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Potential Impact of Rapid Phenotype Identification on Antimicrobial Prescribing

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AMENDED ABSTRACT

Background: Automated microscopy can rapidly measure growth rates of small numbers of immobilized bacteria to identify MRSA.[1] We report the performance of this method using *S. aureus* (SA) clinical isolates, and retrospective chart review to assess potential impact on antimicrobial prescribing. The purpose is to test a model of experimental design for future trials to determine the potential to reduce the choice of inactive or suboptimal initial therapy for critically ill patients.

Methods: Frozen SA isolates were resuspended after 24-h growth on blood agar. After 2-h, 30 μL aliquots of 1E+6 CFU/mL were pipetted into microfluidic flowcells. 200-500 bacteria were immobilized onto poly-L-lysine coated glass. An image analyzer processed microscopy images and measured growth rates after 1-h induction by 1 μg/mL then 3-h of 6 μg/mL cefoxitin. SA was classified as MRSA if growth arrest occurred within a defined observation time, and MSSA if arrest did not occur. Results were compared to the original clinical lab culture reports. 50 charts were abstracted for antimicrobial days of therapy against SA for the 3 days that followed original specimen collection while the clinical lab performed culture analysis. Prescriptions actually used were categorized as active/optimal, suboptimal/redundant, or inactive.

Results: The microscopy method correctly classified 17/19 MSSA and 39/39 MRSA. The 2 incorrectly classified isolates were small colony variants from the same patient. Sensitivity and specificity in identifying MRSA were 100% [CI 88%-100%] and 89% [CI 65%-98%], respectively. For 50 charts reviewed, 208 days of therapy were prescribed *vs*. SA during the 3 days while awaiting culture results. Based on SA susceptibility, 96/208 (46%) days of therapy were considered active/optimal; 55/208 (26%) days were suboptimal/redundant; and 57/208 (27%) days were inactive. If it had been used directly with patient specimens, the new method could have raised the active/optimal rate from 46% to 97%, reduced the inactive rate to zero, and reduced the suboptimal rate to 3%.

Conclusions: The new method effectively identified MRSA within 4 hours using small numbers of bacteria from clinical isolates with an inoculum size compatible with direct extraction from clinical specimens.[2] It shows promise for reducing the high rate of inactive and suboptimal/redundant therapy observed while awaiting results from conventional cultures.

INTRODUCTION

Infections due to methicillin resistant *S. aureus* (MRSA) are increasing in frequency. For critically ill patients, the physician must begin therapy within a few hours of deciding the need for intervention. However, standard clinical lab cultures require 2-3 days. Identifiable risk factors for MRSA are not always evident, prompting empiric use of vancomycin or other inappropriate initial therapy. Inappropriate therapy promotes emergence, selection, and spread of resistant pathogens.

Rapid phenotype identification using automated microscopy could potentially improve antimicrobial prescribing by identifying more appropriate initial therapy. Our study objectives were to —

1. Determine the sensitivity, specificity, and speed of automated microscopy to detect MRSA in clinically significant *S. aureus* isolates.
2. Measure the potential impact of rapid phenotype identification on antimicrobial prescribing.

MATERIALS & METHODS

Study Design. Retrospective chart review and concurrent microbial analysis of stored isolates using automated microscopy.

Setting. Denver Health Medical Center, a 495 bed public teaching hospital and Level 1 Trauma Center.

Study Population. 54 patients with 58 clinically significant *S. aureus* isolates retrievable from -70° C storage. 50 charts were sufficiently complete to permit review.

Study Period. December 2003 through April 2006.

Microbiology. Frozen SA isolates were resuspended in tryptic soy broth after 24-h growth on blood agar.

- After 2-h, 30 μL aliquots of log-phase *S. aureus* at 1E+6 CFU/mL were pipetted into microfluidic flowcells.
- Each flowcell surface had a transparent electrode coating, forming an electrophoresis chamber within each flowcell (***Fig 1***). Application of an electrical field caused bacteria to migrate to the positively-charged lower electrode during a capture step.
- 200-500 bacteria were immobilized to a poly-L-lysine coated surface, permitting subsequent medium exchanges.
- Image analysis software measured growth rates in microscopy images acquired at 10-minute intervals.
- The standard test[1] exposed an isolate to 1 μg/mL cefoxitin (FOX) for 1-h induction, followed by 3-h at 6 μg/mL.
- The system identified MRSA if an isolate's growth arrested after 130 minutes of 6 μg/mL exposure, and MSSA if not. Tests were run in triplicate and results were averaged.

Figure 1: *32-channel flowcell cassette.*

- Each isolate was also tested in separate flowcell channels with no antibiotic (growth control).
- Results were compared to the original culture reported in the clinic microbiology laboratory using standard methods.

