Solving a microbiological quandary: Does MALDI-TOF Mass Spectrometry hold the key to rapid detection of vancomycin-intermediate *Staphylococcus aureus*?

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Background: Vancomycin is a mainstay in the treatment of serious infections due to methicillin-resistant *Staphylococcus aureus* (MRSA). However, vancomycin treatment failure rates are higher in patients with blood-stream infection due to MRSA strains that are either vancomycin-intermediate or have a vancomycin-intermediate sub-population (so-called heterogeneous VISA strains or "hVISA"). Importantly, hVISA strains often go undetected because the vancomycin-intermediate sub-population is present at a frequency lower than what can be detected by standard susceptibility testing methods. Because the vancomycin-intermediate phenotype is related to alterations in the expression of a variety of proteins, the goal of this study was therefore to investigate the potential for MALDI-TOF Mass Spectrometry to rapidly detect VISA and hVISA strains.

Methods: 16 VISA, 15 hVISA and 47 vancomycin-susceptible *S. aureus* (VSSA) strains were inoculated onto Sheep Blood Agar (BA) and Mueller-Hinton agar with 0.5 μg/mL vancomycin (MHV) and incubated at 35°C with 5% CO_2 for 20-24 hours prior to extraction. Vancomycin susceptibility was established for all isolates by broth microdilution minimum inhibitory concentration and by modified population analysis profile (PAP), which is the gold standard for hVISA detection. All isolates were processed using ethanol-formic-acid-acetonitrile extraction. Spectra were acquired using on the Bruker Biotyper system (Bruker Daltonics) and analyzed using the MALDIQuant program (R archive CRAN).

Results: We observed 394 peaks that were present in at least 1 spectrum on both BA and MHV for all isolates tested. Of these, 168 peaks were shared within a class (i.e. VISA, VSSA, or hVISA) with a CV<50% between each media type. We observed 96 peaks that differed in intensity between VSSA and VISA strains on BA, compared with 120 peaks on MHV ($p<0.0001$). Using a training set comprised of 10 VISA & 10 VSSA isolates chosen to maximize differences in PAP values, we were able to correctly identify 100% of the remaining VISA isolates tested (6/6), with 31/37 VSSA strains correctly identified for a specificity of 84%. Interestingly, 47% of hVISA isolates were identified as VISA when grown on MHV compared with only a 27% identification rate when grown on BA.

Conclusions: This is the first study to fully characterize confirmed VISA, hVISA and VSSA strains by MALDI-TOF-MS. Spectral differences, particularly with regard to peak intensity, can indeed be observed between VISA, hVISA and VSSA strains. Although further optimization appears to be required to reliably differentiate these strains by MALDI-TOF-MS, this method offers the potential to dramatically improve the time-to-detection for VISA and hVISA strains.