Evaluation of mannitol salt agar, CHROMagar Staph aureus and CHROMagar MRSA for detection of methicillin-resistant *Staphylococcus aureus* from nasal swab specimens

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Mannitol salt agar (MSA), CHROMagar Staph aureus (CSA) and CHROMagar MRSA (CSA-MRSA) were evaluated with nasal surveillance specimens for their ability to detect *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA). CSA was found to be more sensitive than MSA in detecting *S. aureus* (98 versus 84.3%; $P=0.03$). CSA and CSA-MRSA were equivalent in the ability to detect MRSA at 24 h (89.7 versus 87.2%) and at 48 h (94.9 versus 94.9%).

When combined with Staphaurex slide confirmation testing, both CSA and CSA-MRSA were highly specific (100%) media for detecting MRSA from nasal swab specimens.

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INTRODUCTION

Staphylococcus aureus is a major pathogen responsible for nosocomial and community-acquired infection. Methicillin-resistant *S. aureus* (MRSA) has emerged as a nosocomial pathogen of major worldwide importance and is an increasingly frequent cause of community-acquired infections that cause significant morbidity and mortality (Rubin *et al.*, 1999). Colonized personnel can serve as a reservoir for the nosocomial spread of MRSA. Active surveillance and timely identification of MRSA colonization of patients is an important infection control activity that helps to prevent nosocomial spread and is cost-effective (Muto *et al.*, 2003; Wernitz *et al.*, 2005). Our goal was to compare the recovery of MRSA on CHROMagar MRSA with that on other conventional (mannitol salt agar) and chromogenic media (CHROMagar Staph aureus).

METHODS

From October 2004 to May 2005, we evaluated several culture media for their ability to detect MRSA colonization from surveillance nasal

specimens, including mannitol salt agar (MSA), CHROMagar Staph aureus (CSA) and CHROMagar MRSA (CSA-MRSA) (all from BD Diagnostics). Nasal swabs (BBL CultureSwab Liquid Stuart, Double Swab, BD Diagnostics) were prospectively collected from 656 inpatients at the Hospital of the University of Pennsylvania. CSA was initially compared to MSA medium in phase I of the study, prior to the availability of CSA-MRSA from the manufacturer. CSA is a differential medium for the rapid identification of *S. aureus*. Colonies suggestive of *S. aureus* (mauve phenotype) must be further tested for methicillin resistance to confirm the presence of MRSA. For phase I, 316 nasal swab specimens were collected prospectively from patients in the medical intensive care and transplant units, and were sampled upon admission to the unit and weekly thereafter until positive, and used to compare MSA and CSA.

In phase II of the study, 340 nasal swab specimens were collected prospectively from patients in the medical and surgical intensive care units upon admission to the unit, and weekly thereafter until positive, and were used to compare CSA (with cefoxitin disk test confirmation) and CSA-MRSA. Each double swab was emulsified in 2 ml trypticase soy broth, and an equal number of drops were added to CSA, CSA-MRSA or MSA. The plates were streaked for isolation and incubated at 37 °C without CO₂ for 24–48 h.

After 18–24 h incubation, mauve colonies on CSA as well as CSA-MRSA, and yellow colonies on MSA, were regarded as presumptive *S. aureus* isolates and were subcultured onto 5% sheep blood agar. All negative cultures at 24 h were reincubated for an additional 24 h. *S. aureus* isolates were identified using the catalase test (Humco), Staphaurex slide test (Remel Europe), tube coagulase test (BD

Abbreviations: CNS, coagulase-negative staphylococci; CSA, CHROMagar Staph aureus; CSA-MRSA, CHROMagar MRSA; MRSA, methicillin-resistant *S. aureus*; MSA, mannitol salt agar.

Table 1. Comparison of MSA and CSA for detection of *S. aureus* ($n=51$) from nasal swab specimens

Medium	Incubation time (h)	Sensitivity (%) (95 % CI)*	Specificity (%)† (95 % CI)	Specificity (%)‡ (95 % CI)	P value
CSA	24	90.2 (78.5, 96.7)	99.3 (97.4, 99.9)	99.2 (97.4, 99.9)	0.11§
	48	98.0 (89.5, 99.9)	89.4 (85.1, 92.9)	99.6 (97.9, 99.9)	0.03
MSA	24	76.5 (62.5, 87.2)	99.6 (97.9, 99.9)	100 (97.8, 100)	—
	48	84.3 (71.4, 92.9)	95.8 (92.7, 97.9)	100 (97.8, 100)	—

*CI, 95 % confidence interval.

