

Carol Young, Linda Lapsley, and Duane Newton
Department of Pathology, University of Michigan Hospitals, Ann Arbor, MI

Abstract

Background

MRSA infections are increasing in the hospital and community. Enhanced infection control measures recommend surveillance cultures to prevent nosocomial infections in high risk patients. While current culture techniques require 48-96 hours for completion, newer chromogenic media could decrease this time to 24 hours. We evaluated 4 media to determine their clinical utility for more rapid detection of MRSA.

Methods

Nasal swabs were collected from 370 adult patients in our ICU's. Swabs were vortexed in 1mL saline and 2 drops (~40uL) of this suspension were inoculated onto each of 4 media: Mannitol Salt Agar with 5 ug/mL cefoxitin (MSA-FX), prepared in-house; MRSA*Select*TM (Bio-Rad); CHROMagarTM MRSA (BBL); and SpectraTM MRSA (REMEL-Thermo Fisher Scientific). Plates were incubated in the dark at 35°C and read at 18, 24, and 48 hours. Organisms with appropriate color (yellow halo on MSA-FX, pink on MRSA*Select*TM, mauve on CHROMagarTM MRSA and blue on SpectraTM MRSA) were then subcultured onto Sheep Blood Agar (SBA). Isolates were confirmed as MRSA by coagulase production and presence of the *mecA* gene by PCR.

Results

Of 370 cultures, twenty nine (29) had MRSA (7.8%). At 24 hours, SpectraTM MRSA detected 29 (100%), MRSA*Select*TM, 29 (100%), CHROMagarTM, 26 (90%), and MSA-FX, 21 (72%). Organisms can be called MRSA at 24 hours without further testing from all media except MSA-FX, which required subculture and coagulase testing as other species of *Staphylococcus* ferment mannitol (thereby delaying detection of MRSA). The color of the colonies on SpectraTM MRSA and MRSA*Select*TM are prominent due to the opaque media, whereas the color is less prominent on CHROMagarTM which is clear. However, there was one culture with a light blue colony on SpectraTM MRSA which was determined to be MSSA, therefore colonies with lighter color should be subcultured for confirmation. After an additional overnight incubation CHROMagarTM detected 28 (97%) and MSA-FX, 28 (97%).

Conclusions

MRSA*Select*TM and SpectraTM MRSA can accurately detect the presence or absence of MRSA at 24 hrs. The others are less efficient. CHROMagarTM detected 90% whereas MSA-FX detected 72%. Both require an additional overnight incubation before confirming the absence of MRSA.



Duane W. Newton, Ph.D., D(ABMM)
Clinical Microbiology Laboratories
University of Michigan Hospitals, UH2F461
1500 East Medical Center Drive
Ann Arbor MI 48109-5054
Phone: 734-936-6847 FAX: 734-647-9093
dnewton@med.umich.edu

ACKNOWLEDGEMENTS

We would like to thank Bio-Rad and REMEL - Thermo Fisher Scientific for graciously providing media to support performance of this study.

Introduction

Staphylococcus aureus is an important cause of skin, soft tissue, and bloodstream infections that can be rapidly fatal if not treated effectively. It is the single leading pathogen in health care-associated infections. Nasal carriage of *S. aureus* has been suggested as the source of bacteremia, surgical-site, and other infections, as well as a reservoir of *S. aureus* in hospitals. Early detection followed by decolonization may prevent infections and reduce transmission. Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are increasing in the hospital and community. Enhanced infection control measures recommend surveillance cultures to prevent nosocomial infections in high risk patients

Medicaid has passed regulation that hospitals will not be paid for preventable conditions caused by the hospital, including nosocomial staphylococcal infections. Active surveillance cultures may be one tool, along with hand hygiene, decontamination of the environment and implementation of contact precautions of infected/colonized patients, which can be used in attempting to control MRSA infections.

With our current MRSA surveillance method taking 48-96 hours, our goal in this study was to determine whether any of the available chromogenic media could produce results in 24 hours.

OBJECTIVES:

- Evaluate media to hasten the time of detection of MRSA in the clinical laboratory
- Compare time of detectable growth on 4 media
 - Mannitol Salt Agar w/cefoxitin (prepared in-house)
 - CHROMagar™ (BBL)
 - MRSASelect™ (Bio-Rad)
 - Spectra™ MRSA (REMEL-Thermo Fisher Scientific)

Methods

Nasal swabs were collected from 370 adult surgical ICU patients, then vortexed in 1 ml saline. Aliquots (10 ul) were inoculated onto each media:

- Mannitol salt with 5ug/ml cefoxitin (MSA-FX)
- BBL CHROMAgar™ MRSA
- Bio-Rad MRSASelect™
- REMEL Spectra™ MRSA

Plates were incubated in the dark at 35°C and read at 18, 24, and 42-48 hours and phenotypic characteristics of all colonies on each media were recorded. Plates were examined for appropriate color:

- yellow on MSA-FX
- pink on MRSASelect™
- mauve on CHROMAgar™
- blue on Spectra™ MRSA

For the first 100 cultures, all colony types were subcultured and ID confirmed (coagulase; slide or tube)

- Methicillin resistance confirmed by *mecA* PCR

For the remaining cultures, only MRSA colony types were subcultured and ID confirmed (coagulase; slide or tube)

- Methicillin resistance confirmed by *mecA* PCR

Results

Table 1. Detection of MRSA positive cultures (n = 29) on each type of media

	MSA-FX Yellow must sub-coag	BBL Maive must sub-coag	REMEL blue	Bio-Rad pink
18 hr	11	8	17	17
24 hr	21 reincubate negatives	26 reincubate negatives	29	29
42-48 hr	28	27	N/A	N/A

Figure 1. Representative MRSA-positive cultures on each media after 24 hr incubation



Results

Table 2. Summary of media attributes/limitations and workflow

CHROMagar™ MRSA

- Mauve color is slow to develop
 - If mauve at 24 hrs – call MRSA
- Media is clear – color not as pronounced
- Other bacteria grow as blue or white colonies
- Media is most inhibitory to other bacteria
- Must reincubate negatives for 48 hrs
 - If mauve at 48 hrs – do coagulase

Spectra™ MRSA

- Good color at 24 hrs
- Blue color is easy to see
- Opaque background
- Must develop workflow so media is not incubated too long
 - Non-specific colonies develop with overincubation

MRSASelect™

- Good color at 24 hrs
- Pink color is easy to see
- Opaque background
- Must develop workflow so media is not incubated too long
 - Non-specific colonies develop with overincubation

TODAY

Process Specimen
Incubate until tomorrow

9am
1pm
6pm
10pm

→
→
→
→

TOMORROW

Read Media
Result – Pos or Neg

9am
1pm
6pm*
10pm*

*with new positive blood cultures

Conclusions

- Organisms can be called MRSA at 24 hours without further testing from all media except MSA-FX, which required subculture and coagulase testing as other species of *Staphylococcus* ferment mannitol (thereby delaying detection of MRSA).
- The color of the colonies on Spectra™ MRSA and MRSASelect™ are prominent due to the opaque media, whereas the color is less prominent on CHROMagar™ which is clear. However, there was one culture with a light blue colony on Spectra™ MRSA which was determined to be MSSA, therefore colonies with lighter color should be subcultured for confirmation.
- An additional overnight incubation (48 hours total) was required to achieve maximum sensitivity for CHROMagar™ and MSA-FX.