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Prospective Comparison of a New Chromogenic Medium, MRSA*Select*, to CHROMagar MRSA and Mannitol-Salt Medium Supplemented with Oxacillin or Cefoxitin for Detection of Methicillin-Resistant *Staphylococcus aureus*

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MRSA*Select* agar was compared to CHROMagar, mannitol-salt agar with oxacillin, and mannitol-salt agar with cefoxitin (MSA-CFOX) for the isolation of methicillin-resistant *Staphylococcus aureus* (MRSA). The sensitivities and specificities were 97.3% and 99.8%, 82.9% and 99.1%, 80.2% and 79%, and 99.1% and 84.8%, respectively. MSA-CFOX and MRSA*Select* had a high sensitivity. MRSA*Select*, however, was more specific and proved to be a more reliable and rapid medium for the detection of MRSA.

Hospital-acquired infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) have been reported worldwide. Currently, MRSA is the most common pathogen identified in U.S. hospitals (3, 4). MRSA infections are associated with considerable morbidity, attributable mortality, and attributable excess cost (5). It has been shown that, in most cases, the source of *S. aureus* causing bacteremia is the patient's nose, and colonization with MRSA leads to autoinfection at a higher rate than colonization with methicillin-susceptible isolates (9, 14). Rapid and accurate identification of MRSA with implementation of infection control measures is essential in limiting the nosocomial spread of this organism. We evaluated four rapid MRSA detection methods, mannitol-salt agar (MSA)-oxacillin (6 mg/liter) (MSA-OXA) (Oxoid, Ottawa, Canada), MSA-cefoxitin (MSA-CFOX) (Oxoid, Ottawa, Canada), and two chromogenic media, CHROMagar MRSA (CMRSA) (Becton Dickinson and Company, Sparks, MD) and MRSA*Select* (MRSAS) (Marnes la Coquette, France), a new selective and chromogenic medium marketed by Bio-Rad Laboratories in Canada. All media were commercially obtained.

In our facility, nasal and perineal swabs are routinely collected from all patients admitted to our hospital if they have been admitted to any health care facility in the previous 6 months. Patients in intensive care unit settings are screened weekly. Swabs are placed in transport medium and sent to the Microbiology Laboratory. In this study, on arrival, swabs were placed in 400 μl sterile 0.9% saline and vortexed vigorously for 30 s. Of this suspension, 50 μl was used to inoculate each of the four media. Plates were incubated at 35°C in ambient air. All plate types were read after 18 and 48 h, except MRSAS, which was read only after 18 h of incubation.

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Colonies of MRSA are pink on CMRSA and MRSAS. The manufacturer of MRSAS provided a color chart to differentiate MRSA from non-MRSA colonies. Colonies of *Staphylococcus aureus* including MRSA are usually yellow on mannitol-salt medium. Depending on the growth, up to five suspected colonies from each test medium were harvested and suspended in 250 μl of sterile distilled water. Two microliters of this suspension was used for the detection of MRSA by multiplex PCR amplifying the *nuc*, *femB*, and *mecA* genes, and resulting amplicons were detected by electrophoresis on a 0.1% agarose gel containing ethidium bromide (2, 13). The presence of *mecA* with *nuc* and/or *femB* bands identified MRSA.

For the purpose of this study, if colonies resembling MRSA grew on a medium and were confirmed by PCR as MRSA, the result was considered to be true positive. If MRSA-like colonies grew on medium but could not be confirmed by PCR and MRSA was not isolated from the same sample by any other medium, the result was thought to be false positive. If a sample was negative for MRSA on test media, the result was assumed to be true negative. If a medium failed to grow MRSA but MRSA was confirmed by any other medium, the result was considered to be false negative.

A total of 2,125 (1,243 nasal and 882 perineal) consecutive swabs were processed. Of these, 111 specimens (61 nasal and 50 perineal) were positive for MRSA. Seventy-six strains were recovered from all four media, 27 from three, and 8 from only two of the media tested. The performance of each medium after 18 and 48 h of incubation is shown in Table 1. Tables 2 and 3 show the number of true- and false-positive and true- and false-negative results, sensitivity, specificity, and positive and negative predictive values.

