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Soft Salt-Mannitol Agar–Cloxacillin Test: a Highly Specific Bedside Screening Test for Detection of Colonization with Methicillin-Resistant *Staphylococcus aureus*

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The early detection of colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) of patients in intensive-care units is an essential step in the strategy for preventing MRSA epidemics. In this study, tubes containing soft salt-mannitol agar with cloxacillin (6 μg/ml) (SSMAC) were prepared for inoculation of clinical samples at patients' bedsides by personnel of an intensive-care unit. A total of 1,914 swabs from different sample sites of 81 patients were dipped into SSMAC tubes, and after 24 h of incubation (in an incubator located near the intensive-care unit), an evident color change was considered by the intensive-care-unit personnel to be an MRSA alarm. Sixty-three (3.3%) SSMAC tubes were considered positive for MRSA, 1,827 (95.4%) were considered negative, and 24 (1.2%) were considered intermediate. Compared with values for parallel conventional surveillance cultures for MRSA, excluding tubes with intermediate results, the SSMAC test had a sensitivity of 72.7%, a specificity of 99.2%, a positive predictive value of 76.2%, and a negative predictive value of 99.0%. When intermediate tubes were considered positive, the corresponding values were 75.3, 98.2, 63.2, and 99.0%, respectively. The sensitivity and specificity values of the test to identify MRSA-colonized patients were 89.4 and 100%, respectively. Oropharyngeal and naris specimens were the most reliable samples for MRSA detection. False-negative results were frequent in bronchial aspirates with low (<10^3 to 10^6 CFU/ml) MRSA counts. False-positive results were mainly due to methicillin-resistant *Staphylococcus haemolyticus*. The SSMAC tube is a useful, rapid, and inexpensive tool for the early identification of MRSA-colonized patients and, consequently, for the implementation of measures to prevent the spread of MRSA.

Staphylococcus aureus infections are a major cause of morbidity and mortality, particularly in hospitalized patients. Multidrug-resistant *S. aureus* isolates, especially methicillin-resistant *S. aureus* (MRSA) strains, remain a major clinical and epidemiological problem in hospitals, as they are easily transferred among hospital personnel and patients, particularly in intensive-care units (12). Because MRSA infections are usually preceded by a period of carriage, the rapid identification and isolation of MRSA-colonized patients are essential for the implementation of appropriate control measures (20, 26). Therefore, specific and sensitive tests for early detection are necessary to identify and control these infections in order to prevent the spread of MRSA isolates.

New rapid techniques involving genetic or immunologic assays have been developed for the identification of MRSA (11, 21). Nevertheless, the practical availability of these techniques for routine use with every new patient admitted to an intensive-care unit is limited, due to high costs and the need of trained personnel. Conventional clinical laboratory protocols involve plating specimens on nonselective media and then selecting a few staphylococcus-like colonies for identification and susceptibility testing. Low-level MRSA colonization may be missed due to overgrowth of normal flora or the presence of a mixture of MRSA and methicillin-susceptible strains. Other protocols involve salt-mannitol agar containing methicillin or oxacillin as a screening medium (2, 8, 14, 15, 30). By using these selective and differential media, it is possible to increase the rate of recovery of MRSA isolates.

The aim of this work was to assess the diagnostic value of test tubes with soft salt-mannitol agar incorporating 6 μg of cloxacillin per ml (SSMAC) for early detection of MRSA. The test tubes were designed to be inoculated and read by intensive-care-unit-based personnel. The utility of such a bedside strategy was evaluated in an intensive-care unit with a high prevalence of MRSA (higher than 20%).

MATERIALS AND METHODS

Patients and samples. A total of 1,914 swabs from different sources (bronchial aspirates and oropharyngeal, rectal, hair, naris, axilla, and groin samples) were obtained from 81 long-term-intubated patients from an intensive-care unit from a 400-bed teaching institution, and the specimens were cultured in SSMAC test tubes. The sample distribution is expressed in Table 1. In addition, three times a week, samples from the same locations were recovered in parallel for conventional culture tests. The background prevalence of MRSA colonization in this unit was 22% at the beginning of the study. The ages of the patients ranged from 19 to 97 years (mean ± standard deviation, 66.3 ± 15), with a male/female ratio of 3.5. The mean stay in the intensive-care unit was 14.3 (±16.5) days, and the mortality rate of MRSA-colonized patients was 38.6%.

