Performance of SaSelect, a Chromogenic Medium for Detection of Staphylococci in Clinical Specimens

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In a preliminary study, known staphylococcus (n = 86) and other microbial (n = 12) isolates were plated on three chromogenic media, SaSelect (Bio-Rad, Hercules, CA, USA), CHROMagar Staph. aureus (CHROMagar Microbiology, Paris, France), and S. aureus ID (bioMérieux, Marcy l’Etoile, France). The sensitivities of all the media to detect Staphylococcus aureus after 24 h of incubation were high (100.0%). However, their specificities varied at 93.3% (95% confidence interval [CI], 86.0% to 100.0%) (CHROMagar Staph. aureus), 97.8% (95% CI, 93.5% to 100.0%) (S. aureus ID), and 100.0% (SaSelect). SaSelect also showed the highest sensitivity for recovery and differentiation of other staphylococci. As the best performing chromogenic medium, SaSelect was then prospectively compared to conventional culture and identification tests for the detection of staphylococci from 2,780 clinical specimens. A total of 1,589 staphylococcal isolates were recovered. Of these, 912 were S. aureus and 677 were other staphylococci. The sensitivity and specificity of SaSelect to detect S. aureus in clinical specimens after 24 h of incubation were 99.6% and 99.9% (95% CI, 99.2% to 100.0% and 99.8% to 100.0%, respectively), whereas the sensitivity and specificity using conventional plates combined with laboratory identification methods were 96.8% and 99.5% (95% CI, 95.7 to 99.9% and 99.2% to 99.8%). For the recovery and preliminary identification of other staphylococci, the sensitivity and specificity of SaSelect were 94.4% and 99.9%. SaSelect is a well-performing chromogenic medium that significantly improved the detection of staphylococci, especially S. aureus, compared to conventional culture (P < 0.0001).

Culture-based methods are useful and cost-effective approaches for the recovery and identification of many bacterial pathogens. Therefore, they are widely used in many microbiological laboratories. However, when nonspecific media are used, additional tests for accurate species identification are required, making conventional culture methods rather time-consuming, particularly when several colony morphotypes are seen on the plates. Furthermore, nonselective media allow rapidly growing or swarming species to overgrow or cover all the other microbes.

To improve the recovery and identification of various microbes, several proprietary chromogenic mixtures have been developed (1–3). These media allow the presumptive identification of, e.g., Gram-negative bacilli within 24 h of incubation (1) or yeast within 48 h of incubation (3) on the basis of colonial morphology and distinctive color patterns. In combination with selective substrates, chromogenic culture can be used for the isolation of certain groups of microbes (1) or even for one particular pathogen (4). These media have been shown to improve the culture-based methodology by decreasing the total turnaround time and by increasing the differentiation and detection of the target microbes of interest (2, 4, 5).

The performance of two chromogenic media, CHROMagar Staph. aureus (CHROMagar Microbiology, Paris, France) and S. aureus ID (bioMérieux, Marcy l’Etoile, France), for the isolation and preliminary identification of Staphylococcus aureus has previously been investigated (2, 6–8). In our study, the performance of a third recently described medium, SaSelect (Bio-Rad, Hercules, CA, USA) (9), was compared to those of the two above-mentioned media, comprising not only S. aureus, but other staphylococci and other microbes, as well. Furthermore, the suitability of SaSelect for the isolation and identification of various staphylococci in clinical specimens was assessed.

