Septic transfusion case caused by a platelet pool with visible clotting due to contamination with *Staphylococcus aureus*

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BACKGROUND: Contamination of platelet concentrates (PCs) with *Staphylococcus aureus* is one of the most significant ongoing transfusion safety risks in developed countries.

CASE REPORT: This report describes a transfusion reaction in an elderly patient diagnosed with acute myeloid leukemia, transfused with a 4-day-old buffy coat PC through a central venous catheter. The transfusion was interrupted when a large fibrous clot in the PC obstructed infusion pump flow. Shortly afterward, a red blood cell (RBC) unit transfusion started. After septic symptoms were developed, the RBC transfusion was also interrupted. While the RBC unit tested negative for bacterial contamination, the PC and the patient samples were found to be contaminated with a *S. aureus* strain that exhibited the same phenotypic and genome sequencing profiles. The isolated *S. aureus* forms biofilms and produces the superantigen enterotoxin-like U, which was detected in a sample of the transfused PCs. The patient received posttransfusion antibiotic treatment and had her original central line removed and replaced.

DISCUSSION: As the implicated PC had been tested for bacterial contamination during routine screening yielding negative results, this is a false-negative transfusion sepsis case. Using a point-of-care test could have prevented the transfusion reaction. This report highlights the increasing incidence of *S. aureus* as a major PC contaminant with grave clinical implications. Importantly, *S. aureus* is able to interact with platelet components resulting in visible changes in PCs.

CONCLUSION: Visual inspection of blood components before transfusion is an essential safety practice to interdict the transfusion of bacterially contaminated units.

A 73-year-old female patient with remitted acute myeloid leukemia was transfused with an irradiated 4-day-old buffy coat platelet (PLT) pool at a Canadian hospital for a PLT count of 4×10^9/L. Fourteen minutes after the pool was issued by the blood bank, the transfusion started. After being transfused with two-thirds of the PLT concentrate (PC) a solid obstruction of the infusion line and a large fibrous clot were noticed (Fig. 1A), and the transfusion was interrupted. This change in appearance was not noticed in the blood bank at the time of issuing the PC or at the bedside before the transfusion started. As no reaction was apparent when the PC transfusion was stopped, a transfusion with a 34-day-old unit of red blood cells (RBCs) was commenced immediately for hemoglobin level of 64 g/L. When one-third of the RBC unit had been transfused, the patient developed chills, rigors, hypotension, nausea, and vomiting. These symptoms developed 2 hours 30 minutes

ABBREVIATIONS: CVC = central venous catheter; PC(s) = platelet concentrate(s); PIA = polysaccharide intercellular adhesin; SE = enterotoxin.

