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A Comparative Study of Denim Blue and MRSA-Select Chromogenic Agars for the Detection of MRSA

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Introduction

Methicillin resistant *Staphyococcus aureus* (MRSA) colonization is a risk factor for MRSA transmission. Rapid identification of MRSA will therefore reduce the risk of infection and patient to patient transmission. Benefits would also include improved patient treatment and a reduction in the costly burden of MRSA on institutions. Laboratory screening for MRSA is a complex balance between cost, sensitivity, specificity and speed of detection. Recently developed chromogenic medias specific for this purpose, such as Oxoid's Denim Blue (DB) and Bio-Rad's MRSA Select (MS), combine primary growth and selectivity with differentiation from coagulase negative staphylococci. These products utilize chomogenic substrates and various inhibitors to select for MRSA within 18 - 24 hours of incubation at 35°C and show improved specificity when compared to traditional mannitol salt medias. This study compares the two aforementioned medias in terms of detection time, performance and reliability and their achieved benefits when incorporated into laboratory workflow strategies.

Method

DB and MS were evaluated for detection of MRSA by:

A) Inocuations of diluted and previously identified MRSA strains.

B) Direct inoculation of patient samples.

C) Ability to recover MRSA from various screening sites.

A) Strain recovery and growth characteristics.

Seventy-one archived isolates of primarily CMRSA 2 and 10 recovered from routine screening cultures were prepared to evaluate recovery rates and colonial characteristics. Some of these strains were challenging.

A standardized dilution of 10^4 CFU/plate was acheived as follows;

•Slants were subcultured to 5% SBA

•0.5 McFarland suspensions in 0.9% saline were prepared from each

•0.2mL of this inoculum was transferred to 1.8mL of saline

•1μL of this suspension was inoculated onto each media and streaked for isolation.

Incubation was at 35°C ±2°C. Evaluations occurred hourly between 18 - 24 hours of incubation.

Evaluations included:

•Colony size: using a stereoscopic microscope and digital calipers, sizes of colonies were measured every 2 hours.

Colony count: A general count was taken every 2 hours.

B) Screening Specimens

750 patient swabs received from multiple locations including tertiary care hospitals, nursing homes and correctional institutions were utilized. Sampling sources were primariy rectal/perineum and nasal swabs. These were inoculated directly and alternatively onto each media plate.

Assessments included:

•Comparative amounts of growth of presumptive MRSA/sample

•Amount and identifiaction of background flora able to grow on DB and/or MS

•Assessment of false positive and/or negatives

Method

Identification of chromogenic positive colonies was performed using Pastorex Staph Plus latex agglutination (Bio-Rad) and PBP2' latex agglutination (Oxoid) directly from these plates as suggested by the manufacturers. Identification of chromogenic negative colonies was achieved using gram stain, Pastorex Staph Plus and Vitek GPI cards.

C) Prevalence of MRSA/Site

A secondary evaluation of the prevalence of MRSA in various screening sites was determined for both medias. One instituation routinely submits rectal/perineum, throat and nasal swabs for screening. Statistics from this instiution were compiled using both medias.

All MRSA isolates were submitted to the Ottawa Hospital Microbiology laboratory. Confirmation of methicillin resistance was provided upon request using the Roche LightCycler MRSA detection Kit. Selected isolates were typed by Pulsed-field get electrophoresis as part of molecular epidemiological surveys for the region.

Results

A) Strain Recovery and Growth Characterisctics

All 71 strains of MRSA, after dilution, achieved a 3+ presumptive growth of MRSA. MS presented mauve colonies whereas DB presented blue colonies. In general, DB supported 25% larger colonies than MS throughtout the 18-24 hour incubation period. Because of the larger size and better contrast with respect to background, DB's colonies were more easily visible and an impression was given of a higher yield of growth.