MATERIALS AND METHODS
The performance of CHROMagar Staph. aureus, S. aureus ID, and SaSelect in growing and identifying staphylococci was evaluated using a collection of ATCC (n = 21) and known clinical stock (n = 77) isolates, including 86 staphylococcal and 12 other microbial isolates. The ATCC strains were S. aureus (n = 3) (ATCC 29213, CCGU 35601|Culture Collection, University of Göteborg, Göteborg, Sweden), and ATCC 25923, Staphylococcus auricularis (n = 1) (ATCC 33753), Staphylococcus hominis (n = 2) (ATCC 27844 and ATCC 700236), Staphylococcus intermedius (n = 1) (ATCC 29663), Staphylococcus schleiferi (n = 1) (ATCC 49545), Staphylococcus sciuri (n = 2) (ATCC 29060 and ATCC 29062), Staphylococcus simulans (n = 1) (ATCC 27848), Candida albicans (n = 1) (ATCC 28366), Candida parapsilosis (n = 1) (ATCC 22019), Enterococcus faecalis (n = 1) (ATCC 29212), Enterococcus faecium (n = 1) (ATCC 19434), Moraxella catarrhalis (n = 1) (ATCC 25238), Pseudomonas aeruginosa (n = 1) (ATCC 27852), Streptococcus agalactiae (n = 1) (ATCC 13813), Streptococcus dysgalactiae subsp. equisimilis (n = 2) (ATCC 12394 and ATCC 35666), and Streptococcus pyogenes (n = 1) (ATCC 19615). The clinical stock isolates consisted of S. aureus (n = 50), Staphylococcus capitis (n = 1), Staphylococcus epidermidis (n = 2), Staphylococcus haemolyticus (n = 3), S. hominis (n = 1), S. intermedius (n = 3), Staphylococcus lugdunensis (n = 2), Staphylococcus saprophyticus (n = 5), S. sciuri (n = 4), S. simulans (n = 1), Staphylococcus warneri (n = 2), Staphylococcus xylosus (n = 1), Micrococcus luteus (n = 1), and Stenotrophomonas maltophilia (n = 1). The species identification was performed in Helsinki University Central Hospital Laboratory.

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oratory (HUSLAB), using biochemical tests, e.g., API Staph or Staph ID 32 (bioMérieux, Marcy l’Étoile, France), or using 16S rRNA gene sequencing, as previously described (10). The colors and sizes of colonies were examined on each medium, and the differentiation of staphylococci was based on colony morphology and distinctive color patterns.

The chromogenic medium that performed best in the previous comparison study (SaSelect) was then used, in combination with conventional selective and nonselective agars, for the analysis of 2,780 clinical specimens of various natures, such as wounds, catheters, pus, exudates, joint fluids, superficial swabs, and upper respiratory secretions, as well as blood culture samples containing Gram-positive cocci in clusters. All specimens were prospectively collected between July 2011 and November 2012 in the Vaasa Hospital District, Finland, and streaked at least on chocolate agar plates (Becton, Dickinson, Sparks, MD, USA), Columbia agar plates supplemented with 5% horse blood (Becton, Dickinson) with or without colistin and oxolinic acid, and SaSelect plates. All plates for each specimen were inoculated at the same time and examined after 24 h and 48 h of incubation at 35°C. Fisher’s exact test was used to determine the statistical significance of the differences between SaSelect and conventional cultures.

The criteria for presumptive identification of different staphylococci growing on SaSelect were defined as follows: S. aureus, pink to orange colonies of various sizes; S. epidermidis, small and faint pink colonies; S. intermedius, bulky purple-gray colonies; S. saprophyticus, turquoise colonies; S. simulans, S. cohnii, or S. xylosus, blue to light-blue colonies of various sizes; and S. haemolyticus, S. hominis, S. capitis, S. warneri, S. caprae, S. lugdunensis, or S. sciuri, white to yellow colonies. Colonies on conventional agar plates were suspected to be staphylococcus on the basis of colony morphology, Gram staining, and a positive catalase reaction. Preliminary identification was confirmed using tube coagulase (Labema, Kerava, Finland), Staphaurex Plus (Remel, Lenexa, KS, USA), API Staph or Staph ID 32, or an S. aureus-specific PCR assay (GenomEra MRSA/SA assay; Abacuss Diagnostica, Turku, Finland).

RESULTS

Almost all staphylococcus stock isolates grew well on all three chromogenic media. Only two coagulase-negative staphylococci (CoNS) (S. warneri and S. xylosus) yielded no colonies on CHROMagar Staph. aureus, even after 48 h of incubation. Of the nonstaphylococcal isolates, none produced colonies on any of the media after 24 h to 48 h of incubation.

