Evaluation of Two Chromogenic Agar Media for Recovery and Identification of Staphylococcus aureus Small-Colony Variants


Evaluation of Two Chromogenic Agar Media for Recovery and Identification of *Staphylococcus aureus* Small-Colony Variants

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To identify the most rapid and reliable technique for recovery and identification of *Staphylococcus aureus* small-colony variants (SCVs), the colonial appearance of 106 isolates representing SCVs and the normal phenotype were evaluated on two newly described chromogenic agar media. Although almost all of the SCVs grew on the chromogenic agar media, they did not exhibit a change of color. In comparison with conventional media, *S. aureus* ID agar (SAID; bioMérieux, La Balme Les Grottes, France) showed the most reliable results, with 49 of 53 SCVs tested growing either as an SCV colony or with a normal phenotype after only 24 h of incubation. Growth of SCVs was often not detected before 72 h of incubation on some of the media tested. In conclusion, the most accurate and rapid method to detect both the species *S. aureus* and the SCV phenotype is to inoculate specimens onto both Columbia blood agar and SAID.

Since an association of the presence of *Staphylococcus aureus* small-colony variants (SCVs) and persistent and relapsing infection was reported, a renewed interest in infections due to staphylococcal SCVs has emerged (14, 17). Device-related infections, skin and soft tissue infections, chronic osteomyelitis, and persistent airway infections in patients with cystic fibrosis have been associated with this naturally occurring subpopulation (6, 7, 12, 15, 16, 18, 19). All cases reported impressively illustrate the poor clinical and microbiological response to even prolonged antimicrobial therapy in patients infected with these variants. In view of the frequent recovery of these variants—in patients with cystic fibrosis *S. aureus* SCVs were recovered in 26 of 78 patients (33.3%) and, thus, in about half of the patients harboring *S. aureus* (26 of 53; 49.1%) (6)—a reliable and rapid method for detection of these variants is of utmost importance.

*S. aureus* SCVs have been recognized as thymidine dependent or were characterized as electron transport-deficient bacteria due to their auxotrophies to hemin and/or menadione (6, 14, 19). The ability to interrupt electron transport and to form a variant subpopulation affords *S. aureus* a number of survival advantages beyond just increased resistance to antibiotics (13). The potential of these variants to persist intracellularly within nonprofessional phagocytes shields SCVs from host defenses and decreases exposure to antimicrobial agents (6, 18, 20). In addition, SCVs may not be recovered in the clinical microbiological laboratory due to their fastidious growth characteristics. SCVs produce tiny, mostly nonpigmented and nonhemolytic colonies and also exhibit various other characteristics that are atypical for *S. aureus* including reduced coagulase production; failure to use mannitol; and increased resistance to aminoglycosides, trimethoprim-sulfamethoxazole, cationic peptides, and cell-wall-active antibiotics (6, 12, 15, 18, 20). Consequently, the more resistant subpopulation of microorganisms in the infection will not have been reported (8, 12). These features may help to explain the great difficulties in achieving cures for infections due to staphylococcal SCVs. These variants present a challenge to clinical microbiologists in terms of recovery, identification, and susceptibility testing (13, 17). To avoid misidentification and misinterpretation, a prerequisite for the recovery and isolation of these variants is the application of extended conventional culture and identification techniques. In order to evaluate a potentially more rapid and reliable technique for recovery and identification of *S. aureus* SCVs, this study was designed (i) to test the potential of the two newly described chromogenic agar media, *S. aureus* ID (SAID; bioMérieux, La Balme Les Grottes, France) and CHROMagar Staph aureus (CHRA; Mast Diagnostica, Darmstadt, Germany), for recognition of *S. aureus* SCVs, this study was designed (i) to test the potential of the two newly described chromogenic agar media, *S. aureus* ID (SAID; bioMérieux, La Balme Les Grottes, France) and CHROMagar Staph aureus (CHRA; Mast Diagnostica, Darmstadt, Germany), for recognition of *S. aureus* SCVs by using a well-defined strain collection of *S. aureus* isolates with the SCV phenotype including a group of clinical SCV isolates and their clonally normal phenotype pairs and (ii) to compare the chromogenic agars with conventional media.