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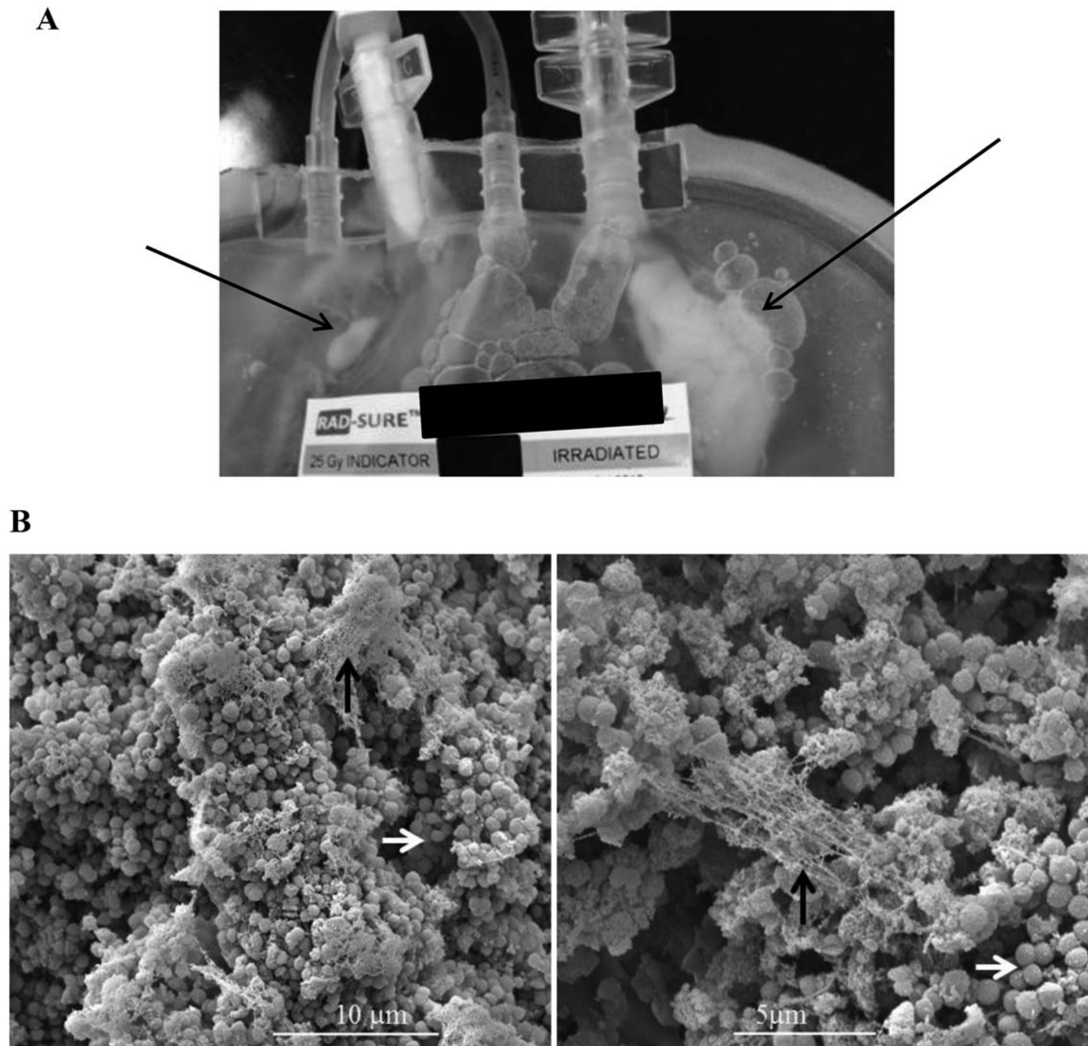

Fig. 1. (A) PCs showing a fibrous clot (arrows). (B) Scanning electron microscopy of a clotted PC sample. *S. aureus* CBS2016-05 aggregates (white arrows) form biofilms with fibrous materials and PLT debris (black arrows).

after the start of the PC transfusion and 1 hour 15 minutes after the transfusion with the RBCs had commenced. At the onset of the symptoms, the RBC transfusion was stopped. It was confirmed that the patient did not have fever or nausea before the transfusion event. Changes in vital signs of the patient during the development of the transfusion reaction are described in Table 1. The patient was admitted with a temperature of 38.8°C, which reached 39.3°C on the morning of the following day. The patient received treatment with ondansetron, dimenhydrinate, and acetaminophen. Hypotension was addressed with a saline bolus and the associated onset of fever and amid neutropenia (absolute neutrophil count, 0.6×10^9/L) led to direct admission with empiric antibiotic treatment (piperacillin/tazobactam and tobramycin). Samples for peripheral blood cultures and analysis of the patient's central venous catheter (CVC) were taken and sent to the hospital microbiology laboratory. The PC and RBC units were also tested for the presence of bacteria at the hospital. The four donors of the implicated PC were not involved in previous transfusion reactions, and all denied recent dental work, skin infections, and signs of systemic illness. Samples taken from the patient, CVC, and PC yielded the presence of *Staphylococcus aureus*. Bacterial cultures of the RBC unit were negative. After speciation and sensitivity results, the regimen was changed to cloxacillin for 4 weeks. The CVC was removed 4 days after the reaction, with a new CVC inserted 7 days later. The patient was discharged from hospital 11 days after admission.