†Without Staphaurex slide test confirmation.

‡With Staphaurex slide test confirmation.

§CSA versus MSA at 24 h.

||CSA versus MSA at 48 h.

Diagnostics) and Vitek 2 (bioMérieux). Confirmed *S. aureus* strains were tested using the cefoxitin disk test according to Clinical and Laboratory Standards Institute (2005) guidelines. Coagulase-negative staphylococci (CNS) were identified using the Vitek 2 ID-GP card.

RESULTS AND DISCUSSION

Among the 316 nasal surveillance specimens that were used to compare CSA and MSA, *S. aureus* was isolated from 51 samples, of which 19 (37.3 %) were MRSA (Table 1). CSA showed higher sensitivity than MSA at 24 h (90.2 versus 76.5 % at 24 h; $P=0.11$), although the difference was not statistically different; CSA was significantly more sensitive at 48 h (98.0 versus 84.3 % at 48 h; $P=0.03$). For CSA and MSA, it was possible to increase the sensitivity by approximately 8 % by incubating negative plates for an additional 24 h. The specificities of CSA and MSA were 99.3 and 99.6 % at 24 h and 89.4 and 95.8 % at 48 h, respectively, without the use of Staphaurex slide test confirmation. The specificities improved, however, to 99.2 and 100 % for CSA and MSA, respectively, at 24 h and 99.6 and 100 % at 48 h, respectively, when the Staphaurex slide test confirmation was used.

Of the 340 nasal surveillance specimens plated on CSA and CSA-MRSA in the phase II analysis, *S. aureus* MRSA was isolated from 39 samples (Table 2). CSA recovered 89.7 and 94.9 % MRSA at 24 and 48 h, respectively. CSA-MRSA

recovered 87.2 and 94.9 % of the MRSA isolates at 24 and 48 h, respectively (CSA versus CSA-MRSA; P not significant). The specificity of both media combined with the Staphaurex slide test was 100 %. One MRSA strain was missed by CSA plus cefoxitin disk-diffusion testing but picked up by CSA-MRSA. This was due to a culture containing both MRSA and methicillin-susceptible *S. aureus* (MSSA), and only the MSSA was picked from the CSA medium for cefoxitin disk-diffusion testing.

False positives which occurred at 24 h on CSA/CSA-MRSA were all CNS ($n=2$). False positives at 48 h on CSA/CSA-MRSA were mostly CNS, *Staphylococcus epidermidis* ($n=5$), *Staphylococcus hominis* ($n=1$), *Staphylococcus intermedius* ($n=1$), seven unidentified, and *Corynebacterium* spp. ($n=2$). The false-positive yellow colonies on MSA were *Bacillus* spp. ($n=2$) and CNS ($n=9$).

Growth from CSA can be used directly for Staphaurex slide test and susceptibility testing according to the manufacturer's instructions. Performing the Staphaurex slide test directly from mauve colonies increased the specificity of CSA from 99.2 to 99.6 % at 24 h and from 89.4 to 99.2 % at 48 h.

We also tested 32 random MRSA and 32 CNS from frozen storage. All MRSA and CNS isolates gave the expected phenotypes on both CSA and CSA-MRSA at 24 h

Table 2. Comparison of CSA and CSA-MRSA for detection of MRSA ($n=39$) from nasal swab specimens

Medium	Incubation time (h)	Sensitivity (%) (95 % CI)*	Specificity (%)† (95 % CI)	Specificity (%)‡ (95 % CI)	P value
CSA	24	89.7 (75.7, 97.1)	Not done	100 (97.8, 100)	0.7§
	48	94.9 (82.7, 99.4)		100 (97.8, 100)	1.0
CSA-MRSA	24	87.2 (72.6, 95.7)	99.3 (97.6, 99.9)	100 (97.8, 100)	—
	48	94.9 (82.7, 99.4)	94.3 (91.1, 96.7)	100 (97.8, 100)	—

*CI, 95 % confidence interval.

