

Use of BBL CHROMagar MRSA Medium for Identification of Methicillin-Resistant *Staphylococcus aureus* Directly from Blood Cultures

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We evaluated the ability of BBL CHROMagar MRSA medium (Becton Dickinson, Sparks, MD) to identify methicillin-resistant *Staphylococcus aureus* (MRSA) directly upon subculture from positive blood culture bottles. There were 124 MRSA isolates recovered from blood cultures in the study. BBL CHROMagar MRSA medium was highly sensitive (97.6% [121/124] at 18 to 24 h of incubation and 100% [124/124] at 48 h) and 99.9% specific for identifying MRSA from positive blood cultures.

Identification of methicillin-resistant *Staphylococcus aureus* (MRSA) from blood cultures usually takes 1 to 2 days following detection in semiautomated blood culture systems. Bloodstream infections with MRSA are associated with significant morbidity and mortality and have a significant impact on health-related costs (1, 3). Therefore, early detection of MRSA in blood cultures plays an important role in patient management. A selective and differential medium, CHROMagar MRSA medium (C-MRSA) (Becton Dickinson [BD], Sparks, MD), has been evaluated for identifying MRSA from pure cultures as well as from nasal swab specimens and found to have excellent performance characteristics (5, 7, 8, 12). To determine whether this medium could be useful for rapid identification of MRSA directly from blood cultures, we evaluated the ability of CHROMagar MRSA medium to accurately detect MRSA directly from subcultures of positive blood cultures.

The study was performed in the Clinical Microbiology Laboratory at the Hospital of the University of Pennsylvania from May 2005 through January 2006. All blood culture bottles (Bactec Plus aerobic/anaerobic media; BD) containing gram-positive cocci in clusters, consistent with staphylococci, were prospectively subcultured to blood agar plates (Trypticase soy agar with 5% sheep blood; BD, Sparks, MD) in addition to C-MRSA and incubated at 35°C without CO₂ for 18 to 24 h before examination. Only the first set of blood cultures containing gram-positive cocci in clusters from a particular patient was included in the study. C-MRSA plates were reincubated for an additional 24 h if there was no growth on the plates. Tube coagulase tests (BD) were performed directly on samples from positive blood culture bottles. Colonies exhibiting the mauve phenotype on C-MRSA were further identified using the catalase test (HUMCO, Texarkana, TX) and the Staphaurex slide test (Remel Europe Ltd., Dartford, Kent, United Kingdom),

and susceptibility testing was performed using the Vitek 2 system (GP-61 card; BioMerieux Inc., Hazelwood, MO).

One hundred twenty-four MRSA isolates were recovered from blood cultures during the time period (Table 1). C-MRSA detected 121 isolates after 18 to 24 h of incubation (97.6% sensitivity) and 124 after 48 h of incubation (100% sensitivity). Eighty-seven methicillin-susceptible *S. aureus* (MSSA) isolates were recovered, and none of the MSSA isolates grew on C-MRSA (Table 2). Of 657 coagulase-negative staphylococci (CNS) isolated from blood cultures, 188 did not grow on C-MRSA at 24 or 48 h, 431 grew on C-MRSA at 24 h and 48 h but did not form mauve colonies (i.e., they were colorless), and 31 isolates did not grow after 24 h but appeared as nonmauve colonies at 48 h. Additionally, five CNS isolates grew on C-MRSA along with other bacteria, and all of these were nonmauve colonies. There were two false-positive results. One isolate that grew on C-MRSA and appeared as mauve colonies at 18 to 24 h was identified as *Staphylococcus epidermidis*. One additional isolate grew on C-MRSA at 48 h, appeared as mauve colonies, and was identified as *Staphylococcus warneri*. Blood cultures that met the definition for subcultures that grew bacteria other than CNS but either did not grow on C-MRSA or exhibited nonmauve colonies included anaerobes ($n = 3$; no growth), *Micrococcus* spp. ($n = 6$; no growth), group B strep-

