 10⁷ CFU/mL are associated with severe and often fatal outcomes.

Although the FDA requires reporting fatalities related to transfusion, there is no requirement for reporting milder reactions. From 1976 to 1985 in the United States, there were 256 deaths directly related to transfusion and approximately 10% of these were traced to bacterial contamination of a blood product. In another report from the years 1986 to 1991, there were 182 transfusion-related deaths documented with 29 of those deaths due to bacterial contamination. Over the past several years in Canada there have been 11 severe reactions associated with bacterially-contaminated components; seven with platelet pools and four with RBC.

ORGANISMS IN RBC COMPONENTS
The overall fatality rate as a result of contaminated RBC units is approximately one in every million units transfused. Y. enterocolitica, Serratia spp., and Pseudomonas spp. represent more than 50% of the implicated organisms that are reported to FDA, with Y. enterocolitica being the most frequently identified.

Y. enterocolitica causes an acute enteritis characterized by fever, nausea, and diarrhea as well as a transient bacteremia associated with an asymptomatic period before or after the episode of enteritis. If a donor is drawn during this asymptomatic period, the phagocytized organism within the donor’s white blood cells is released into the unit when the white cells disintegrate. Y. enterocolitica is capable of growth at 4 °C in the presence of iron and glucose and subsequently produces endotoxin. After an initial lag phase of 10 to 20 days the organism rapidly reproduces throughout the shelf-life of blood to concentrations of greater than 10⁸ CFU/mL. Therefore the longer blood is stored, the more likely that the concentration of endotoxin will be high enough to induce sepsis.

Between 1985 and 1996 there were 21 cases of sepsis due to Y. enterocolitica contaminated RBC, with a total of 12 deaths. Ten of these cases and five fatalities occurred between 1991 and 1996. When implicated donors were questioned in follow up interviews, several reported diarrheal episodes within a few weeks before or after donation; but other donors reported no symptoms. The first case of Y. enterocolitica contaminated platelets has been reported from a patient who received pooled platelets. The associated unit of RBCs, which had not been transfused, also grew Y. enterocolitica.

The genus Serratia contains opportunistic organisms that grow in moist areas including the respiratory and gastrointestinal tract of animals, as well as the environment. They are associated with nosocomial infections such as urinary tract infections or wound infections. S. marcescens and S. liquefaciens have been isolated from blood components. These organisms are capable of growth over a wide range of temperatures including 4 °C, and like Y. enterocolitica, produce endotoxin. The organisms can adhere to plastic transfer bags and derive nutrition from carbon sources in the water soluble plastics of blood bags. In the last ten years, S. liquefaciens has gained increased prominence as a cause of transfusion-associated sepsis. Between 1992 and 1999 there were five cases of S. liquefaciens sepsis (80% fatality rate) – three from infusion of RBC, one from a platelet transfusion, and one from autologous blood. I solation of S. liquefaciens from the blood bag and culture of the recipient’s blood confirmed a fatal case of sepsis in the United Kingdom. In the cases of RBC contamination, several of the contaminated units appeared hemolyzed or had an unusual dark color. Symptoms started after as little as 20 mL to 50 mL of blood were infused and recipients developed septic shock due to an increased endotoxin level.
Even autologous blood carries a risk for bacterial contamination. Several patients who developed transfusion-transmitted Y. enterocolitica infection had received autologous transfusions.23,33 In a Japanese study, the most common contaminating organism in autologous blood was coagulase-negative Staphylococcus with the highest contamination rate found in intra-operative salvage units.34

**Organisms in Platelet Concentrates**

The risk of a bacterially contaminated platelet transfusion is 50 to 250 times higher than risk of virally contaminated one.5 Because of the rapid growth of organisms at room temperature and increased risk of resultant sepsis in the patient, platelet storage is limited to five days. In most cases, the units implicated in bacterial sepsis are those that are four to five days old and have bacterial counts well in excess of $10^6$ CFU/mL.18,35,36 The causative organisms in platelet contamination are more varied than those found in RBC. They are primarily normal skin flora with coagulase-negative Staphylococcus (usually S. epidermidis), accounting for more than 50% of the isolates.18,35-38

