Identification of *Staphylococcus aureus* from enriched nasal swabs within 24 h is improved with use of multiple culture media

Nasal carriage of *Staphylococcus aureus* is commonly evaluated via culture-based methods. We found that parallel use of two media, Baird-Parker and CHROMagar™ Staph aureus, increased detection of *S. aureus* from a healthy population by 29%. We suggest use of both media for optimal identification of *S. aureus* from healthy cohorts.

*S. aureus* is an opportunistic pathogen found in the nares of approximately 25–30% of the human population, as well as on the skin, hair, and perineum (Gorwitz et al., 2008). *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA), is capable of causing a wide range of infections. Nasal carriage of *S. aureus* and MRSA is commonly evaluated for active surveillance of hospital and community populations. Healthy cohorts are evaluated to determine risk factors for carriage, to identify populations prone to colonization, and to assess the dissemination of specific resistance patterns and sequence types (Creech et al., 2005; Gorwitz et al., 2008; Klyutmans et al., 1997; Melles et al., 2004; Shopsin et al., 2000; Tenover et al., 2008). Culture-based methods are typically the cheapest and most reliable option for detection, and enable subsequent phenotypic and molecular characterization of isolates. However, many culture-based studies use only one type of media to detect *S. aureus* from enriched nasal swabs. Few studies have evaluated whether use of single versus multiple media can impact the sensitivity of detection of *S. aureus* from colonized individuals, possibly leading to underreporting of prevalence.

Recently, we conducted an epidemiological study assessing nasal carriage of *S. aureus* in an occupational cohort of 234 healthy individuals who reported direct or indirect contact with livestock production facilities in North Carolina (Rinsky et al., 2013). Due to a lack of consensus in the literature regarding which commercial media is ideal for isolating *S. aureus*, we conducted a sub-study that aimed to evaluate two differential media, Baird-Parker with Egg Yolk Tellurite Enrichment and BBL™ CHROMagar™ Staph aureus (Beckton, Dickinson and Company), in parallel. Baird-Parker is a conventional medium that has been used for decades for *S. aureus* isolation (Baird-Parker, 1962), while CHROMagar™ Staph aureus is a more recently developed chromogenic medium (Gaillot et al., 2000). Both media have demonstrated high sensitivity and specificity for *S. aureus* in previous studies (Carricajo et al., 2001; Gaillot et al., 2000; Kim & Oh, 2010; Kircher et al., 2002), though Baird-Parker recommends 24–48 h of incubation while CHROMagar™ Staph aureus recommends only 24 h.

Briefly, study personnel obtained nasal swabs from both anterior nares of each participant using a BD BBL (Beckton, Dickson and Company), which was inserted into Stuart’s medium and stored at 4 °C for up to 5 days until arrival at the laboratory. Swabs were then inoculated into 10 ml Mueller–Hinton broth containing 6.5% NaCl (MHB +) to increase sensitivity prior to plating (Safdar et al., 2003), and incubated overnight at 37 °C. A 10 μl loopful of MHB + was streaked onto both types of media to isolate presumptive *S. aureus*. Due to time constraints imposed by our epidemiological study, all plates were incubated at 37 °C for 24 h. Colonies with phenotypic characteristics for *S. aureus* growth on Baird-Parker (black, shiny, with clearing) and CHROMagar™ Staph aureus (mauve, matte, with halo) were streaked to isolation on the same media from which they were isolated, then archived in brain heart infusion broth with 15% (by volume) glycerol added for cryopreservation at −80 °C. *S. aureus* identification was confirmed through catalase testing, tube coagulase testing with rabbit plasma (BD BBL™), PCR detection of a *Staphylococcus*-specific region of the 16S rRNA gene, and PCR detection of the *S. aureus*-specific *nuc* gene (Poulsen et al., 2003). MRSA strain Mu50 was used as a positive control for all confirmatory assays (courtesy of Dr Melissa Miller, University of North Carolina at Chapel Hill). For a randomly selected subset of colonies, the 16S rRNA amplicon was sequenced for additional confirmation.

