Chromagar Staph aureus Versus Blood Agar and Mannitol Salt Agar for Isolation and Identification of Staphylococcus aureus from Suppurative Skin Lesions

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Chromagar Staph aureus (CHROMagar Company, Paris, France) is a chromogenic agar medium proposed for the detection of Staphylococcus aureus by incorporation of a chromatic substrate into a suitable isolation medium and then detection of the activities of a specific bacterial enzymes by color changes thus eliminating the need for time consuming and costly biochemical identification. To evaluate this medium, a total of 105 suppurative skin lesion swabs were cultured onto CHROMagar Staph aureus agar, blood agar (gold standard) and mannitol salt agar. After 24 h of incubation a total of 83 S. aureus strains were recovered on blood agar. Chromagar Staph aureus succeeded to isolate 82 strains with mauve color in the first 24 h with a sensitivity of 98.79 % and a specificity of 95.45 %, with no further isolation after extending the incubation time to 48 h. As regards mannitol salt agar, 43 (51.8%) strains were recovered as yellow colonies after 24 h of incubation compared with 61 (73.4%) strains after incubation for 48 h. These results were associated with a sensitivity of 50.60% after 24 reaching up to 73.49% after 48 h. We conclude that CHROMagar Staph aureus compared favorably to conventional media for rapid detection of S. aureus in clinical samples and achieved a higher sensitivity and specificity than mannitol salt agar for the isolation and presumptive identification of S. aureus from suppurative skin lesions.

Key words:
Chromagar Staph aureus, Blood Agar, Mannitol Salt Agar, Staphylococcus aureus

INTRODUCTION
Staphylococcus aureus (S. aureus) is an important and frequent cause of of skin and soft tissue infection and is consequently one of the most common pathogens sought in clinical microbiology laboratories. Reliable and rapid methods to identify these organisms are crucial in any clinical laboratory.

Diagnosis of S. aureus skin infection is generally performed by culture of swabs onto nonspecific media and confirmation of suspected colonies by biochemical and/or serological tests. Most commonly, this involves testing colonies of staphylococci for the coagulation of plasma, the fermentation of mannitol (mannitol salt agar [MSA]), the production of thermostable nuclease (DNase), egg yolk lipase hydrolysis (lipovitellin-salt-mannitol agar [LSM]), and the production of natural pigment.

Colonies of S. aureus may be atypical and difficult to differentiate from coagulase-negative staphylococci, a large number of agglutination tests may be required to rule out the presence of S. aureus. A number of culture media have been developed to increase the specificity of S. aureus detection, including mannitol-salt agar and Baird-Parker medium.

A more recent approach has been the use of CHROMagar Staph aureus, which employs chromogenic enzyme substrates in a selective agar medium, allowing the detection of S. aureus with a high degree of sensitivity and specificity.

The present study examines the use of a chromogenic plate medium, CHROMagar Staph aureus (CAS), and Mannitol Salt Agar (MSA) versus the gold standard Blood agar (BA) for the isolation and identification of S. aureus from pyoderma (suppurative skin lesions). The criteria for medium evaluation included colony growth reaction, color reproducibility for the identification of S. aureus.

MATERIAL AND METHODS
Culture media.
CHROMagar Staph aureus was provided by the CHROMagar Company, Paris, France. The medium contained agar (15 g/liter), peptones (40 g/liter), NaCl (25 g/liter), and a proprietary chromogenic mix (3.5 g/liter). The medium was prepared as instructed by the manufacturer by avoiding heating at over 100°C. Columbia blood agar
base (Acumedia, Baltimore, Md.) supplemented with 5% human blood (blood agar) and mannitol-salt agar (Oxoid-CM85), were prepared according to the manufacturer's instructions.

Clinical samples:
Suppurative skin lesions of a total of 105 pyoderma patients admitted to the outpatient clinic of the Main University Hospital, Alexandria, Egypt, were included in this study. Swabs were used to collect suppurative exudates from skin lesions, then immediately delivered to the lab for processing. Each swab was inoculated and spread onto each of the three types of media (BA, MSA and CAS). All plates were aerobically incubated at 37°C, BA for 24 h\(^{(10)}\), CAS for 48 h (according to manufacturer instructions) and MSA for 48 h.\(^{(10)}\)

Identification of \textit{S. aureus}.
Golden yellow hemolytic or non hemolytic colonies on blood agar, yellow mannitol fermenting colonies on MSA and mauve colonies on CAS were regarded as presumptive \textit{S. aureus} and were confirmed with Gram stain reactions, reactions to catalase (3% [wt/vol] hydrogen peroxide, reactions to slide and tube coagulase (rabbit plasma), and DNase activity were used for identification.\(^{(10)}\)

\section*{RESULTS}
Eighty three confirmed strains of \textit{S. aureus} were isolated (from 105 swabs taken from suppurative skin lesions) on one or more agar culture media within 48 h of incubation.
A total of 83 strains were isolated on BA after 24 h of incubation.
On CSA, 83 strains were recovered as mauve colonies after 24 h and no further growth was detected after incubation for 48 h. One of these mauve colony was not proved to be \textit{S. aureus} (false positive). These results were associated with sensitivity of 98.79 % and a specificity of 95.45 %. (Table 1)
As regards MSA, 43 strains were recovered as yellow colonies after 24 h of incubation compared with 62 strains after incubation for 48 h. One yellow colony was not proved to be \textit{S. aureus} (false positive). These results were associated with a sensitivity of 50.60% and 73.49 % after 24 and 48 h. respectively. (Table 2, 3)

