

Comparison of the BBL CHROMagar Staph aureus Agar Medium to Conventional Media for Detection of *Staphylococcus aureus* in Respiratory Samples

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Screening for *Staphylococcus aureus* has become routine in certain patient populations. This study is the first clinical evaluation of the BBL CHROMagar Staph aureus agar (CSA) medium (BD Diagnostics, Sparks, Md.) for detection of *S. aureus* in nasal surveillance cultures and in respiratory samples from cystic fibrosis (CF) patients. *S. aureus* colonies appear mauve on CSA. Other organisms are inhibited or produce a distinctly different colony color. *S. aureus* was identified from all media by slide coagulase, exogenous DNase, and mannitol fermentation assays. Susceptibility testing was performed using the agar dilution method. A total of 679 samples were evaluated. All samples were inoculated onto CSA. Nasal surveillance cultures were inoculated onto sheep blood agar (SBA) (BD Diagnostics), and samples from CF patients were inoculated onto mannitol salt agar (MSA) (BD Diagnostics). Of the 679 samples cultured, 200 organisms produced a mauve color on CSA (suspicious for *S. aureus*) and 180 were positive for *S. aureus* on SBA or MSA. Of 200 CSA-positive samples 191 were identified as *S. aureus*. Nine mauve colonies were slide coagulase negative and were subsequently identified as *Staphylococcus lugdunensis* (one), *Staphylococcus epidermidis* (three), *Staphylococcus haemolyticus* (one), and *Corynebacterium* species (four). CSA improved the ability to detect *S. aureus* by recovering 12 *S. aureus* isolates missed by conventional media. Of the 192 *S. aureus* isolates recovered, 122 were methicillin susceptible and 70 were methicillin resistant. Overall, the sensitivity and specificity of CSA in this study were 99.5 and 98%, respectively. There was no difference in the performance of the slide coagulase test or in susceptibility testing performed on *S. aureus* recovered from CSA compared to SBA or MSA. Our data support the use of CSA in place of standard culture media for detection of *S. aureus* in heavily contaminated respiratory samples.

Staphylococcal infections cause significant morbidity and mortality in both the community and hospital settings (3). Screening for staphylococci among various patient populations has become important for appropriate therapeutic management and for epidemiological reasons. An example of the former scenario is the routine inoculation of mannitol salt agar (MSA) in the workup of respiratory specimens from cystic fibrosis (CF) patients who may be infected with *Staphylococcus aureus*, including thymidine-dependent strains (12). These organisms, which are felt to contribute to chronic lung infection (especially in the pediatric CF population), may be overlooked in cases of mixed infection with other potential pathogens (5).

Identifying patients who are colonized with staphylococci by using active-surveillance cultures has become a useful practice in reducing nosocomial spread during outbreaks of methicillin-resistant *S. aureus* (MRSA) (6, 14). Likewise, in outbreak settings samples from health care workers may also be cultured. Many hospitals routinely screen patients from long-term care facilities for staphylococcal colonization, in particular that caused by MRSA, upon admission to the acute care facility. Patients found to be colonized with MRSA are placed on contact precautions to prevent nosocomial spread (6). Colo-

nized health care workers may be treated. While it is not a widespread practice, some institutions routinely screen patients at high risk for staphylococcal nasal carriage prior to invasive procedures. Patients identified as carriers are treated with topical antimicrobial agents either to prevent the spread of infection to others or to prevent dissemination or infection at the time of surgery (8). It is likely that the need for surveillance cultures will increase as institutions struggle with the rise in staphylococcal nosocomial infections (13), thereby increasing the burden of rapid identification and susceptibility testing on clinical laboratories. Systems that allow for easy distinction between *S. aureus* and other gram-positive cocci could reduce the time to identification, decrease laboratory costs, and potentially lead to earlier definitive patient management.

This study compared the BBL CHROMagar Staph aureus selective and differential agar (CSA) medium to conventional isolation and identification methods for the detection of *S. aureus* from clinical specimens of patients with CF and those patients routinely screened for staphylococcal nasal carriage. The study also evaluated the ability to perform slide coagulase testing and susceptibility testing directly from the CSA medium. In addition, the cost of implementation of chromogenic medium was also assessed.

