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Performance of the Chromogenic Medium CHROMagar Staph Aureus and the Staphychrom Coagulase Test in the Detection and Identification of *Staphylococcus aureus* in Clinical Specimens

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CHROMagar Staph aureus (CSAM) (CHROMagar Microbiology, Paris, France) is a new chromogenic medium designed to enable detection of colonies of *Staphylococcus aureus* by their pink color. A total of 775 specimens were cultured in parallel on CHROMagar Staph aureus and conventional media. Among the 267 *S. aureus* strains recovered on at least one medium, 263 were isolated on CSAM medium (sensitivity, 98.5%), and 245 (sensitivity, 91.8%) were isolated on conventional media. The specificity of presumptive identification of *S. aureus* on the basis of pink colony color on CSAM medium was 97% (493 of 508). This specificity increased to 100% when coagulase detection with the Staphychrom coagulase test was added and to 98.8% when *S. aureus* surface components were detected by agglutination in the Pastorex Staph Plus test. Susceptibility testing of 67 *S. aureus* strains, performed in parallel on pink CSAM colonies and on colonies grown on blood agar, gave similar results. Thus, rapid and accurate recognition and identification of *S. aureus* isolates were achieved with CSAM as the primary isolation medium, followed by the staphylocoagulase Staphychrom test. Antimicrobial susceptibility testing (disk-diffusion method or ATB STAPH System) can be performed directly on pink CSAM colonies.

Staphylococcus aureus causes severe suppurative infections associated with high morbidity and mortality. Its isolation from a patient with an infectious syndrome usually leads to specific antibiotic treatment. *S. aureus* can be missed when the clinical specimen contains a mixed flora. This is especially the case when other staphylococcal species with an identical colony appearance are present or when swarming colonies of *Proteus* or *Pseudomonas* cover those of *S. aureus*. Misidentification of *S. aureus* in a clinical sample can have serious clinical repercussions.

S. aureus colonies grown on a chromogenic medium, such as CHROMagar Staph aureus (CHROMagar Microbiology, Paris, France) (CSAM) are pink, unlike colonies of other *Staphylococcus* species. CSAM has been reported to yield a higher detection rate for *S. aureus* in plurimicrobial samples. The reported sensitivity of the CSAM method is 95.5%, compared to 81.9% with conventional methods (Gaillot et al. [6]). Pink colonies grown on CSAM can be rapidly confirmed to be *S. aureus* by using agglutination kits, such as Pastorex Staph Plus, which simultaneously detects the clumping factor, protein A, and capsular antigens. The specificity of this test is only 75.5%, however (13), as species like *Staphylococcus schleiferi* and *Staphylococcus lugdunensis,* which are sometimes involved in human diseases, may also give positive reactions (2, 4, 7, 12). *S. aureus* is also identified by coagulase testing, but other staphylococcal species are also coagulase positive (e.g., *Staphylococcus intermedius*) even if they are rarely detected in human specimens (9, 14). The Staphychrom coagulase test is a fluorogenic staphylocoagulase test based on human prothrombin and protease inhibitors and specifically detects *S. aureus* coagulase (8; A. Treny, M. Bes, N. Fonsale, A. Carricajo, M. E. Reverdy, and A. M. Freydiere, Abstr. 11th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P1512, p. 326–327, 2001).

We assessed the performance of CSAM by culturing 775 clinical samples; all pink colonies were then submitted to the Staphychrom coagulase test. Results were compared with those obtained with conventional culture media, the Pastorex Staph Plus agglutination kit, and the conventional coagulase tube test.

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MATERIALS AND METHODS

Clinical specimens. From August 1999 to June 2000 we tested 775 clinical specimens, comprising 431 wound samples, 6 urine samples, 4 stool samples, 98 blood culture supernatants, 5 bronchoalveolar lavage samples, 3 sputum samples, 198 tracheal aspirates, 5 drainage fluid samples, and 65 nasal specimens. Four hundred sixty-eight specimens were studied at Antiquaille hospital (Lyon, France), and 307 were studied at Bellevue hospital (Saint Etienne, France), using the same protocol. Nonfluid specimens were suspended in physiological (0.85%) saline, and 0.01 ml of this suspension was streaked on the different plates.

