Predicting the hVISA Phenotype Using a Novel *in vitro* Real-time Assessment of *S. aureus* Division Rates

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AMENDED ABSTRACT

Background: Vancomycin has been the primary treatment for serious methicillin-resistant *Staphylococcus aureus* (MRSA) infections including life threatening bacteremia. However, MRSA isolates with reduced susceptibility to vancomycin have emerged in vancomycin intermediate *S. aureus* (VISA), vancomycin-resistant *S. aureus* (VRSA) and heterogeneous vancomycin intermediate *S. aureus* (hVISA). All three of these phenotypes are associated with a high degree of persistent infections and treatment failure. However, hVISA remains particularly problematic since there is no standard or rapid means of detecting these organisms. This study evaluated *S. aureus* strain growth kinetics as a means to detect hVISA directly from positive blood cultures.

Methods: Triplicate runs of 11 hVISA isolates - as determined by conventional population area under the curve (AUC) methodology - and control isolates ATCC® 29213 (VSSA), Mu3 (hVISA), and Mu50 (VISA) were performed on the Accelerate Pheno™ system on separate days. Single replicate runs of 8 vancomycin susceptible isolates were run as additional controls. Isolates were seeded into blood culture bottles containing healthy donor blood and incubated until positive. Post positivity, samples were loaded in the system and run with a custom consumable and script. Cells were introduced into multiple, independent flowcell channels and for susceptibility testing were exposed to growth media containing 8 separate vancomycin concentrations ranging from 0 to 4 µg/mL. The system counted the number of growing clones that increased in mass 4-fold and measured the median growth rate of isolates in vancomycin for each test condition. Data collection was complete in less than 7 hours. The fraction of surviving growing clones and the fractional depression of division rates relative to the antibiotic free control were used to construct separate vancomycin population analysis profiles (PAP). An algorithm combined the PAP information and generated an AUC measure for each replicate. A minimum of 250 growing clones in the growth control was required to report a valid AUC.

Results: All 3 replicates of one hVISA isolate did not have sufficient growing clones for analysis. 3 runs failed to run to completion and were not included in the analysis leaving 39 runs for scoring. An AUC cutoff value of 2.25 was determined using the data set, and 21 of the 24 hVISA replicates were scored correctly. Of the 3 failures across 2 isolates, 1 of 3 replicates and 2 of 3 replicates were characterized correctly. Mu3 and Mu50 were characterized as having vancomycin AUCs significantly higher than the cutoff value (4.8 and 7.0 respectively).

Conclusions: Division rate and clone counting analysis represents a promising approach for the detection of hVISA and VISA directly from positive blood cultures in under 8 hours. Additional optimization is needed to improve the reliability of detection.

INTRODUCTION

Staphylococcus aureus is one of the most frequently isolated pathogens from patients with bloodstream infections (BSI). Methicillin resistant *S. aureus* (MRSA) continues to increase in prevalence worldwide and vancomycin remains the drug of choice for treatment of these infections. However, an increase in failure rates to vancomycin has been reported in patients that have *S. aureus* strains demonstrating MIC values within the suceptible range. One potential explanation for these failures are *S. aureus* that demonstrate an intermediate susceptibility phenotype to vancomycin. These organisms are known as heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA). Currently, clinical labs do not screen for hVISA. The gold standard for testing for hVISA is by Population Analysis Profiling – Area Under the Curve (PAP-AUC) analysis, which is a laborious and lengthy process (i.e. >48 hours). Conventional antibiotic susceptibility testing methods (e.g. broth microdilution, disk diffusion, etc.) that are routinely performed in clinical labs do not reliably detect hVISA because these strains will often show up as susceptible to vancomycin when using these testing methods. This leads to inadequate patient treatment with vancomycin. This study evaluated the ability of the Accelerate Pheno™ system to identify the hVISA phenotype directly from positive blood cultures.

