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Performance of a New Chromogenic Medium, BBL CHROMagar MRSA II (BD), for Detection of Methicillin-Resistant *Staphylococcus aureus* in Screening Samples[▽]

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Two chromogenic media for the detection of MRSA were compared: BBL CHROMagar MRSA II (BD) and MRSA ID agar (bioMérieux). Following overnight nonselective enrichment, 1,919 screening samples were inoculated on both chromogenic agars. After 24 h, the sensitivities of both media were high and comparable. Both media showed an important decrease in specificity after 48 h of incubation (decreases of 8% for MRSA II and 10% for MRSA ID), but MRSA II was significantly more specific at both time points.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections worldwide. Infection with MRSA is associated with important clinical and financial implications (7). Screening of high-risk populations and subsequent isolation of carriers are a cost-effective measure to prevent transmission in the hospital, at least if screening results are reliable and readily available (14). A wide range of culture methods and, more recently, molecular methods have been used for MRSA screening (1, 10, 13). Selective media such as mannitol salt agar supplemented with oxacillin have shown limited sensitivity (5) and specificity (4). Broth enrichment culture has been recommended, resulting in a significantly higher yield (15). The incorporation of cefoxitin in culture media has proven to be superior to the incorporation of oxacillin in culture media in the detection of MRSA (12). Recently, chromogenic media became available, permitting the direct detection and identification of MRSA through the presence of specific chromogenic substrates and incorporated antibiotics (6, 8, 9, 11, 12, 16).

The purpose of this study was the evaluation of a new chromogenic medium, BBL CHROMagar MRSA II (Becton Dickinson) (hereafter, MRSA II), in comparison to a second, already established chromogenic agar, MRSA ID (bioMérieux), for the detection of MRSA in screening samples after nonselective enrichment. On this new medium, MRSA is visualized as mauve colonies by the presence of a specific chromogenic substate (proprietary formulation) and cefoxitin (5.2 mg/liter). Additional selective agents are added for the inhibition of Gram-negative organisms, yeasts, and other Gram-positive cocci. MRSA II is a modified version of the existing BBL CHROMagar MRSA (cefoxitin, 6 mg/liter). On MRSA ID, MRSA is detected as green colonies through the presence of a chromogenic substrate targeting the α-glucosidase enzyme of *S. aureus* and cefoxitin (4 mg/liter). Previous studies have shown that MRSA ID is a highly valuable medium for the detection of MRSA, with sensitivities up to 96.4% and specificities up to 99.5% after 24 h of incubation (5, 6, 11).

A multicenter prospective evaluation was set up in 5 microbiology laboratories. A total of 1,919 samples submitted for MRSA screening were included, consisting of 588 nares swabs, 394 perianal swabs, 320 throat swabs, 430 pooled swabs (throat, nose, and perianal), and 187 wound swabs. All specimens were enriched overnight in 5 ml nonselective tryptic soy broth (TSB) at 35°C in ambient air. After plating 10 μl of the TSB broth on MRSA II and MRSA ID medium using a three-streak dilution method, all media were incubated at 35°C in ambient air. All plates were read at 24 h and 48 h of incubation, and colony color, size, and growth intensity were scored. Identification of suspicious colonies (characteristic color and morphology) was assessed with at least a coagulase tube test with rabbit plasma or slide agglutination in combination with the detection of DNase. Screening tests for MecA-mediated oxacillin resistance were used according to CLSI guidelines (3). When confirmation tests of suspicious colonies showed no growth of MRSA, the sample was considered false positive. No growth or the absence of suspicious colonies was considered negative. In case of MRSA detection on only one chromogenic medium, the sample was inoculated again on both media starting from TSB to check the reproducibility of the obtained data ($n = 8$). Quality control testing was successfully performed on each lot of chromogenic media before use in the study (plating a standardized inoculum of ATCC 43300 or ATCC 29213, an in-house methicillin-susceptible *S. aureus* [MSSA] strain with a cefoxitin MIC of 4 μg/ml).