Selective SSMAC cultures and conventional cultures. SSMAC test tubes were prepared weekly in the laboratory. The culture medium included D-mannitol (1%; Merck, Darmstadt, Germany), NaCl (10%), Bacto Peptone (1%; Difco, Detroit, Mich.), beef extract (0.1%; Oxoid, Basingstoke, United Kingdom), and agar (0.8% at pH 7.4; Pronadisa, Madrid, Spain) and was supplemented with cloxacillin (6 μg/ml; SmithKline Beecham, Madrid, Spain). Phenol red was used as a pH indicator. Test tubes were stored at 4°C for a maximum of 15 days until they were required. Cotton swabs from different sources were deeply introduced into the SSMAC test tubes, and the tubes were incubated at 35°C for 24 h in a small incubator located near the intensive-care unit. The intensive-care-unit personnel both performed the inoculation and read the results. The appearance of a bright-yellow color in the test tube was presumptively considered positive for MRSA colonization. An orange to pale-yellow color was considered an intermediate result, and a pale-red color was considered a negative test. Conventional

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TABLE 1. Diagnostic yields of SSMAC test tubes by sampling site

Sampling site (no. of samples)	No. of SSMAC test tubes with indicated result				
	True positive	False positive	True negative	False negative	Intermediate
Bronchial aspirate (329)	5		314	9	1
Nares (314)	9	1	300	1	3
Oropharynx (334)	24	3	295	4	8
Groin (207)	1	4	197	1	4
Axilla (205)	1	1	200		3
Hair (211)	1		210		
Rectum (314)	7	6	293	3	5
Total (1,914)	48	15	1,809	18	24

cultures were performed by direct inoculation of duplicate swabs on Columbia sheep blood (5%) agar and salt-mannitol agar (BioMerieux, Marcy l'Etoile, France). A test was considered a true positive when the SSMAC test was positive and subculture yielded an MRSA isolate. If subculture was negative for MRSA, the result was considered a false positive. A test was considered a true or false negative when the SSMAC test was negative and conventional culture was negative or positive for MRSA, respectively.

Confirmatory tests for MRSA. All positive and intermediate SSMAC test tubes were subcultured on Columbia sheep blood (5%) and salt-mannitol agar to obtain the organisms responsible for growth and color change. Colonies suspected to be *S. aureus* were first identified by means of Gram stain, catalase reaction, coagulase, and DNase tests. Biochemical identification was completed with a semiautomatic identification system (PASCO system; Difco).

Susceptibility testing. Methicillin resistance was investigated by the standard disk diffusion method with 1 μg of oxacillin of the National Committee for Clinical Laboratory Standards (22) and confirmed with oxacillin MICs determined by the microdilution PASCO system (Difco). MRSA ATCC 33591 and methicillin-susceptible ATCC 29213 and ATCC 25923 were used as quality control strains. MRSA identification was confirmed by hybridization with a specific *mec* probe as previously published (7).

MRSA detection threshold in SSMAC test tubes. Different bacterial suspensions (ranging from 10^2 to 10^9 CFU/ml) were prepared with overnight brain heart infusion broth cultures of (i) MRSA ATCC 33591, (ii) β-lactamase-positive methicillin-sensitive *S. aureus* ATCC 29213, and (iii) methicillin-resistant *Staphylococcus haemolyticus* 58HPA/94. Test tubes were inoculated with a wet swab from each dilution. Swabs were dipped into the bacterial suspension, and the excess fluid was removed by pressing the swab against the side of the tube. Then swabs were introduced into the SSMAC test tubes.

RESULTS

According to the confirmed cultures obtained with both the SSMAC screening test tubes and the conventional cultures, 19 of the 81 intubated intensive-care-unit patients (23.4%) included in this study were MRSA colonized. A total of 1,914 SSMAC test tube cultures were performed: 63 (3.3%) were considered positive for MRSA, 24 (1.2%) were considered intermediate (i.e., doubtful), and 1,827 (95.4%) were considered negative for MRSA.

Table 1 shows diagnostic yields by sampling site. The corresponding values for sensitivity, specificity, and positive and negative predictive values, excluding intermediate results, were 72.7, 99.2, 76.2, and 99.0%, respectively (Table 2). Fifteen test tubes (0.7%) yielded false-positive results. Methicillin-resistant coagulase-negative staphylococci (MRCNS), consisting mainly of *S. haemolyticus*, accounted for these results and in most cases were recovered from rectum, groin, and oropharynx samples (Tables 1 and 3). A comparison of results of screening by the SSMAC test tube method and conventional cultures demonstrated that 18 test tubes (0.9%) accounted for false-negative results. Nine and four were from bronchial-aspirate and naris samples, respectively (Table 3).

Only 1.2% of test tubes (24 of 1,914) yielded doubtful results. When the contents of these intermediate test tubes were considered positive or negative for MRSA, the specificity values of the test (98.2 or 99.2%, respectively) were similar to those obtained when intermediate test tubes were excluded (99.2%). The corresponding values for sensitivity and positive and negative predictive values when intermediate test tubes were considered positive or negative were, respectively, 75.3 or 65.7%, 63.2 or 76.1%, and 99.0 or 98.6% (Table 2). MRCNS (58.3% of cultures with intermediate results) and MRSA (29.1% of cultures with intermediate results) were the organisms recovered most frequently from test tubes with intermediate results and were isolated mainly from oropharynx, rectum, and groin samples. In addition, *Proteus* spp. plus MRCNS were also observed in subcultures of 12.5% of these test tubes (Table 4).

