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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Background

- *S. aureus* is one of the most prevalent opportunistic pathogens and is the primary cause of bacterial related deaths in 135 countries (1)
- Bacteraemia fatality rates in the UK were 22.1% MSSA and 26.3% MRSA in 2022 (1)
- Many virulence factors of *S. aureus* are secreted proteins, such as TSST-1 and PVL.
- Antibiotic resistance in *S. aureus* poses a serious threat globally (2) responsible for around one million deaths worldwide (1)
- Reducing the burden of infection-related fatality is a critical global health objective with WHO including *S. aureus* in its priority list of antibiotic-resistant pathogenic bacteria (2)
- PVL and TSST-1 *S. aureus* infections result in a poorer patient outcome (3), these toxins are not routinely screened for within the NHS (3)
- *S. aureus* adapts and responds depending on its environment, regulating virulence genes and toxin expression, hence it is crucial to select optimal diagnostic media for *S. aureus* culture (6)
- Matrix Assisted Laser Desorption Ionisation time of flight (MALDI-TOF) has been used as a diagnostic technique for microbial identification for many years, reducing time to detection (4), however it has not been used for detection of MRSA, PVL and TSST (5)

Aims

To investigate a range of culture media, multiple preparation methods and different matrices to determine if MALDI-TOF can be used as a novel method for the detection of MRSA, TSST-1 and PVL strains of *S. aureus*

Methods

Strain Selection

S. aureus isolates (n = 427) were analysed using control strains (NCTC 13435 PVL, 11963 MRSA (*mec*A)12493, *S. aureus* 12973 TSST-1) and clinical isolates (James Cook Hospital, Middlesborough)

Liquid cultures: CCY, TSB, NB and BHI incubated at 37˚C, 180rpm for 16-18 hours.

Solid culture: Columbia agar with 5% horse blood, aerobic conditions, 37˚C, 18-24 hours

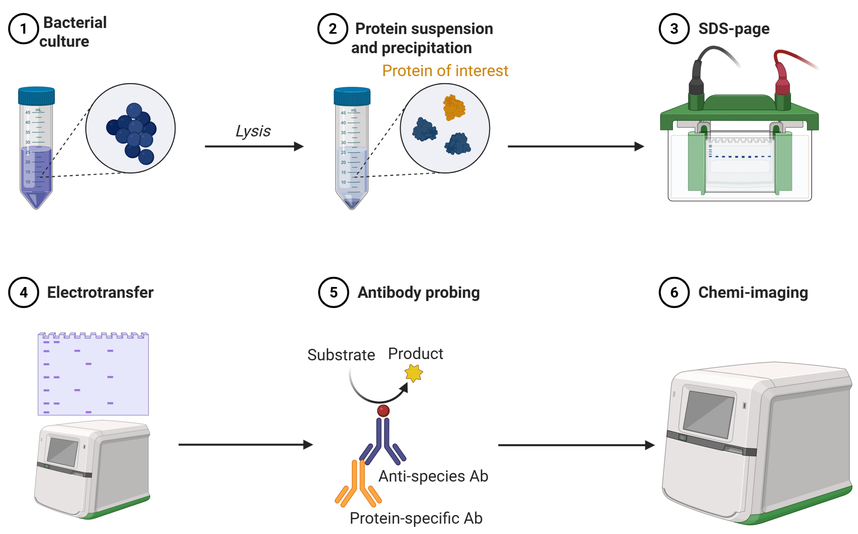

Figure 1: Western blot workflow performed on all liquid cultures and purified LukS-PV and TSST-1 proteins. Bacterial strains were cultures in TSB, CCY and BHI, Log OD measured at 600nm (~1.7), samples prepared using protein precipitation, SDS page and western blot performed with primary antibodies LukS-PV and TSST-1, secondary antibodies Anti-rabbit HRP

