Comparison of Mannitol Salt Agar and Blood Agar Plates for Identification and Susceptibility Testing of *Staphylococcus aureus* in Specimens from Cystic Fibrosis Patients

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Antimicrobial susceptibilities of *Staphylococcus aureus* strains can be determined accurately by using isolates from mannitol salt agar, and yellow isolates on mannitol salt agar at quantities of $>1$ can be reported as *S. aureus*. These methods decrease the time to identification/antimicrobial susceptibility testing of *S. aureus* and decrease costs through eliminating additional testing.

According to a report from the Cystic Fibrosis Foundation in 2005, *Staphylococcus aureus* is recovered very frequently from the airways of cystic fibrosis (CF) patients (on average, ~52% of patients [60% of children and 30% of adults] and is one of the first pathogens recovered from infants and children suffering from CF (1, 3). The methods used to detect and identify this organism in a timely fashion and to produce accurate susceptibility patterns are important to the critical care of and outcomes for CF patients.

Those laboratories that perform respiratory cultures for CF patients are well aware of the time necessary to rule out or rule in pathogens and provide appropriate susceptibility data for these pathogens. *S. aureus* is only one among several pathogens considered to be crucial for detection in this patient population; others include *Pseudomonas aeruginosa*, *Burkholderia* and related spp., *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*, as well as mycobacteria and viruses. Since cultures performed on specimens collected from CF patients are labor-intensive and involve the use of many medium types, selective media are often utilized. These media minimize growth of normal flora and allow potential pathogens to be recovered more easily from the complex microflora of these respiratory specimens. One of these selective media is mannitol salt agar (MSA), which is used for selectively and differentially recovering isolates of *S. aureus* (which will appear yellow on this agar; coagulase-negative staphylococci will remain the color of the agar [red]). However, noneselective/non differential media are currently recommended for use in performing identification methods for *S. aureus* and preparing suspensions for use in susceptibility testing on a Vitek-2 instrument. In order to decrease the time and expense associated with identification and susceptibility testing of *S. aureus* from respiratory cultures from CF patients, this study was undertaken to determine if MSA could be used for definitive identification as well as for preparing suspensions for susceptibility testing in the Vitek-2 system.

(Received, the most recent respiratory culture reports for all CF patients with specimens submitted in the year 2005 were reviewed. This review was performed in order to determine how often yellow colonies detected on the MSA plate were definitively identified as *S. aureus*.

Ninety-seven percent of the 69 oxacillin-resistant and 31 oxacillin-susceptible *S. aureus* isolates recovered from CF patients showed exact agreement with susceptibility interpretations, whether they were taken from the MSA or BA medium. Only three discrepancies were noted upon comparison of susceptibility testing results obtained with MSA or BA plates.

Two isolates had discrepant results regarding clindamycin. In one case, the isolate tested from the BA plate was resistant while testing intermediate from the MSA plate; in the second case, the reverse was seen (erythromycin intermediate on BA plate and erythromycin resistant on MSA plate). In the third and final case, the discrepant result was with clindamycin; in this case, the isolate was resistant to clindamycin when tested from the BA plate and intermediate when tested from the MSA plate. All three isolates were D test positive and there-
fore would have been reported as clindamycin and erythromycin resistant based on the disk diffusion D test. All other antimicrobials, which included ampicillin-sulbactam, chloramphenicol, gentamicin, quinupristin-dalfopristin (Synercid), rifampin, oxacillin, cefazolin, linezolid, and vancomycin, showed the same results on the GP-61 Vitek-2 card, regardless of which medium was used for testing.

The most recent culture reports for all CF patients with specimens submitted in the year 2005 (only one report per patient was reviewed in order to exclude duplicates within the year’s time) were reviewed to determine if yellow colonies seen on MSA plates were in fact isolates of S. aureus. Two hundred seven reports were reviewed, and it was found that in >98% of cases, if yellow colonies were found at quantities of >1+ (1+ is defined as growth only in the first quadrant of streaking) on the MSA plate, then the organism was ultimately identified as S. aureus after being subcultured onto a BA plate and tested by routine laboratory methods (a slide coagulase test followed by a tube coagulase test if necessary).

MSA has been used for many years as a selective, differential medium for the isolation of S. aureus and has long been recommended as one of the selective media for use with CF patients’ respiratory specimens (4). Recently, a new selective agar, CHROMagar Staph aureus, was also reported to be useful for selective and differential use with S. aureus isolates (2, 4). Flayhart et al. (2) reported that the identification of S. aureus could be reported from this medium without additional testing with 100% specificity, exceeding the 95% accuracy requirement of the CLSI (formerly NCCLS) M35-A document for use as an acceptable single rapid test for the identification of organisms (2, 5). With regard to this statement from the study of Flayhart and colleagues, a recent Cumitech authored by Gilligan et al. (4) states that “It is very likely that similar results would be obtained with MSA but that evaluation has not been recently done.” Over 200 patient reports were reviewed in the present study to evaluate the performance of MSA for the detection of S. aureus from CF respiratory cultures, and the review showed that MSA can also accurately identify S. aureus (>98%) if the proper chromogenic reaction takes place and there is a >1+ level of appropriate yellow colonies isolated on the medium. Infrequently, yellow colonies in quantities of ≤1+ were identified as either coagulase-negative staphylococci or Corynebacterium species. If yellow colonies are grown on MSA in quantities of ≤1+ or if a mixture of organisms is grown, then one should proceed with routine isolation and identification procedures to rule out the presence of S. aureus in these cultures.

The ability to perform accurate susceptibility testing in a Vitek-2 instrument with isolates taken directly off MSA plates was also assessed. Agreement was achieved for 97% of isolates, with only three discrepant isolates noted and with only one antibiotic per isolate in question. These three organisms showed deviations in either erythromycin (2 isolates) or clindamycin (1 isolate) susceptibility, and no discrepancies were noted for determining the presence of oxacillin resistance (69 isolates) or oxacillin susceptibility (31 isolates). Since all of our S. aureus isolates were also tested for the presence of inducible clindamycin resistance with the D test, the isolates were properly reported as resistant to both clindamycin and erythromycin, as all three discrepant organisms were D test positive. This is similar to reports of accurate susceptibility results from the evaluation of utilizing colonies directly from CHROMagar Staph aureus medium carried out by Flayhart and colleagues (2).

In conclusion, although the study isolates of S. aureus were subcultured onto MSA and BA plates prior to being tested for susceptibility, one could reasonably summarize from these findings that susceptibility testing of appropriately pure, isolated, and incubated colonies directly from MSA inoculated with specimens could be achieved with similar certainty without the need for subculture on a nonselective medium prior to testing. Additional prospective studies utilizing clinical specimens are required to validate these findings. In addition, organisms showing the characteristic yellow colonies on MSA plates at quantities of >1+ can be reported as S. aureus without further identification testing, with >98% confidence. These methods not only may decrease the time needed for detection and susceptibility testing of S. aureus for this patient population but may also decrease costs to the laboratory through the elimination of some subculture media and identification reagents currently used in the reporting of S. aureus from CF specimens. Additionally, in utilizing MSA to its maximum potential regarding CF respiratory cultures, there is no need for the addition of newer, more expensive selective media.