On the other hand, the small percentage of intermediate results made them irrelevant for determining the validity of the SSMAC test in detecting patients with MRSA. With the exception of two patients (11.7%), MRSA-colonized patients had at least one positive SSMAC test tube result. The sensitivity, specificity, and positive and negative predictive values for the SSMAC test in detecting colonized patients (as opposed to sampling sites), assuming the above-mentioned two patients were MRSA positive, were 89.4, 100, 100, and 96.8%, respectively.

The sampling sites with the highest yield for MRSA colonization detection with SSMAC test tubes were the nares, oropharynx, and rectum. Specificity and negative predictive values for these sites were higher than 98% (Table 2). Moreover, the

TABLE 2. Diagnostic values of the SSMAC test, indicating the most efficient sampling sites

Sampling site(s)	Diagnostic value (%)			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
All sample sites				
Excluding intermediate results	72.7	99.2	76.2	99.0
Including intermediate results as positive results	75.3	98.2	63.2	99.0
Nares[a]	90.0	99.6	90.0	99.6
Oropharynx[a]	85.7	99.0	88.8	98.7
Rectum[a]	70	98	53.8	98.7
Bronchial aspirate[a]	35.7	100	100	97.2
Nares + oropharynx + rectum				
Excluding intermediate results	83.3	98.9	80.0	99.1
Including intermediate results as positive results	87.5	98.9	89.8	99.1

[a] Excluding intermediate results.

TABLE 3. Isolates recovered from SSMAC test tubes with positive results

Organism(s) (no. of positive test tubes)	No. of positive test tubes from sample from:						
	Bronchial aspirate	Nares	Oropharynx	Groin	Axilla	Hair	Rectum
MRSA (48)	5	9	24	1	1	1	7
MRCNS[a] (15)	0	1	3	4	1	0	6
Total (63)	5	10	27	5	2	1	13

[a] Including *S. haemolyticus.*

TABLE 4. Isolates recovered from SSMAC test tubes with intermediate results

Organism(s) (no. of intermediate test tubes)	No. of intermediate test tubes from sample from:						
	Bronchial aspirate	Nares	Oro-pharynx	Groin	Axilla	Hair	Rectum
MRSA (7)	1	1	4	1	0	0	0
MRCNS[a] (14)	0	2	3	3	3	0	3
MRCNS[a] + *Proteus* spp. (3)	0	0	1	0	0	0	2
Total (24)	1	3	8	4	3	0	5

[a] Including *S. haemolyticus.*

oropharynx (47.4%) and nares (21.0%) were the sites yielding the earliest positive results. The SSMAC test provided earlier detection than conventional cultures of most MRSA-colonized patients: MRSA in 89.5% of the colonized patients was detected with SSMAC tubes 24 h earlier than by conventional plating procedures.

The ATCC 33591 MRSA strain produced a bright-yellow color when it was inoculated into SSMAC test tubes. For this strain, the lowest MRSA bacterial concentration reliably detected, under the conditions described in Materials and Methods, was 10^6 CFU/ml. Negative results were always observed with methicillin-sensitive *S. aureus* ATCC 29213. With the methicillin-resistant strain *S. haemolyticus* 58HPA/94, a high inoculum (10^9 CFU/ml) was required to produce a positive result in the SSMAC test tubes.

DISCUSSION

There are many studies comparing the abilities of different methods to detect methicillin resistance in *S. aureus*. They usually employ colonies of previously identified *S. aureus* isolates (9, 23–25, 31) that have been screened for growth on selective agar with methicillin or oxacillin (1, 2, 8, 10, 13–15, 27, 28, 30), or they employ different standardized susceptibility testing methods for the discrimination of MRSA from susceptible or borderline-resistant *S. aureus* (6, 16, 29). A few studies have evaluated the simultaneous detection and identification of MRSA isolates by plating clinical surveillance specimens in selective agar media (8, 14, 30, 32). To our knowledge, however, none of these studies used screening methods for bedside detection of MRSA-infected or -colonized patients. An SSMAC test was therefore prospectively evaluated by directly culturing clinical samples in test tubes. Samples were recovered from 81 long-term-intubated patients from an intensive-care unit. Seven samples per patient were obtained daily from different locations, and in addition, samples from the same locations were recovered in parallel three times a week for conventional cultures. The prevalence of MRSA colonization in this intensive-care unit during the study period was 23.4%, slightly higher than that obtained during the 4 months before we started the study (22%).

