Journal of Microbiological Methods 161 (2019) 71–73

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Note

Chromagar™ requires secondary confirmation strategies to minimize false positive/negative results for detection of *Staphylococcus aureus*

Sirisha Sirobhushanam[a], Navya Parsa[b], Tamra J. Reed[a], J. Michelle Kahlenberg[a,*]

[a] Department of Internal Medicine, Division of Rheumatology, University of Michigan, Ann Arbor, MI 48109, USA
[b] University of Toledo, Toledo, OH, USA

ARTICLE INFO

Keywords:
Chromagar
Colonization
Staphylococcus aureus

ABSTRACT

Chromagar is a medium that is used in culture-based colonization studies of *Staphylococcus aureus*. We have found that *S. aureus* negative colonies often fit the color recommendations for *S. aureus* identification and can cause overestimation of colonization rates. Confirmation of suspect colonies is important to minimize false negative/positive results.

Staphylococcus aureus is a pathobiont that colonizes ~30% of the healthy adult population and is the leading cause of skin and soft tissue infections in the world (Balasubramanian et al., 2017; Wertheim et al., 2005). Skewing of the skin microbiome to promote colonization by *S. aureus* is associated with cutaneous disease severity for diseases such as atopic dermatitis (Meylan et al., 2017; Williams et al., 2017). This has resulted in resurgence in *S. aureus* colonization studies (Conti et al., 2016; Hajialilo et al., 2015; Ng et al., 2017; Williams and Gallo, 2017; Wollina, 2017). These investigations often require isolation of the bacterium from survey populations performed by individuals who lack in-depth knowledge of *S. aureus* and its growth characteristics. Thus, methodology that is quick, inexpensive for large numbers of samples, and reliable in results is required for these studies.

Growth of bacteria on blood agar is effective but does not allow for selection of staphylococci. Growth of bacteria in Mannitol Salt Agar (MSA) selects for staphylococci (high salt media) and differentiates *S. aureus* (mannitol fermentation). Unfortunately, false positive results derived from coagulase negative *staphylococci* cause overestimation of colonization rates (Shittu et al., 2006). Chromagar™ is recognized as an effective medium for identifying *S. aureus* in comparison to MSA by various studies (Carricajo et al., 2002; Flayhart et al., 2004; Gaillot et al., 2006; Han et al., 2007; Luteijn et al., 2011).

We employed rigorous methodology to survey our patient population for *S. aureus* colonization. Patients from the University of Michigan Rheumatology clinic ($n = 51$) were consented according to IRBMED #HUM00116841 and swabbed in the nares and on various areas of their skin using sterile FLOQSwabs (Copan Diagnostics, Murrieta, CA) wetted with sterile PBS. Swabs were placed in PBS and vortexed, 100 µl was spread on plates (Chromagar) and incubated at 35 ± 2 °C for 24 h. Mauve colored colonies were restreaked onto MSA plates and incubated for 16–20 h at 37 °C and observed for mannitol fermentation. All colonies were restreaked on Chromagar to present clear photographic evidence (Figs. 1 and 2). This was followed by colony PCR for *S. aureus* specific region in the thermonuclease gene using the primers SaNuc forward 5′-GCGATTGATGGTGATACGGTT -3′ and reverse 5′-AGCCAA GCCTTGACGAACTAAAGC -3′ as a gold standard to confirm the presence of *S. aureus* (Zhang et al., 2004). Our approach has been summarized as a flowchart in Fig. 1A. Each step of the pathway was accompanied by positive and negative controls using *S. aureus* strain USA300, a community acquired methicillin-resistant *S. aureus*, generously shared by Dr. Alexander R. Horswill, Department of Immunology and Microbiology, University of Colorado, and *Staphylococcus epidermidis* H140 obtained from BEI resources.

S. aureus positive colonies that displayed the expected color on Chromagar are presented in Fig. 1B and *S. aureus* negative colonies that were easily identified are presented in Fig. 1C. However, several colonies did not fit the expected color characteristics. Fig. 2A shows *S. aureus* positive colonies (by PCR) that were atypical in appearance at 24 h. Further incubation resulted in persistence of this appearance in some colonies while others showed intense mauve color at 48 h (Fig. 2B). The most problematic results were those *S. aureus* negative colonies (confirmed by PCR) that exhibited varying shades of the color mauve/pink and were found to be indistinguishable from typical *S. aureus* positive colonies at both 24 and 48 h incubation (Fig. 2C and D).