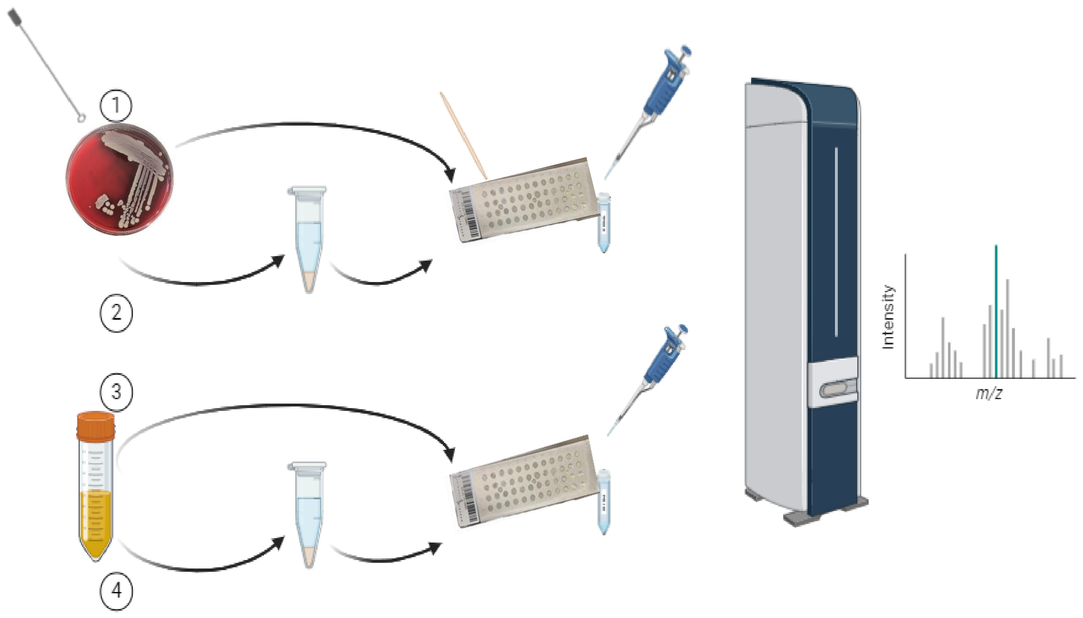

Figure 2: Workflow of MALDI-TOF MS showing the 4 different methods of culture, preparation and analysis. 1, Direct transfer; 2, Full extraction; 3, Liquid culture direct; 4, Culture supernatant treat using full extraction method and protein precipitation method. All samples were analysed in triplicate, 1µl of CHCA, DHB or Sinapinic acid applied to each sample and air dried prior to MALDI-TOF analysis

Results

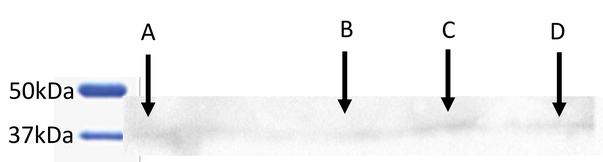

Figure 3 PVL Western blot of liquid culture supernatants from *S. aureus* strains in CCY, TSB and BHI. Primary antibody used was anti-LukS-PV, secondary antibody anti-rabbit IgG. **A** points to band seen at ~37kDa in lane 2 containing LukS-PV purified protein. **B** points to faint band seen in lane 6 containing PVL positive strain cultured in TSB media, **C** points to band seen at ~37kDa in lane 10 containing PVL positive strain cultured in CCY media. **D** points to band seen at ~37kDa in lane 14 containing PVL positive strain cultured in BHI.

Figure 4 TSST-1 Western blot of liquid culture supernatants from *S. aureus* strains in CCY, TSB and BHI. Primary antibody used was anti-TSST1, secondary antibody anti-rabbit IgG. **A** points to band seen at ~22kDa in lane 3 containing TSST-1 purified protein. **B** points to faint band seen in lane 11 containing TSST1 positive strain cultured in CCY media, **C** points to band seen at ~22kDa in lane 15 containing TSST1 positive strain cultured in BHI media.

