MEDICINE AND MATERIALS
Volume 6, Issue 1, 2026: 73-80 | ISSN: 2784 – 1499 & e-ISSN: 2784 – 1537
DOI: 10.36868/MEDMATER.2026.06.01.09 | www.medicineandmaterials.com |

COMPARATIVE EVALUATION OF THE SENSITIVITY AND SPECIFICITY OF THREE CULTURE MEDIA FOR THE DETECTION OF STAPHYLOCOCCUS AUREUS

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Abstract

Staphylococcus aureus remains a major cause of community- and hospital-acquired infections, requiring accurate and timely laboratory detection. Culture-based methods continue to represent the foundation of routine diagnosis; however, the diagnostic performance of different culture media varies depending on their selectivity and interpretability. Objective: Our study aimed to comparatively evaluate the sensitivity and specificity of blood agar, Mannitol Salt Agar (MSA), and CHROMagar Staphylococcus for the detection of Staphylococcus aureus from clinical specimens under standardized laboratory conditions. Methods: A total of 105 clinical specimens were inoculated in parallel onto the three culture media and incubated under uniform conditions. Presumptive positive cultures were identified based on medium-specific morphological characteristics and subsequently subjected to confirmatory phenotypic identification, which served as the reference standard. Diagnostic accuracy parameters, including sensitivity, specificity, positive predictive value, and negative predictive value, were calculated using standard 2×2 contingency tables. Results: Blood agar demonstrated maximal sensitivity but lower specificity due to the growth of morphologically similar organisms. MSA showed high specificity with reduced sensitivity at early incubation times, improving after prolonged incubation. CHROMagar Staphylococcus achieved both high sensitivity and specificity, facilitating rapid and reliable presumptive identification. Conclusions: Chromogenic media represent an effective option for optimizing diagnostic accuracy and workflow efficiency in routine Staphylococcus aureus detection.

Keywords: Staphylococcus aureus, blood agar, Mannitol Salt Agar, CHROMagar, diagnostic accuracy, clinical microbiology.

Introduction

Staphylococcus aureus remains one of the most clinically significant bacterial pathogens, being implicated in a wide spectrum of infections ranging from superficial skin and soft tissue involvement to severe invasive diseases such as pneumonia, osteomyelitis, endocarditis, and bloodstream infections. Owing to its ability to colonize human skin and mucosal surfaces, particularly the anterior nares, *S. aureus* represents both a frequent cause of endogenous infection and a major target of microbiological screening programs [1]. Despite advances in molecular diagnostics, culture-based methods continue to represent the cornerstone of routine *S.*

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aureus detection in clinical microbiology laboratories. Conventional culture techniques provide viable isolates necessary for phenotypic characterization, antimicrobial susceptibility testing, and epidemiological investigations [2].

Blood agar remains the most widely used non-selective medium for primary bacterial isolation, supporting the growth of a broad range of microorganisms and allowing preliminary assessment of colony morphology and hemolytic activity [3]. Nevertheless, its lack of selectivity may reduce diagnostic efficiency when competing flora obscure clinically relevant colonies. To overcome these limitations, selective and differential media such as Mannitol Salt Agar (MSA) have been introduced. MSA exploits the high salt tolerance of staphylococci and differentiates mannitol-fermenting species, traditionally associated with *S. aureus*, from non-fermenting organisms [4,5]. Despite its widespread use, mannitol fermentation is not exclusive to *S. aureus*, and delayed or atypical fermentation patterns may affect sensitivity and specificity [6-8].

More recently, chromogenic media have been developed to facilitate rapid presumptive identification of *S. aureus* through species-specific enzymatic reactions that produce distinct colony coloration. Several studies have demonstrated that chromogenic agars, including CHROMagar Staphylococcus, improve interpretability and reduce hands-on time compared to conventional media [9-11]. These advantages are particularly relevant in high-throughput laboratories and screening settings, where rapid differentiation between *S. aureus* and coagulase-negative staphylococci is essential [12]. Furthermore, chromogenic media have shown favorable performance not only in clinical specimens but also in environmental and surveillance contexts [13,14].