Chart Reviews. Medical records were reviewed for —

- Infection diagnosis: abscess, bacteremia, necrotizing fasciitis, cellulitis, osteomyelitis, pneumonia, empyema, septic joint, endocarditis.
- Clinical laboratory susceptibility results reported for *S. aureus*.
- Patient allergies.
- Antibiotics administered in 1st 72 hours after collecting original specimen.

Analysis.

- Sensitivity, specificity, and speed in detecting MRSA.
- Appropriateness of therapy in the 1st 72 hours: active/optimal, suboptimal/redundant, inactive/inappropriate.
  - Vancomycin active/optimal when MRSA isolated or if severe β-lactam allergy; suboptimal when MSSA isolated.
  - Use of a β-lactam was active/optimal *vs*. MSSA.
  - Clindamycin considered active/optimal for MRSA or MSSA when susceptibilities documented activity without inducible resistance in skin infection, osteomyelitis, pneumonia. In necrotizing fasciitis, clindamycin considered suboptimal if not used with β-lactam, and inappropriate *vs*. bacteremia or endocarditis.
  - Fluoroquinolones were considered inappropriate unless used in the presence of rifampin when isolate susceptible to both agents.
  - Linezolid active/optimal for MRSA when no other option.
  - TMP-SMX active/optimal for MRSA when susceptibilities documented activity in uncomplicated skin infection.
- Calculate potential impact of rapid identification in prescribing by increase in percentage of active/optimal prescribing.

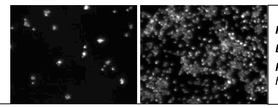

Figure 2.
Left: *MRSA, start of growth period.*
Right: *MRSA, after induction and 3 hours of growth in 6 μg/mL FOX.*

RESULTS

Table 1: Analytical Test Performance

Automated Microscopy	Clinical Lab Report		
	MRSA	MSSA	
MRSA	39	2*	Sensitivity: **100%** [88%-100% CI]
MSSA	0	17	Specificity: **89%** [65%-98% CI]

** Two false-positive isolates appeared to be small colony variants, based on culture plates and growth rates.*

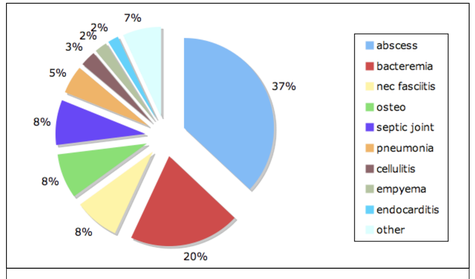

Figure 3: *Sites of infection for isolates from reviewed cases.*

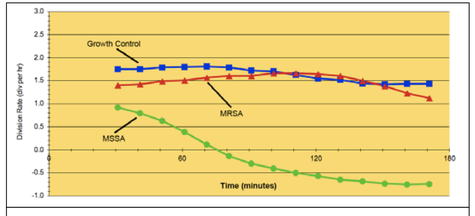

Figure 4: *Examples of growth control, MRSA, and MSSA growth curves.*

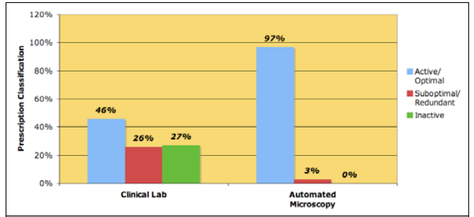

Figure 5: *Projected effect of rapid microbiological phenotyping on appropriateness of initial prescribing. Appropriateness of empiric therapy, 1st 3 days after specimen obtained.*

CONCLUSIONS

- Sensitivity and specificity of the automated microscopy method was comparable to that for standard clinical lab culturing methods for MRSA phenotype identification. Time to result using clinical isolates was less than 4 hours. The cell number used for analysis was consistent with that previously shown adequate to rapidly identify pathogens[2,3] from organisms extracted directly from a polymicrobial patient specimen.
- The MRSA analytical method therefore offers potential to integrate into a rapid diagnostic with same-day time to result.
- If applied in a direct-from-specimen analysis, the method has the potential to significantly reduce the use of inactive and suboptimal/redundant initial therapy in critically ill patients.

Limitations of the study include its retrospective design, due to current unavailability of an automated system approved for use in clinical trials. However, previous studies[2] with direct-from-specimen BAL fluid have shown the potential for test integration.

References

1. Metzger S., *et al.* 2008. Direct Identification of MRSA and MLS$_B$ Phenotypes in *Staphylococcus aureus* Using Small Numbers of Immobilized Cells. 108th ASM General Meeting, Poster C-005.
2. Hance K., *et al.* 2007. Rapid Identification of Live *Acinetobacter* spp. in Bronchoalveolar Lavage Specimens by Automated Immunofluorescence Microscopy. 47th ICAAC, Poster K-392.
3. Metzger S., *et al.* 2007. Direct Detection and Enumeration of Viable Bacteria in Human Bronchoalveolar Lavage Specimens Using Automated Growth Rate Analysis. 108th ASM General Meeting, Poster C-145.