As previously mentioned, several fatal cases were due to organisms such as Y. enterocolitica and S. liquefaciens that are usually associated with contaminated RBC. Other organisms that have been reported in fatal sepsis cases due to infusion of contaminated platelets include methicillin resistant S. aureus, Clostridium perfringens, S. epidermidis, Salmonella enteritidis, and S. marcescens.35,39-43

Most often however, transfusion of units contaminated with normal flora is not fatal but rather causes symptoms similar to those of a febrile, non-hemolytic transfusion reaction. The frequency of contamination of units is much higher than episodes of sepsis. Reports of incidence of bacterial contamination of platelets ranges widely with most estimating contamination as approximately 1 in 2,000 to 1 in 3,000 units, regardless of whether the source is single donor (apheresis) platelets or platelets made from whole blood.11,18,36,44,45 Risk for contamination in pooled platelets, because of the pooling, is higher than the risk in apheresis platelets.44 Septic reactions are estimated to occur in one-quarter to one-sixth of

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contaminated transfusions with estimates for overall mortality as high as 26%. Several national studies have looked at the organisms involved in transfusion-related incidents and the fatalities that occurred. Table 1 summarizes the number of incidents and fatalities reported. The SHOT study in England was a voluntary reporting study designed to track complications associated with blood transfusions. Clinicians responsible for transfusions reported 366 cases of complications between 1996 and 1998. Of these, 12 were confirmed as transfusion-transmitted infections, with four being bacterial in origin, seven viral, and one malarial. The three non-fatal bacterial infections were due to: Serratia liquefaciens (from RBC) and Escherichia coli and Bacillus cereus (from platelets). The single fatal bacterial infection was due to Staphylococcus aureus in a unit of platelets.

The French Hemovigilance Study was conducted from 1994 to 1999 in an effort to collect and analyze information from transfusion-related incidents. In this program, all transfusion-related incidents regardless of severity were required to be reported to the French Blood Agency (now the French Agency of Medical Safety of Health Products). Of the 730 incidents suspected to be caused by bacteria, 185 were confirmed as bacterially-related with 89 from platelet components and 113 from RBC. There were 18 fatalities. The overall incidence of bacterial contamination was 12.6 per million components. Fifty-eight percent of the bacteria isolated in RBC were gram-positive cocci - primarily Staphylococcus sp and Streptococcus sp., with gram-negative bacilli identified in 32% of the cases, and the remaining 10% of cases were due to other types of bacteria. In platelet concentrates, gram-negative organisms were isolated in 36% of the units; gram-positive cocci in 42%, and other bacteria in 22%.

The Bacthem Study was conducted within the French Hemovigilance Network from 1996 to 1998. This matched case control study assessed risk factors associated with transfusion-associated bacterial contamination. During this time, 41 cases of transfusion associated bacterial contamination met the criteria for inclusion in the study. Of these, 25 were due to contamination in red cell components and 16 due to contamination in platelets. There were six fatalities - four due to RBC contamination and two due to apheresis platelet contamination. Gram-negative bacilli were responsible for 52% of contaminants in red cell components versus 37% of contaminants in platelets. Based on the number of units transfused, the risk of contamination was three times higher with platelets than with RBC transfusions and increased to 12 times when platelets were pooled. In addition, the risk of contamination increased if the platelet concentrates had been stored longer than one day or RBC longer than eight days.

One of the conclusions from this study was that there is a strong association among type of component, age of component at transfusion, and the risk of transfusion-associated bacterial contamination.