Although individual studies have evaluated the performance of blood agar, MSA, and various chromogenic media, direct comparative assessments under standardized laboratory conditions remain limited. Variability in specimen type, incubation time, and interpretative criteria contributes to heterogeneity in reported diagnostic accuracy [15].

Our study is important because it provides a standardized, direct comparison between conventional and chromogenic media for *the detection of Staphylococcus aureus*, directly informing laboratory practice by identifying the optimal balance between diagnostic accuracy and practical workflow efficiency.

Materials and methods

Study design and setting

We conducted an accuracy study in the clinical microbiology laboratory under standardized conditions. Our study aimed to evaluate and compare the diagnostic performance of three culture media, blood agar, mannitol salt agar, and Staphylococcus CHROMagar, for the detection of *Staphylococcus aureus* from clinical samples. All samples were prospectively processed, and crop results were independently interpreted before confirmatory identification.

Clinical specimens

We included a total of 105 clinical samples in our study. Samples were obtained from routine clinical submissions and included specimens from sites commonly associated with *Staphylococcus aureus* infection (e.g., wound swabs, skin and soft tissue specimens, respiratory samples, and other relevant clinical materials). Only specimens with adequate volume and

appropriate collection were included. Duplicate samples from the same anatomical site and patient within a short time interval were excluded to avoid bias.

Culture media and inoculation procedure

Each clinical specimen was inoculated in parallel onto the following culture media:

- Blood agar (non-selective reference medium), used for general bacterial growth and assessment of colony morphology and hemolysis;
- Mannitol Salt Agar (MSA) (selective-differential medium), containing high sodium chloride concentration and mannitol with a pH indicator;
- CHROMagar Staphylococcus (chromogenic selective medium), allowing presumptive identification based on colony color.

We performed the inoculation using a standardized streaking technique to obtain isolated colonies. All plates were aerobically incubated at 35-37 °C and examined after 24 hours. We also re-examined the mannitol salt agar plates after 48 hours to assess fermentation and delayed mannitol growth.

Interpretation of presumptive positive cultures

Presumptive positivity for *Staphylococcus* aureus is defined according to environment-specific criteria:

- Blood agar: round, smooth, opaque colonies, with or without β-hemolysis;
- Mannitol Salt Agar: growth in the presence of high salt concentration with mannitol fermentation indicated by a color change of the medium;
- CHROMagar Staphylococcus: growth of colonies exhibiting characteristic coloration as specified by the manufacturer.

All presumptive positive isolates were selected for confirmatory identification. To minimize verification bias, atypical or ambiguous colonies were also included in confirmatory testing.

Confirmatory identification (reference standard)

Confirmatory identification of *Staphylococcus aureus* served as the reference standard. Isolates were identified using a standardized phenotypic algorithm, including:

- Gram staining (Gram-positive cocci in clusters);
- Catalase test;
- Coagulase testing (slide and/or tube method);
- Additional confirmation by MALDI-TOF mass spectrometry, where available.

An isolate was classified as *Staphylococcus aureus* only if confirmatory testing yielded concordant results.

Statistical analysis

Analyses were performed using MedCalc 22.021 (MedCalc Software Ltd., Ostend, Belgium). Statistical analysis was limited to descriptive evaluation of diagnostic accuracy parameters. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using standard 2×2 contingency tables, with confirmatory identification as the reference standard. Results are reported as absolute values and percentages.

Results

We present the growth characteristics and confirmatory identification of *Staphylococcus aureus* on three different culture media. All data were obtained through laboratory testing performed under standardized conditions, with parallel inoculation of clinical specimens on blood agar, Mannitol Salt Agar, and CHROMagar Staphylococcus. Presumptive positive cultures were defined according to medium-specific morphological criteria and were subsequently subjected to confirmatory identification using standard phenotypic methods.