For the nasal isolates, the detection rates with MSA-CFOX, MRSAS, and CMRSA were similar and significantly higher than the detection rate with MSA-OXA ($P < 0.001$). For the perineal isolates, the detection rate with MSA-CFOX was significantly higher than detection rates with MSA-OXA

TABLE 1. Number of MRSA strains detected by test media

Medium	No. (%) of MRSA strains detected					
	Nasal swab (1,243 swabs)			Perineal swab (882 swabs)		
	18 h	48 h	Total	18 h	48 h	Total
MSA-OXA	11	35	46 (75)	7	36	43 (86)
MSA-CFOX	48	12	60 (98)	35	15	50 (100)
CMRSA	53	5	58 (95)	30	5	35 (70)
MRSAS	60		60 (98)	48		48 (96)
Total			61 (100)			50 (100)

($P < 0.01$) and CMRSA ($P < 0.0001$) but not significantly higher than the detection rate with MRSAS ($P = 0.3$). The chi-square test with one degree of freedom was used to compare the proportions of MRSA-positive cultures detected with the different culture media. The test was two sided at the 0.05 level of significance.

A wide range of techniques has been used to detect and identify MRSA from clinical specimens (7, 11, 12, 15). Selective and differential culture media, especially MSA supplemented with oxacillin, are most widely employed. However, all MSA media are not the same: they differ in their salt and antibiotic contents, and their performances may not be comparable. In general, MSA media are not sensitive enough to be used for detection of *Staphylococcus aureus* including MRSA in clinical specimens (8). This was confirmed by Blanc et al. in a large study of 1,427 clinical specimens; they showed that the sensitivity and specificity of oxacillin resistance screening agar base (Oxoid Ltd.), a modification of MSA, were only 74% and 47%, respectively, when this product was used alone as a primary culture medium (1).

The phenotypic expression of the *mecA* gene can be heterotypic or homotypic; thus, the detection of methicillin resistance may require induction of PBP 2a when expressed heterogeneously. Alteration of growth conditions, such as temperature and salt concentration, markedly affects resistance expression. In 1989, Okonogi et al. demonstrated that cephamycins are better inducers of PBP 2a than noncephamycin antibiotics (10). Several investigators have demonstrated the superiority of cefoxitin for the identification of MRSA (6, 11).

In two in vitro studies using mostly the same MRSA and methicillin-susceptible *Staphylococcus aureus* isolates, the sensitivity and specificity of MSA medium were 90.7 and 96.0%, respectively, but improved to 100% when MSA medium with 4% cefoxitin was used as a screening medium. In the present study, cefoxitin-supplemented media, MSA-CFOX, CMRSA (cefoxitin, 6 mg/liter), and MRSAS, performed better than MSA-OXA. The formulation of MRSAS is proprietary, and it is not known if MRSAS contains a cephamycin. Almost all MRSA strains were recovered from MSA-CFOX (110 of 111) and MRSAS (108 of 111). The only strain not isolated with MSA-CFOX failed to ferment mannitol. MRSAS failed to identify three MRSA-positive samples. One of these strains was a small-colony variant isolated from the nose and perineum of the same patient, and it grew on all of the other three media. MSA-OXA was least sensitive, and specificities of MSA-OXA and MSA-CFOX were low; from these plates, 511 and 415 PCR had to be performed to confirm 89 and 110 MRSA strains, respectively. CMRSA and MRSAS were highly specific. However, MRSAS detected 108 of 111 MRSA strains, whereas CMRSA failed to identify 19 strains. Performance of CMRSA for nasal swabs was similar to that of MRSAS and MSA-CFOX, but the detection rate from perineal swabs was only 70%. CMRSA and MRSAS do not inhibit the growth of enterococci. Enterococcal colonies are dark blue on CMRSA and colorless on MRSAS; therefore, growth of MRSA, especially when present in small numbers, may be obscured on CMRSA but not on MRSAS. Perry and coinvestigators also noted that CMRSA detected 52% of the MRSA strains from rectal swabs but was comparable (sensitivity, 83%) to other detection media tested for nasal specimens (11).

In conclusion, MSA-CFOX and MRSAS were equally sensitive in detection of MRSA carriage, but the specificity of MSA-CFOX was inferior to that of MRSAS. The added advantage of MRSAS over MSA-CFOX was that results of MRSAS were available within 24 h, whereas almost one-quarter of MRSA strains required 48 h of incubation time before these could be identified with MSA-CFOX. In addition, pink colonies on MRSAS can be regarded as MRSA, as recommended by the manufacturer, or may be confirmed by simple tests, such as Gram smear and latex agglutination.

TABLE 2. Number of true- and false-positive and true- and false-negative results for test media for 2,125 specimens

Medium	No. of results			
	True positive	False positive	True negative	False negative
MSA-OXA	89	422	1,592	22
MSA-CFOX	110	305	1,709	1
CMRSA	92	17	1,997	19
MRSAS	108	3	2,011	3
Total	111		2,014	

TABLE 3. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the selective media for all isolates

Medium	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
MSA-OXA	80.2	79.0	17.4	98.6
MSA-CFOX	99.1	84.8	26.5	99.9
CMRSA	82.9	99.1	84.4	99.9
MRSAS	97.3	99.8	97.3	99.9

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