In the U.S. the BaCon study was undertaken to assess the rate of adverse reactions due to bacterial contamination of blood products, to identify the organisms associated with the reactions, and to identify the risk factors for contamination. It was conducted from 1998 through 2000 as a voluntary joint effort among the American Red Cross, Department of Defense, and the American Association of Blood Banks. During the first two years there were a total of 103 reports of reactions suspected to be due to bacterial contamination. Of these, 34 reports met the criteria for the study (Table 1). There were nine fatalities reported. The most common gram-positive organism isolated was Staphylococcus epidermidis (eight isolates), followed by S. aureus (four isolates). Of the gram-negative organisms, five isolates were Escherichia coli and five Serratia sp., (three S. marcescens, two S. liquefaciens). Patients who received units contaminated with gram-negative organisms were more likely to have severe reactions than those who received units contaminated with gram-positive organisms. Although some authors had previously shown that bacterial contamination and septic reactions occurred up to five times more frequently with pooled platelet concentrates than with single donor platelets, in the BaCon study there were four fatalities due to single donor platelets and two due to pooled platelets.

Methods to prevent or detect bacterial contamination
With the ultimate aim of preventing serious reactions due to bacterial sepsis, the American Association of Blood Banks (AABB) has added a new standard to the proposed 22nd edition of Standards for Blood Banks and Transfusion Services. This standard requires blood banks or transfusion services to have methods that limit and detect bacterial contamination in all platelet components. Facilities are expected to implement this by March 1, 2004.

Over the years, a variety of methods has been suggested to limit and/or detect bacterial contamination. These include changes in aseptic technique for phlebotomy, diversion of the initial aliquot of blood collected, leukoreduction of blood, use of single donor apheresis platelets, use of gram stain or automated blood culture to detect bacterial growth, and
pathogen inactivation. Although RBC and platelets are the most commonly contaminated components, fresh frozen plasma and cryoprecipitate may also be contaminated. Because of the relatively infrequent reports of bacterially-contaminated plasma products, this section will focus on methods to detect and/or prevent bacterial contamination of RBC and platelets. The detection of bacteria in units post-collection is necessary, but the improvement of methods to decrease contamination during collection should also be addressed. The entry of normal skin flora with the initial skin plug or from a skin flap formed by the needle bore during donation are two methods theorized to induce contamination. Comparative studies of donor skin disinfection methods have shown that a combination of isopropyl alcohol and povidone iodine would give the greatest reduction in bacterial skin counts (greater than 99% reduction) or platelet contamination rates.47-49

A second prevention approach is to decrease the number of skin bacteria that reach the blood unit. Model systems were developed to compare bacterial concentration in the first few milliliters of blood into the blood bag versus the concentration in the latter part of the flow. Results from several studies showed that the initial 10 to 15 mL draw had the highest bacterial contamination and that by diverting this from the unit, there was a significant drop in percentage of contaminated units.50-52 DeKorte noted that, after diversion of this initial aliquot, the prevalence rate of bacteria in units decreased to 0.21% compared to a 0.35% rate in a previous study in which blood was collected using a standard collection method. A significant drop in contamination by staphylococcal species (from 0.14% to 0.03%) was seen after diversion.53 Diverted blood is collected in an attached pouch and can be used for serologic testing or in some cases sampled for bacterial growth. Within the last six months, the FDA has given approval to market a diversion pouch on blood bags in the United States.

Leukoreduction has been instituted in many countries to decrease the risk of HLA immunization, cytomegalovirus transmission, and the incidence of febrile, non-hemolytic transfusion reactions. It has also been suggested that use of prestorage leukoreduction will decrease the growth of Y. enterocolitica in blood units because white cells containing phagocytized bacteria will be removed before cells disintegrate and release the bacteria. A number of studies have shown decreases in rates of Y. enterocolitica contamination using prestorage leukoreduction.11,20,53 The predominance of organisms other than Y. enterocolitica in contaminated RBC in the Bacthem study may be due to use of leukoreduced units in the study.11

Results from a study by Holden in which 19 strains of coagulase negative Staphylococcus were inoculated into whole blood prior to leukoreduction and component preparation showed that leukoreduction reduced, but did not eliminate bacterial contamination.37 The impact of leukoreduction was also studied as part of the Hemovigilance project. Prevalence of contamination was compared in 18-month periods prior to, and just after, leukoreduction was instituted. There was a significant decrease in both the percentage of contaminated units (3.8% pre-leukoreduction to 1.7% post-leukoreduction) and in the number of septic reactions (71 to 24).38