MATERIALS AND METHODS

Clinical samples. From November 2002 to March 2003, we tested 679 consecutive clinical samples, comprising 459 nasal surveillance culture samples and

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220 respiratory samples. Samples were obtained from inpatients and outpatients seen at The Johns Hopkins Hospital, Baltimore, Md. The nasal samples were collected for the detection of MRSA carriage from patients at high risk for nosocomial infection, including intensive care unit patients and transplant recipients. The respiratory samples were obtained from pediatric and adult CF patients for the detection of *S. aureus*, *Pseudomonas aeruginosa*, and other respiratory pathogens. The CF samples included 129 throat samples, 89 expectorated sputa, and two bronchoalveolar lavage specimens.

Media. CSA (BD Diagnostics, Sparks, Md.) contains the following ingredients: chromopeptone, 40.0 g/liter; sodium chloride, 25.0 g/liter; agar, 14.0 g/liter; a proprietary chromogen mix, 0.5 g/liter; and 0.07 g of inhibitory agents/liter including colistin, nalidixic acid, and an antifungal agent. This medium is designed for the isolation and identification of *S. aureus* directly from clinical sources. *S. aureus* colonies produce a light mauve to mauve pigmentation, while coagulase-negative staphylococci appear as white, beige, or light blue colonies. Other organisms are inhibited or grow poorly.

Nasal swabs were planted onto 5% sheep blood agar (SBA; BD Diagnostics) and CSA. CSA was planted after routine media. The plates were streaked for isolation and incubated at 37°C with 5 to 10% CO₂ for 18 to 20 h.

Respiratory specimens obtained from CF patients were planted to chocolate agar (BBL), SBA (BBL), MacConkey agar (BBL), *Burkholderia cepacia* selective agar (Oxoid, Nepean, Ontario, Canada), MSA (BBL), and CSA (Becton Dickinson Inc., Sparks, Md.). CSA was planted after routine media. The plates were streaked for isolation and incubated at 37°C with 5 to 10% CO₂ for 18 to 20 h.

Presumptive identification of *S. aureus*. After 20 h of incubation, plates were examined for *S. aureus* as follows. On CSA, all mauve colonies had a slide coagulase test performed. All other colonies (white, colorless, blue, and green) were not identified. On MSA, all yellow colonies were subcultured for further identification as outlined below. On SBA, all white, yellow, or gold colonies with or without beta-hemolysis were further characterized to rule out *S. aureus*. A slide coagulase test was completed on all suspicious colonies. All cultures negative for *S. aureus* at 20 h were reincubated for an additional 24 h. MSA was incubated for a total of 72 h.

Identification. (i) *S. aureus*. All *S. aureus* isolates were identified using the following biochemical assays: slide coagulase, exogenous nuclease, and fermentation of mannitol. A tube coagulase test, polymyxin B susceptibility, and ornithine decarboxylation were used if other tests did not provide a conclusive identification. All reactions were read at 24 h with the exception of the slide coagulase test. Sugar fermentation was determined using a peptone agar base with phenol red indicator. An isolate positive by slide coagulase, exogenous nuclease, and mannitol fermentation assays was identified as *S. aureus* (1).

(ii) Coagulase-negative staphylococci. A combination of conventional biochemicals and cell wall fatty acid analysis was used to identify coagulase-negative staphylococci to the species level. Biochemical assays completed included fermentation of sucrose, lactose, mannitol, arabinose, turanose, trehalose, and mannose; urease detection; and novobiocin and polymyxin B susceptibility. Cellular fatty acid analysis was performed using the Microbial Identification System (MIS) (MIDI, Newark, Del.). Organism identification was based on the computer comparison of the unknown organism's fatty acid methyl ester profile with predetermined fatty acid methyl ester library profiles of the MIS with the use of version 3.1 software.

