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Performance of CHROMagar Selective Medium and Oxacillin Resistance Screening Agar Base for Identifying *Staphylococcus aureus* and Detecting Methicillin Resistance

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Two new selective media, oxacillin resistance screening agar base (ORSAB) and CHROMagar Staph aureus (CSA), were evaluated for identification of *Staphylococcus aureus* and for screening of methicillin resistance by addition of antimicrobial agents to these media. A well-defined collection consisting of 1,140 staphylococci was used. A total of 624 were *S. aureus*, of which 358 were methicillin susceptible and 266 were methicillin resistant, and 516 were coagulase-negative staphylococci. The methicillin-resistant *S. aureus* (MRSA) strains were selected based on the results of phage typing; 247 different types were included in the analysis. For identification of *S. aureus*, both media performed better after 24 h than after 48 h. The sensitivities at 24 h were comparable (CSA, 98.6%; ORSAB, 97.1%), but the specificity of CSA was significantly higher (CSA, 97.1%; ORSAB, 92.1%). For screening of methicillin resistance, antibiotic supplements were added to both media. The sensitivity was lower after 24 h (CSA, 58.6%; ORSAB, 84.2%) and increased significantly after 48 h (CSA, 77.5%; ORSAB, 91.4%). At both time intervals ORSAB was significantly more sensitive than CSA. However, the specificities of both media were high after 24 h (CSA, 99.1%; ORSAB, 98.3%) and decreased significantly after 48 h of incubation (CSA, 94.7%; ORSAB, 95.5%). In conclusion, for identification of *S. aureus*, CSA is more accurate than ORSAB because of a significantly higher specificity. For screening of MRSA, ORSAB performs better than CSA, but the usefulness in clinical practice is limited because a significant number of strains are not detected.

Staphylococcus aureus is one of the most frequently isolated pathogens in clinical specimens. In fact, *S. aureus* is currently the most common cause of infections in hospitalized patients (1). It causes a variety of serious diseases associated with a high mortality (12). Since methicillin resistance is now widespread in hospitals all over the world (17), therapy has become cumbersome. A new and even more threatening development is the emergence of strains with a reduced susceptibility to glycopeptides (13, 14). Therefore, control of *S. aureus* in the hospital has now become more important than ever before.

Rapid isolation and identification of *S. aureus* in clinical samples are essential for appropriate patient care and control of this microorganism in the hospital. In the clinical laboratory routine *S. aureus* is usually isolated on nonspecific media and then presumptively identified before definitive overnight characterization (6, 7). Selective media have been developed to achieve isolation and presumptive identification in a single step. Mannitol-salt agar (MSA) is one of the most widely used selective media for isolation of *S. aureus*. It is also recommended for selective screening of methicillin-resistant *S. aureus* (MRSA) (4). It consists of mannitol to indicate the presence of *S. aureus* and a variable concentration of sodium chloride to inhibit the growth of other microorganisms. Some strains of *S. aureus*, however, are also inhibited by the salt component, which reduces the sensitivity of this method (5). In addition, a beta-lactam antibiotic can be added to the MSA for selective isolation of MRSA.

The purpose of this study is to evaluate the in vitro sensitivity and specificity of a recently developed MSA, oxacillin resistance screening agar base (ORSAB), and a new selective medium, CHROMagar Staph aureus (CSA), for identification of *S. aureus* and screening for methicillin resistance by addition of antimicrobial agents to these media.

MATERIALS AND METHODS

Bacterial strains. A well-defined collection consisting of 1,140 staphylococci was used. A total of 624 were *S. aureus*, of which 358 were methicillin-susceptible *S. aureus* (MSSA) and 266 were MRSA, and 516 were coagulase-negative staphylococci (CNS). The isolates were stored at −70°C until they were tested.