The use of single donor apheresis platelets instead of pooled platelets has been suggested as another way to decrease risk of septic events. Data from a 12-year study in which platelet units implicated in a febrile, non-hemolytic reaction were cultured indicated that as the percentage of single donor platelet transfusions increased, the rate of septic platelet reactions decreased.44 Several studies using gram stain and bacterial culture demonstrated that bacterial contamination was higher in pooled platelet concentrate than in single donor apheresis platelets, while one study showed higher rates of contamination in apheresis units.15,20,54

Some initial platelet surveillance programs used Gram's stain and bacterial culture at the time of platelet transfusion and compared results with storage age of the platelets. The contamination rate was lowest in units stored less than four days. The authors concluded that risk of bacterial contamination was related to duration of storage.18 A major limitation is that the Gram's stain is usually not positive until bacteria reach levels of 10^4 to 10^5 CFU/mL, which is often on the fourth or fifth day of storage. Another microscopy approach involves the use of a fluorescent nucleic stain applied to the platelet sample with enumeration of the bacteria performed using an epifluorescent microscope. This method is able to detect bacteria at concentrations similar to that of the Gram's stain.55 Screening methods using visual changes or measurement of biochemical parameters to detect bacterial contamination have also been studied.56-60 One screening method suggested for detecting possible bacterial contamination in RBC was visual observation of the unit.57,58 RBC units with large numbers of bacteria often show hemolysis or darkening of plasma as compared to blood in the attached segments. Brecher inoculated RBC with either Y. enterocolitica or S. liquefaciens and observed for the presence of hemolysis. Aliquots were sampled for glucose concentration. As bacterial growth increased, the presence of hemolysis increased and the glucose concentration decreased.57
Although bacterial contamination of RBC is usually from the donor, external causes cannot be excluded. Case reports have linked contamination of RBC by Burkholderia cepacia (an environmental organism) to contaminated chlorhexidine solution used in disinfection of donor arm prior to phlebotomy as well as the presence of S. marcescens to a contaminated lot of blood bags.\textsuperscript{61,62}

Platelet concentrates are normally cloudy, therefore visual evidence of bacterial growth is not a useful screening method. Surrogate tests such as decreased glucose concentration (less than 250 mg/dL) or a pH less than 7.0 in platelet concentrate as well as changes in platelet swirling patterns have been suggested as results indicative of bacterial contamination.\textsuperscript{15,51,60}

Several studies using a number of different bacterial strains associated with platelet contamination were conducted to evaluate changes in platelet plasma glucose and pH using qualitative and semi-quantitative dipstick techniques.\textsuperscript{15,51,60} The swirling pattern of normal discoid platelets, which is disrupted by platelet shape alterations due to plasma pH changes from bacteria, was visually evaluated. Bacterial concentration reached $10^7$ to $10^8$ CFU/mL before any of these methods detected changes. Results indicated that these methods were less sensitive than microscopic examination of a Gram's stained smear.\textsuperscript{15,51,60}

A number of studies have evaluated the use of an automated blood culture system that detects increased CO$_2$ as an indicator of bacterial contamination in platelet concentrates.\textsuperscript{63-67} A variety of organisms (normal flora and pathogens), inoculum sizes, and spiking techniques were used in these studies. Some studies used a special blood collection system containing an integral bag used for sampling that allowed sequential sampling.\textsuperscript{63,65} Brecher inoculated apheresis units with either 10 CFU/mL or 100 CFU/mL of one of 15 strains of bacteria. All but one of the organisms (P. acnes) were detected within 10 to 26 hours.\textsuperscript{63} Results from a study comparing growth of normal skin flora and pathogens at inoculum levels of 10 to 100 CFU/mL in both apheresis platelets and random donor platelets demonstrated that bacteria were detected in 98% of the platelet units within 24 hours and in all units within 48 hours, regardless of initial inoculum size.\textsuperscript{66}