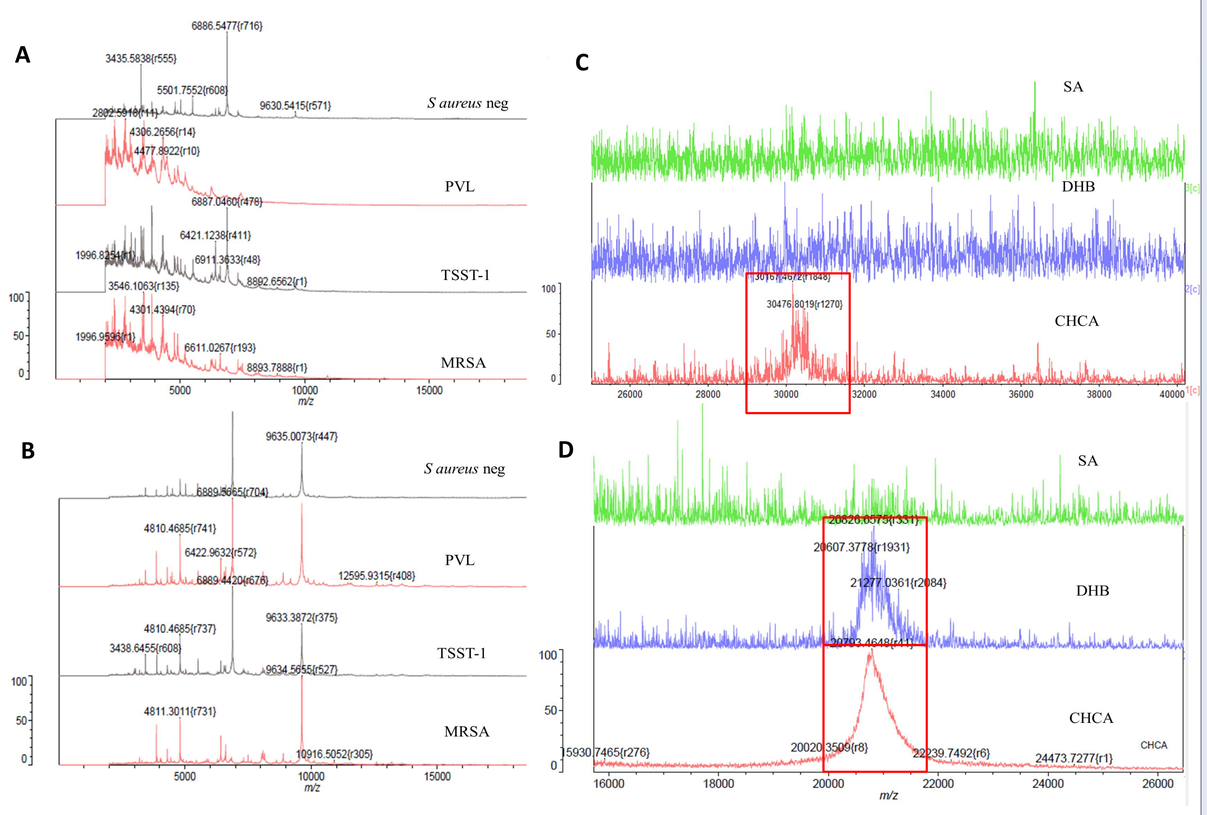

Figure 5. Mass spectra produced from various extraction methods using whole cells and purified proteins, demonstrating peak analysis between the different strains of *S. aureus*. Methods analysed using Shimadzu Axima ID plus confidence in linear negative mode, laser power at 72, spectrometer parameters at 2-20kDa m/z and 1-100kDa > 400 spectra were produced and analysed. **A** Example spectra produced from whole cell method for all strains. **B** Example spectra full extraction spectra produced from all strains **C** Example spectra at 25 – 40kDa from PVL purified protein direct on target plate with CHCA, DHB and sinapinic acid (SA) matrix with red box indicating the peak associated with PVL protein **D** Example spectra at 16 – 26 kDa from TSST-1 purified protein direct on target place with CHCA, DHB and SA matrix with the red box indicating peak associated with TSST-1 protein.

Conclusions

- Use of TSB, CCY and BHI media results in protein secretion of both PVL and TSST-1 proteins from *S. aureus* (Figure 3&4) , previous research suggests this is the case with CCY due to the addition of pyruvate (6)
- Full extraction produced the highest confidence score for identification of *S. aureus*, allowing for better resolution and defined peaks in spectra produced (Figure 5), however there was no difference between strain analysis
- Whole cell or liquid culture analysis on MALDI-TOF did not result in differentiation between strains
- Analysis of isolates and liquid cultures using alternative matrices did not allow for differentiation between strains
- PVL and TSST-1 protein are detectable on MALDI-TOF
- Analysis using CHCA and additional matrices (DHB and SA) with purified proteins showed proteins can be detected using MALDI-TOF with CHCA giving peaks ~ 20-22kDa for TSST-1 protein (Figure 2, D) and ~ 30kDa for PVL protein (Figure 2, C) compared to expected values of 22kDa and 37kDa
- Further work will involve analysis of *S. aureus* cultured in BHI medium on MALDI-TOF using the different matrices
- Use of novel matrices will be explored and analysed with culture supernatants processed using both full extraction method and protein precipitation

References

1.Ikuta KS, Swetschinski LR, Robles Aguilar G, Sharara F, Mestrovic T, Gray AP, et al.. The Lancet [Internet]. 2022 Dec;400(10369):2221-48. 2.World Health Organisation (2017) Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis 3.McGuire E, Neill C, Collin SM, Higgins H, Guy R, Ganner M, et al. J Med Microbiol. 2023;72(4). 4.Alizadeh M, Yousefi L, Pakdel F, Ghotaslou R, Rezaee MA, Khodadadi E, et al. Hindawi Limited; 2021 . 5. Marina Oviaño, Belén Rodríguez-Sánchez, MALDI-TOF mass spectrometry in the 21st century clinical microbiology laboratory, Enfermedades infecciosas y microbiologia clinica (English ed.),, Volume 39, Issue 4,, 2021,, Pages 192-200, ISSN 2529-993X, https://doi.org/10.1016/j.eimce.2020.02.016.. 6.Harper L, Balasubramanian D, Ohneck EA, Sause WE, Chapman J, Mejia-Sosa B, et al. mBio. 2018 Jan 1;9(1). 7.Krismer B, Liebeke M, Janek D, Nega M, Rautenberg M, Hornig G, et al. PLoS Pathog. 2014 Jan;10(1). 8.Drevinek M, Dresler J, Klimentova J, Pisa L, Hubalek M. Lett Appl Microbiol. 2012;55(1):40-6