The 266 MRSA isolates were collected in The Netherlands between 1989 and 1998 and are part of the MRSA strain collection of the National Institute of Public Health and Environmental Protection (RIVM), Bilthoven, The Netherlands. Identification as *S. aureus* and methicillin resistance were determined by duplex PCR for the *mecA* gene and coagulase gene as described previously (15). Strains were selected on the basis of their different phage types. Bacteriophage typing was performed as described previously by using (i) the international set of phages at 1× and 100× routine test dilution concentrations, (ii) an additional set of Dutch phages, and (iii) a set of experimental MRSA phages (10, 16; J. A. Rost, unpublished data). The 266 MRSA isolates included in the study comprised 247 different phage types. Three isolates were not typeable.

The 358 MSSA and 516 CNS isolates were isolated from cultures of blood collected between May 1996 and June 1999 from consecutive patients at the following six hospitals: St. Elisabeth Hospital and Tweesteden Hospital, Tilburg, The Netherlands; Pasteur Hospital, Oosterhout, The Netherlands; Tweesteden Hospital, Waalwijk, The Netherlands; and St. Ignatius Hospital and Hospital de Baronie, Breda, The Netherlands. Only one isolate was included per patient per admission period. Isolates were identified by a latex agglutination test (Staphau-

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rex Plus; Murex Diagnostics Ltd., Dartford, United Kingdom), by the detection of free coagulase by the tube coagulase test with rabbit plasma (6), and by the detection of DNase (DNase agar; Oxoid Ltd., Basingstoke, United Kingdom). If the results of these tests were discordant, an AccuProbe culture identification test (Gen-Probe, San Diego, Calif.) was performed according to the manufacturer's instructions. The result of the AccuProbe test was considered the "gold standard" for the identification of *S. aureus*. The blood culture isolates were classified as methicillin susceptible (MIC of oxacillin, ≤2 μg/ml) at the time of collection by broth microdilution susceptibility testing performed according to the National Committee for Clinical Laboratory Standards (NCCLS). Furthermore, no growth was observed by the oxacillin agar screen test according to the NCCLS standard (9).

CSA. CSA (CHROMagar Microbiology, Paris, France) is a new chromogenic medium for identification of *S. aureus.* The medium contains the following ingredients: peptone, 40.0 g/liter; agar, 15.0 g/liter; sodium chloride, 25.0 g/liter; and a chromogenic mix, 3.5 g/liter. The composition of the chromogenic mix is proprietary. This agar base was tested with (CSA+) and without (CSA) the addition of oxacillin at 4.0 mg/liter as suggested by the manufacturer and others (8).

The isolates were inoculated on Columbia agar plates with 5% sheep blood and incubated for 24 h at 35°C. From the resulting cultures a suspension at 0.5 McFarland standard was made, and subsequently a swab was dipped in the suspension and streaked on a CSA or CSA+ plate. The results were read after 24 and 48 h of incubation at 35°C. Growth of colonies showing any pink or mauve coloration was considered to be positive (indicating *S. aureus*). The procedure is as recommended by the manufacturer.

ORSAB test. ORSAB (Oxoid Ltd.) is a medium for screening for MRSA directly from routine swab samples. It uses aniline blue to detect mannitol fermentation, resulting in intense blue colonies of *S. aureus*. The agar base without supplement was used in this study to identify *S. aureus*. The formula of the agar base is as follows: peptone, 11.8 g/liter; yeast extract, 9.0 g/liter; mannitol, 10.0 g/liter; sodium chloride, 55.0 g/liter; lithium chloride, 5.0 g/liter; aniline blue, 0.2 g/liter; and agar, 12.5 g/liter. This agar base can be supplemented with selective ingredients to detect oxacillin resistance (ORSAB+). These consist of polymyxin B at 50,000 IU/liter and oxacillin at 2.0 mg/liter. The agar base with supplement was used to identify MRSA. The isolates were inoculated on Columbia agar plates with 5% sheep blood and incubated for 24 h at 35°C. From the resulting cultures a suspension of a 0.5 McFarland standard was made, and subsequently a swab was dipped in the suspension and streaked on the agar plate (with or without supplement). The results were read after 24 and 48 h of incubation at 35°C. Growth of colonies showing an intense blue coloration was considered to be positive (indicating *S. aureus*). The procedure is as recommended by the manufacturer.