TABLE 1. Performance characteristics of BBL CHROMagar MRSA medium for identification of MRSA from blood cultures

Measurement	Result after the following incubation time:	
	24 h	48 h
No. of MRSA isolates detected	121	124
Sensitivity (%) (95% CI) ^a	97.6 (0.93–0.99)	100 (0.97–1.00)
Specificity ^b (%) (95% CI)	99.9 (0.99–1.00)	99.9 (0.99–1.00)
Positive predictive value (%) (95% CI)	99.2 (0.96–0.99)	99.2 (0.96–0.99)
Negative predictive value (%) (95% CI)	99.6 (0.98–0.99)	100 (0.99–1.00)

^a CI, confidence interval.

^b There were 2 false-positive cultures among 758 non-MRSA isolates (see Table 2).

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TABLE 2. Other bacteria isolated from blood cultures and characteristics on BBL CHROMagar MRSA medium

Organism	No. of isolates	Characteristic(s)
<i>S. aureus</i> , not MRSA	87	No growth
Coagulase-negative staphylococci	188	No growth
	467	Growth, nonmauve
	1	Growth, mauve colonies, identified as <i>S. epidermidis</i>
	1	Growth, mauve colonies, identified as <i>S. warneri</i>
<i>Micrococcus</i> spp.	6	No growth
Group B streptococcus	2	No growth
Viridans streptococcus	1	Growth, nonmauve
<i>Enterococcus</i> spp.	4	Growth, nonmauve
<i>P. aeruginosa</i>	1	Growth, nonmauve

tococci ($n = 2$; no growth), viridans streptococcus ($n = 1$; growth, nonmauve colonies), *Enterococcus* species ($n = 4$; growth, nonmauve colonies), and *Pseudomonas aeruginosa* ($n = 1$; growth, nonmauve colonies). Thus, the overall specificity for C-MRSA was 99.9%.

C-MRSA has been shown to be a highly sensitive and specific medium for identification of MRSA once isolated colonies are obtained on a traditional laboratory medium (5, 8). C-MRSA has also been shown to be an effective medium for identifying MRSA directly from a variety of other clinical specimens, including nasal swabs, sputum samples, and perineal swabs (7, 12). Several other studies have used both phenotypic and genotypic methods to rapidly identify MRSA from blood cultures, including direct identification and susceptibility testing, DNA probes and real-time PCR, and immunologic approaches (4, 6, 9, 10). Each approach has advantages and disadvantages, including turnaround time, technical requirements, and cost. Semiautomated systems, such as the Vitek 2 system used in this study, have been shown to accurately identify MRSA; however, pure isolates are required for analysis, adding to the time for reporting results (11). A simple culture-based approach for rapidly detecting MRSA in blood cultures is therefore desirable for routine use in clinical microbiology laboratories.

Our study shows that use of C-MRSA as a primary medium for subculture of positive blood cultures is rapid, highly sensitive, and specific. Further, it is an easy method for rapid identification of MRSA and can easily fit into the routine workflow of both small and large clinical microbiology laboratories. False-positive results have been observed using C-MRSA with more complex clinical samples such as nasal swabs (7, 12; Z. Han et al., submitted for publication) but are rarely encountered when pure cultures are used (5, 8). In the current study, there were two false-positive cultures caused by CNS. Although the specificity of C-MRSA in our study was 99.9%, the number of false positives can be limited if the use of C-MRSA medium is restricted to blood cultures containing *S. aureus* as

determined by direct tube coagulase testing. In our study, direct tube coagulase testing had a sensitivity and specificity of 92.6% and 100%, respectively. Although direct tube coagulase testing may give false-negative results, it has been found to be highly specific for *S. aureus* in previous studies (4, 13). Alternatively, colonies exhibiting the mauve phenotype on C-MRSA can be confirmed as *S. aureus* directly from C-MRSA plates before reporting (2). Since the vast majority of MRSA isolates were detected after 18 to 24 h (97.6%), use of C-MRSA to subculture positive blood cultures containing gram-positive cocci should decrease the time to reporting positive results compared with conventional identification and susceptibility testing.