Media. Specimens were streaked on CSAM agar plates (CHROMagar Microbiology), Columbia agar plates supplemented with 5% horse blood (bioMérieux, Marcy-l'Etoile, France), and chocolate agar plates (bioMérieux). All the plates were randomly inoculated at the same time and examined after 24 and 48 h of incubation at 37°C. CSAM plates and conventional agar plates were read independently by different technicians.

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TABLE 1. Number of *S. aureus* isolates recovered from 775 clinical specimens and number of false negatives by using CSAM and a standard method

Method	No. of isolates identified as *S. aureus*	No. of false negatives	Sensitivity (%)
Standard method[a]	245	22	91.8
CSAM[b]	263	4	98.5
Any media	267[c]		100

[a] Suggestive colony morphology and Gram staining, positive catalase test, and positive latex agglutination.
[b] Mauve colony and suggestive Gram staining.
[c] Total number of *S. aureus* isolates recovered by at least one method.

Identification of *S. aureus*. All pink colonies grown on CSAM agar were Gram stained and agglutinated with the Pastorex Staph Plus kit; coagulase production was detected using the Staphychrom test according to the manufacturer's recommendations. Colonies grown on conventional agar were suspected to be staphylococcal on the basis of their morphology, Gram staining, and catalase positivity. Catalase-positive colonies and gram-positive cocci were agglutinated with the Pastorex Staph Plus kit, and coagulase production was detected with EDTA-rabbit plasma (Difco Laboratories, Detroit, Mich.). When the result of the latex agglutination test differed from that of the coagulase test, identification was performed with the API ID32 STAPH gallery (bioMérieux) or the Accuprobe test (bioMérieux), which detects *S. aureus*-specific rRNA sequences.

Susceptibility testing. Antimicrobial susceptibility testing was performed by picking colonies directly from CSAM and conventional media. The disk-diffusion method on Mueller-Hinton agar (bioMérieux) was used at Antiquaille hospital according to the recommendations of the Comité Français de l'Antibiogramme (3). The methicillin susceptibility test was performed with a 5-μg oxacillin disk, a 10^8-CFU/ml inoculum, and incubation at 30°C for 24 h. The automated ATB STAPH System (bioMérieux) was used at Bellevue hospital, according to the manufacturer's recommendations. Briefly, a 0.5× McFarland emulsion of isolated colonies in sterile saline was added to 7 ml of ATB medium (Mueller-Hinton broth supplemented with 5% NaCl). The final inoculum was transferred into an oxacillin (2 μg/ml) well and incubated for 24 h at 35°C. The antibiotics tested were penicillin G, oxacillin, erythromycin, lincomycin, pristinamycin, tetracycline, kanamycin, tobramycin, gentamicin, rifampin, fusidic acid, fosfomycin, pefloxacin, cotrimoxazole, vancomycin, and teicoplanin.

RESULTS

Detection of *S. aureus*. A total of 267 *S. aureus* strains (25% methicillin-resistant strains) were isolated from CSAM and/or conventional media (Table 1). Two hundred sixty-three isolates grew on CSAM: 242 (92%) grew after 24 h and 21 (8%) after 48 h. Two hundred forty-three isolates grew on conventional media. Four isolates were not detected on CSAM and 22 were not detected on conventional media (13 isolates were masked or inhibited by a gram-negative species, 7 were mixed with several other coagulase-negative staphylococci, and 2 isolates corresponded to samples containing too few colonies). The sensitivities of CSAM and conventional media for growing *S. aureus* were 98.5 and 91.8%, respectively.

Fifteen pink colonies that grew on CSAM were subsequently identified as species other than *S. aureus*. None of them produced coagulase in the Staphychrom coagulase test, while six agglutinated in the Pastorex Staph Plus test and were identified as *Staphylococcus simulans*, *Staphylococcus intermedius*, *S. schleiferi,* or *Staphylococcus warneri* by using the API STAPH gallery (Table 2). The isolates which were neither coagulase positive nor agglutination positive belonged to other coagulase-negative *Staphylococcus* species or were micrococci (Table 2). Five of these 15 strains were detected after 48 h of incubation. In addition, 55 *Corynebacterium* spp., 3 *Candida albicans* isolates, and 1 unidentified gram-negative bacillus developed a pink aspect on CSAM medium. Except for one *C. albicans* isolate and one *Corynebacterium* isolate, which were detected after 24 h of incubation, all the other strains took 48 h to grow. For gram-positive cocci, the specificity of CSAM for the presumptive identification of *S. aureus* was 97%. This specificity increased to 98% with the Pastorex Staph Plus test, and to 100% with the Staphychrom coagulase test (Table 2).