The use of cloxacillin and high concentrations of NaCl prevented the growth in test tubes of methicillin-sensitive *S. aureus* isolates and enteric organisms, respectively. A high salt concentration (7.5%), as described originally by Chapman (5), facilitates the isolation of *S. aureus* from clinical specimens where colonization with commensal flora makes isolation difficult. The increase of NaCl to 10% in the test tube was to reduce the number of intermediate test results. Preliminary testing of the method with a 7.5% NaCl content showed that an intermediate result in test tubes, mostly those inoculated with rectal swabs, was caused mainly by the presence of *Enterococcus* spp. organisms. By increasing the NaCl to 10%, *Enterococcus* spp. organisms were no longer recovered. The rate of test tubes positive for MRSA did not change for test tubes containing 10% NaCl (data not shown). In addition, NaCl has been shown to increase penicillin-binding protein 2a production in *S. aureus* and thus enhance the expression of methicillin resistance in heterogeneous strains (4, 17). Moreover, the addition of NaCl to the medium might improve the stability of penicillinase-stable penicillins during storage (3). One month of storage in a conventional refrigerator did not decrease the ability of the tubes to detect MRSA. Nevertheless, to avoid false-positive results due to possible cloxacillin deterioration, test tubes were routinely replaced by fresh ones 15 days after their manufacture. In our hands, a 24-h incubation of test tubes was preferable to a 48-h incubation, as has been recommended by other authors (19), with different agar media for recognition of methicillin resistance and also for standardized susceptibility testing (16, 22) of penicillinase-stable penicillins against *S. aureus*. False-positive results were observed when incubation was prolonged for 48 h. These results were due mainly to the growth of both methicillin-susceptible and -resistant *S. haemolyticus* and methicillin-susceptible *S. aureus.*

Results obtained from our study demonstrate that SSMAC medium in test tubes is a highly specific screening medium for MRSA colonization (>99%). Moreover, a low number of false-positive results were obtained and the negative predictive value was nearly 100%. However, sensitivity and positive predictive values were lower: 72.7 and 76.2%, respectively. The sensitivity score and positive predictive values were higher for naris and oropharynx samples, with no decline in the specificity and positive predictive values, indicating that they were the most efficient sampling sites for detection of MRSA colonization with test tubes. These sampling sites, in addition to surgical wounds, endotracheal or tracheostomy tubes, burns, or other extensive cutaneous lesions, have been observed to provide the most efficient and useful clinical samples for MRSA screening (12). It is interesting that a three-sample test, including samples from the nares, oropharynx, and rectum, increased the sensitivity of the study to 87.5% and provided a specificity of 98.9% (Table 2).

In our experience, bronchial aspirates had a low sensitivity for detection of MRSA colonization with test tubes, as a high number of false-negative results were observed. This result is related to the low bacterial inoculum, as all bronchial aspirates with false-negative SSMAC test tube results had MRSA counts ranging from $<10^3$ to 10^6 CFU/ml in conventional quantitative cultures. Moreover, all but one patient with a false-negative bronchial aspirate had positive SSMAC oropharyngeal test tube results.

Some isolates of coagulase-negative staphylococci (e.g., *S. haemolyticus*, *Staphylococcus saprophyticus*, and *Staphylococcus intermedius*) have been found to use mannitol. In our study, high rates of false-positive results and intermediate results were due to the recovery of methicillin-resistant *S. haemolyticus*. A similar observation was made with other screening test media; methicillin-resistant *S. haemolyticus* isolates produced numbers of colonies in mannitol-salt-oxacillin-tellurite medium similar to those produced by MRSA isolates (18).

The usefulness of the SSMAC test as a screening test for the early detection of patients colonized with MRSA is based on the high sensitivity (89.4%) and specificity (100%) values obtained in this study. The SSMAC test tube can be a helpful complement to MRSA clinical microbiology surveillance by standard methods. Such methods require a minimum of 2 to 4 days from specimen collection to obtain the laboratory alert for

MRSA. The SSMAC test tube detection strategy provides reliable data in only 24 h. Communication time is also saved, as the clinical personnel in the intensive-care unit, who are present 24 h/day, perform inoculation and take readings. Intensive-care-unit personnel can adopt immediate isolation procedures after an MRSA alert provided by a positive SSMAC test tube. The most critically exposed patients are those with high bacterial counts in different samples ("cloud patients"), and most of them can be reliably detected by the SSMAC test. Nevertheless, all positive and intermediate SSMAC test tubes should be sent to a microbiology laboratory to confirm the MRSA colonization.

In summary, the SSMAC test tube technique is an easy, rapid, and highly specific test for MRSA detection at the bedside of the patient and may lead to the development of prompt strategies to prevent the spread of MRSA among patients.

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