We also observed that samples that contain high numbers of *S. aureus* were easy to screen and less likely to display false negative

* Corresponding author.
E-mail addresses: sisirobh@med.umich.edu (S. Sirobhushanam), Navya.Parsa@utoledo.edu (N. Parsa), tamrar@med.umich.edu (T.J. Reed), mkahlenb@med.umich.edu (J.M. Kahlenberg).

https://doi.org/10.1016/j.mimet.2019.04.013
Received 8 April 2019; Received in revised form 18 April 2019; Accepted 18 April 2019
Available online 20 April 2019

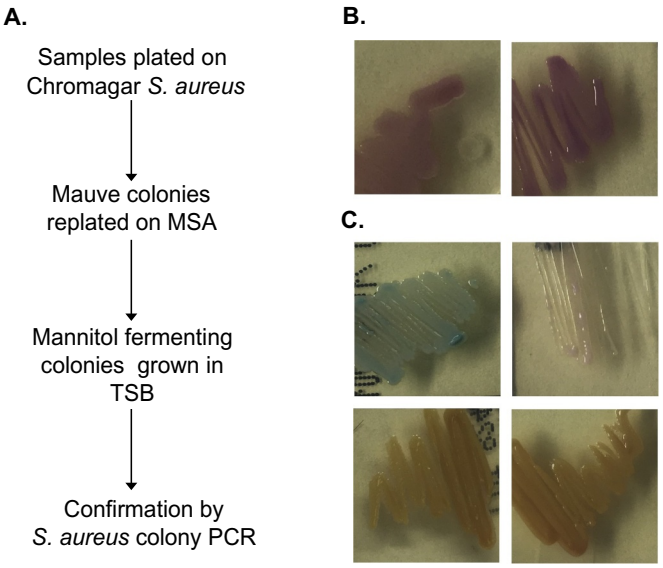

Fig. 1. A. Flowchart for *S. aureus* confirmation methodology. B. Single colonies of confirmed *S. aureus* positive isolates were re-streaked on Chromagar to clearly show color differences. C. Single colonies of *S. aureus* negative (via PCR) isolates were restreaked on Chromagar to clearly show color differences. Photographs were taken at 24 h post inoculation.

results. Presence of other staphylococci including *S. epidermidis* in high numbers made *S. aureus* identification more difficult resulting in higher false negative and false positive results. False positive results were lower in Chromagar (7%) than MSA (11.5%) at 24 h but increase dramatically by 48 h (13%) (Table. 1). This indicates that although chromagar was more specific than MSA at 24 h further incubation results in misidentification. However, false negative results were fairly high for both media types (31% Chromagar and 39% on MSA). This is likely due to *S. aureus* positive colonies that did not show typical appearance and Coagulase negative Staphylococci that ferment mannitol.

In summary, we have identified Chromagar as an excellent media for *S. aureus* identification in line with other groups (Carricajo et al., 2002; Han et al., 2007; Luteijn et al., 2011). However, we also report a 7% false-positive rate and 31% false-negative rate when colonies are judged by color alone. This is in line with published work (both report ~20% false-negative results) (D'Souza and Baron, 2005; Luteijn et al., 2011). In this work we also pursued colonies that had typical *S. aureus* appearance on the media, although not the required color, with colony PCR and identified several that were in fact *S. aureus*. We recommend that colonies exhibiting other features of *S. aureus* colony morphology such as yellow color and circular, pinhead shape (1–2 mm) with a smooth margin, that appear on the agar ought to be pursued by confirmatory methods (Ribeiro de Souza da Cunha, 2017). Confirmation could involve plating on MSA to ensure growth in high salt media that is typical of staphylococci followed by PCR confirmation.