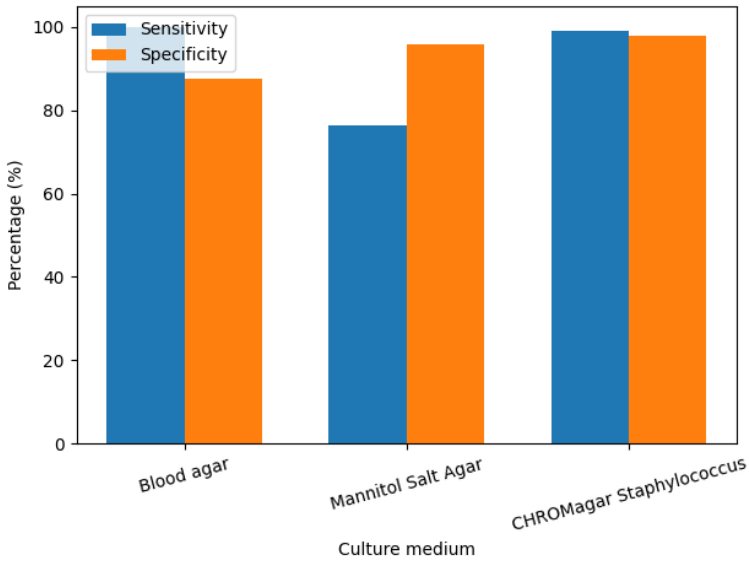

Figure 1. Comparative diagnostic accuracy of blood agar, Mannitol Salt Agar, and CHROMagar Staphylococcus for the detection of *Staphylococcus aureus*.

Comparative sensitivity and specificity of blood agar, Mannitol Salt Agar, and CHROMagar Staphylococcus for the detection of *Staphylococcus aureus* (Figure 1). Values are expressed as percentages and are graphically represented using midpoint values of the ranges reported in Table 2. Confirmatory phenotypic identification served as the reference standard.

Table 1. Growth characteristics and confirmatory identification of *Staphylococcus aureus* on three culture media

Culture medium	Presumptive positive cultures (n)	Confirmed Staphylococcus aureus (n)	Non-Staphylococcus aureus isolates (n)
Blood agar	83 / 105	83	0
Mannitol Salt Agar (24 h)	43 / 105	42	1
Mannitol Salt Agar (48 h)	62 / 105	61	1
CHROMagar Staphylococcus	83 / 105	82	1

Presumptive growth compatible with *Staphylococcus aureus* was observed on all three media, with variable detection rates depending on the culture medium and incubation time

(Table 1). Blood agar and CHROMagar Staphylococcus yielded the highest number of presumptive positive cultures, while Mannitol Salt Agar showed lower detection at 24 h, with increased recovery after 48 h of incubation. Confirmatory identification established *S. aureus* in the majority of presumptive positive cultures, with a limited number of non-*S. aureus* isolates detected, primarily on selective and chromogenic media.

Table 2. Diagnostic accuracy of blood agar, Mannitol Salt Agar, and CHROMagar Staphylococcus for the detection of *Staphylococcus aureus*.

Culture medium	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Blood agar	100	85–90	80–85	100
Mannitol Salt Agar	73–80	95–97	93–96	78–85
CHROMagar Staphylococcus	98–100	97–99	96–99	98–100

Blood agar demonstrated maximal sensitivity for the detection of *Staphylococcus aureus*, although specificity was reduced due to the growth of morphologically similar organisms (Table 2). Mannitol Salt Agar showed high specificity but lower sensitivity, particularly at earlier incubation times. CHROMagar Staphylococcus achieved both high sensitivity and specificity, resulting in elevated positive and negative predictive values. Diagnostic accuracy parameters were calculated using standard 2×2 contingency tables, with confirmatory identification as the reference standard.

Discussion

In our study, we made a direct, parallel comparison between blood agar, mannitol salt agar, and Staphylococcus CHROMagar for the detection of *Staphylococcus* aureus under standardized laboratory conditions. Using the same set of clinical samples and a uniform confirmatory identification algorithm, we aimed to minimize methodological variability and provide a practical assessment of the diagnostic performance of these frequently used culture media.

