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Optimal detection of *Staphylococcus aureus* from clinical specimens using a new chromogenic medium

Zmira Samra[a,*], Orit Ofir[a], Judi Bahar[b]

[a]*Department of Microbiology, Rabin Medical Center, Tel Aviv University, Tel Aviv, Israel*
[b]*Hy-Laboratories Ltd., Rehovot, Israel*

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Abstract

The new chromogenic medium CHROMagar Staph aureus (CASA) was evaluated for its ability to detect and presumptively identify *Staphylococcus aureus*. Nine hundred forty-two clinical specimens (742 wound, 200 sputum and bronchoalveolar lavage) were cultured on CASA, tryptic soy blood agar (TSBA), and mannitol salt agar (MSA). Of the 153 *S. aureus* isolates from wounds on any media, 151 grew on CASA and TSBA and 146 on MSA. Sensitivity after 24 hours was 93.5%, 94%, and 77%, respectively, and increased after 48 hours to 99% for CASA and TSBA and to 95% for MSA. Of the 41 isolates recovered from sputum and lavage, all grew on CASA, 27 on TSBA, and 36 on MSA. Sensitivity after 24 hours was 93%, 66%, and 81%, respectively, and 100%, 66%, and 88% after 48 hours. All specimens revealed 99% sensitivity for CASA, 92% for TSBA, and 94% for MSA. Specificity for CASA was 100%. Antimicrobial susceptibility tests showed full agreement between isolates from CASA and from reference media. In conclusion, CASA has a high sensitivity and can identify isolates undetected on conventional media (p value for CASA vs. TSBA was 0.001 and vs. MSA, 0.006). This difference is particularly notable when mixed flora are present. The simplicity of the colony recognition increased the medium specificity, allowing a reliable and rapid method for the detection of *S. aureus* on the primary plate.

1. Introduction

Staphylococcus aureus is one of the pathogens most frequently identified in clinical laboratories. It is responsible for severe suppurative infections associated with high morbidity and mortality and is a leading cause of nosocomial infection (Sheagren, 1984; Archer, 1998). Over the last several decades, *S. aureus* has developed resistance to many common antibiotics (Sabath, 1982; Samra et al., 2001). Resistance to methicillin has reached global proportions (Voss and Doebbeling, 1995), and a decreasing susceptibility to vancomycin has been reported since 1996 (Hiramatsu et al., 1997; Tenover et al., 1998). The prompt isolation and identification of *S. aureus* is essential for appropriate patient care and control in the hospital setting. However, the microorganism can be missed on routine clinical laboratory testing if the specimen contains mixed flora, especially when staphylococcal species with an identical colony appearance are present or when swarming colonies of *Pseudomonas* or *Proteus* cover the *S. aureus* colonies (Carricajo et al., 2001). Therefore, reliable and rapid identification methods are crucial.

S. aureus is usually isolated on nonspecific media (e.g., blood agar) and then identified. Although immunoenzymatic and genetic assays are available (Brakstad et al., 1992; Guzman et al., 1992), the identification of suspect colonies relies mostly on the detection of specific determinants, such as plasma coagulation, fermentation of mannitol, thermo-stable nuclease (DNase) production, egg yolk lipase hydrolysis, or agglutination of sensitized latex particles (Fournier et al., 1993; Kloos and Bannermann, 1995; Wilkerson et al., 1997). These methods, though, become costly and time-consuming when more than one suspect microorganism is present on the primary plate. To screen specifically for *S. aureus* in potentially contaminated samples, clinical laboratories use mannitol salt agar (MSA) or other highly selective differential media. However, their average sensitivity compared to blood agar is too low for coagulase-positive staphylococci in clinical specimens (Koneman et al., 1997); a more specific medium with a similar sensitivity to blood agar is still needed for one-step isolation and presumptive identification.

* Corresponding author. Tel.: +97-23-937-6725; fax: +97-23-921-8466.
E-mail address: zsamra@clalit.org.il (Z. Samra).

