Evaluation of a Cost Effective Broth and Selective Agar Combination for the Detection of MRSA and *Staphylococcus aureus* from Surveillance Specimens Using Regular Workflow

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ABSTRACT:

OBJECTIVE: To evaluate the use of selective agar and broth combination in a regular laboratory daily workflow.

DESIGN: Swabs from 173 surveillance specimens were inoculated onto half of the Bio-Rad MRSA*Select* (M), Sa*Select* (S) and Sheep Blood agars (SBA) and the swab placed in the LIM broth. After overnight incubation, 10 µL of the LIM broth was inoculated onto the other half of the three agars and incubated overnight. All the agars were examined and worked up after approximate14-18 hours of incubation for day one and two according to the regular workflow of the laboratory, without incubating for the full 24 hours for each incubation day. M agar and SBA were evaluated for Methicillin-Resistant *Staphylococcus aureus* (MRSA), while the S agar was evaluated for *Staphylococcus aureus* (SA) based on typical colony morphology development. Colonies on the SBA were picked and processed for definitive identification and cefoxitin susceptibility result.

SETTING: Trinity Medical Center, a community hospital with network hospitals

PATIENTS: Patient admitted to the hospital submitted swab for surveillance culture

RESULTS: There were a total of 29 MRSA isolated in the study. On day one, both M agar and SBA detected 14 MRSA (48.3%) and on day two, M agar detected 10 (82.7%), while SBA detected 8 (75.8%) additional MRSA. LIM broth added 5 more MRSA to both agars on day 2, to give M agar a total of 29 (100%) and SBA agar a total of 27 (93.1%) of MRSA from the 173 specimens.

There were a total of 62 SA isolated. Both the S agar and SBA isolated 34 (54.8%) on day one and 15 more (79%) on day two. The LIM broth added an additional 13 SA for both agars on day two.

CONCLUSION: Using half of the agar plate for the initial swab and the other half for the broth creates an economic strategy for the detection of MRSA using the M agar and SA using the S agar. Both the M and S agars provided excellent identification and recovery of MRSA or SA based on color and colony morphology unless the colony was too young for color development. The color morphology from the M and S agars was distinguishable overnight after being subcultured from LIM broth. Working up the specimen according to the workflow of the laboratory without having to wait for each plate to incubate a full 24 hours, can still detect all the targeted organisms within 2 workdays using this cost effective strategy.

ABBREVIATIONS: MRSA = Methicillin resistant *Staphylococcus aureus,* MSSA = Methicillin susceptible *Staphylococcus aureus*, SA = *Staphylococcus aureus*

INDEX TERMS: MRSA, *Staphylococcus aureus*, surveillance culture

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INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) infections have become a major public health as well as healthcare burden. The increasing incidences of infections caused by community-acquired MRSA and healthcare-associated MRSA have caused many healthcare facilities to implement MRSA screening for in-coming or pre-admission patients. Early screening methods involve using agar and/or broth to culture for MRSA. Culture methods usually take 1-2 days to grow and identify the organism. The use of selective and chromogenic agars helps to select and identify MRSA from culture in 24 hours. The addition of an overnight broth culture has been shown to increase the sensitivity of the culture results[1] but also increases the turn-around–time (TAT). In the past few years, the FDA has approved several molecular assays for detection of MRSA with processing time as short as two hours. The sensitivity of these assays ranges between 68 to 100% and specificity ranges from 64-100%.[2] Most of these assays are approved only for nares and some for blood cultures or skin and soft tissue. False positive and false negative results with molecular assays have been reported. False positives can be due to mecA genes dropping out of the staphylococcus cassette chromosome mec (SCCmec).[3] These SCCmec variants can range from 3.4 to 74% in different geographical regions. False negatives can result in cross contamination if positive patients with false negative results were put together with true negative patients in the same room. In addition to the technical issues, the major challenge for facilities to adopt molecular testing in their laboratories is cost, which can be 5 to 7 times the cost of culture.

Recent studies have shown that screening for MRSA may not be sufficient for surgical patients.[4,5] There may also be a need for screening for both MRSA and methicillin susceptible *S. aureus* (MSSA). Bio-Rad's MRSASelect agar for the culturing of MRSA from nasal and wound specimens has been commercially available for some time. The newly released SaSelect agar is for culture and identification of *S. aureus* (SA). Both are chromogenic agars, the MRSASelect will identify MRSA with the development of a pink colony after 18-28 hours of incubation, while the SaSelect agar will identify *S. aureus* (both MRSA and MSSA) with a pink to orange colony within 18-24 hours according to the manufacturer's package insert. However, patient specimens come into the clinical laboratory all through the day and evening, and for the technologist having to time the plate for a full 24 hour incubation before working up the plates would interrupt the workflow of the laboratory. The goal of this study is two-fold; first, to evaluate the combination of chromogenic agar and broth using half a plate for each day for the detection of MRSA or SA in comparison with Blood agar/broth combination. Second, the effect of working-up these specimens according to our current laboratory workflow without constantly checking each plate after a full 24 hours of incubation for the detection of MRSA and *S. aureus* from surveillance samples that are submitted to the clinical microbiology laboratory for culture.