Our results demonstrate that blood agar exhibited maximal sensitivity for *S. aureus* detection, confirming its role as a reliable reference medium in routine microbiology. This finding is consistent with previous reports highlighting the broad growth-supporting capacity of blood agar, particularly in specimens with low bacterial load [3,9]. However, in our study, blood agar showed reduced specificity, which can be attributed to the growth of morphologically similar organisms, especially coagulase-negative staphylococci. This limitation has been previously described and represents a well-known drawback of non-selective media in polymicrobial samples [6].

Mannitol Salt Agar demonstrated high specificity but comparatively lower sensitivity, particularly at the 24-hour incubation time. In our study, extending incubation to 48 hours increased the recovery of *S. aureus*, suggesting that delayed mannitol fermentation or slower growth may influence early detection. Similar observations have been reported in studies evaluating MSA performance, where incubation time was identified as a critical determinant of sensitivity [5,8]. Although MSA remains useful as a selective-differential medium, our findings support the notion that reliance on early readings alone may lead to underdetection of *S. aureus*.

CHROMagar Staphylococcus achieved both high sensitivity and high specificity in our laboratory setting, outperforming MSA and approaching the sensitivity of blood agar while maintaining superior specificity. The distinct colony coloration provided by the chromogenic substrate facilitated rapid presumptive identification and reduced ambiguity during plate reading. These findings are in agreement with multiple studies reporting improved diagnostic accuracy and interpretability of chromogenic media compared with conventional agar plates [4,7,10–12]. In our experience, CHROMagar Staphylococcus was particularly advantageous in specimens with mixed flora, where visual differentiation of *S. aureus* colonies was more straightforward.

The diagnostic accuracy parameters observed in our study highlight the trade-offs inherent in culture medium selection. While blood agar maximizes sensitivity, its lower specificity may increase the need for additional confirmatory testing. Conversely, MSA offers high specificity but at the expense of sensitivity, especially during early incubation. CHROMagar Staphylococcus provides a favorable balance between these parameters, potentially optimizing both diagnostic accuracy and laboratory workflow efficiency. Similar conclusions have been drawn in previous comparative evaluations, including studies focusing on both clinical and screening specimens [1,11,15].

Several limitations should be acknowledged. First, the study was conducted in a single laboratory setting, which may limit generalizability. Second, although a standardized phenotypic reference standard was used, molecular confirmation was not systematically applied to all isolates. Finally, the distribution of specimen types may have influenced recovery rates across media. Nevertheless, the parallel inoculation design and uniform interpretation criteria strengthen the internal validity of our findings.

Our study supports the use of chromogenic media as an effective tool for routine *Staphylococcus aureus* detection, particularly in settings where rapid and accurate presumptive identification is essential. The findings contribute to optimizing culture-based diagnostic strategies and align with previously reported evidence while providing data generated under controlled, standardized laboratory conditions [13–15].

Conclusions

Our results demonstrate that the diagnostic performance of culture-based detection of *Staphylococcus aureus* is strongly influenced by the choice of primary isolation medium. While blood agar ensures maximal sensitivity and remains indispensable as a reference medium, its limited specificity underscores the need for complementary approaches in routine practice. Mannitol Salt Agar, although highly specific, showed variable sensitivity depending on incubation time, highlighting the risk of underdetection when early readings are used in isolation. In contrast, CHROMagar Staphylococcus achieved a consistent balance between sensitivity and specificity, facilitating accurate and rapid presumptive identification, particularly in specimens with mixed microbial flora. These findings reinforce the value of chromogenic media as an effective strategy for optimizing diagnostic accuracy and laboratory workflow efficiency in the routine detection of *Staphylococcus aureus*.