All 53 S. aureus stock isolates grew as mauve colonies on CHROMagar Staph. aureus, green on S. aureus ID, and pink to orange on SaSelect after 24 h of incubation. This implied high (100.0%) and consistent sensitivity for the detection of S. aureus. However, one of the four S. intermedius stock isolates also yielded green colonies on S. aureus ID, and two S. epidermidis and one S. schleiferi stock isolates yielded mauve colonies on CHROMagar Staph. aureus, causing a false-positive presumptive detection of S. aureus. With SaSelect, no false-positive detection of S. aureus occurred. Consequently, the specificities for the detection of S. aureus were 93.3% (95% confidence interval [CI], 86.0% to 100.0%) with CHROMagar Staph. aureus, 97.8% (95% CI, 93.5% to 100.0%) with S. aureus ID, and 100.0% with SaSelect.

Although CoNS stock isolates produced different shades of blue, green, yellow, pink, or white colonies on all three chromogenic media tested, variation in species detection was observed. For example, both S. epidermidis isolates yielded distinctive pale-pink colonies on SaSelect and pink colonies on CHROMagar Staph. aureus and thus could be differentiated from all other CoNS. On S. aureus ID, however, S. epidermidis produced white colonies similar to those of S. haemolyticus, S. hominis, and S. saprophyticus. All S. intermedius isolates produced unique purple-gray colonies on SaSelect and purple colonies on CHROMagar Staph. aureus, whereas on S. aureus ID, they produced green to turquoise green colonies close to S. aureus. In addition, S. saprophyticus produced distinctive blue-green colonies only on SaSelect, whereas on S. aureus ID it yielded white colonies, as mentioned above, and green colonies similar to those of S. sciuri on CHROMagar Staph. aureus, S. sciuri, on the other hand, grew as deep-yellow colonies on SaSelect and as ink blue on S. aureus ID. S. lugdunensis produced light-yellow colonies on SaSelect, white on CHROMagar Staph. aureus, and pale pink on S. aureus ID. Altogether, the differentiation and preliminary identification of CoNS stock isolates was most definite with SaSelect, and therefore, it was selected for the second part of this study.

During the 18-month clinical study period, a total of 1,589 (57.1%) specimens yielded one or more staphylococci on at least one of the conventional media used. Of these, 1,564 (98.4%) grew on SaSelect. Moreover, 528 (33.2%) specimens were polymicrobial, also containing Gram-negative bacilli on conventional media. Of the 1,589 specimens containing staphylococci, 912 included S. aureus. After 24 h of incubation, 908 (99.6%) S. aureus isolates were recovered and correctly identified with SaSelect, whereas 883 (96.8%) isolates were recovered on conventional culture plates. The 95% CI of sensitivity were 99.2% to 100.0% for SaSelect and 95.7% to 97.9% for conventional culture. All 29 S. aureus isolates not detected with conventional culture after 24 h of incubation were from highly polymicrobial specimens. When subcultured from the mixed growth on conventional agar plates onto additional plates and also on SaSelect, all these S. aureus isolates were finally recovered. Thus, the sensitivity of SaSelect for the recovery of S. aureus after 24 h of incubation was significantly higher than that of conventional culture (P < 0.0001). Of the four S. aureus isolates not detected by SaSelect, one produced only a pink slime without visible colonies after 24 h or 48 h of incubation. The strain produced small colonies on conventional agar plates, resembling a small-colony variant of S. aureus. Two of the four isolates produced faint pink colonies on SaSelect after 24 h of incubation and were first misidentified as S. epidermidis. However, the colonies became more intensely colored after 48 h of incubation and were finally correctly identified. The fourth S. aureus isolate was present in small numbers and grew on only one conventional agar plate. One additional misidentification occurred with SaSelect after 24 h of incubation when the growth of S. maltophilia was suspected to be S. aureus. A total of 10 S. maltophilia isolates were recovered from the clinical specimens with conventional agar plates, but only one of them yielded pink colonies on SaSelect; however, they turned compact, dark pink, and slimy, clearly distinct from S. aureus, after 48 h of incubation.

The rest of the 677 specimens, containing non-S. aureus staphylococci, consisted of 180 S. epidermidis, 471 white to yellow group staphylococci (S. haemolyticus, S. hominis, S. capitis, S. warneri, S. caprae, S. lugdunensis, or S. sciuri), 22 blue to light-blue group staphylococci (S. simulans, Staphylococcus cohnii, or S. xylosus), and four S. intermedius. All four S. intermedius isolates were recovered and correctly identified after 24 h of incubation using SaSelect but misidentified as probable S. aureus with conventional culture using S. aureus-like colony morphology and positive tube coagulase tests as criteria. After 48 h of incubation and additional biochemical tests, S. intermedius was identified properly, also with conventional culture.