MATERIALS AND METHODS

Swabs from 173 surveillance specimens were sent to the Clinical Microbiology laboratory throughout the day and evening for the 2 month study period. Most specimens were from nares, some were from other body sites according to our hospital infection control admission protocol.

Each swab was inoculated in no particular order onto half of a plate of the MRSA*Select* (M), Sa*Select* (S) (Bio-Rad Laboratories, Redmond, WA) and Sheep Blood agars (SBA) and then placed into the LIM broth. After overnight incubation at 35°C in ambient air (CO_2 for SBA), 10 µL of the LIM broth was inoculated onto the other half of the three agars and plates were incubated for another 14-18 hours (Figure 1).

Each day one technologist was assigned to work up the surveillance specimens as part of the bench workload together with other bench duties. All the agars were examined and worked up after 14-18 hours of incubation for each day 1 and 2. M agar and SBA were evaluated for MRSA, while the S agar was evaluated for SA based on the colony morphology according to the manufacturer's package insert. If the colonies from the selective agars were not typical on the first day, the plates were reincubated and worked on the next day (day 2). Colonies were identified based on Gram stain, coagulase (slide or tube) and cefoxitin disc diffusion test and were taken from colonies on the SBA as the final identification and susceptibility standard. The SBA served as the standard to confirm or not confirm the

typical morphology from the M or S agar. Based on our initial study, typical pink colonies from the M agar were MRSA and pink to orange colonies from the S agar were *S. aureus*, and were not further tested. If there were any discrepancies between the colonies from the selective agars and the SBA, colonies from both media were picked for identification and susceptibility. Questionable color or colony morphologies from M and S agars were also tested for identification and susceptibility testing. Cefoxitin disc diffusion susceptibility testing for determination of methicillin resistant was done on all *S. aureus* isolated according to the latest Clinical Laboratory Standard Institute[6] recommendations.

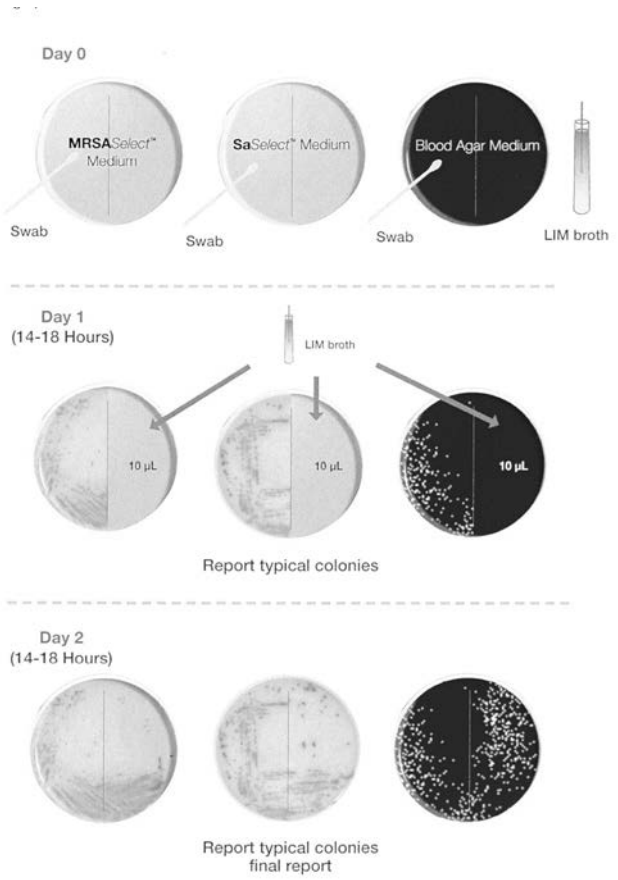

Figure 1. Inoculation, subculture and reading of each media on Day 1 and 2.

RESULTS

There were a total of 29 MRSA and 62 *S. aureus* isolated from the 173 swabs submitted for the study. After the first day of incubation, both M agar and SBA detected 14 MRSA (48.3%) using the typical morphology as identification for the M agar. On the second day, M agar detected 10 (82.8% total), while SBA detected 8 (75.8% total) additional MRSA. From the LIM broth, 5 more MRSA were added to both agars, giving the M agar a total of 29 (100%) and SBA 27 (93.1%) MRSA from the 173 specimens. Colonies that grew directly from the inoculation of the swab took a longer time to develop the typical pink colony for detection. However, colonies that grew from the overnight LIM broth appeared pink within 8-12 hours, and they were easily distinguishable to be MRSA. The SBA is a standard agar in most Clinical Microbiology laboratories and experienced technologists are familiar with staphylococcal colony morphology. Because the SBA is not a special medium for MRSA or *S. aureus*, most colonies required further testing to identify MRSA from other staphylococci, which usually took another day.