The Public Health Agency of Canada recommends retention of residual blood components for potential investigations of posttransfusion events.[1] Following this practice, the remaining PC and RBCs transfused to the patient, as well as 3 RBC units and 1 plasma unit associated with the transfused PC, were sent to the Canadian Blood Services Centre for Innovation microbiology

TABLE 1. Changes in vital signs of the patient during the development of the transfusion reaction

Vital sign	Pretransfusion	At the time when PC transfusion was interrupted and RBC transfusion started	Onset of symptoms of transfusion reaction
Temperature (°C)	36.7	36.7	36.9*
Heart rate	97	87	89
Blood pressure	150/80	118/58	167/79
Respirations	18	16	18
Oxygen saturation on room air (%)	99	96	98

* The patient quickly developed fever having a temperature of 38.8°C at the time of admission, which reached 39.3°C on the morning of the following day.

laboratory in Ottawa (Canada) for further testing. Scanning electron microscopy imaging of a sample from the PC containing visible clots showed the presence of bacterial aggregates (biofilms) formed by staphylococcal cells, PLT debris, and what seem to be fibrin sheaths (Fig. 1B). The *S. aureus* isolates obtained from the patient, CVC, and PC were subjected to phenotypic characterization and antibiotic susceptibility testing using Analytical Profile Index (API) Staph strips and Epsilometer test (Etest) strips, respectively (bioMérieux). Genomic DNA was extracted from all isolates for whole genome sequencing (Illumina), which revealed that there were 1,761,267 bp of predicted coding sequence overlapping between all genomes with only 1995 single-nucleotide differences indicating the close relatedness of the genomes. These phenotypic and genetic analyses demonstrated that the same *S. aureus* strain was isolated from the patient samples and PC (named *S. aureus* CBS2016-05). Investigation on the ability of this isolate to form biofilms was performed using a semiquantitative crystal violet assay, slime formation on Congo red agar, and immunologic detection of the biofilm matrix polysaccharide intercellular adhesin (PIA) using established protocols.[2,3] Results showed that CBS2016-05 is a strong biofilm former in PCs (Fig. 2A) although atypically, it does not produce PIA or slime (Figs. 2B and 2C), suggesting a biofilm matrix composed of proteins and/or extracellular DNA.

The bacterial concentration in the PC at the time it was received at Canadian Blood Services was approximately 3×10^9 colony-forming units/mL, which is considered to be clinically significant[4] and has the potential of high superantigen content. Superantigen exotoxins are important virulence factors produced by *S. aureus* and include the toxic shock syndrome toxin-1, enterotoxins (SE) A to E and G, and SE-like toxins types H to X, with unconfirmed enterotoxic activity.[5] Superantigens levels as low as 0.01 μg/mL can cause fever and hypotension.[6] Investigation of superantigen production by *S. aureus* CBS2016-05 and superantigen presence in the PC revealed that this isolate produces SEG and SE-like H, I, M, N, S, U,