Statistical analysis. Technicians who were blinded for the characteristics of the strains performed the evaluations. To determine statistical significance, the chi-square test was used. Statistical significance was accepted when the P value was <0.05.

RESULTS

The results obtained with CSA and ORSAB without a supplement containing antibiotics are shown in Table 1. The sensitivities of both CSA and ORSAB to detect *S. aureus* after 24 h were high, i.e., 98.6 and 97.1%, respectively. After 48 h the sensitivity of ORSAB was identical and that of CSA was slightly lower. The specificities of both media were highest after 24 h of incubation. For CSA the specificity after 48 h was significantly lower than that after 24 h. Also, the specificities of ORSAB after 24 and 48 h were significantly lower than that of CSA. Table 2 shows the results obtained with a supplement containing antibiotics. The sensitivity of CSA+ for detecting MRSA was significantly lower than of ORSAB+ at both times. For both media the sensitivity was higher after 48 h of incubation. The specificity for detecting MRSA, as measured with MSSA and CNS, was high for both media after 24 h. After 48 h it was lower (Table 2).

TABLE 1. Results with CSA and ORSAB without supplement after 24 and 48 h of incubation

Organisms	Medium	No. of strains with a positive test result/total number of strains (%)[a] after:	
		24 h	48 h
MSSA	CSA	352/358 (98.3) a	341/358 (95.3) a
	ORSAB	347/358 (96.9)	347/358 (96.9)
MRSA	CSA	263/266 (98.9)	266/266 (100) b
	ORSAB	259/266 (97.4)	259/266 (97.4) b
All *S. aureus*	CSA	615/624 (98.6)	607/624 (97.3)
	ORSAB	606/624 (97.1)	606/624 (97.1)
CNS	CSA	15/516 (2.9) ce	31/516 (6.0) cf
	ORSAB	41/516 (7.9) de	61/516 (11.8) df

[a] Values with the same letter are significantly different ($P < 0.05$).

DISCUSSION

In this in vitro evaluation, CSA and ORSAB were tested for their capacities to detect *S. aureus* and to screen for methicillin resistance. The CSA performed better than ORSAB for identifying *S. aureus*. The sensitivities of both media were comparable, but the specificity of CSA was significantly higher, with the best results for both media obtained after 24 h of incubation. The results with ORSAB were highly consistent after 24 or 48 h of incubation. CSA showed some isolates with inconsistencies. Sometimes, strains which were considered positive after 24 h were negative after 48 h. As the results were read blindly, this may be due to variability in the interpretation of the observer or to a true discoloration after prolonged incubation. Whatever the cause may be, we recommend reading CSA plates after 24 h, as this gives a more accurate and faster result.

To screen for MRSA, antibiotic supplements were added to the plates. The results obtained were less favorable. For CSA+ a very high specificity was observed, as none of the MSSA isolates grew on the plates and only 1.6% of the CNS gave false-positive results after 24 h. However, after 24 h only 58.6% of the MRSA isolates gave positive results. Prolonging of the incubation period improved the sensitivity to 77.5%, but the percentage of CNS that gave false-positive results also increased to 8.9%. ORSAB+ was more sensitive than CSA+,

TABLE 2. Results with CSA+ and ORSAB+ after 24 and 48 h of incubation

Organisms	Medium	No. of strains with a positive test result/total number of strains (%)[a] after:	
		24 h	48 h
MSSA	CSA+	0/358 (0)	0/358 (0) b
	ORSAB+	3/358 (0.8) a	19/358 (5.3) ab
MRSA	CSA+	156/266 (58.6) ce	206/266 (77.5) cf
	ORSAB+	224/266 (84.2) de	243/266 (91.4) df
CNS	CSA+	8/516 (1.6) g	46/516 (8.9) gh
	ORSAB+	12/516 (2.3)	20/516 (3.9) h