Of the total 62 *S. aureus* isolated, both S agar and SBA were able to detect 34 (54.8%) SA on day one and 15 (79% total) more on day two. The LIM broth added the final 13 (100%) SA on day two (Table 1). Again, organisms in the inoculum from the broth put onto the S agar took less time to develop the typical color colony morphology than organisms that grew from the swab.

Table 1. The detection of each targeted organism in each media for Day 1 and 2.

	Day 1	Day 2		
	Plate	Plate	Broth	Total
M (MRSA)	14 (48.3%)	10	5	29 (100%)
SBA (MRSA)	14 (48.3%)	8	5	27 (93%)
S (SA)	34 (54.8%)	15	13	62
SBA (SA)	34 (54.8%)	15	13	62

Using this half plate chromogenic agar and broth combination in our regular workflow, we were able to detect the targeted MRSA and SA within two days, 48.3% and 54.8% respectively on day one. The workflow was not interrupted by having to constantly check plates that had been incubated for 24 hours. The technologist was able to finish both regular duties as well as the surveillance samples in a timely manner.

DISCUSSION

There is sufficient published data to demonstrate that MRSA is a major pathogen that causes healthcare-associated as well as community-associated infection. However, the approach to prevent spread of MRSA is

controversial. Many publications have shown active surveillance or "search and destroy" reduces hospital MRSA infection rates and increases cost effectiveness,[7,8] while others did not find that active surveillance for MRSA had any significant reduction of MRSA infection.[9,10] As a matter of fact, two recent articles from the New England Journal of Medicine have completely different findings.[11,12]

Another concern is which site is appropriate for screening. Most institutions use nares as the major or only site for culture or detection, while other studies demonstrated throat,[13,14] perineum or rectum[15,16] are also major sites for carriers.

The other controversy regarding surveillance screening is which method is appropriate. Culturing methods will usually take 2 to 3 days to detect the organism, while molecular tests are rapid and results can be obtained in as short as two hours. The disadvantages with the culture method include lack of sensitivity if agar is used alone and a long turn-around time (TAT). A point-counterpoint discussion suggested that using a molecular assay screening with sensitivity of >80% and a reporting time of less than 15 hours will be effective to reduce MRSA infection, while the counterpoint questioned the effectiveness of "active detection and isolation" and emphasis on infection control.[17]

Some states have legislation that requires hospitals to screen patients for MRSA as part of the admission requirement for infection control.[18] To be able to offer molecular tests and staff around the clock to provide timely TAT can be very costly to the hospital's budget. The alternative to molecular testing is culture, which may lack sensitivity (agar alone) and has a longer TAT, but is much more affordable for most laboratories.

The use of selective Chromogenic agars with or without broth culture improves the detection of MRSA from patient specimens. The M agar has been studied and proven to have high sensitivity and specificity when compared with several other culture agars.[19] Most of the initial studies used 24 to 48 hours incubation to fully detect all the MRSA. In the current study, both the M agar and SBA detected 14 MRSA on the first day, and 10 more by the M agar and 8 by the SBA the next day. This demonstrates that the M agar can detect 8.3% more MRSA than SBA based on the agar alone in two working days. Adding the overnight incubated broth to the other half of the agar recovered 5 more MRSA from both M agar and SBA. This is expected, as the overnight incubated broth allows the bacteria to multiply into large numbers and when inoculated onto the solid media, this large number of bacteria will produce large numbers of the colonies rapidly. However, the MRSA from the broth culture will produce its distinguishable colony morphology in 8-12 hours, a much shorter timeframe than from swab inoculation. This could be due to a larger number of bacteria that reacted with more chromogens in the agar to produce more pink colored colonies. According to our regular workflow on the workbench, 48.3% (14/29) of the MRSA were detected on the first day. 82.8% (24/29) were detected on the second day based on agar alone. Inoculating the other half of the agar with the overnight incubated broth increased the recovery of MRSA from our surveillance samples to 100%. This is different from another study that detected 98% of MRSA when plates were read after 24 hours of incubation.[20] However to monitor all the specimens so that they are read after 24 hours would interrupt the workflow of the technologist greatly and is more suitable for a research setting. Most of our plates were incubated around 14-18 hours by the time the technologists were reading them for the first day and another 14-18 hours by the second reading on the second day. The broth half of the agar made up for most of the second day's work, because if there were MRSA that did not grow or morphology that was not typical on the first day, the other side of the agar where the broth was inoculated grew with the typical morphology by the time the second day reading took place.

