MALDI-TOF analysis for the detection of Panton-Valentine leukocidin (PVL), toxic shock syndrome toxin 1 (TSST-1) and methicillin resistant *Staphylococcus aureus* (MRSA)

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Natalie Lamont, Lewis Bingle, Callum Cooper

[1]Faculty of Health Sciences and Wellbeing, University of Sunderland, Sciences Complex, Sunderland, UK
Natalie.lamont@sunderland.ac.uk

University of Sunderland

Background and Aims

Staphylococcus aureus is one of the most prevalent opportunistic pathogens that secretes many virulence factors, such as Toxic Shock Syndrome Toxin (TSST-1) and Panton-Valentine leucocidin (PVL), is responsible for around one million deaths worldwide and is the primary cause of bacterial related deaths in 135 countries (1).
Fatality rates for bacteraemia were 22.1% for methicillin sensitive *Staphylococcus aureus* (MSSA) and 26.3% for MRSA in 2022 (1).
Antibiotics resistance in *S. aureus*, such as methicillin resistance, poses a serious threat worldwide. Reducing the burden of infection-related mortality is a critical global health objective with WHO including *S. aureus* in its priority list of antibiotic resistant pathogenic bacteria (2).
Both PVL and TSST-1 expression in *S. aureus* infections result in a poorer patient outcome, however, analysis for these toxins is not standard NHS practice with samples sent to the national reference laboratory in London on clinical suspicion(3).

Matrix Assisted Laser Desorption Ionisation time of flight (MALDI-TOF) has been used as a diagnostic technique for microbial identification for many years and is becoming increasingly common within diagnostic laboratories (4).

Aims
This research aims to investigates a range of culture media and environments and determine if MALDI-TOF can be used as a novel method for the detection of MRSA, TSST-1 and PVL.

Methods

Strain Selection
S. aureus strains (NCTC 12973, 13435, 11963) clinical isolates (James Cook Hospital, Middlesborough).
Liquid cultures: CCY, TSB and NB, incubated at 37˚C, 180rpm for 16-18 hours.
Solid culture: 5% horse blood media, aerobic conditions, 37˚C, 18-24 hours.
PVL&TSST-1: Western blot, primary antibodies anti-LukS-PV and anti-TSST-1, secondary antibody Rabbit polyclonal HRP and imaged using Biorad software.

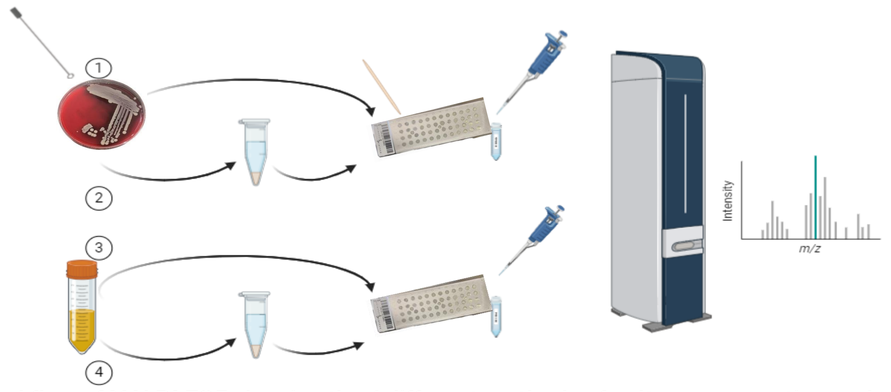

Figure 1: Workflow of MALDI-TOF showing the 4 different methods of culture, preparation and analysis. 1. Direct transfer; Isolates from solid agar picked & smeared onto target plate. **2.** Full extraction; 300µl ultra-pure water adding 1-2 colonies & emulsified, 900µl pure ethanol mixed & centrifuged (13000rpm, 2 minutes), supernatant removed & re-centrifuged & air dried. 30µl 70% formic acid added & scratch mixed, 30µl 100% acetonitrile added, vortexed & centrifuged (13000rpm, 2 minutes), 1µl extracted supernatant added to target plate. **3.** Liquid culture supernatant was centrifuged at 4000g, 4°c for 10 minutes, 1µl of supernatant applied to target plate. **4.** Centrifuged culture supernatant was subject to protein precipitation using 10% TCA and acetone. All methods performed in triplicate, MALDI Fleximass target plate was used, 1µl of CHCA applied to each sample & air dried prior to MALDI-TOF analysis