B) Screening Specimens

Of the 750 patient samples inoculated onto DB and MS media, 45 were positive for MRSA. DB recovered all 45 whereas MS recovered 44. Very light growths were easily missed using MS at 18 hours, but colonies became more visible at 20 hours. Both media grew a haze of their respective chromogenic colors if certain strains of *Enterococcus sp.* were present. With some experience these became easier to differentiate from true MRSA colonies.

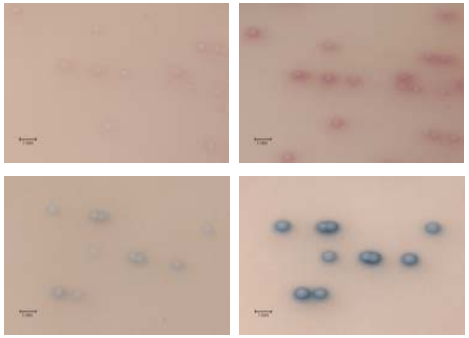
Media	Incubation Time			
	18 hrs	20 hrs	22 hrs	24 hrs
MS	.42mm	.45mm	.50mm	.57mm
DB	.56mm	.61mm	.67mm	.79mm

Average size of MRSA colonies..

Media	Growth			
	±	1+	2+	3+
MS n=34	17	8	14	5
DB n=35	12	10	16	7

Relative Amounts of Growth of MRSA..

Results



MS colonies (top) and DB colonies (bottom) at 18 hrs. (left) and 24 hrs. (right).

Media	Growth			
	±	1+	2+	3+
MS n=62	16	30	12	4
DB n=23	16	1	4	2

Comparative Amounts of Background Flora.

Generally, background flora grew as white colonies and these could be differentiated from the chromogenically positive colonies. However, this becamse more difficult if a predominance of larger coagulase negative white colonies were present coupled with only a very light growth of chromogenic positive colonies. In particular, this difficulty was more pronounced with MS media. MS media also yielded higher amounts and types of background flora (MS n=62; DB n=23). Strains of *S. sciuri* and *S. epidermidis* grew as white colonies on MS. Strains of *S. hemolyticus* and *S. hominis* grew as white colonies on both medias.

C) Prevalance of MRSA/site

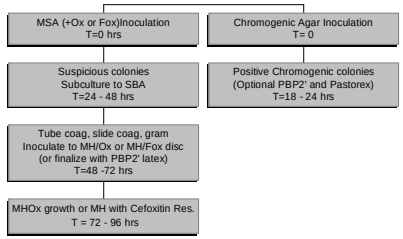
Both media recovered MRSA from each screening site equally well. The following is data from the tertiary care hospital submitting all 3 screening sites; rectal/perineum, throat and nasal. Between throat and rectal/perineum swabs, recovery of MRSA was 100%. No nasal swabs were the sole source of MRSA colonization from any patient.

	Source		
	Rectal	Throat	Nasal
MS + DB n=29	26	23	19
% of recovery	89.6%	79.3%	65.5%

Rate of recovery from 3 screening sites.

Results

Workflow Strategies and Processing Time Comparison for Mannitol Agars (+Ox or Fox) vs. Chromogenic Agars for Positive MRSA



Conclusions

Both medias selected for and differentiated MRSA effectively between 18 - 24 hours.

Oxoid's Denim Blue was superior in demonstrating larger, more distinct MRSA colonies. This is especially important when considering low colony counts at 18 hour readings. Technologists unanimously found a better contrast of color with Denim Blue.

Background flora was more prevalent in MS media and it may, in some cases, have a masking effect on visibility of a very light growth of MRSA at 18 hours.

Several articles as well as an unpublished in house evaluation suggests chromogenic agars are superior to mannitol screening agar/MH + OX or FOX combinations. The most valuable benefit of these agars is a reduction of TAT from 2-4 days to 1 day. A further reduction of technologist time (-40%) and consumables suggest that laboratories may want to consider the use of MRSA chromogenic agars.

Of interest also is that rectal and throat colonization of MRSA was found to be more frequent than nasal carriage.

Acknowledgements

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