[a] Values with the same letter are significantly different ($P < 0.05$).

after both 24 and 48 h. The specificity was highest after 24 h. Only 0.8% of MSSA isolates and 2.3% of CNS gave false-positive results at that time. Apparently, the higher concentration of oxacillin in the CSA+ inhibited the growth of several MRSA isolates. Also, the higher salt concentration in the ORSAB+ may have stimulated the expression of the *mecA* gene. On the other hand, a high salt concentration by itself is reported to inhibit certain strains (5). However, all MSSA and MRSA isolates showed growth, blue or white, on the ORSAB plates without supplement. Therefore, we could not confirm that the inhibitory effect of salt at a concentration of 5.5% played a role.

Other investigators have studied the clinical performance of these selective media. Simor et al. compared ORSAB+ with a conventional MSA plate supplemented with 2.0 mg of oxacillin per liter for the detection of MRSA in clinical specimens (11). The sensitivities were comparable, but the specificity of ORSAB+ was much higher. A comparison with nonselective media was not made. Gaillot et al. compared CSA with conventional detection of *S. aureus* on 5% horse blood agar plates with 2,000 consecutive clinical samples (3). The sensitivity of CSA was significantly higher (95.5 versus 81.9%), whereas the specificities were comparable (approximately 99%). Carricajo et al. compared CSA with conventional media, which were not further specified, with 775 clinical samples (2). Again the sensitivity of CSA was better than that of the conventional media (98.5 versus 91.8%). The specificity also was high (97%). Finally, Merlino et al. performed a study on a small sample of 136 staphylococci, of which 114 were *S. aureus* and 22 were CNS (8). CSA was compared with conventional MSA, DNase agar plates, and the tube coagulase test. All media had a high sensitivity (98% or higher). CSA and the tube coagulase test gave no false-positive results, whereas the DNase and MSA had 4.6 and 36.5% false positives, respectively. Those authors also supplemented the medium with methicillin at a concentration of 4.0 mg/liter. None of the MSSA isolates grew on the plates, and all 36 high-level-resistant MRSA isolates grew suspect colonies. However, a collection of 12 community-acquired MRSA strains were also tested. Only 4 of these 12 strains (33.3%) grew on the supplemented CSA. This seems to be consistent with our findings that a substantial proportion of MRSA strains do not grow on CSA supplemented with 4.0 mg of oxacillin or methicillin per liter. Our collection of MRSA strains consists of 266 strains that are part of the national collection of RIVM of The Netherlands. In The Netherlands, the incidence of MRSA is still very low (<1%), and all MRSA strains isolated are sent to RIVM for surveillance, typing, and further testing. Strains were selected based on having a unique phage type. Therefore, all strains can be considered unique. In this respect the present evaluation is totally different from studies using consecutive clinical samples in an setting of endemicity, where only a limited number of types will be tested. Since MRSA is a prime example of clonal spread of resistance, a clinical survey will likely yield only a few different types. If one of these types is not detected by the test under investigation, this will have great impact on the performance. On the other hand, if the local strain is correctly identified, it may overestimate the performance in other settings. In the present study a strain was included only once and many different strains were tested. Therefore, this study can show how the test performs with a broad array of different strains. The translation of these results to a local situation still can be influenced significantly if certain epidemic clones of MRSA exist. However, the results are easier to interpret and translate.

In conclusion, both CSA and ORSAB are rapid and highly sensitive for identifying *S. aureus*. However, the specificity of CSA was significantly higher than that of ORSAB. The addition of antibiotics to screen for MRSA resulted in a low sensitivity for CSA+. ORSAB+ was more sensitive and was highly specific after 24 h. However, approximately 15% of the MRSA isolates tested were not detected at that time.

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