A total of 53 well-characterized SCV phenotype *S. aureus* isolates recovered from clinical specimens were included in this study. In addition, 53 corresponding isolates with normal phenotype which were recovered in the same or in subsequent clinical specimens as the SCVs (strain pairs) were also included in the study (6, 7, 15, 18, 19). Clonal identity of strain pairs was demonstrated by pulsed-field gel electrophoresis with Smal digests of total bacterial DNA as described previously (5). Furthermore, two laboratory-derived mutants mimicking the SCV phenotype as well as their parent strains were tested (1, 20). These mutants were constructed by interrupting one of the...
hemin (hemB) or menadione (menD) biosynthetic genes, respectively.

Apart from two reference strains (S. aureus ATCC 25923 and methicillin-resistant S. aureus ATCC 43300) and the defined mutants with SCV phenotype, all isolates were collected from patients with persistent and/or recurrent infections such as chronic osteomyelitis and chronic skin and soft tissue infections or from respiratory secretions of patients with cystic fibrosis (6, 7, 15, 18, 19).

S. aureus SCVs were identified as reported in the aforementioned case reports or prospective studies. Auxotrophy for hemin, menadione, and/or thymidine was determined on chemically defined medium as previously described (17). Identification as S. aureus was based on conventional criteria, including the coagulase tube test and the ID 32 STAPH system (bioMérieux, Marcy-l’Etoile, France). In addition, all strains with SCV phenotype were confirmed as S. aureus by testing for the S. aureus-specific nuc gene (2).

The colonial appearance, i.e., growth and color of colonies, was evaluated on the two chromogenic agar media, SAID and CHRA, in comparison with four conventional media, i.e., mannitol salt phenol red agar (MSA; Merck, Darmstadt, Germany) and Baird-Parker agar (BPA; Heipha Dr. Müller, Heidelberg, Germany) as media for differentiation and tryptic soy agar (TSA; Difco, Becton Dickinson, Sparks, Md.) and Columbia blood agar (CBA; BBL, Becton Dickinson) as primary media for cultivation.

Testing was performed as previously described, except with modifications of the incubation times due to the fastidious growth characteristics of SCVs (4, 9, 11). In brief, saline suspensions were prepared from freshly grown colonies and adjusted to an 0.5 McFarland turbidity. Subsequently, all suspensions were inoculated in parallel on eight different plates by spreading 100 µl on each plate. Plates were examined after 24, 48, and 72 h of incubation at 37°C. Growth of colonies showing any pink or mauve coloration on CHRA or green coloration on SAID was considered to be positive (indicating S. aureus) (4, 11). All plates were read independently by two investigators.

The results obtained with SCV phenotype S. aureus isolates are summarized in Table 1. Both growth and color of colonies are documented in separate columns because both the phenotype (growth with SCV phenotype or growth with normal phenotype) and the identification of S. aureus (appropriate color change on chromogenic agar) are of utmost importance for the recognition and treatment of the infection.

Following 24 h of incubation, 34 of 53 SCVs (64%) tested exhibited the typical variant phenotype on SAID, while only 19 isolates (36%) showed this phenotype on MSA and CHRA. Of interest, 12 (23%) isolates initially displaying SCV phenotype when inoculated grew as normal colonies (with normal phenotype) on SAID (19% showed normal phenotype on BPA) following this incubation period. Fifty-one percent of the SCVs (n = 27) did not grow at all on CHRA in comparison to only 3% (n = 2) that failed to grow on CBA after 24 h.