Most of the 180 S. epidermidis isolates (91.7%) produced pale-pink colonies on SaSelect after 24 h of incubation. However, by...
extending the incubation from 24 h to 48 h, the number of isolates yielding pale-pink colonies increased from 165 to 172 (95.6%). Thus, the sensitivity of SaSelect for preliminary detection of S. epidermidis varied from 91.7% (after 24 h of incubation) (95% CI, 87.7% to 95.7%) to 95.6% (after 48 h of incubation) (95% CI, 92.6% to 98.6%). The specificity for detection of S. epidermidis was 99.9% (95% CI, 99.8% to 100.0%) after 24 h of incubation due to the two S. aureus misidentifications mentioned above. Of the 471 white to yellow group staphylococci, 430 (95.5%) grew on SaSelect after 24 h or 48 h of incubation. The majority (97.8%) yielded white colonies and were identified by confirmatory tests as S. haemolyticus, S. hominis, S. capitis, S. warneri, or S. caprae. Ten isolates yielded yellow colonies on SaSelect and were identified as S. lugdunensis (n = 5) or S. sciuri (n = 5). The colonies of S. lugdunensis were slightly smaller and lighter colored than the colonies of S. sciuri. The rest of the 22 isolates were identified as S. simulans (n = 8), S. cohnii (n = 4), or S. xylosus (n = 10). Of these, 20 grew on SaSelect after 24 h or 48 h of incubation, yielding blue to light-blue colonies. The overall sensitivity of SaSelect for the recovery of various non-S. aureus staphylococci in clinical specimens after 24 h of incubation was 96.6% (n = 654) (95% CI, 92.7% to 96.1%).

**DISCUSSION**

Staphyloccoci are a diverse group of bacteria that constitute part of the normal human flora but can also cause diseases ranging from minor skin infections to life-threatening bacteremia. They are classified into coagulase-positive staphylococci (e.g., S. aureus and S. intermedius) and CoNS by their ability to produce coagulase (11). Within the genus Staphylococcus, S. aureus is the most important human pathogen, while the CoNS play roles mainly in opportunistic infections (12–15).

Although rapid detection of S. aureus in clinical specimens is essential for appropriate patient care, the recovery and identification of other staphylococci is also important, especially from catheters and other foreign-body samples and blood cultures (16–21). Culture-based detection methods are cost-effective and useful, especially when various microbe species are examined. Disadvantages of conventional culture, however, are the need for additional tests for accurate species identification and the difficulty in differentiating various microbes if different species produce similar colonies, as may be the case with staphylococci. Furthermore, swarming or rapidly growing bacteria, such as Gram-negative bacilli, may cover or overgrow all other species present in the specimen unless selective supplements are used.

All the chromogenic media tested in the first stage of this study proved to be highly sensitive for the recovery of S. aureus, as has been demonstrated previously (2, 7, 9). Their specificities, however, varied, and SaSelect proved to be far more specific (100.0%) than S. aureus ID (97.0%) or CHROMagar Staph. aureus (90.9%) for the detection of S. aureus. In a recent study by Perry et al., the performances of CHROMagar Staph. aureus and S. aureus ID for screening of S. aureus were compared, with results similar to ours (7). As the performance results in our study were conclusive in favor of SaSelect, it was chosen to be compared to conventional nonchromogenic media in clinical specimen analysis.

The selective medium in SaSelect, optimized for rapid growth of staphylococci, particularly of S. aureus, allowed significant improvements in the recovery of staphylococcus isolates compared to conventional culture. This was observed especially with wound specimens containing polymicrobial growth of S. aureus and Escherichia coli, Proteus spp., or P. aeruginosa. Among these specimens, S. aureus was recovered and identified more quickly with SaSelect (after 24 h of incubation) than using conventional culture (after 48 to 72 h of incubation). On conventional media, rapidly growing Gram-negative bacilli covered the colonies of S. aureus, which could not be isolated unless subcultured into additional plates. Moreover, the specific chromogenic substrates in SaSelect allowed rapid and reliable identification of S. aureus, decreasing the need for further testing. This clear superiority of selective chromogenic media over conventional media has previously also been demonstrated with CHROMagar Staph. aureus and S. aureus ID (2, 6, 7). However, despite the encouraging findings, two S. aureus isolates were undetected with SaSelect. One missed isolate was from a specimen containing small quantities of microbes. This specimen yielded only three S. aureus colonies on the chocolate agar plate cultured first, whereas all other conventional agar plates were negative, including blood agar and fastidious anaerobe agar (Lab M Ltd., Lancashire, United Kingdom). The other isolate resembled an S. aureus small-colony variant on conventional media and yielded only pink slime on SaSelect. In this case, the color was typical, but because no proper colonies were seen on SaSelect, it was first interpreted as nonspecific growth of nonstaphylococci species and only after Gram staining and a catalase test as a possible staphylococcus species.

