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Evaluation of a Novel Medium for Screening Specimens from Hospitalized Patients To Detect Methicillin-Resistant *Staphylococcus aureus*

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A novel medium, Oxacillin Resistant Screening Agar (ORSA) medium, was evaluated for the screening of specimens for methicillin-resistant *Staphylococcus aureus* (MRSA) in the hospital setting. Screening swabs (swabs of the nose, throat, perineum, and infected sites) were inoculated onto the new ORSA medium and into an enrichment broth (Muller-Hinton broth supplemented with NaCl and oxacillin). After 24 h of incubation, the enrichment broth was subcultured onto one ORSA plate and one lipovitellin Chapman salt agar plate. The sensitivities for the detection of MRSA were calculated for each medium alone and for the media in combination. A low sensitivity (74%) was obtained when ORSA medium was used alone as a primary culture, whereas the sensitivity was 88% when a single selective enrichment broth was used. Among the 414 blue colonies observed on ORSA plates, only 47% were found to be MRSA, 40% were coagulase-negative staphylococci, 7% were *Enterococcus* species, and 2% were methicillin-sensitive *S. aureus*. The optimal incubation time for the ORSA plates was evaluated. On primary culture, 38% of the blue MRSA colonies were visible only after 48 h of incubation (no blue colonies were not seen after 24 h of incubation), whereas 94% of the colonies were already visible at 24 h when ORSA plates were used for subcultures. In conclusion, the advantage of the novel ORSA medium is the ease of recognition of mannitol-fermenting bacteria, but further identification tests are needed to confirm the identification of *S. aureus*. An enrichment broth is still needed to ensure a good sensitivity for the recovery of MRSA, and an incubation time of 48 h is required for primary culture on ORSA medium.

The incidence of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) continues to increase worldwide. Rapid assessment of clinical specimens for the presence of MRSA is an important part of the infection control measures taken to control the spread of MRSA and, thus, to decrease hospitalization costs. In hospitals with low rates of MRSA, it is probably important to detect each patient colonized or infected with MRSA. Thus, the negative predictive value of the screening test should be high. Although PCR-based methods have recently been developed for the direct detection of MRSA in specimens (2, 3), these methods remain expensive and cannot be done in every laboratory. Evaluation of their results, as well as their costs and benefits in terms of patient management and infection control procedures, should be undertaken. Thus, new screening media for the improved detection of MRSA have recently been developed (6–8). In this study, we evaluated the usefulness of one of these novel media, the Oxacillin Resistant Screening Agar (ORSA) medium (Oxoid Limited, Basingstoke, England), for the screening of specimens for MRSA in a hospital setting. This medium uses aniline blue to detect mannitol fermentation in staphylococci. The antibiotic supplement (100,000 IU of polymyxin B and 2.0 mg of oxacillin per liter) and sodium chloride concentration (5.5%) have the potential to reduce the growth of nonstaphylococcal bacteria and select for MRSA. The study was designed to evaluate (i) the sensitivity of the procedure for the recovery of MRSA from patient specimens by using ORSA base medium alone as a primary culture medium or as an enrichment broth medium for subculture, (ii) the proportion of samples that do not contain MRSA but that show the growth of blue colonies on ORSA medium, and (iii) the optimal incubation time.

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MATERIALS AND METHODS

Setting. The University Hospital of Lausanne is an 870-bed tertiary-care hospital. The proportion of MRSA isolates among all *S. aureus* isolates in the hospital was previously found to be <5% (1) and can thus be considered low. Screening samples were obtained from the following individuals: (i) rehospitalized patients known to have been positive for MRSA during a previous hospitalization, (ii) patients transferred from a foreign hospital, (iii) the roommates of a patient known to be infected or colonized with MRSA, (iv) staff members and/or patients from a ward with a suspected cluster of cases of MRSA infection, and (v) patients screened after treatment for MRSA eradication. The screening samples obtained from each patient included swabs (sterile transport swab; Copan, Brescia, Italy) of the nose, the throat, the perineum, and all infected sites. The nose, throat and perineal swabs from all patients with a negative or unknown history of MRSA infection or colonization were pooled for each analysis.