The S agar is a newly marketed chromogenic agar that is selective for *S. aureus*. It will detect *S. aureus* (both MRSA and MSSA) in 18-24 hours with a pink to orange colony morphology. According to the package insert, *S. epidermidis* will give a faint pink small colony; *S. saprophyticus, S. simulans, S. cohnii* and *S. xylosus* will give blue to turquoise colonies; and *S. intermedius* will give purple-grey colonies. The recovery of *S. aureus* is similar for both S agar and SBA. In our study, there were 62 *S. aureus* isolated from both S agar and SBA. The agars detected 34 (54.8%) on day 1 and 15 more on day 2 (79%). The broth detected 13 (21%) more on day 2. Again, colonies from the broth half of the

agar grew rapidly and gave the distinguished morphology by the time the second day reading took place. This new medium will be useful when screening for *Staphylococcus aureus* and is critical to help reduce infection rates in patients, such as surgical patients[4,5].

Overnight broth enrichment is critical for increased sensitivity of detection. One study showed that overnight broth enrichment increased the bacterial load by 20 fold when compared with direct plating with swab or a 4 hours pre-enrichment treatment.[21] That may be the reason why the broth inoculated on half of the agar grew the characteristic colony morphology by the time plate reading took place. Using this broth enrichment and selective agar combination, studies have shown similar sensitivity to Xpert MRSA PCR[22] as well as BD GeneOhm assay.[23]

We believe this is the first study using selective chromogenic agars in combination with broth to detect MRSA and *S. aureus* using a regular laboratory workflow. Most studies involved waiting a full 24 or 48 hours of incubation time before reading, which is difficult for most clinical laboratories to do. The reason behind this study was that most hospital specimens come into the laboratory all through the day and night. We have three shifts, including night shift staffed by generalists who rotate throughout the laboratory and process the specimens as they come, which is part of the regulatory requirement for patient care. However, for technologists who work on surveillance specimens as part of their regular assignment during the day shift, constantly checking plates when the 24 hours incubation period is up is very disruptive to their other bench work. Another factor that helped to develop this combination is that the MRSASelect agar has been studied extensively giving over 97% in sensitivity and specificity.[19,21] Inclusion of the broth can further improve the detection rate to close to the sensitivity of molecular assays.[22] Using half of a plate for each day will reduce the number of plates used, reducing the economic impact on the cost of media. Based on our study, almost half of the specimens with MRSA or *S. aureus* were reported on the first day and the rest reported within 2 working days based on the colony morphology on the selective agars. The same was true for *S. aureus* using the S agar colony morphology. The advantage of this strategy is that it is cost-effective (much reduced cost in comparison with molecular assays), and has comparable sensitivity and high specificity (due to mecA gene dropouts missed by molecular assays). Other advantages include the fact that the use of broth and agar is not limited to nasal specimens only, and clinical isolates are available for future studies if needed. Work up can be done within the regular workflow of most laboratories. The disadvantage of this method is a one day longer TAT. However according to a 2009 review from Lancet Infectious Diseases "our data does not support use of the rapid test by itself to identify the MRSA carrier or to reduce acquisition rate in wards in which active screening with enrichment cultures linked to contact isolation are already in place."[24]

For the financial comparison between this approach and using a molecular-based assay, each plate costs about $5, while the molecular tests cost between $35 to $45 for reagents alone. Using the most conservative number of $35 for the molecular assay, if a hospital is doing 100 samples per month, there will be a savings of approximately $3,000 on reagents alone, more if more samples are tested, and yet the sensitivity of detection remains similar to that of the molecular-based assay. Half of the results were reported on the first day. In our case, we did not add extra manpower to implement this workflow.

The limitation of this study is we did not have a molecular assay with which to compare with our culture results. A molecular assay for MRSA or *S. aureus* is not available in our laboratory. This study is not intended to measure the sensitivity and specificity of these agars. The clinical performance of these agars has been established by others.[19,21,22] Studies have shown that MRSASelct agar with/without broth enrichment has similar sensitivities to molecular assays.[22,23] In this current study, the agar/broth combination detected more MRSA than just the agar alone. The sensitivity of our agar/broth combination could be close to molecular assays.

In conclusion, we have introduced a cost-effective strategy using a combination of M agar and broth and within the regular workflow of the laboratory to enhance the recovery of MRSA from surveillance specimens. Culture results can be finalized within two working days. The same goes for *S. aureus* using the S agar and broth combination using this strategy. This workflow and agar/broth combination should enhance

laboratory screening for MRSA or *S. aureus* without affecting the workflow of the bench with minimal cost.

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