One novel approach to detect contaminated platelets involves incubating a sample with a fluorescent-labeled vancomycin probe and then examining it by microvolume fluorometry. The method detected contamination at $10^2$ CFU/mL.\textsuperscript{68}

Based on results of studies, the most rapid effective and sensitive method (to one CFU/mL) to detect bacterial contamination in platelets is use of an automated blood culture system. Major limitations to this method are that platelets must be stored at least one day for bacterial growth to begin before samples can be inoculated into the blood culture medium and growth must be monitored for another 24 hours.

Within the last year, the FDA has given approval for one company to market the newest system to detect bacterial contamination in platelets—the Pall bacterial detection system (BDS\textsuperscript{®} System). The method uses O$_2$ levels as a surrogate marker of bacterial contamination. A small aliquot of platelet concentrate is passed over an in-line filter that allows bacteria to move into a small pouch containing growth media but keeps cells back. The pouch is incubated at 35 °C for 24 hours and then tested at room temperature for oxygen concentration. Decreased levels are indicative of bacterial contamination and results are reported as ‘pass’ or ‘fail’.

**INACTIVATION OF BACTERIA**

Ideally, instead of having to detect and discard units that are contaminated, any organism present could simply be inactivated. Methods using photochemical treatment focus on inactivating bacteria, viruses, and parasites that are present in the component. Many of the compounds described are targeted for platelet concentrates since these components carry the highest rate of bacterial contamination but there are methods reported for RBC.\textsuperscript{69-73} The most common procedure for platelets involves the combination of a psoralen compound which intercalates with DNA and RNA in the organism, and ultraviolet A (UVA) light. The combination of compound and light causes crosslinking of molecules, which in turn, inhibits replication and transcription. The process does not appear to affect the hemostatic function of platelets.\textsuperscript{70} Other similar procedures use thiocarbamide and short wavelength ultraviolet light.\textsuperscript{69} The psoralen methods have also been used to successfully inactivate the intracellular bacterium Orientia tsutsugamushi (the etiologic agent of scrub typhus) in platelet concentrates.\textsuperscript{74}

**A FINAL LOOK—THE OLDEST AND THE NEWEST**

Syphilis is perhaps the oldest known transfusion-transmitted disease and when blood was transfused directly from donor to recipient it was the most common transfusion-transmitted infectious disease. But now, due to improved donor screening tests, medical history questions to identify high-risk donors, refrigeration of units, and the decrease in syphilitic
lis in the general population, cases of transmission are rare. In fact, the last reported case was in 1969.75 Despite these facts and the lack of consensus about need to retain the test, there is concern about the remote possibility of survival of the organism in platelet concentrate, so that syphilis screening of donors continues to be required.76,77

The May 2003 issue of Transfusion published a study by Hedin that investigated the transfusion-transmission potential of Chlamydia pneumoniae.78 This intracellular bacterium has the potential to be transmitted in white blood cells via transfusion. Although results of this initial study using leukodepleted blood did not yield serological evidence of transfusion-transmission, the authors point out that further investigation should be conducted before adding it to or deleting it from the ever-growing list of transfusion-transmitted organisms.

CONCLUSION

In summary, bacterial contamination remains a major cause of sepsis in the transfused patient. Psychrophils such as Y. enterocolitica predominate as causative organisms in RBC components and normal flora in platelet components. As a result of data from national studies on prevalence and studies on optimizing detection methods, new AABB standards for prevention and detection of bacterial contamination have been developed. An AABB Association Bulletin from May 2003 has summarized procedures that can be used to help facilities implement the new standards dealing with detection of bacterial contamination.79 The methods include those discussed in this article including improved disinfection, use of diversion techniques, and various methods to detect contamination. Implementation of these methods provide the transfusion community with another challenge in the on-going effort to make a transfusion as safe as possible for the patient.

REFERENCES

FOCUS: TRANSFUSION RISKS

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<tr>
<th>Title</th>
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