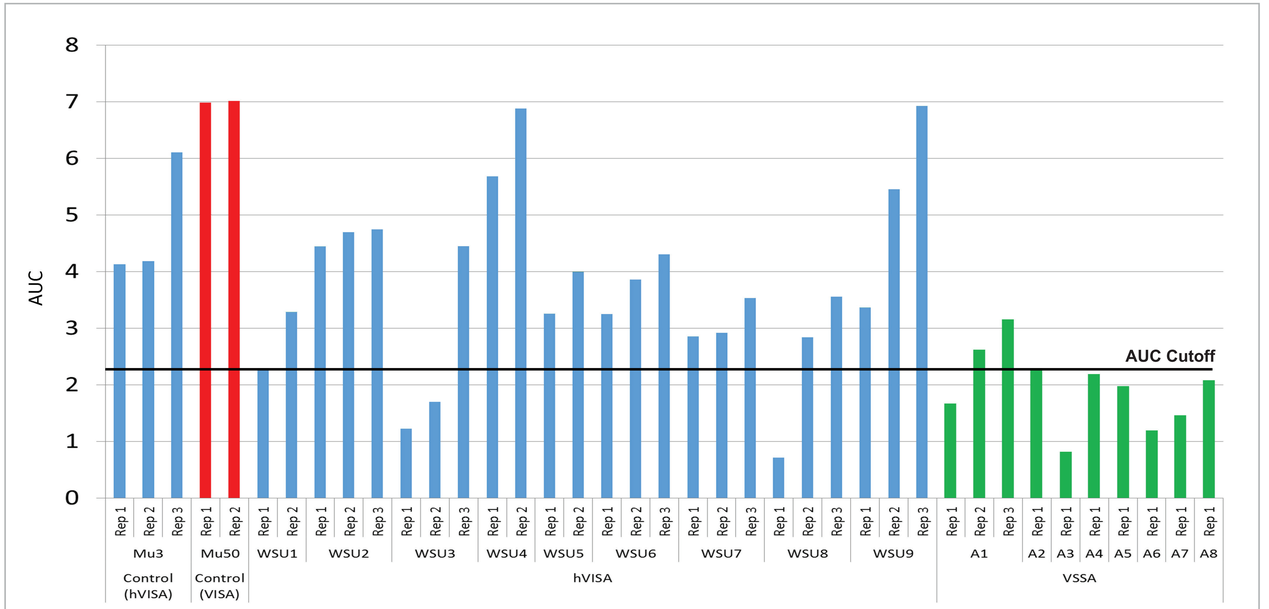

Figure 1: *AUC of each run in relation to 2.25 AUC cutoff*

METHODS

A total of 45 experiments were run over the course of 3 days using 20 different isolates. The isolates consisted of 11 hVISA, 1 vancomycin-intermediate *Staphylococcus aureus* (VISA) and 8 VSSA. Control isolates used were ATCC® 29213 (VSSA), Mu3 (hVISA), and Mu50 (VISA). The 10 hVISA, 1 VISA and ATCC® 29213 (VSSA) were run in triplicate over the 3 days. The 7 remaining VSSA were run once each as additional controls. All isolates were grown on Brain Heart Infusion agar and inoculated at 26 CFU/mL into 50 mL BACTEC™ Plus Aerobic/F Medium bottles (Becton Dickenson, Baltimore, MD) containing healthy human donor blood to simulate a human blood sample. Post-positivity, 1 mL of blood culture was aliquoted into a sample vial and run on the Accelerate Pheno™ system using a custom RUO reagent cartridge and assay.

Sample preparation, cell dilution and cell capture were performed automatically by the system. A custom antibiotic plate on the reagent cartridge contained different concentrations of vancomycin which were added to 8 separate flowcells in the 48-channel test cassette using a modified assay. All isolates were introduced to, and challenged against the resulting panel of various increasing final concentrations of vancomycin ranging from 0 to 4 µg/mL.

Image analysis software analyzed the growth rates of individual bacterial clones to develop a median division rate. The Accelerate Pheno™ system counted the number of growing clones that increased in mass 4-fold and measured the median growth rate of the isolates in varying concentrations of vancomycin. This data was analyzed for each of the 8 flowcells. The fraction of surviving growing clones and the fractional depression of division rates relative to the antibiotic-free control were used to construct separate vancomycin population analysis profiles (PAP). A custom algorithm combined with the PAP information, generated an AUC measure for each replicate.

RESULTS

The division rate and clone counting analysis method time to result was under 8 hours. Forty-five experiments were run in total for the study. Three experiments for 1 hVISA isolate did not have sufficient growing clones for analysis. Three other hVISA runs failed to complete. All 6 runs were excluded from the experiment data.

Figure 1 shows the AUC calculated by the Accelerate Pheno™ system for each isolate. Thirty-nine runs in total were scored. An AUC cutoff value of 2.25 was determined based on the data. Using this cutoff, 21 of the 24 hVISA replicates were scored correctly (87%). For 2 hVISA isolates, 1 of 3 reps (WSU8) and 2 of 3 reps (WSU3) fell into the VSSA range causing a false-negative result (3 total runs) while the remaining reps for these isolates were called correctly. Two VSSA isolates were called as hVISA (false-positives, A1 and A2).

CONCLUSION

Results support the feasibility of the Accelerate Pheno™ system using division rate and clone counting analysis as a promising rapid approach to detect the presence of hVISA and VISA directly from positive blood cultures in under 8 hours. The Accelerate Pheno™ system offers a promising alternative with respect to time to detection compared to the current PAP-AUC method, providing faster results that can potentially allow for more accurate patient treatment. Correct identification of the hVISA phenotype was 87%, however, run-to-run variability could be improved upon. All phenotypes were identified correctly in at least one run throughout the study. Additional testing using current isolates as well as a more diverse panel of hVISA isolates will help to modify the existing assay to improve reportability and reliability of the assay.