(iii) *Corynebacterium* species. All *Corynebacterium* isolates were identified using the following tests: Gram stain, catalase, and cellular fatty acid analysis. Cellular fatty acid analysis was performed using the MIS, according to the manufacturer's recommendations. Organism identification was based on the computer comparison of the unknown organism's fatty acid methyl ester profile with the predetermined fatty acid methyl ester library profile of the MIS with the use of version 3.1 software.

Susceptibility testing. MICs were determined by the reference agar dilution method per NCCLS guidelines (10). Agents tested included: oxacillin, penicillin, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, tetracycline, nitrofurantoin, gatifloxacin, and vancomycin. Twofold dilutions were tested (ranges determined by antibiotic) using Mueller-Hinton agar. Agar used for oxacillin testing was supplemented with 2% (wt/vol) NaCl. MICs were determined after incubating plates at 37°C for 20 to 24 h.

RESULTS

Table 1 presents the overall results. A total of 679 specimens were inoculated onto conventional media and CSA as described above. There was a 98% correlation between the results with CSA medium and those obtained by conventional

TABLE 1. Overall results

Source ^a	Total no. of specimens	No. (%) of specimens with result for <i>S. aureus</i>	
		Negative	Positive
Nasal	459	333 (73)	126 (27)
CF-throat	129	89 (69)	40 (31)
CF-spt/BAL ^a	91	65 (71)	26 (29)
Total	679	487 (72)	192 (28)

^a spt/BAL, sputum-bronchoalveolar lavage.

media. One hundred ninety-two cultures (28%) were positive for *S. aureus*. One hundred twenty-six *S. aureus* isolates were recovered from the nasal surveillance cultures, and 66 *S. aureus* isolates were recovered from the CF respiratory specimens. From the nasal surveillance cultures (Table 2), CSA detected 120 of the 121 *S. aureus* isolates recovered from conventional media. An additional five isolates (four of methicillin-susceptible *S. aureus* [MSSA] and one of MRSA) were recovered on CSA alone. From the CF throat cultures, CSA detected 35 of the 35 *S. aureus* isolates recovered from MSA. An additional five isolates (three of MSSA and two of MRSA) were recovered on CSA alone. For the CF respiratory cultures, CSA detected 24 of the 24 *S. aureus* isolates recovered by MSA. An additional two isolates (one of MSSA and one of MRSA) were recovered on CSA alone.

Incubation of negative plates for an additional 24 h (48 h total) resulted in the increased recovery of 10 isolates of *S. aureus*. For CF specimens, 48 h of incubation resulted in an 8% increase of *S. aureus* recovery on MSA and an 11% increase of *S. aureus* recovery on CSA. For nasal specimens, 48 h of incubation resulted in an 0.8% increase on SBA and a 2.4% increase on CSA. In summary, CSA detected an additional 12 isolates missed by conventional media (eight at 24 h and four at 48 h). CSA failed to recover one isolate recovered on conventional media.

There were nine mauve colonies, which when confirmed were species other than *S. aureus*. The isolates misidentified after 24 h of incubation included four of *Corynebacterium* species, one of *Staphylococcus epidermidis*, and one of *Staphylococcus lugdunensis*. At 48 h of incubation, two mauve colonies were later identified as *S. epidermidis* and one was identified as *Staphylococcus haemolyticus*. From the CF patients' respiratory cultures, the CSA medium detected all *S. aureus* isolates recovered from conventional media and did not misidentify any non-*S. aureus* species. The specificity of CSA for nasal speci-

TABLE 2. Numbers of *S. aureus* isolates detected by various media

Source	Total no. of <i>S. aureus</i> isolates	Total no. (%) of <i>S. aureus</i> isolates detected by:	
		CSA	Conventional media
Nasal	126	125 (99.2)	121 (96)
CF-throat	40	40 (100)	35 (87.5)
CF-respiratory	26	26 (100)	24 (92.3)
Total	192	191 (99.4)	180 (93.7)

TABLE 3. Sensitivity and specificity by specimen type of CSA compared to conventional Media

Specimen type	Sensitivity (%)	Specificity (%)
Nasal surveillance ^a	99	97
CF-throat ^a	100	100
CF-sputum-BAL ^{b,c}	100	100

^a An additional five isolates were detected by CSA alone.