Results

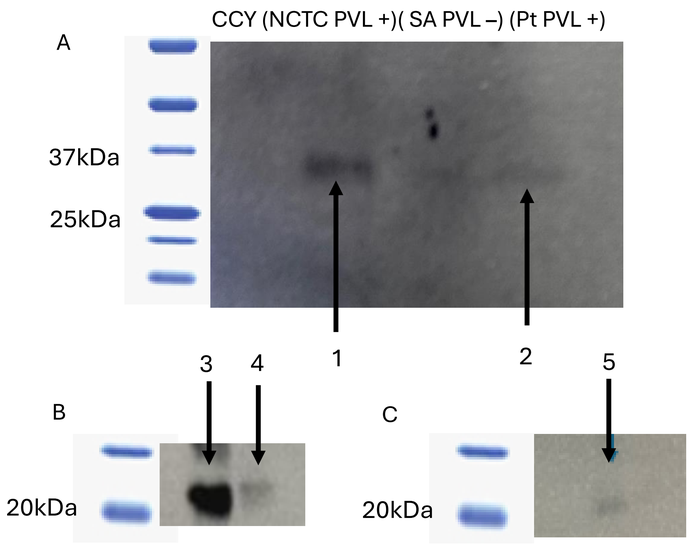

Figure 2 Western blot analysis of liquid culture supernatants from *S. aureus* in CCY, TSB and NB. A Western blot of PVL negative and positive culture supernatants with bands seen at ~ 35kDa. 1 shows band in CCY NCTC PVL positive strain, 2 shows faint band in CCY PVL positive patient isolate. **B** Western blot of TSST-1 with bands in the region of 20kDa. 3 shows NCTC positive strain from CCY culture, 4 shows patient positive TSST-1 strain in CCY. **C** Western blot NCTC TSST-1 positive strain with faint band.

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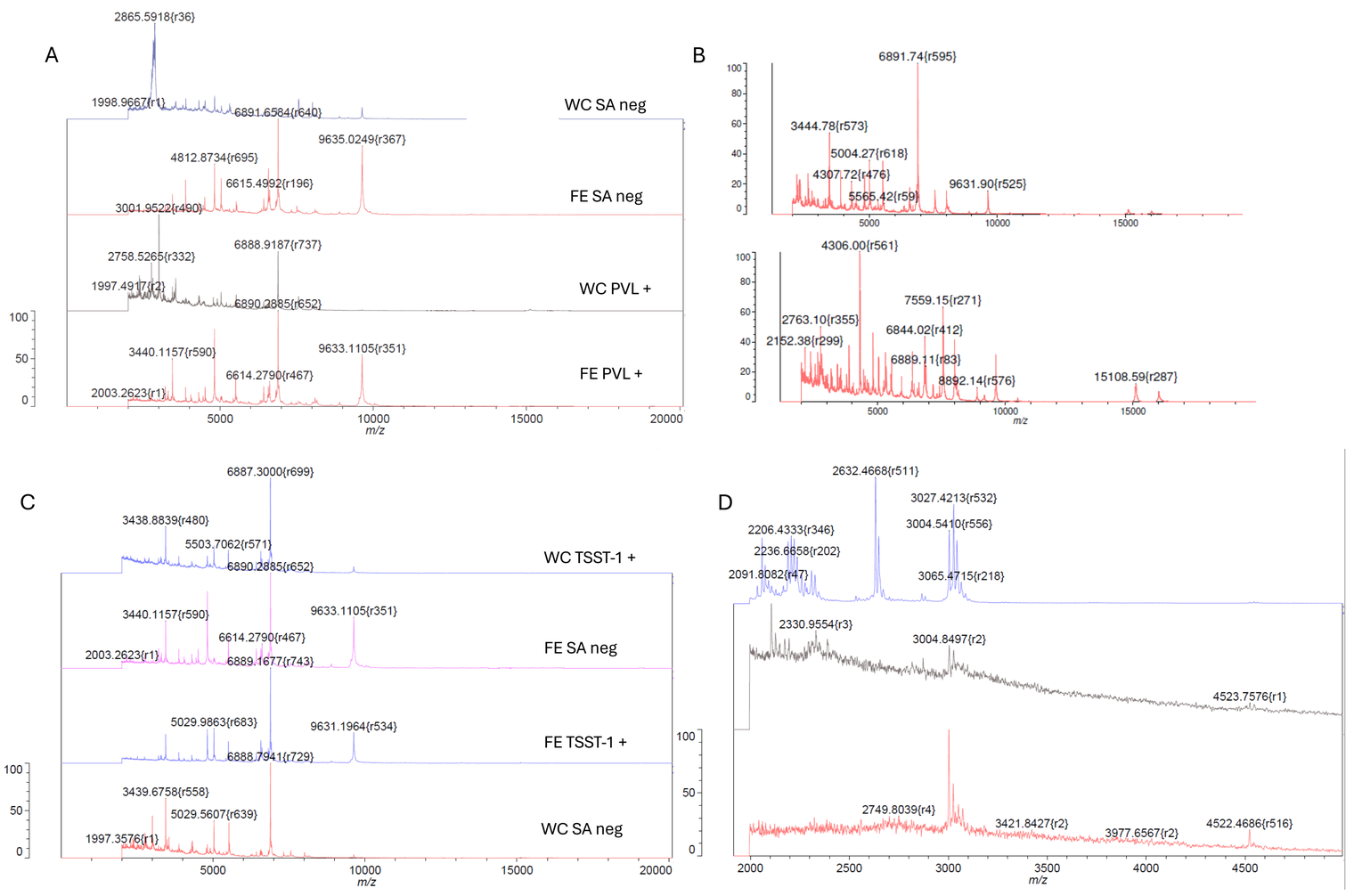