Side-by-side comparison of Baird-Parker and CHROMagar™ Staph aureus performance was conducted for 210 out of 234 participants from our epidemiological study. Of these, 83 (40%) carried *S. aureus* in their nostrils. The sensitivity and specificity of each medium were calculated using PCR confirmation of both the *nuc* gene and a *Staphylococcus*-specific region of the 16S rRNA gene as the gold standard. The sensitivity and specificity for correctly isolating and identifying *S. aureus* using Baird-Parker media after 24 h of incubation were 80.7% and 96.9%, respectively (Table 1). The sensitivity of Baird-Parker in this study was lower than some previous reports (Kim & Oh, 2010), likely due to reduced incubation times. The sensitivity and specificity for correctly isolating and identifying *S. aureus* using CHROMagar™ Staph aureus after 24 h of incubation were 90.4% and 96.9%, respectively (Table 1). These findings are comparable to previous reports (Carricajo et al., 2001; Gaillot et al., 2000; Kircher et al., 2002). Each medium incorrectly identified four isolates as *S. aureus* (Table 1); one isolate was incorrectly identified by both media and may have been another *Staphylococcus* species known to cause false positives (e.g. *S. schleiferi*), although this was not investigated further. The other three false positives were not shared between tested media. Overall, we found substantial agreement between the two media in their capacity to detect *S. aureus* from nasal swabs (kappa coefficient = 0.69; 95% confidence interval: 0.59, 0.80) (Landis & Koch, 1977).
Despite substantial agreement between the two media according to Cohen’s kappa (Landis & Koch, 1977), each medium identified \textit{S. aureus}-positive individuals that the other medium did not. Of the 83 \textit{S. aureus}-positive participants for whom side-by-side comparison was possible, 16 (19\%) were identified with CHROMagar\textsuperscript{TM} Staph aureus, but not Baird-Parker. Conversely, 8/83 (10\%) individuals were identified with Baird-Parker, but not CHROMagar\textsuperscript{TM} Staph aureus. Overall, only 59/83 (71\%) of \textit{S. aureus}-positive participants for whom side-by-side comparison was possible were identified by both Baird-Parker and CHROMagar\textsuperscript{TM} Staph aureus; the remaining 24/83 (29\%) were identified by one type of media or the other.

Additionally, while there was no conclusive difference between media in capacity to detect specific clonal complexes (CCs) identified in this study (Table 2), 8/11 (73\%) isolates belonging to CC182, a community-associated strain, were uniquely detected using CHROMagar\textsuperscript{TM} Staph aureus. A novel singleton most closely related to CC398, a livestock-associated strain, was also only detected by CHROMagar\textsuperscript{TM} Staph aureus. Our findings suggest that some strains may systematically be better detected by CHROMagar\textsuperscript{TM} Staph aureus and improved agreement between the two media (Table 1).

This study is the first to our knowledge to report increased identification of \textit{S. aureus} from enriched nasal swabs through parallel use of two types of media, rather than just one. However, our findings must be interpreted within the context of the limitations of this study. Importantly, we incubated Baird-Parker plates for 24 h, not up to 48 h as recommended by the manufacturer. Additional incubation time may have reduced the number of unique positives detected by CHROMagar\textsuperscript{TM} Staph aureus and improved agreement between the two media (Table 1). However, some studies may report \textit{S. aureus} prevalence based on Baird-Parker results after only 24 h of incubation (Plano et al., 2011; van de Giessen et al., 2009; Waters et al., 2011). Thus, our findings are

\textbf{Table 1.} Sensitivity and specificity of two media for identifying and isolating \textit{S. aureus}, using PCR confirmation as the gold standard