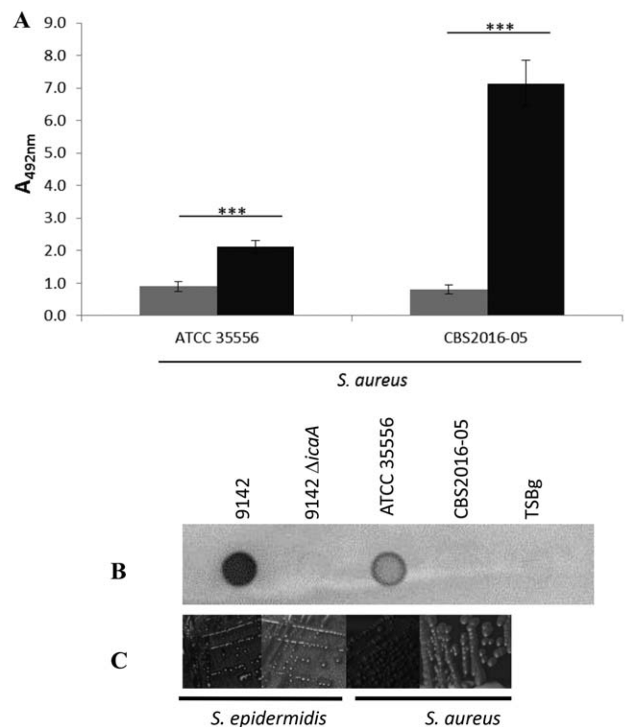

Fig. 2. Biofilm formation by *S. aureus* CBS2016-05. (A) Semi-quantitative crystal violet assay: the bars reflect the mean ± SD of two independent experiments. (■) Bacterial biofilms grown in media (trypticase soy broth supplemented with 0.5% glucose, TSBg); (■) bacterial biofilm formation in PCs. Positive biofilm formation is considered for absorbance (A_{492nm}) of more than 0.05 as reported previously[2] (*$p < 0.0001$, t tests). Strain ATCC 35556 was used as a positive control. (B) Detection of the polysaccharide biofilm matrix PIA by dot-immunoblotting using anti-*S. epidermidis* PIA antibodies is indicated by a dark dot. *S. epidermidis* 9142 and *S. aureus* ATCC 35556 were used as positive controls while *S. epidermidis* 9142 Δ*icaA* was used as a negative control. (C) Colony morphology and pigmentation on Congo red agar: positive controls *S. epidermidis* 9142 and *S. aureus* ATCC 35556 formed black crusty colonies, while the negative control *S. epidermidis* 9142 Δ*icaA* and test strain CBS2016-05 formed creamy colonies.**

and X. This profile is consistent with the clonal group USA100 as defined by the Centers for Disease Control and Prevention, a common strain found in the anterior nares of humans. An exotoxin that could be SEB, SEC, or SE-like U was detected in the PC. As these three superantigens have immunologic cross-reactivity, they cannot be distinguished by Western blotting.[5] However, *S. aureus* CBS2016-05 does not produce SEB or SEC, and therefore the superantigen present in the PC sample was likely SE-like U.

DISCUSSION

The clinical outcome of bacterial sepsis acquired during transfusion depends on the virulence and load of the causative agent and the patient's immune status. Despite the implementation of mitigation strategies to reduce bacterial contamination in blood products, transfusion-associated infections are still reported.[7-9] Surveillance at Canadian Blood Services from 2006 to 2016 revealed that approximately 90,000 PCs are transfused per year, of which 70% are buffy coat pools and 30% are apheresis units. The rate of bacterial contamination in PCs is approximately one in 10,000 with a septic transfusion reaction rate of approximately one in 125,000 and a fatality rate of approximately one in 500,000. The aerobic organisms most commonly isolated from contaminated PCs are staphylococci,[7-10] with increasing isolation of pathogenic *S. aureus* in recent years.[7,10] This bacterium is a human commensal and has become a persistent colonizer in 20% to 25% of the human population.[11] It is a common causative agent of hospital-acquired infections resulting in high morbidity and mortality[12] and has been involved in severe septic transfusion reactions.[7,10,13,14]

In 2015, a total of 41 transfusion-related fatalities were reported to the Food and Drug Administration with four involving bacterially contaminated PCs. In three of these four fatalities, *S. aureus* was identified as the causing organism.[7] Since implementation of PC screening by the National Health System Blood and Transplant (NHSBT, UK) in 2011, approximately 1,200,000 PCs have been tested.[10] At the NHSBT, PCs are stored for 36 hours before sampling for sterility testing. Despite this long quarantine period, four contaminated units have been missed, all containing *S. aureus,* showing the characteristic slow growth of this bacterium. While one of the PC units was transfused causing patient morbidity, transfusion of the other three units was prevented because the presence of aggregates was noted during visual inspection before transfusion.[10] Héma-Québec was also able to avoid the transfusion of a PC contaminated with *S. aureus* due to the presence of cotton-like aggregates.[13] In the case of the septic case described herein, PLT aggregates were noticed during the transfusion, which was fortunately interrupted. If a rapid point-of-care bacterial detection test had been performed at the blood bank, it is possible that this PC pool would not have been issued for transfusion.

At this point, it is not clear if biofilm formation or another mechanism is responsible for the formation of aggregates in PCs contaminated with *S. aureus*. Importantly, bacterial biofilm formation during PC storage might result in missed detection during routine screening and increased pathogenicity,[15-17] which could be exacerbated by the presence of pyrogenic toxins in the contaminated PCs. This is exemplified by the detection of the superantigen SE-like U, one of the superantigens frequently implicated in human disease, in the PC of the case described herein. Two other cases of PCs contaminated with superantigen-producing *S. aureus* causing fatal transfusion reactions have been reported in the literature.[14] In conclusion, this report highlights the increased reporting of pathogenic *S. aureus* as a predominant PC contaminant and the importance of visual inspection of blood products before transfusion, which is essential to interdict contaminated units and prevent septic transfusion reactions.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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