After incubation for 48 h, 14 SCVs (26%) grew with the variant phenotype on CHRA, while 44 SCV isolates (83%) showed normal colony size on BPA following this incubation period. However, 7 of these 44 isolates did not show a color change on the respective plate, thus not allowing identification of the isolate as S. aureus.
Most SCVs grew with normal phenotype following incubation for 72 h on the respective plates, with only one strain (2%) not detected on BPA and SAID and 9% not detected on TSA. In general, clinical isolates and mutants exhibiting the SCV phenotype did not lead to the identifying color changes which were obvious when testing the normal phenotype. Growth or color of colonies with SCV phenotype did not depend on the auxotrophisms of SCVs tested. When the genetically defined color of colonies with SCV phenotype did not depend on the

In general, clinical isolates and mutants exhibiting the SCV phenotype were tested, no major differences were found from clinical SCV isolates with regard to the colonial appearance.

Sensitivities with regard to growth on the respective agar media after incubation for 24, 48, and 72 h, respectively, were as follows: SAID, 92, 98, and 98%; CHRA, 49, 89, and 94%; MSA, 66, 89, and 94%; BPA, 85, 96, and 98%; TSA, 74, 85, and 91%; CBA, 97, 100, and 100%.

Among the isolates with normal phenotype clonal to those with SCV phenotype, rapid and accurate recognition and identification were achieved with both chromogenic agar media; however, normal isolates were identified as S. aureus more rapidly on SAID (91, 96, and 96% after 24, 48, and 72 h of incubation, respectively) than on CHRA (77, 96, and 98%, respectively).

In past studies evaluating new chromogenic agar media for detection and identification of S. aureus, SAID as well as CHRA was shown to be highly sensitive and specific (3, 4, 9–11). The rapid and accurate recognition of S. aureus isolates and lack of necessity for additional testing have been emphasized. However, in none of the aforementioned studies were SCVs of S. aureus systematically studied on chromogenic agar media. In only one study was a single isolate with the SCV phenotype (auxotrophic for thymidine) tested (4). In addition, despite the high prevalence of SCVs in defined patient populations such as patients with chronic osteomyelitis or patients with cystic fibrosis (6, 7, 19), no study so far has compared a substantial number of different media for cultivation and/or differentiation of microorganisms for their ability to detect and to identify SCVs of S. aureus. Laboratories should be specifically looking for S. aureus SCVs in samples from patients who have received long-term therapy or when an infectious disease has been unusually recurrent, antibiotic refractory, or persistent by application of extended conventional culture and special identification techniques (6, 7, 12, 15, 16, 18, 19).

In summary, we compared the colonial appearance of S. aureus SCVs and of clonal isolates with the normal phenotype on two newly described chromogenic agar media, SAID and CHRA, with that on conventional media for primary cultivation (TSA and CBA) and for differentiation (MSA and BPA). Nearly all S. aureus isolates with the SCV phenotype grew on the media tested; however, depending on the medium, it was often not before 72 h of incubation. Of the chromogenic agars, SAID yielded the most reliable results for initial detection, with 49 of 53 (92.5%) SCVs tested growing either as SCV or as normal phenotype after incubation for only 24 h. Although SCVs grew on the chromogenic agar media tested, the colony color specific for S. aureus was not observed for strains exhibiting the SCV phenotype. The SCV phenotype was most stable on CBA, i.e., SCVs were still perceived as isolates with the SCV phenotype (76% grew as SCVs after 24 h). On CBA, of course, such a phenotype is not distinguishable as S. aureus without further characterizing tests. In at least one observation, an SCV isolate from a cystic fibrosis patient’s sample was readily distinguishable on a chromogenic S. aureus medium not evaluated in this study (E. J. Baron and H. A. D’Souza, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. D-1681, 2003).

Based on our in vitro data with these two chromogenic formulas, we conclude that the most accurate and rapid method to detect both the species S. aureus and the SCV phenotype is to inoculate specimens onto both CBA (to detect the SCV phenotype) and SAID (to confirm that these SCVs are a subpopulation of the species S. aureus). Further studies are warranted to evaluate if this is the best strategy for detection and identification of S. aureus SCVs directly from clinical specimens of various origins and if other formulations of chromogenic agars show equivalent or better results.

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REFERENCES