†Without Staphaurex slide confirmation test.

‡With Staphaurex slide confirmation test.

§CSA versus CSA-MRSA at 24 h, $P=0.7$.

||CSA versus CSA-MRSA at 48 h, $P=1.0$.

(sensitivity and specificity, 100 %); this was consistent with the result reported by Diederer *et al.* (2005), which demonstrated that CSA-MRSA was 100 % sensitive in detecting frozen strains of MRSA. Gaillot *et al.* (2000) plated 100 frozen strains of *S. aureus* and 45 CNS on CSA and found that all strains showed the desired colony morphology at 24 h. However, the sensitivity and specificity of CSA were 95.5 and 99.4 %, respectively, when clinical specimens were plated, and our data were consistent with that study.

Isolates that exhibited false-positive reactions in the phase I and phase II studies on CSA and/or CSA-MRSA were frozen and subsequently retested. Nineteen CNS [*S. epidermidis* ($n=11$), *S. hominis* ($n=4$), *Staphylococcus caprae* ($n=1$), not identified ($n=3$)] and five *Corynebacterium* spp. isolates that exhibited false-positive reactions on CSA and/or CSA-MRSA were retrieved and replated to CSA and CSA-MRSA. Five of 24 isolates exhibited mauve colonies (one isolate of *Corynebacterium* spp. at 24 h on both CSA and CSA-MRSA, three isolates of *Corynebacterium* spp. and one isolate of *S. caprae* at 48 h on CSA). This suggests that the bacterial factors that contribute to false-positive reactions on CSA and/or CSA-MRSA are unstable.

CSA-MRSA was found to be comparable with CSA plus the cefoxitin disk-diffusion test in detecting MRSA. It has high sensitivity and specificity when combined with the Staphaurex slide test and allows reporting of MRSA within 1 day. Additionally, CSA-MRSA requires less hands-on time than CSA combined with disk confirmation. Further, using CSA-MRSA medium offers additional time savings (~1 day) compared with CSA combined with cefoxitin disk testing. One limitation of the current study is that we did not confirm all MRSA with testing directly for *mecA* using molecular methods. The cefoxitin disk test, however, has been shown to be highly sensitive and specific for *mecA*, and to have an excellent correlation with *mecA* testing for the detection of MRSA (Swenson *et al.*, 2005).

The results from our study are consistent with the findings from a multicentre study using CHROMagar MRSA medium for detecting MRSA in nasal swab specimens (Flayhart *et al.*, 2005). In that study, CHROMagar MRSA medium had 95.2 % sensitivity and >96.7 % specificity. CNS and *Corynebacterium* spp. also occasionally exhibited false positives, as we noted in our survey. Similarly, Stoakes and colleagues compared CHROMagar MRSA medium with other selective media, and for nasal swab specimens, found a sensitivity of 95 % and a high specificity, but did not comment on false positives with this medium (Stoakes *et al.*, 2006). Other chromogenic media, such as *S. aureus* ID (bioMérieux) (Perry *et al.*, 2003) are also available for detecting *S. aureus*. *S. aureus* ID supplemented with cefoxitin (MRSA ID) has been developed by the same manufacturer, and shows comparable sensitivity and specificity to those of CSA-MRSA (Perry *et al.*, 2004).

Several groups have used molecular methods for the rapid detection of MRSA from clinical specimens within a few

hours (Francois *et al.*, 2003; Warren *et al.*, 2004). Although molecular methods provide same-day results, they have certain disadvantages, including the need to batch clinical specimens, greater technical demands than culture, expensive reagents, and the need for specialized laboratory equipment.

In summary, our results indicate that CSA-MRSA, or CSA plus the cefoxitin disk diffusion test, are highly effective for detecting MRSA from nasal swab specimens, and this is consistent with the performance characteristics reported in several recent studies that have used direct inoculation of nasal swab specimens. The excellent inhibition of resistant Gram-negative organisms as well as nasopharyngeal flora allowed for a clear visualization of highly specific mauve colonies. However, mauve colonies on CSA/CSA-MRSA should be confirmed directly from plates using the Staphaurex slide test or the tube coagulase test before the presumptive reporting of *S. aureus*/MRSA.

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