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

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Background: Vancomycin has been the primary treatment for serious methicillin-resistant Staphylococcus aureus (MRSA) infections including the threatening bacteria with the hVISA phenotype. hVISA, MRSA susceptible to vancomycin, shows heterogeneous vancomycin-intermediate (VISA) strain growth kinetics as a phenotype directly from positive blood cultures. These strains will often show up as susceptible to vancomycin when using these current assays. 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 Mu60 (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 as 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/BioLog Medium bottles (Becton Dickinson, 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.

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 Mu60 (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 as 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/BioLog Medium bottles (Becton Dickinson, 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.

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 vancomycin-resistant S. aureus (VISA) and VISA directly from positive blood cultures in under 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 VISA phenotype was confirmed for additional experiments to improve upon. Additional testing using current isolates as well as a more diverse panel of VISA isolates will help to modify the existing assay to improve reportability and reliability of the assay.

AUC of each run in relation to 2.25 AUC cutoff

Methods: Triplicate runs of 11 hVISA isolates – as determined by conventional population analysis profiles (PAP) – were performed on the Accelerate Pheno™ system on separate days. Single replicate runs of 8 vancomycin susceptible isolates were run as positive and negative 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 control and sample. Cells were introduced into multiple, independent flow channels and for susceptibility testing were exposed to growth media containing 8 separate vancomycin concentrations ranging from 0 to 4.0 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 division rate of isolates 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 260 growing clones in the growth control was required to report a valid AUC.

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

Within the susceptible range, one potential explanation for those 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.

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 these organisms. This study evaluated S. aureus strain growth kinetics as a means to detect VISA directly from positive blood cultures.

AMENDED ABSTRACT

Background: Vancomycin has been the primary treatment for serious methicillin-resistant Staphylococcus aureus (MRSA) infections including the threatening bacteria with the hVISA phenotype. hVISA, MRSA susceptible to vancomycin, shows heterogeneous vancomycin-intermediate (VISA) strain growth kinetics as a means to detect VISA directly from positive blood cultures. 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 Mu60 (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 as 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/BioLog Medium bottles (Becton Dickinson, 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.

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