MRSA was detected in 274 samples (14.3%) on one or both chromogenic media (Table 1). There was no difference in sizes and growth intensities of positive colonies on both chromogenic media. After 24 h, MRSA was detected in 261 samples on MRSA II and in 257 samples on MRSA ID. After 48 h, 271 samples were positive on MRSA II and 269 on MRSA ID. This

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TABLE 1. Number of MRSA strains isolated from 1,919 screening samples

Medium	No. of MRSA strains detected (no. after 48 h)	% sensitivity		% specificity[a]	
		24 h	48 h	24 h	48 h
MRSA II	261 (271)	95.3	98.9	97.4*	89.4*†
MRSA ID	257 (269)	93.8	98.2	94.8	84.7†

[a] *, Statistically significant difference between MRSA II and MRSA ID (McNemar, $P < 0.05$); †, statistically significant difference between 24 h and 48 h for each medium (McNemar, $P < 0.05$).

resulted in comparable sensitivities after 24 h of incubation for MRSA II and MRSA ID (95.3% and 93.8%, respectively; McNemar $P = 0.42$) as well as after 48 h (98.9% and 98.2%, respectively; McNemar $P = 0.72$). For both chromogenic media, the sensitivities were significantly higher after 48 h of incubation versus 24 h of incubation (MRSA II, $P = 0.002$; MRSA ID, $P = 0.0005$). However, when we excluded the results of one center, we observed nearly identical sensitivities after 24 and 48 h of incubation (98.3% and 99.1%, respectively, for MRSA II, $P = 0.5$; 97.8% and 99.1%, respectively, for MRSA ID, $P = 0.25$), with a positivity rate of 13.2% in 1,759 samples. This one center included 160 samples (positivity rate of 26.2%), with significantly higher sensitivities for both chromogenic media after 48 h compared to 24 h. Sensitivity for MRSA II increased from 78.5% to 95.2% ($P = 0.0078$), and that for MRSA ID increased from 71.4 to 92.8% ($P = 0.0039$). It was stressed that the same procedure was followed in the 5 centers, with extra care for a complete 24-h incubation before the first reading of the chromogenic agars. However, this center reported a possible too cautious early assignment of suspected colonies after 24 h, as they use selective instead of TSB enrichment in their daily routine.

After 24 h of incubation, 43 samples were false positive on MRSA II and 85 on MRSA ID, resulting in specificities of 97.4% and 94.8%, respectively, for MRSA II and MRSA ID. The specificity decreased after 48 h of incubation, most notably for MRSA ID: 174 and 251 samples were false positive, respectively, resulting in specificities of 89.4% for MRSA II and 84.7% for MRSA ID. At both time points, MRSA II was significantly more specific ($P < 0.0001$). As we observed in this study, some experience is needed for the recognition of suspected colonies. Whereas no methicillin-susceptible *S. aureus* (MSSA) was detected after 24 h of incubation, longer incubation led to breakthrough of MSSA isolates on both media in a few samples. We did not study the specific effects of enrichment, as no direct inoculation was performed. In a previous study, Böcher et al. (2) showed no increase in yield after selective enrichment, whereas other studies showed that an enrichment step in combination with chromogenic agars led to increased sensitivities of 14 to 26% (11) and 12% (16), respectively.

This study is one of the first evaluations of the new chromogenic medium BBL CHROMagar MRSA II for MRSA detection in screening samples. We prospectively compared the performance of this medium to MRSA ID (bioMérieux) after nonselective enrichment. In conclusion, both chromogenic media permit a fast and sensitive detection of MRSA in screening samples after nonselective enrichment. Growth on MRSA II was significantly more specific than that on MRSA ID. After 24 h of incubation, MSSA was not detected on the chomogenic media, permitting confirmation of the presence of MRSA in case of growth of *S. aureus*.

Despite a moderate increase in yield, a prolonged incubation should be avoided as it results in an important reduction in specificity for both media and an essential delay in reporting the results. In the era of chromogenic media, optimalization of culture methods for MRSA screening is still important, along with investigation of the ideal incubation time and the need for enrichment.

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