Bacteriology. Each specimen was inoculated onto two primary culture media: an ORSA base medium plate composed of (per liter) peptone (11.8 g), yeast extract (9.0 g), mannitol (10.0 g), NaCl (55.0 g), lithium chloride (5.0 g), aniline blue (0.2 g), and agar (12.5 g) selectively supplemented with 100,000 IU of polymyxin B and 2.0 mg of oxacillin (Oxoid Limited) and Mueller-Hinton broth composed of (per liter) beef extract (3.0 g), acid hydrolysate of casein (17.5 g), and starch (1.5 g; BBL Becton Dickinson, Heidelberg, Germany) supplemented with 6 mg of oxacillin per liter and 45 g of NaCl per liter (OX-MH broth). After

TABLE 1. Sensitivities for detection of MRSA in each medium alone or in combination

Procedure		No. with MRSA[a]	Sensitivity (%)
Primary culture	Subculture of OX-MH broth		
ORSA plate		823	74
OX-MH broth	ORSA plate	979	88
OX-MH broth	LSM plate	1,002	90
OX-MH broth	ORSA and LSM plates	1,049	94
ORSA and OX-MH broth	ORSA plate	1,092	98
ORSA and OX-MH broth	ORSA and LSM plates	1,112	100

[a] The number of specimens found to be positive for MRSA among the 1,112 MRSA-positive specimens.

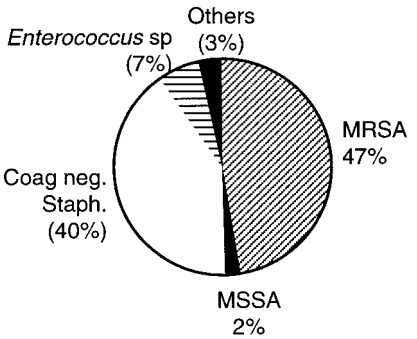

FIG. 1. Distributions of the principal species or genera of the 414 blue colonies from 1,427 specimens observed on ORSA medium. MSSA, methicillin-sensitive *S. aureus*; Coag. neg., coagulase negative.

24 h of incubation, the OX-MH broth was subcultured onto one ORSA plate and one lipovitellin salt agar (lipovitellin salt-mannitol [LSM]) plate composed of (per liter) pancreatic digest of casein (5.0 g), peptic digest of animal tissue (5.0 g), beef extract (1.0 g), NaCl (75.0 g), D-mannitol (10.0 g), phenol red (25 mg), and agar (15.0 g; BBL Becton Dickinson) supplemented with 20 g of egg yolk per liter (5). All agar plates were incubated aerobically for 48 h at 35°C and were read every day.

Each morphotype of mannitol-fermenting colonies was subcultured onto a blood agar plate and identified by conventional biochemical tests, including Gram staining and catalase, DNase, and latex agglutination (Pastorex Staph Plus; Bio-Rad, Redmond, Wash.) tests. Resistance to methicillin was determined by the disk diffusion method and the oxacillin agar screen test according to the guidelines of the National Committee for Clinical Laboratory Standards (4). The MRSA-Screen (Denka-Seiken, Tokyo, Japan) was also used on several occasions to confirm the resistance. Species identification of isolates other than *S. aureus* was performed with the Vitek 2 automated system (bioMérieux, Marcy l'Etoile, France) and the ID32 Strep test (bioMérieux).

RESULTS

In order to optimize the procedure for the detection of MRSA, the sensitivity of each medium alone or in combination was analyzed. From February 2001 to January 2002, 7,929 specimens or pools of specimens were analyzed. These specimens were obtained from 2,157 patients or staff members. Among these specimens, 1,112 (14%) were positive for MRSA. This served as the reference value to define a sensitivity of 100%. The sensitivity for the detection of MRSA was then calculated for each medium alone or the two media in combination (Table 1). A low sensitivity (74%) was obtained when ORSA medium alone was used as a primary culture. The sensitivity increased with the number of media included in the procedure.

