Peer Review File

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Reviewer comments

Reviewer A

In this paper, Chi and colleagues reported a case of transfusion-transmitted infection due to staphylococcus aureus (SA) contaminated platelet concentrates (PCs) which was overlooked by routine PC screening. This issue should be taken into account in respect to platelet transfusion quality and safety.

Comment 1) It is not clear to me how routine PC screening could miss this bacterial contamination. In the result section (clinical findings) the authors mentioned positive results in less than 3.5 hours of incubation in the BAC/ALERT system. Did they use different method for the routine screening?

Reply 1: These were two different BACT/ALERT cultures. The routine culture performed on PCs at Canadian Blood Services was done as per standard procedures yielding negative results. The second BACT/ALERT culture was performed at the hospital on the residual product after the transfusion event.

As Stated in the last sentence of "Case Presentation", lines 119-121, the BACT/ALERT testing result of the implicated PC was negative. We have clarified that the result was obtained after 7 days of bottle incubation as per protocol of reference 6. Therefore, the result is "false negative". The BACT/ALERT culture referred by the Reviewer was performed on the remaining PC after the transfusion event. As stated in the first sentence of "Clinical findings" (lines 124-125), "residual infused PC was analyzed at the hospital microbiology laboratory". Further clarification that this BACT/ALERT testing was performed in residual product was added on on lines 128-129 of the revised manuscript.

Comment 2) The quantification of SA in the residual PC revealed a concentration of 10*9 CFU/ml. Very high? citation?

Reply 2: Quantification of bacteria was performed in the Microbiology Lab at Canadian Blood Services as part of the investigation reported in this case, therefore there is not citation for the actual result. Clarification that this concentration is clinically significant (new reference #16) has been provided on lines 141-142 of the revised manuscript.

Comment 3) ATR-20003; citation?

Reply 3: ATR-20003 is the identification number of the strain that was assigned at Canadian Blood Services. It is the ID number of the strain isolated in this case, therefore there is not a relevant citation. Clarification that the ID number was assigned as per

Canadian Blood Services procedures has been provided on lines 143-144 of the revised manuscript.

Comment 4) Result and legend figure 1: add abbreviations SEs and SE-like into the legend.
Reply 4: Abbreviations have been added to the figure legend of figure 1 as recommended.

Comment 5) The quality of the figure 1 should be improved.
Reply 5: We think that the Reviewer is referring to Figure 2.

Comment 6) The numbering is blurry and the position of the band in PC is not clear.
Reply 6: Labeling of lanes of Figure 2 has been revised as a new Figure 2 was prepared and included in the revised manuscript.

Comment 7) The band should have similar migration as TSB?
Reply 7: The migration of the band seems different, but this is due to the way that proteins run in this lane of the gel. The difference is likely due to the fact that the samples of the two lanes were prepared from different matrices, media (TSB) vs PCs. This assay was performed in a very small volume of the residual PC (post-transfusion). A revised Figure 2 has been prepared and included in the revised manuscript.

Comment 8) The anti-SEG protein?
Reply 8: We thank the Reviewer for pointing out this error. The figure legend of Figure 2 has been revised.

Comment 9) Could we compare the concentrations of SEG protein in TSB and PC by immunoblot to gain any information about the content of SEG in the contaminated PC.
Reply 9: This would require titration of purified SEG toxin to prepare a standard curve, which is not available in our lab. Furthermore, there is not residual PC sample of the unit involved in the transfusion reaction to perform this type of experiments. Quantification of the toxin is beyond the scope of the study. Our intent is to report a qualitative assessment of the presence of SEG in the implicated PC.

Comment 10) Finally, could we have a hint by the laboratory analysis wheather the PCs are actually low or high contaminated with SA.
Reply 10: This is the assessment that was done during quantification of bacteria performed on the residual product as stated in Clinical Findings. The PC has a high contamination with *S. aureus,* the bacterium was found to be 10^9 CFU/mL, which is clinically significant (see lines 141-142 of the revised manuscript).

Reviewer B
The manuscript by Chi et al, entitled “Transfusion of a Platelet Pool Contaminated with

Exotoxin-Producing Staphylococcus aureus: A Case Report" is well presented case report of a Staphylococcus aureus contamination of a platelet concentrates not detected with routine culture methods. This report highlights the continued threat of PC bacterial contamination and presents a reminder of the clinical ramifications that may occur.

Comment 1) Page 4 line 110: Please mention whether or not empiric antibiotics administered during the onset of the signs and symptoms from the reaction.
Reply 1: Antibiotic treatment was administrated when the patient was stabilized but still febrile on the day of the transfusion. Please see new information on lines 114-115 of the revised manuscript.

Comment 2) Page 4 line 115: Please include the BACT/ALERT test result day 7 of 7 or 5 of 5 (depending on your policy) to demonstrate the culture remained negative for the life of the product.
Reply 2: The bottles were incubated for 7 days. The culture bottles unload report is available and was reviewed by the Lab Supervisor as per Canadian Blood Services standard procedures. A statement mentioning the length of bottle incubation has been added on line 121 of the revised manuscript.

Comment 3) Figure 2: Replace current figure with an image with improved resolution and quality.
Reply 3: Figure 2 has been replaced.

Comment 4) Table 1: modify table to clearly delineate vital signs during pre, during and post reaction. Perhaps moving the post to its own column.
Reply 4: Table 1 has been updated as recommended.

Reviewer C
This is a well written case report detailing a septic transfusion reaction from false negative sampling error. While this is a well-known problem, the authors characterized the organism and identified that it produced an exotoxin (SEG), which is of interest when considering virulence. A greater understanding of bacterial virulence is needed to better understand septic reaction risk (along with recipient factors and inoculum size). Some comments and questions for the authors are listed below:

Comment 1) When in the timeline did the patient receive antibacterials (e.g. cefazolin or other)?
Reply 1: The patient received treatment with Tazocin the day of the septic reaction when he was still febrile. Treatment was stopped the day after once he was afebrile. Please see updated information on lines 114-115 of the revised manuscript.

Comment 2) Please state the time of sampling, volume, culture bottle types for the

BacT/Alert screening (LVDS/primary culture) that was performed.
Reply 2: Please see updated information on lines 119-120 of the revised manuscript. PC testing at Canadian Blood Services is done with a LVDS algorithm described in reference 6.

Comment 3) How was the subsequent RBC sampling for culture performed? Could these have been negative due to false negative sampling error and a low overall concentration of contaminating bacteria?
Reply 3: RBC units were tested following established procedures (new reference #15 has been added); please see updated information on lines 137-139 of the revised manuscript. It is possible that cultures are negative due to sampling error or self-sterilization of the microorganism in this blood component.

Comment 4) Additional discussion about quantitative risks associated with false negative sampling error would be useful. Please expound upon this further in the introduction/discussion.
Reply 4: Additional information on false negative results has been provided in the Introduction (lines 69-71) and Discussion on page 3 (lines 166-170 and new reference #17).