SaSelect also provided excellent assistance in the case of mixed growth of different staphylococci. We observed several specimens containing abundant growth of S. aureus and S. epidermidis, which could be distinguished only on SaSelect but not on universal rich medium after 24 h of incubation. In all these cases, S. aureus isolates did not produce the characteristic yellow pigment on conventional media, yielding, however, distinctive pink/mauve colonies on SaSelect (Fig. 1). The colonies of S. epidermidis, on the other hand, were white to pale pink (Fig. 1). Thus, SaSelect reduced the risk of mixed testing of polymicrobial growth. This was an important observation, as in all these cases, S. epidermidis was methicillin resistant (MR), containing the mecA gene, while S. aureus was methicillin sensitive (MS), lacking the mecA gene. We have previously seen in our laboratory that polymicrobial growth of MS S. aureus (MSSA) and MRCoNS may cause inconveniences and disadvantageous exclusion of MR S. aureus (MRSA) when using only universal rich medium for primary plating of various specimens.

**FIG 1** Colonial appearance of S. aureus and S. epidermidis on SaSelect agar after 24 h of incubation. The black arrows point to the colonies of S. aureus, and the white arrows point to the colonies of S. epidermidis.
Apart from *S. epidermidis*, several other non-*S. aureus* species could also be identified using SaSelect alone. An important finding was that the growth of *S. intermedius*, which yielded distinctive purple-gray colonies on SaSelect, was easily identified and distinguished from that of *S. aureus*. This coagulase-positive species may be misidentified as *S. aureus* with conventional culture media and, e.g., tube coagulate or latex agglutination tests (6). In a study by Carricajo et al. using CHROMagar *Staph. aureus* for detection of *S. aureus* in clinical specimens, *S. intermedius* yielded colonies that resembled *S. aureus* on that particular chromogenic medium, and the two species were indistinguishable without additional tests (6). Other staphylococci producing clumping factor, i.e., *S. lugdunensis*, *S. schleiferi*, and *S. sciuri*, were also well differentiated from *S. aureus* by SaSelect. Furthermore, *S. sciuri* and *S. lugdunensis* yielded characteristic yellow colonies on SaSelect, which was useful for preliminary identification of the two species. However, definite conclusions for the reliable identification of the species by SaSelect alone cannot yet be made, as the number of isolates investigated in this study was small.

In addition, SaSelect can be helpful for the preliminary screening of *S. saprophyticus* in urine specimens (data not shown). *S. saprophyticus* yields characteristic bulky and deep-green colonies on SaSelect, which are easily recognizable after 24 h of incubation.

In conclusion, SaSelect proved to be a well-performing culture medium for the primary isolation of various staphylococci. It greatly improved the recovery of *S. aureus* and the detection of other staphylococcus isolates compared to conventional culture, also providing assistance with the differentiation of staphylococci in polymicrobial specimens. Although chromogenic media may be more expensive than conventional media, their use in primary plating of specimens may be greatly advantageous, since the need for identification tests for various isolates decreases and presumptive results are obtained sooner. Our results indicated that SaSelect was highly sensitive and specific, especially for *S. aureus*, thereby reducing the requirement for additional testing and confirmation of suspected *S. aureus* growth. Although the sensitivity and specificity of SaSelect for detection of, e.g., *S. epidermidis*, *S. intermedius*, *S. lugdunensis*, and *S. sciuri* proved to be high as well, the requirement for additional identification tests could not be excluded with certainty due to the small number of these isolates in this study.

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REFERENCES