Susceptibility testing was performed on 67 *S. aureus* isolates (47 by the disk-diffusion method and 20 with the ATB STAPH system); 11 of these 67 *S. aureus* strains (16%) were methicillin-resistant strains. Full agreement was obtained between pink colonies on CSAM media and corresponding colonies on conventional media, for all the antibiotics tested.

DISCUSSION

The use of CSAM medium for the detection of *S. aureus*, followed by Staphychrom coagulase testing, yielded an overall sensitivity of 98% and a specificity of 100%. The four *S. aureus* isolates which were not detected on CSAM but grew on conventional media corresponded to samples containing only one to five colonies per plate, and the lack of growth on CSAM might have been a consequence of random seeding. CSAM medium permitted the detection of 22 *S. aureus* isolates that were not detected on conventional media, notably in plurimicrobial samples. The good visibility of the pink colonies on CSAM facilitates the recognition of potential *S. aureus* isolates and thus increases the detection rate.

These results confirm data reported by Gaillot et al. (6), who isolated 310 *S. aureus* strains on CSAM from among 2,000 clinical samples; they obtained a sensitivity of 95.5% for CSAM versus 81.9% for a conventional method. CSAM has also been used for the detection of *S. aureus* nasal carriage: Laudat et al. (P. Laudat, A. Gendre, and C. Chillou, Abstr. 20th Interdiscip. Meet. Anti-Infect. Chemother., Soc. Fr. Microbiol. Section Agents Antimicrob. Soc. Pathol. Infect., abstr. 343/P2, 2000) detected 26 *S. aureus* carriers with CSAM and

TABLE 2. Number and nature of false-positive *S. aureus* strains identified using CSAM alone, CSAM plus the Pastorex Staph Plus test, and CSAM plus the Staphychrom coagulase test

Strain(s)	No. of strains with false-positive *S. aureus* identification using[a]:		
	CSAM[b]	CSAM[b] + Pastorex Staph Plus test	CSAM[b] + Staphychrom coagulase test
Micrococcus spp.	2	0	0
Staphylococcus epidermidis	3	0	0
Staphylococcus simulans	3[c]	3[c]	0
Staphylococcus intermedius	1	1	0
Staphylococcus warneri	1	1	0
Staphylococcus schleiferi	1	1	0
Other coagulase-negative *Staphylococcus* spp.	4	0	0
Total	15	6	0

[a] Specificities were the following: for CSAM, 97%; for CSAM + Pastorex Staph Plus test, 98.8%; for CSAM + Staphychrom coagulase test, 100%.
[b] Mauve colony and suggestive Gram staining, but not an *S. aureus* strain.
[c] These three strains were isolated from different specimens from the same patient.

only 22 with blood agar plates. Higher-than-normal detection rates have also been described with several other chromogenic media designed for urinary tract pathogens, *C. albicans*, and salmonellae (1, 5, 10, 11).

The rapid Staphychrom coagulase test was more specific than the latex-agglutination test Pastorex Staph Plus for *S. aureus* identification, since the six non-*S. aureus* strains that yielded both pink colonies and a positive latex agglutination test were correctly identified as belonging to other staphylococcal species. Thus, the use of CSAM combined with the rapid Staphychrom coagulase test for confirmation of *S. aureus* identification seems to be an optimal strategy, since it avoids the frequently recommended combination of two tests for accurate identification of *S. aureus* (an agglutination test and the tube coagulase test) (13; Treny et al., 11th ECCMID).

Although CSAM medium is more expensive than conventional medium, it may be cost effective, since it eliminates the need for numerous catalase and latex agglutination tests for non-*S. aureus* strains grown on conventional media (A. M. Freydiere, M. Bès, C. Roure, and A. Carricajo, Abstr. 100th Gen. Meet. Am. Soc. Microbiol., abstr. C230, p. 183, 2000). On CSAM, only *Staphylococcus* strains yielding pink colonies require further testing, thereby reducing handling and reagent costs. The Staphychrom coagulase test is slightly more fastidious and less rapid (2 h) than the Pastorex Staph Plus test.

Finally, susceptibility testing performed directly on pink CSAM colonies yields results that are in perfect agreement with those obtained with colonies grown on conventional media.

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