Figure 3. Spectra produced from MALDI-TOF analysis of *S. aureus* strains. Methods analysed using Shimadzu Axima ID plus confidence in linear positive mode, laser power at 72, spectrometer parameters at 2-20kDa m/z range. **A** Spectra produced from whole cell method and full extraction method from both *S. aureus* negative and PVL positive strains. **B** Whole cell MSSA (bottom) and MRSA (top) spectra. **C** Whole cell method and full extraction for *S. aureus* negative and TSST positive strains. **D** PVL positive *S. aureus* comparing CCY (top), NB (middle) and TSB (bottom).

Discussion and further work

- *S. aureus* adapts and responds depending on its environment regulating virulence genes and toxin expression. It is crucial to select optimal media for *S. aureus* culture (5).
- CCY media results in protein secretion of both PVL and TSST-1 proteins (Figure 2), this aligns with previous research suggesting the addition of pyruvate upregulates PVL (5).
- Further analysis is required for protein quantification.
- Synthetic nasal medium (SNM) for culture of *S. aureus* has been reported to upregulate TSST-1 protein, mimicking the composition of human nasal secretions (6) and will be analysed to determine its suitability for secretion of PVL and TSST-1.
- Full extraction method for MALDI-TOF produced the highest confidence score for identification of *S. aureus,* allowing for better resolution and less noise in spectra produced (Figure 3, A and C), suggesting full extraction of whole cells directly from solid culture would be the optimal method (7).
- Spectra produced so far does not allow for differentiation between strains (Figure 3, A, B and C).
- Analysis of culture supernatant determined CCY produced cleaner, higher resolution spectra in comparison to TSB and NB, with NB being unsuitable for MALDI-TOF analysis due to low resolution and high noise (Figure 3, D).
- Protein precipitation method did not improve spectra quality, requiring further steps and being more time consuming this would be difficult to bring into diagnostic practice.
- Analysis of results from supernatant visually and with mass comparison did not allow for differentiation between strains.
- Further work for this research involves increasing the m/z ratio outside of 2-20kDa to 1-100kDa, allowing for wider range of peaks to be detected. The aim is to explore alternative approaches for peak analysis and discrimination between spectra.

References

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