^b An additional two isolates were detected by CSA alone.

^c BAL, bronchoalveolar lavage.

mens and for CF respiratory samples was 97 and 100%, respectively (Table 3).

The ability to perform slide coagulase testing and susceptibility testing directly from CSA was also evaluated. The performance of slide coagulase testing was not affected by growth on CSA. Susceptibility testing was completed on all isolates of *S. aureus* recovered from all media. Seventy of the isolates were MRSA, and 122 were MSSA. There were no differences in MIC susceptibility results observed between isolates from the same patient recovered from CSA and those recovered from conventional media (data not shown).

DISCUSSION

This study evaluated the performance of the newly formulated BBL CSA medium for the detection of MSSA and MRSA isolates from nasal surveillance and CF respiratory specimens. Typically in our CF patient population sputum and throat samples contain heavy amounts of multiple morphologies of *P. aeruginosa* and *S. aureus* as well as mixed respiratory flora. The nasal surveillance cultures contain variable amounts of primarily *Corynebacterium* sp. strains and coagulase-negative staphylococci. CSA detected 99.5% of all *S. aureus* isolates recovered from conventional media. In addition, it detected 12 isolates missed by SBA or MSA. The sensitivity did not vary by specimen type.

This is the first study to evaluate the BBL formulation of CHROMagar Staph aureus. Our results are similar to the work of other groups who have evaluated the formulation produced by CHROMagar Microbiology, Paris, France (2, 4, 7, 9). In contrast to our study, two of these groups tested stock isolates only (7, 9). Carricajo et al. (2) compared CHROMagar Staph aureus to Columbia agar plates with 5% horse blood and chocolate agar medium for the detection of *S. aureus*. An analysis of 775 clinical samples yielded a 98.5% sensitivity and 97% specificity based on colony pigmentation (2). The addition of the Staphychrom coagulase test (International Microbio, Signes, France) increased the specificity to 100% (2). They also recovered several additional isolates compared to traditional medium (2). Gaillot et al. (4) completed an evaluation of 2,000 clinical samples, and their findings were similar to our evaluation. However, unlike that study, we did not encounter difficulties in obtaining expected pigmentation from the atypical *S. aureus* strains isolated from CF patients (4).

The cost of CSA (\$3.07/plate list price) is higher than that of nonselective conventional media. However, there are some efficiencies gained with the use of this medium. The more rapid visualization of *S. aureus* allows technologists to work through cultures more quickly. There is no need to subculture organ-

isms for antimicrobial susceptibility testing. This is in contrast to other selective media that require subculturing prior to susceptibility testing, thus delaying results. The mauve pigmentation is specific for *S. aureus*; therefore, slide coagulase tests can be substantially reduced or eliminated. Additional biochemical tests or identification methods that may be needed on occasion for final identification can also be reduced or eliminated. CF cultures require the use of selective media for the isolation of *S. aureus*. The cost of selective media such as MSA (\$1.31/plate) is higher than that of nonselective media. The additional costs incurred with the use of the MSA and required subculture plates (\$1.03/plate), slide coagulase, and additional biochemicals narrow the cost differential and make the change from a selective medium to CHROMagar Staph aureus closer to cost-neutral.

Our results indicate that primary plating of CSA improves recovery of *S. aureus*. The heavily contaminated samples challenged both the selective and differential capacity of the media. The excellent inhibition of resistant gram-negative organisms as well as nasopharyngeal flora allowed for a clear visualization of highly specific mauve colonies.