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
 & Blood agar & Chromagar \\
\hline
 & positive & negative & total \\
\hline
positive & 82 & 1 & 83 \\
\hline
negative & 1 & 21 & 22 \\
\hline
total & 83 & 22 & 105 \\
\hline
\end{tabular}
\caption{Chromagar Staph aureus versus blood agar for detection of \textit{S. aureus} after 24 incubation hours.}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
 & MSA & \\
\hline
 & positive & negative & total \\
\hline
positive & 42 & 41 & 83 \\
\hline
negative & 1 & 21 & 22 \\
\hline
total & 43 & 62 & 105 \\
\hline
\end{tabular}
\caption{Mannitol salt agar versus blood agar for detection of \textit{S. aureus} after 24 incubation hours.}
\end{table}

\* After extending incubation to 48 hours mauve color of colonies start to fade away in most of the plates
Sensitivity = \( \frac{T+ve}{T+ve+F-ve} = \frac{82}{82+1} = 98.79\% \)
Specificity = \( \frac{T-ve}{T-ve+F+ve} = \frac{21}{21+1} = 95.45\% \)
Table 3: Mannitol salt agar versus blood agar for detection of *S. aureus* after 48 incubation hours.

<table>
<thead>
<tr>
<th></th>
<th>Blood agar</th>
<th>MSA</th>
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<tr>
<td></td>
<td>positive</td>
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<tr>
<td>positive</td>
<td>61</td>
<td>22</td>
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<td>negative</td>
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<tr>
<td>total</td>
<td>62</td>
<td>43</td>
<td>105</td>
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Sensitivity = 61/61+22 = 73.49%
Specificity = 21/21+1 = 95.45%

**DISCUSSION**

Isolation of *S. aureus* is usually accomplished with the use of conventional media such as blood agar. The disadvantage of such media is the need of confirmatory tests to differentiate *S. aureus* from colonies with identical colony appearance or when swarming colonies of *Proteus* cover those of *S. aureus* on such ordinary media.\(^\text{(2)}\)

Performing such tests on all colonies resembling staphylococci can be time-consuming and labor intensive.\(^\text{(8,11)}\)

The use of chromogenic media can potentially reduce the number of confirmatory tests that are necessary for the detection of *S. aureus*.\(^\text{(4,8)}\)

The ideal characteristics of any candidate chromogenic medium are the detection of *S. aureus* with high specificity and an isolation rate at least comparable to conventional media after 18 to 20 h of incubation.\(^\text{(4,8)}\)

In this study, CAS was highly sensitive (98.79%) for *S. aureus*, where it succeeded to detect 82 out of the total 83 *S. aureus* strains after 24 h incubation. The same sensitivity (98.5%) was obtained by Carricajo et al.,\(^\text{(11)}\) 2001; and a near results were obtained by Gaillot et al.,\(^\text{(9)}\) 95.5%, 2000, Perry et al.,\(^\text{(4)}\) 96.2% , 2003, and D’Souza et al.,\(^\text{(12)}\) 69%, 2005. Slightly higher sensitivity (99.5%) was obtained by Flyhart et al.,\(^\text{(13)}\) 2004.

In the present study, CAS had a low sensitivity of 73.49%, in contrast to the reports of other authors who reported a sensitivity reaching up to 96% and 97.1% respectively.\(^\text{(12,14)}\)

The low sensitivity of MSA in this study was due to the high false negative rate which we could not find an explanation to, except that these strains might be inhibited by the high salt component of this medium.\(^\text{(16)}\)

MSA showed a sensitivity of 50.60% after 24 h incubation which markedly increased to 73.49% after further 24 h. This seems to be consistent with the findings of D’Souza and Baron\(^\text{(12)}\) who mentioned that the sensitivity of MSA increased from 71% after 24 h to 96% after extending the incubation to another 24 h.

In conclusion, CSA compared favorably to conventional media for rapid detection of *S. aureus* in clinical samples and achieved a higher sensitivity and specificity than MSA for the isolation and presumptive identification of *S. aureus* from suppurative skin lesions.

The use of CSA is much less labor intensive than conventional methods and requires fewer reagents for confirmation of suspect colonies of *S. aureus*.\(^\text{(12,17)}\)

Sometimes, strains which were considered positive after 24 h were negative after 48 h. This may be due to variability in
the interpretation of the observer or to a true discoloration after prolonged incubation. Whatever the cause may be, we recommend reading CSA plates after 24 h, as this gives a more accurate result.\(^{(13)}\)

The cost of CSA is higher than that of nonselective conventional media. However, there is some efficiency gained with the use of this medium. The more rapid visualization of the specific mauve pigmentation of \textit{S. aureus} allows working through cultures more quickly; 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. Moreover, there is no need to subculture organisms for antimicrobial susceptibility testing.\(^{(13)}\)

So we recommend the use of CSA agar medium for the isolation and the specific identification of \textit{S. aureus} from pyodermal suppurative skin lesions.

**REFERENCES**


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