In order to evaluate the proportion of blue colonies growing on ORSA plates that were not MRSA, we analyzed all specimens or pools of specimens (n = 1,427) recovered from April to August 2001 for which complete data were available after 24 and 48 h of incubation. They were obtained from 540 patients or staff members. Blue colonies grew from 399 of these specimens. Among the 414 blue colonies (some specimens showed the presence of different morphotypes), 47% were found to be MRSA, 40% were coagulase-negative staphylococci, 2% were methicillin-sensitive *S. aureus*, and 7% were *Enterococcus* species (*Enterococcus faecalis* or *Enterococcus gallinarum*) (Table 2; Fig. 1). Among the 166 coagulase-negative staphylococci, a subset of 76 isolates was further identified to the species level. Thirty-seven (48.7%) were *Staphylococcus haemolyticus*, 11 (14.4%) were *Staphylococcus warneri*, 3 (3.9%) were *Staphylococcus epidermidis*, 2 (2.6%) were *Staphylococcus cohnii*, 1 (1.3%) was *Staphylococcus hominis*, and 22 (28.9%) were *Staphylococcus* species not identified to the species level with the Vitek 2 automated system.

Twenty-two (52%) of the nonpooled nose, throat, and perineal swab specimens from which blue colonies that were not *S. aureus* (n = 42) grew were perineal swabs. In addition, 78 primary ORSA medium cultures of pooled specimens could be retrospectively analyzed since photographs were taken at 24

TABLE 2. Distributions of blue colonies on ORSA plates at 48 h of incubation (primary cultures and subcultures) by species and sampling site (one morphotype per analysis)

Sample(s)	No. (%) of specimens[a]						
	Total	Blue colonies	MRSA	MSSA	Coag. neg. staphylococci	*Enterococcus* sp.	Others
Nose, throat, and perineal swabs	570	169	13	4	127	14	11
Nose swab	166	52	41	1	6	4	0
Throat swab	166	69	58	1	4	4	2
Perincal swab	165	60	38	0	18	4	0
Wound swab	126	38	31	2	4	1	0
Urine	192	17	10	0	5	1	1
Other	42	9	5	1	2	1	0
Total	1,427	414 (100)	196 (47.3)	9 (2.1)	166 (40.1)	29 (7.0)	14 (3.4)

[a] MSSA, methicillin-susceptible *S. aureus*; Coag. neg., coagulase negative.

and 48 h of incubation. According to the positions of the blue colonies on the plate, 68 of them (79%) could be attributed to perineal swabs. Among the blue colonies that grew from the pooled nose, throat, and perineal swab specimens, 152 of 169 (89.9%) were not *S. aureus*, whereas among the blue colonies that grew from nonpooled swab specimens of the same sites, only 42 of 181 (23.2%) were not *S. aureus*. This difference might be explained by the fact that nonpooled specimens were mainly obtained from patients colonized or infected with MRSA after treatment for MRSA eradication.

The optimal incubation time for the ORSA plates was evaluated with the data for the 196 MRSA colonies recovered during the period from April to August 2001. Among the 144 MRSA colonies that were detected on primary culture, 55 (38%) were visible only after 48 h of incubation (no blue colonies were observed after 24 h of incubation). However, among the 79 MRSA colonies detected on the ORSA plate used for subculture, 94% were detected after only 24 h of incubation.

