

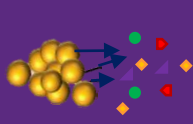
Detection of *Staphylococcus aureus* Exotoxins as Potential Biomarkers for Platelet Concentrate Contamination with *S. aureus*

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BACKGROUND

- Staphylococcus aureus* is a major contaminant of platelet concentrates (PCs), causing false-negative transfusion sepsis due to its ability to¹;
 - evade detection during routine PC screening, owing to slow growth and biofilm formation.
 - secrete exotoxins in PCs, including staphylococcal superantigen-like proteins (SSLs) and staphylococcal enterotoxins (SEs)^{2,3}.



Exfoliative toxins
Hemolysins
SSLs (inhibit host immune response)
SEs (responsible for septic shocks in PC patients)

AIM: To study the expression of *S. aureus* exotoxins as plausible biomarkers for determining PC contamination with this bacterium.

Research Questions

- QR1 Do the studied *S. aureus* produce SE & SSL exotoxin genes?
- QR2 Are the exotoxin genes expressed at RNA & protein levels?
- QR3 Do the exotoxins possess biomarker characteristics?

EXPERIMENTAL PROCEDURES

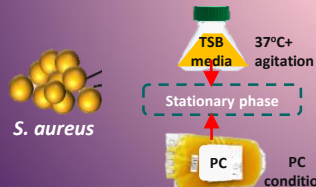
| <i>S. aureus</i> strain | PC screening description |
|-------------------------|--------------------------|
| CBS2016-05 | False-negative (Canada) |
| CI/BAC/25/13/W | Near-miss |
| PS/BAC/169/17/W | Confirmed positive |
| PS/BAC/317/16/W | Confirmed positive |

Genome analyses

DNA isolation

Whole genome sequencing & PCR screening for SE & SSL genes

Transcriptome sequencing for comparative analyses



RNA extraction & RNAseq

Validation by RT-qPCR, WB & Mutagenesis

Differential expression analyses of SE & SSL genes in TSB vs PC

RESULTS

- All strains encode exotoxin genes: that include SEs, SE-like and SSL genes as shown by genome analyses (Table 1).

Table 1: Exotoxin genes encoded by tested *S. aureus*

| <i>S. aureus</i> strain | CBS2016-05 | CI/BAC/25/13/W | PS/BAC/169/17/W | PS/BAC/317/16/W |
|-------------------------|--|---|---|--|
| Exotoxin genes encoded | <i>se g, h, i, m, n, o</i> <i>se-like s, u, w, y</i> <i>ssl 1, 5, 9-11, 14</i> | <i>se b, c, p</i> <i>se-like w, x</i> <i>ssl 3, 5, 7, 10-14</i> | <i>se g, h, i, m, n, o</i> <i>se-like u, w</i> <i>ssl 3-5, 7, 10-14</i> | <i>se g, i, m</i> <i>se-like w</i> <i>ssl 1-5, 7, 9-14</i> |

- RNAseq reveals upregulation of exotoxin genes in PC vs TSB: Most of the SSL and half of SE genes were significantly upregulated (>1 to 6.7 folds) in PCs compared to TSB (Figure 1). QRT-PCR assays has been initiated for validation.

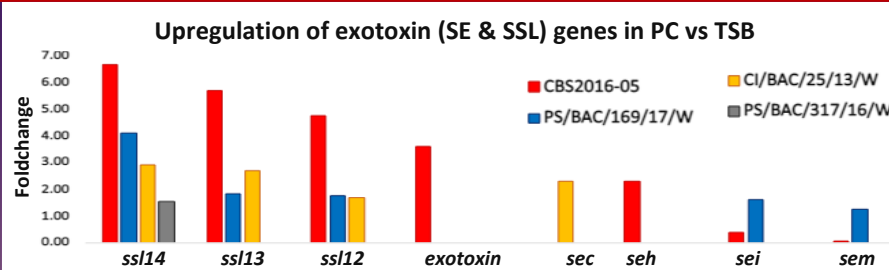


Figure 1: Upregulation of exotoxin genes in TSB compared PC. Shown are *ssl* genes in all strains and *se* genes in strains CBS2016-05 and PC/BAC/169/17/W. Log₂fold-change ≥ or ≤ 1, p<0.05.

- Exotoxin proteins detected in culture supernatants: Western blot analyses indicated the presence of SE-type G (SEG) and SE-type H (SEH) proteins in TSB and PC culture supernatants (Figure 2).

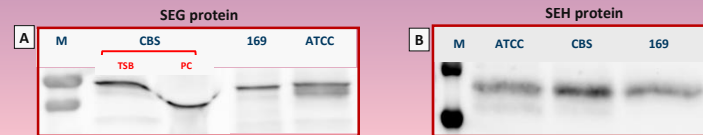


Figure 2: Exotoxin proteins probed by western blotting from culture supernatants: (A) SEG (B) SEH. M; marker, CBS; CBS2016-05, 169; PS/BAC/169/17/W and ATCC; ATCC25923 (positive control).

DISCUSSION & CONCLUSION

The expression of SEs and SSLs in PCs could potentially cause sepsis in PC recipients and prevent host immune clearance, respectively^{2,3}. For example CBS2016-05 was involved in a false-negative septic transfusion and SE-type U was detected in the residual PC sample.

- Staphylococcal exotoxins exhibit biomarkers characteristics⁵;
- secreted into the environment and therefore easily detected from TSB and PC supernatants (Figure 2).
 - SSLs & SEs expression were significantly different between both conditions (Figure 1).
 - SEs are highly effective as concentrations of <0.1pg/mL can induce septic shocks in immune-compromised patients⁴.

The presence and upregulation of SSL and SE RNA transcripts in PCs, and the probed SEG and SEH proteins, highlight the possibility of using exotoxins as indicators of *S. aureus* contamination in PCs.

FUTURE WORK

- Complete RT-qPCR validation of candidate genes and protein detection by Western blotting.
- Functional studies and cytokine release assays.
- Further biomarker characterization of selected exotoxins.

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