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CHROMagar Staph aureus (CASA) (CHROMagar Microbiology, Paris, France) is one of the new selective chromogenic media designed for the presumptive detection of pathogenic organisms in clinical specimens. It identifies colonies of *S. aureus* by their mauve color (Gaillot et al., 2000; Carricajo et al., 2001). The aim of this study was to evaluate the sensitivity and specificity of CASA and its ability to detect and identify *S. aureus* in specimens with potential microbial contamination.

2. Materials and methods

2.1. Study population and specimens

The study was conducted at two major university-affiliated tertiary care facilities (total 1150 beds). Testing was performed on 742 clinical wound specimens and 200 sputum and bronchoalveolar lavage specimens. Each specimen was derived from a different patient.

2.2. Media

CASA medium consists of a selective base containing chromogenic substrates revealing metabolic enzymes from *S. aureus* yielding the growth of typical pink-mauve colonies. The CHROMagar company (Paris, France) provided the dehydrated powder, and Hy-Laboratories (Rehovot, Israel) prepared the medium according to the manufacturer's instructions. The medium was poured into light-protected 90-mm Petri dishes, stored at 4°C, and used within 4 weeks.

Tryptic soy blood agar (TSBA) (No. 2, Difco) with 5% defibrinated sheep blood, MSA (Difco), and Mueller Hinton II agar (BBL) (all products supplied by BD Bioscience, Madison, WI, USA) were used as reference media. Phenylethyl-alcohol agar with 5% defibrinated sheep blood was used for the selective isolation of *S. aureus* from mixed flora. All media were prepared by Hy-Laboratories according to the manufacturers' instructions and supplied in 90-mm Petri dishes.

2.3. Quality control

Each batch of medium was tested for sterility and performance with American Type Culture Collection (ATCC) and wild microorganisms, according to specific standard operation procedures. All standard operation procedures followed the recommendations in the National Committee for Clinical Laboratory Standards (NCCLS, 2001) "Quality Control for Commercially Prepared Microbiological Culture Media" (1996) and "Performance Standards for Antimicrobial Disk Susceptibility Testing" (2001). Additional wild strains were added to the challenge microorganisms with a view of tightening the test's conditions. Sampling of media for sterility testing was performed according to the recommendations of the American National Standard: MIL STD 105E.

The ability of CASA to grow the *S. aureus* challenge strain was evaluated in comparison to the reference TSBA medium. The acceptance criterion for releasing each batch was at least 70% recovery relative to the control plates. The two media were checked in parallel by the plate-counting method for culture response to minimum inoculum (10–100 cfu) with *S. aureus* ATCC 25923.

The specificity of the medium for the detection of *S. aureus* (by mauve colony pigmentation) was evaluated by testing the growth and morphology of *S. aureus* ATCC 43300, *S. aureus* ATCC 25923, *S. xylosus* ATCC 29971, *S. saprophyticus*, wild strain, and *Streptococcus uberis* ATCC 277958. Fresh colony cultures of these bacteria were streaked on the agar medium.

The inhibitory effect of CASA on *S. epidermidis* and gram-negative bacteria was tested by streaking *S. epidermidis* ATCC 12228, *P. aeruginosa* ATCC 27853, and *E. coli* ATCC 25922. Fresh bacterial cultures were diluted in saline solution and adjusted to a 10^7 cell concentration. The actual inoculum on CASA was monitored by the plate-counting method on TSBA.

2.4. Bacteriological procedures

The efficiency of CASA was evaluated in comparison to standard reference media. To inoculate uniform samples on the different media, the nonfluid specimens were first suspended in sterile physiological saline (0.85%). The specimens were inoculated simultaneously on the three media with a 10 μL calibrated loop and then incubated aerobically at 36 ± 2°C and tested after 24 and 48 hours.

All pink-mauve colonies growing on CASA and suspected *S. aureus* colonies on conventional media were Gram-stained and tested with the slide agglutination test (Slidex Staph Plus, bioMerieux, Lyon, France) and DNAse agar test (Hy-Labs). When the results of the two tests differed, final identification was performed with AP ID 32 Staph Gallery (bioMerieux).