<table>
<thead>
<tr>
<th>Media</th>
<th>(n^*)</th>
<th>True positives</th>
<th>False positives</th>
<th>Unique positives(^\dagger)</th>
<th>Sensitivity(^\ddagger)</th>
<th>Specificity(^\§)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(no.)</td>
<td>(no.)</td>
<td>(no.)</td>
<td></td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>Baird-Parker</td>
<td>83</td>
<td>67</td>
<td>4</td>
<td>8</td>
<td>80.7</td>
<td>96.9</td>
</tr>
<tr>
<td>CHROMagar\textsuperscript{TM}</td>
<td>83</td>
<td>75</td>
<td>4</td>
<td>16</td>
<td>90.4</td>
<td>96.9</td>
</tr>
</tbody>
</table>

\(^*\) Number of samples analysed using both media.

\(^\dagger\) Unique positives; \textit{S. aureus}-positive participants identified solely with this media.

\(^\ddagger\) Sensitivity = \frac{true positives}{(true positives + false positives)} \times 100.

\(^\§\) Specificity = \frac{true negatives}{(true negatives + false negatives)} \times 100.

\textbf{Table 2.} Strain diversity among \textit{S. aureus} isolated on both media, Baird-Parker only, and CHROMagar\textsuperscript{TM} Staph aureus only

\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
Clonal complex\(^*\) & Total (\(n=83\)) & Isolated on both media (\(n=59\)) & Baird-Parker only (\(n=8\)) & CHROMagar\textsuperscript{TM} only (\(n=16\)) \\
\hline
 & \(n\) & \(n\) & \%\(^\dagger\) & \(n\) & \%\(^\dagger\) & \(n\) & \%\(^\dagger\) & \\
\hline
1 & 4 & 4 & 100.0 & – & – & – & – & \\
5 & 13 & 10 & 76.9 & 3 & 23.1 & – & – & \\
8 & 5 & 5 & 100.0 & – & – & – & – & \\
9 & 2 & 2 & 100.0 & – & – & – & – & \\
15 & 2 & 2 & 100.0 & – & – & – & – & \\
30 & 18 & 13 & 72.2 & 2 & 11.1 & 3 & 16.7 & \\
45 & 7 & 7 & 100.0 & – & – & – & – & \\
59 & 4 & 2 & 50.0 & 2 & 50.0 & – & – & \\
97 & 2 & 1 & 50.0 & 1 & 50.0 & – & – & \\
182 & 11 & 3 & 27.3 & – & – & 8 & 72.7 & \\
398 & 14 & 10 & 71.4 & – & – & 4 & 28.6 & \\
Unknown\(^\ddagger\) & 1 & – & – & – & – & 1 & 100.0 & \\
\hline
\end{tabular}

\(^*\) Method for assigning clonal complexes to isolates from this study is described elsewhere (Rinsky et al., 2013).

\(^\dagger\) Number of identified isolates as a percentage of the row total.

\(^\ddagger\) Novel singleton most closely related to CC398.

\(^\ddagger\) – indicates 0.
relevant in interpreting previous work as well as in informing methods for future studies. Also of importance is that S. aureus identification in this study was based on anterior nares swabs only; our finding that S. aureus recovery is improved by use of multiple media may differ if samples from the throat, skin, axillae and/or perineum are collected instead (Nilsson & Ripa, 2006; Solberg, 2000). Finally, as broth enrichment of swabs may not be routine in all clinical microbiology laboratories, it is important to note that our findings may differ if samples are plated directly.

Despite these limitations, we conclude that conventional use of only one type of medium may result in underreporting of S. aureus prevalence and reduced power in studies of risk factors for carriage, symptoms of infection, or other adverse health outcomes. We suggest use of both media for optimal recovery of S. aureus from healthy cohorts.

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Abbreviations: BD, Beckton, Dickinson and Company CC, clonal complex; MHB+, Mueller-Hinton broth containing 6.5% NaCl; MRSA, methicillin-resistant *Staphylococcus aureus*. 


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