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REFERENCES

- Abramson, M. A., and D. J. Sexton. 1999. Nosocomial methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* primary bacteremia: at what costs? *Infect. Control Hosp. Epidemiol.* **20**:408–411.
- Becton Dickinson. 2005. BD BBL CHROMagar MRSA. package insert. Becton Dickinson, Sparks, Md.
- Blot, S. I., K. H. Vandewoude, E. A. Hoste, and F. A. Colardyn. 2002. Outcome and attributable mortality in critically ill patients with bacteremia involving methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *Arch. Intern. Med.* **162**:2229–2235.
- Chapin, K., and M. Musgnug. 2003. Evaluation of three rapid methods for the direct identification of *Staphylococcus aureus* from positive blood cultures. *J. Clin. Microbiol.* **41**:4324–4327.
- Diederer, B., I. van Duijn, A. van Belkum, P. Willemse, P. van Keulen, and J. Kluytmans. 2005. Performance of CHROMagar MRSA medium for detection of methicillin-resistant *Staphylococcus aureus*. *J. Clin. Microbiol.* **43**:1925–1927.
- Eigner, U., M. Weizenegger, A. M. Fahr, and W. Witte. 2005. Evaluation of a rapid direct assay for identification of bacteria and the *mecA* and *van* genes from positive-testing blood cultures. *J. Clin. Microbiol.* **43**:5256–5262.
- Flayhart, D., J. F. Hindler, D. A. Bruckner, G. Hall, R. K. Shrestha, S. A. Vogel, S. S. Richter, W. Howard, R. Walthers, and K. C. Carroll. 2005. Multicenter evaluation of BBL CHROMagar MRSA medium for direct detection of methicillin-resistant *Staphylococcus aureus* from surveillance cultures of the anterior nares. *J. Clin. Microbiol.* **43**:5536–5540.
- Hedin, G., and H. Fang. 2005. Evaluation of two new chromogenic media, CHROMagar MRSA and *S. aureus* ID, for identifying *Staphylococcus aureus* and screening methicillin-resistant *S. aureus*. *J. Clin. Microbiol.* **43**:4242–4244.
- Marlowe, E. M., A. J. Linscott, M. Kanatani, and D. A. Bruckner. 2002. Practical therapeutic application of the oxid BBP2' latex agglutination test for the rapid identification of methicillin-resistant *Staphylococcus aureus* in blood cultures. *Am. J. Clin. Pathol.* **118**:287–291.
- Paule, S. M., A. C. Pasquariello, R. B. Thomson, Jr., K. L. Kaul, and L. R. Peterson. 2005. Real-time PCR can rapidly detect methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* directly from positive blood cultures. *Am. J. Clin. Pathol.* **124**:404–407.
- Sakoulas, G., H. S. Gold, L. Venkataraman, P. C. DeGirolami, G. M. Eliopoulos, and Q. Qian. 2001. Methicillin-resistant *Staphylococcus aureus*: comparison of susceptibility testing methods and analysis of *mecA*-positive susceptible strains. *J. Clin. Microbiol.* **39**:3946–3951.
- Stoakes, L., R. Reyes, J. Daniel, G. Lennox, M. A. John, R. Lannigan, and Z. Hussain. 2006. Prospective comparison of a new chromogenic medium, MRSASelect, to CHROMagar MRSA and mannitol-salt medium supplemented with oxacillin or cefoxitin for detection of methicillin-resistant *Staphylococcus aureus*. *J. Clin. Microbiol.* **44**:637–639.
- Varettas, K., C. Mukerjee, and P. C. Taylor. 2005. Anticoagulant carryover may influence clot formation in direct tube coagulase tests from blood cultures. *J. Clin. Microbiol.* **43**:4613–4615.