Antimicrobial susceptibility was tested by the disk-diffusion technique on Mueller Hinton II agar (BBL) according to the recommendations of the NCCLS (2001). The accuracy of antimicrobial susceptibility was evaluated by picking isolates directly from CASA and comparing the results with those from parallel tests of isolates picked from blood agar.

Student's t test, χ^2 test, or Fisher's exact test was used for statistical analysis.

3. Results

The quality control of media assay results of the 5 CASA and TSBA batches evaluated in comparison by plate counting gave mean colony counts of 63.6 ± 4.67 and 71.0 ± 5.6,

Table 1
Ability of different media to detect *S. aureus* isolates from 943 clinical specimens

Positive specimens	Incubation time (hours)	CASA (a)	MSA (b)	TSBA (c)	*p* value*	
					(a) vs. (b)	(a) vs. (c)
Wound (n = 153)	24	143	118	144	0.001	0.813
	48	151	146	151	0.173	1.000
Sputum (n = 41)	24	38	33	27	0.105	0.003
	48	41	36	27	0.055	0.001
Total (n = 194)	24	181	151	171	0.110	0.080
	48	192	182	178	0.006	0.001

CASA, CHROMagar *S. aureus*; MSA, mannitol salt agar; TSBA, blood agar.
* p value was calculated by χ^2 test or Fisher exact test.

respectively. These figures show a good percentage of recovery (90%) on CASA, of the challenge microorganisms, although the difference with the reference medium was statistically significant (p = 0.04, paired t test).

The two *S. aureus* strains challenged in the trial yielded typical mauve colonies surrounded by a matte halo. *S. xylosus* yielded dark steel-blue colonies and *S. saprophyticus*, blue colonies. *Streptococcus uberis* yielded small steel-blue colonies. *S. epidermidis* was highly inhibited, and the scanty colonies that did grow were colorless or blue shadowed, smaller and distinct from those of *S. aureus*. *P. aeruginosa* and *E. coli* were completely inhibited on the medium. Colony morphology and pigmentation were consistent for all test microorganisms throughout the shelf-life of the plates in all the batches used in the study.

A total of 194 *S. aureus* strain isolates were recovered from the 942 specimens tested in this study. One hundred ninety-two grew on CASA (99%), 182 (93.8%) on MSA, and 178 (91.7%) on TSBA (Table 1). *S. aureus* was isolated from 153 of the 742 wound specimens on at least one of the three media. One hundred fifty-one isolates grew on CASA, 151 on TSBA, and 146 on MSA. One hundred twelve isolates grew after 24 hours' incubation on all three media. Four of the 151 isolates grown on TSBA were recovered only after subculture on blood phenylethyl-alcohol medium, owing to the overgrowth of other bacteria on the primary plate. Of the 41 isolates recovered from sputum and bronchoalveolar lavage, all grew on CASA, and 36 grew on MSA; only 27 grew on TSBA, owing to the overgrowth of competitive swarming bacteria on this agar. Twenty-three of the 41 isolates grew after 24 hours' incubation on all three media.

Of the total isolates recovered on each medium, 5.7%, 17%, and 3.9% grew on CASA, MSA, and TSBA, respectively, only after 48 hours of incubation. The sensitivity of the different media for detecting *S. aureus* from clinical specimens is given in Table 2. Specificity of CASA and MSA was 100% and 86.7%, respectively.

Antimicrobial susceptibility testing was performed on 150 *S. aureus* isolates for 16 relevant antimicrobial agents; 38 (25%) were methicillin-resistant strains. Equivalent susceptibilities were obtained in all cases, and very few differences between zone diameters (1 to 2 mm) were detected at random. It is noteworthy that none of these differences were out of the range specified by the NCCLS criteria for the disk-diffusion method of susceptibility testing. The number of susceptible isolates picked from CASA was exactly the same as the number picked from TSBA. No significant differences (paired t test) were found between the number of *S. aureus* isolates that tested intermediate or resistant (p = 0.3332, paired t test).