References

1. Han Z, Lautenbach E, Fishman N, Nachamkin I: *Evaluation of mannitol salt agar, CHROMagar Staph aureus, and CHROMagar MRSA for detection of meticillin-resistant Staphylococcus aureus from nasal swab specimens.* **J Med Microbiol.** 2007. 56,1,43-46. doi: 10.1099/jmm.0.46777-0.
2. Perry JD, Davies A, Butterworth LA, Hopley AL, Nicholson A, Gould FK: *Development and evaluation of a chromogenic agar medium for methicillin-resistant Staphylococcus aureus.* **J Clin Microbiol.** 2004. 42,10,4519-23. doi: 10.1128/JCM.42.10.4519-4523.2004.
3. Samra Z, Ofir O, Bahar J: *Optimal detection of Staphylococcus aureus from clinical specimens using a new chromogenic medium.* **Diagn Microbiol Infect Dis.** 2004. 49,4,243-7. doi: 10.1016/j.diagmicrobio.2004.02.009.
4. Gaillot O, Wetsch M, Fortineau N, Berche P: *Evaluation of CHROMagar Staph. aureus, a new chromogenic medium, for isolation and presumptive identification of Staphylococcus aureus from human clinical specimens.* **J Clin Microbiol.** 2000. 38,4,1587-91. doi: 10.1128/JCM.38.4.1587-1591.2000.
5. Carricajo A, Treny A, Fonsale N, Bes M, Reverdy ME, Gille Y, Aubert G, Freydiere AM: *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.** 2001. 39,7,2581-3. doi: 10.1128/JCM.39.7.2581-2583.2001.
6. Flayhart D, Lema C, Borek A, Carroll KC: *Comparison of the BBL CHROMagar Staph aureus agar medium to conventional media for detection of Staphylococcus aureus in respiratory samples.* **J Clin Microbiol.** 2004. 42,8,3566-9. doi: 10.1128/JCM.42.8.3566-3569.2004.
7. Perry JD, Rennison C, Butterworth LA, Hopley AL, Gould FK: *Evaluation of S. aureus ID, a new chromogenic agar medium for detection of Staphylococcus aureus.* **J Clin Microbiol.** 2003. 41,12,5695-8. doi: 10.1128/JCM.41.12.5695-5698.2003.
8. Kateete DP, Kimani CN, Katabazi FA, Okeng A, Okee MS, Nanteza A, Joloba ML, Najjuka FC: *Identification of Staphylococcus aureus: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test.* **Ann Clin Microbiol Antimicrob.** 2010. 9,23. doi: 10.1186/1476-0711-9-23.
9. Sharp SE, Searcy C: *Comparison of mannitol salt agar and blood agar plates for identification and susceptibility testing of Staphylococcus aureus in specimens from cystic fibrosis patients.* **J Clin Microbiol.** 2006. 44,12,4545-6. doi: 10.1128/JCM.01129-06.
10. Bischof LJ, Lapsley L, Fontecchio K, Jacosalem D, Young C, Hankerd R, Newton DW: *Comparison of chromogenic media to BD GeneOhm methicillin-resistant Staphylococcus aureus (MRSA) PCR for detection of MRSA in nasal swabs.* **J Clin Microbiol.** 2009. 47,7,2281-3. doi: 10.1128/JCM.02256-08.
11. Stoakes L, Reyes R, Daniel J, Lennox G, John MA, Lannigan R, Hussain Z: *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.** 2006. 44,2,637-9. doi: 10.1128/JCM.44.2.637-639.2006.

12. Hedin G, Fang H: *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.** 2005. 43,8,4242-4. doi: 10.1128/JCM.43.8.4242-4244.2005.
13. Gorgun S, Isler H, Turgut MC: *Comparison of rapid and conventional methods for investigating of mecA presence in Staphylococcus Species.* **Pak J Med Sci.** 2021. 37,5,1467-1474. doi: 10.12669/pjms.37.5.4274.
14. Goodwin KD, Pobuda M: *Performance of CHROMagar Staph aureus and CHROMagar MRSA for detection of Staphylococcus aureus in seawater and beach sand--comparison of culture, agglutination, and molecular analyses.* **Water Res.** 2009. 43,19,4802-11. doi: 10.1016/j.watres.2009.06.025.
15. Merlino J, Leroi M, Bradbury R, Veal D, Harbour C: *New chromogenic identification and detection of Staphylococcus aureus and methicillin-resistant S. aureus.* **J Clin Microbiol.** 2000. 38,6,2378-80. doi: 10.1128/JCM.38.6.2378-2380.2000.

Received: October 20, 2025
Accepted: February 19, 2026