In summary, CHROMagar Staph aureus compared favorably to conventional media for rapid detection of *S. aureus* in clinical samples. The NCCLS M35-A document for the abbreviated identification of bacteria and yeast (11) includes chromogenic medium among the acceptable single rapid tests for organism identification as long as the accuracy is greater than 95%. Our data exceed the 95% accuracy requirement and indicate that the reactions on the CHROMagar Staph aureus medium can be used for final identification. This medium has the potential to more rapidly and accurately identify patients and health care workers who may spread resistant staphylococci in the hospital environment. In CF specimens, due to the high specificity (100%), we recommend reporting of *S. aureus* if mauve colonies are present on CSA at 24 or 48 h. In nasal surveillance specimens, the specificity was 97%. This improved to 100% with the addition of the slide coagulase test.

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REFERENCES

- Bannerman, T. L. 2003. *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci that grow aerobically, p. 385–404. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 8th ed. ASM Press, Washington, D.C.
- Carricajo, A., A. Treny, N. Fonsale, M. Bes, M. E. Reverdy, Y. Gille, G. Aubert, and A. M. Freydiere. 2001. Performance of the chromogenic medium CHROMagar Staph aureus and the Staphychrom coagulase test in the detection and identification of *Staphylococcus aureus* in clinical specimens. *J. Clin. Microbiol.* **39**:2581–2583.
- Cosgrove, S. E., G. Sakoulas, E. N. Perencevich, M. J. Schwaber, A. W. Karchmer, and Y. Carmeli. 2003. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. *Clin. Infect. Dis.* **36**:53–59.
- Gaillot, O., M. Wetsch, N. Fortineau, and P. Berche. 2000. Evaluation of CHROMagar Staph. aureus, a new chromogenic medium, for isolation and presumptive identification of *Staphylococcus aureus* from human clinical specimens. *J. Clin. Microbiol.* **38**:1587–1591.
- Gilligan, P. H., P. A. Gage, D. F. Welch, M. J. Muszynski, and K. R. Wait. 1987. Prevalence of thymidine-dependent *Staphylococcus aureus* in patients with cystic fibrosis. *J. Clin. Microbiol.* **25**:1258–1261.
- Jernigan, J. A., M. G. Titus, D. H. Groschel, S. Getchell-White, and B. M. Farr. 1996. Effectiveness of contact isolation during a hospital outbreak of methicillin-resistant *Staphylococcus aureus*. *Am. J. Epidemiol.* **143**:496–504.

7. **Kluytmans, J., A. Van Griethuysen, P. Willemse, and P. Van Keulen.** 2002. Performance of CHROMagar selective medium and oxacillin resistance screening agar base for identifying *Staphylococcus aureus* and detecting methicillin resistance. *J. Clin. Microbiol.* **40**:2480–2482.
8. **Laupland, K. B., and J. M. Conly.** 2003. Treatment of *Staphylococcus aureus* colonization and prophylaxis for infection with topical intranasal mupirocin: an evidence-based review. *Clin. Infect. Dis.* **37**:933–938.
9. **Merlino, J., M. Leroi, R. Bradbury, D. Veal, and C. Harbour.** 2000. New chromogenic identification and detection of *Staphylococcus aureus* and methicillin-resistant *S. aureus*. *J. Clin. Microbiol.* **38**:2378–2380.
10. **National Committee for Clinical Laboratory Standards.** 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th ed. Approved standard M7-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
11. **National Committee for Clinical Laboratory Standards.** 2003. Abbreviated identification of bacteria and yeast. Approved standard M35. National Committee for Clinical Laboratory Standards, Wayne, Pa.
12. **Saiman L., J. Siegel, and Cystic Fibrosis Foundation.** 2003. Infection control recommendations for patients with cystic fibrosis: microbiology, important pathogens, and infection control practices to prevent patient-to-patient transmission. *Infect. Control Hosp. Epidemiol.* **24**(Suppl. 5):S6–S52.
13. **Salgado, C. D., D. P. Calfee, and B. M. Farr.** 2003. Interventions to prevent methicillin-resistant *Staphylococcus aureus* transmission in health care facilities: what works? *Clin. Microbiol. Newsl.* **25**:137–144.
14. **Thompson, R. L., I. Cabezudo, and R. P. Wenzel.** 1982. Epidemiology of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus*. *Ann. Intern. Med.* **97**:309–317.