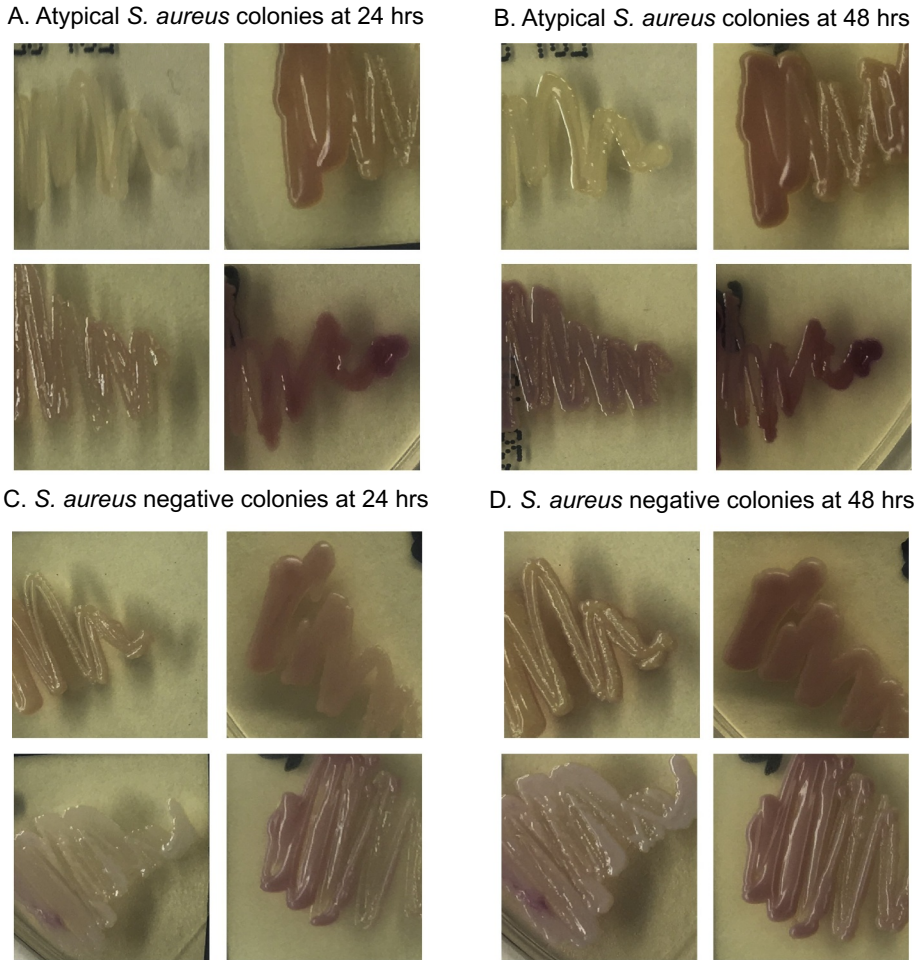

Fig. 2. A. Single colonies of confirmed *S. aureus* positive isolates that were atypical on Chromagar were restreaked to show color differences at 24 h. B. Single colonies of confirmed *S. aureus* positive isolates that were atypical on Chromagar were restreaked to show color differences at 48 h. C. *S. aureus* negative colonies that were atypical on Chromagar were restreaked to show color differences at 24 h. D. *S. aureus* negative colonies that were atypical on Chromagar were restreaked to show color differences at 48 h.

Table 1
Comparison of Chromagar, MSA and *S. aureus nuc* Colony PCR for detection.

Selection on MSA			False positive	false negative
MSA (+) 26	Chromagar (+) 48 h 23	PCR (+) 23	11.5%	
MSA (−) 28	Chromagar (+) 48 h 8	PCR (+) 11		39.3%

Selection on Chromagar			False positive	False negative
Chromagar (+) 24 h 28	MSA (+) 23	PCR (+) 26	7.1%	
48 h 31	23	27	12.9%	
Chromagar (−) 24 h 26	MSA (+) 3	PCR (+) 8		30.8%
48 h 23	3	7		30.4%

Total number of samples tested 54.

Secondary confirmation strategies proved particularly useful in our study since our initial screen yielded very high positive rates that were later corrected due to our rigorous methodology. A brief search in Pubmed with the term "Chromagar *Staphylococcus aureus*" yielded ~110 publications demonstrating its widespread use. However, addition of terms associated with confirmation such as tube coagulase, catalase or PCR resulted in significantly lower number of results indicating that secondary confirmation was not widely employed. Thus, Chromagar, while useful in screening large cohorts for *S. aureus*, should not be considered a confirmatory test for *S. aureus* and other methods such as PCR must be employed to yield accurate results.

Conflict of interest

J.M.K. serves on advisory boards for AstraZeneca and Eli Lilly. The other authors have no financial conflicts of interest.

Acknowledgements

We appreciate the generosity of the patients from the Michigan Rheumatology Clinics for sharing their samples. This work was funded by the Physician Scientist Development award from the Doris Duke Foundation (PI: JMK) and by the National Institute of Arthritis and Musculoskeletal Diseases via R01 AR071384 (PI: JMK).

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