4. Discussion

The first step of this study was performed with stock cultures of different microorganisms to evaluate the ability of CASA to grow ATCC *S. aureus* strains from minimum inoculum (10–100 cfu) in mauve-pigmented colonies. The medium sensitivity, checked by the plate count technique, showed a more than 90% recovery of the challenge strain compared to the reference medium (TSBA). The specificity of CASA in our clinical study was shown to be very high (100%). This result confirms the findings reported by Gaillot et al. (2000) and Carricajo et al. (2001), 99.4% and 97%, respectively. The growth of *S. aureus* in typical mauve colonies surrounded by a matte halo was distinct from the growth of *S. epidermidis* (few, smaller, distinct colonies

Table 2
Sensitivity of different media in detecting *S. aureus* from clinical specimens

Specimens	Incubation time (hours)	CASA	MSA	TSBA
Wound	24	93.5%	77.1%	94.1%
	48	98.7%	95.4%	98.7%
Sputum	24	92.7%	80.5%	65.8%
	48	100%	87.5%	65.8%
Total	24	93.3%	77.8%	88.1%
	48	98.9%	93.8%	91.7%

CASA, CHROMagar *S. aureus*; MS, mannitol salt agar; TSBA, blood agar.

with or without a blue shadow) and *S. saprophyticus* and *S. xylosus* (blue colonies). Gram-negative bacteria were completely inhibited. These results were consistent for all five CASA batches. All the isolated mauve colonies were confirmed to be *S. aureus*. The appearance of a matte halo surrounding the colony aided the recognition of the suspected isolates, although this parameter was not mentioned by the manufacturer of the powder in the accompanying pamphlet.

In the second step of the trial, we tested the ability of CASA to detect and identify *S. aureus* organisms on routine analysis of clinical specimens. The results showed a clear advantage for CASA over conventional media. CASA successfully detected 99% of the organisms directly from the specimen (Table 1), compared to 93.8% for MSA and 91.7% for TSBA. The conventional media also did not always detect the organisms on the primary plating. Gaillot et al. (2000) reported that *S. aureus* is difficult to recover from low inoculum on primary plating on CASA medium, probably because of the set order of inoculation (first blood agar and then CASA). We overcame this problem by a standardization of the inoculation method. All nonliquid specimens were homogenized in sterile saline before their inoculation, and all the samples were inoculated by a quantitative technique. The aim of this treatment was to assure a consistent and equivalent inoculum on all media. The higher sensitivity of CASA relative to conventional media appears to be due to the combination of its complete inhibition of gram-negative bacterial growth and the good visibility of the mauve colonies. This advantage was more noticeable in plurimicrobial samples: CASA recovered 151 of the 153 *S. aureus* isolates from wound specimens on the primary plating. By contrast, 4 of the 151 *S. aureus* isolates from wound specimens on TSBA were recovered only after subculture on selective blood phenylethyl-alcohol agar, because of the overgrowth of other bacteria on the primary plate. This lower sensitivity of TSBA for plurimicrobial samples was especially notable in the specimens from patients with cystic fibrosis, which were associated with abundant gram-negative flora, such as swarming strains of *Pseudomonas* spp. and *Proteus* spp. The high prevalence of *S. aureus*, *Pseudomonas* spp., and other bacteria in sputum of cystic fibrosis patients has been reported previously (Burns et al., 1998).

TSBA was unable to grow 14 of the 41 *S. aureus* isolates from sputum in this study, whereas CASA was able to recover all of them (100%). However, 8% of the positive results were detected only after 48 hours' incubation. This longer incubation period may explain the difference between our results and those of Gaillot et al. (2000).

Susceptibility tests to antimicrobial agent results from the isolates picked from CASA showed full agreement with those from microorganisms picked from reference media.

Our results showed that CASA allows for the detection of *S. aureus* isolates that may go undetected on conventional media, and this difference was notable on plurimicrobial samples. The selectivity of the medium and simplicity of colony recognition increased the detection rate and the specificity. This method is also cost-effective, because it eliminates the need for numerous catalase and latex agglutination tests for non–*S. aureus* strains grown on conventional media. We conclude that CASA is a reliable medium for the rapid detection of *S. aureus*. We recommend its use as a primary plating method for specimens that are likely to be both plurimicrobial and contain *S. aureus* as a significant pathogen, such as wounds or respiratory specimens from cystic fibrosis patients.

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