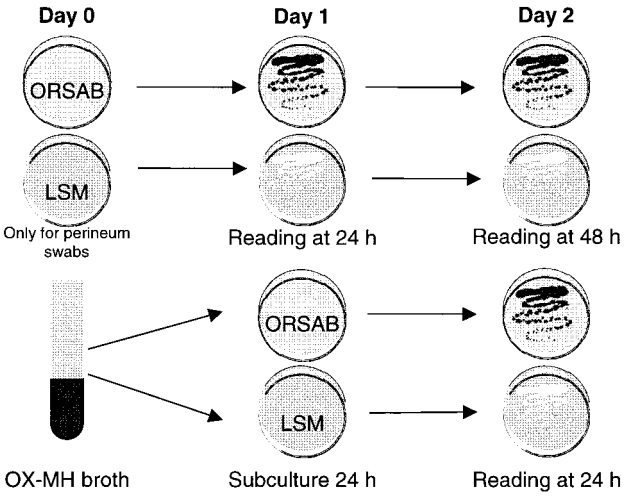

FIG. 2. Laboratory procedure for the screening of MRSA with ORSA medium. ORSAB, ORSA base medium. (Darker and lighter streaks on the plates represent blue and yellow colonies on ORSA and LSM plates, respectively.)

DISCUSSION

The use of a laboratory procedure that is sensitive for the detection of MRSA from patient specimens is crucial if one wants to limit the spread of this multiresistant microorganism. The use of an enrichment and selective broth has always been proposed for that purpose. The drawback of this method, which has a high sensitivity, is a prolonged delay between the time of specimen collection and the time that results are available (in general, a minimum of 3 days). Thus, methods should be optimized in order to reduce the laboratory workload and decrease this delay between the time of collection and the time that results are available without a loss of the sensitivity of the method. Our results show that one cannot avoid the use of an enrichment broth without a substantial loss of sensitivity. However, use of the ORSA plates as primary culture allowed the earlier detection of MRSA on several occasions (data not shown). Thus, ORSA plates and enrichment broth should be used for primary culture.

The advantage of the ORSA medium is the easy detection of mannitol-fermenting colonies (the appearance of a blue color). However, in our study, about half of the blue colonies were not MRSA. This proportion is higher than that reported in another study (26%) (7). This might be because in the latter study, fewer specimens from colonized sites (nares and perineum) were investigated. Indeed, our results show that most of these non-*S. aureus* mannitol-fermenting bacteria were isolated from perineal swabs.

As reported in the previous study (7) and confirmed in the present study, an incubation time of 24 h for a primary culture with ORSA plates was not sufficient for the detection of MRSA and the incubation time should be extended to 48 h. However, an incubation time of 24 h for a subculture in an enrichment broth is sufficient, probably because the bacteria are already in the growth phase in the broth.

These results have allowed us to optimize our protocol for the detection of MRSA in screening specimens (Fig. 2). The major advantage of this protocol is the reduced amount of time for the procedure (2 days instead of 3 days) because the ORSA subculture is incubated for only 24 h. The ORSA medium also has the advantage of easy interpretation, especially for specimens other than perineal swabs. The latter are now treated separately from the pool, as most of the non-MRSA blue colonies were observed in these specimens. Even though LSM did not significantly increase the sensitivity of the method, this medium was kept for the subculture of all specimens and for primary culture of perineal specimens. Indeed, the growth of mannitol-fermenting and lipase-positive colonies on the LSM plate together with the growth of blue colonies on the ORSA plates is highly suggestive of the presence of MRSA and thus offers the technician help with the identification of blue colonies on the ORSA plates. However, to reduce the workload in the laboratory, further tests for the identification of MRSA are performed only with suspected colonies growing on ORSA medium. The addition of the LSM plate was useful because it had not been possible to make a presumptive identification of *S. aureus* on ORSA plates on the basis of the morphologies of the blue colonies.

In conclusion, the main advantage of the novel ORSA medium is the ease of recognition of mannitol-fermenting bacteria. However, further identification tests are needed to confirm the identification of *S. aureus* since half of the blue colonies were not *S. aureus*. Moreover, an enrichment broth is still needed to ensure a good sensitivity of the method for the recovery of MRSA, and an incubation time of 48 h is required for the primary culture. Thus, the procedure still requires a minimum of 2 days before final results can be obtained.

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