USE OF A NOVEL SELECTIVE MEDIUM TO DETECT METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN COLONIZED PATIENTS OF AN INTENSIVE CARE UNIT

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ABSTRACT

BACKGROUND: Detection of colonized patients is important for implementing control measures for methicillin-resistant *Staphylococcus aureus* (MRSA). Laboratory detection of MRSA carriers is increased by the use of selective screening media, helping control dissemination of such organisms.

OBJECTIVE: To evaluate three different media, including selective and nonselective media, in the detection of MRSA from clinical specimens of patients of an intensive care unit (ICU).

PATIENTS: Adult patients in the ICU of the Hospital Mãe de Deus, Porto Alegre, Brazil.

METHODS: A total of 224 specimens were obtained from the nares of patients and plated on blood agar, mannitol salt agar containing 2 µg/mL of oxacillin (MSAO), and oxacillin resistance screening agar base (ORSAB). The presence of MRSA was investigated in typical colonies growing on the three types of media. Discrepant results were resolved by detection of the *mec*A gene by polymerase chain reaction and the modified penicillin binding protein known as PBP2'.

RESULTS: MRSA was detected in 32 (14.3%) of 224 specimens. Blood agar, MSAO, and ORSAB detected MRSA in 17, 31, and 28 specimens, respectively. After the coagulase test, no false resistance was observed.

CONCLUSION: MSAO and ORSAB performed equivalently in the detection of MRSA of colonized patients and require a single supplementary test (coagulase) to confirm MRSA (*Infect Control Hosp Epidemiol* 2004;25:130-132).

Nosocomial infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) are a worldwide problem, causing significant morbidity and mortality.[1] In a multicenter study in Brazil, *S. aureus* was the pathogen most frequently isolated in bloodstream and soft tissue infections and the rate of resistance to methicillin was 34.0%.[2] Aggressive control efforts toward MRSA are justified and deserve special attention. One component of such efforts is the identification of reservoirs for spread among patients and healthcare workers.[3,4]

Culture methods used to detect MRSA carriers directly from clinical specimens are based on enrichment media, selective media, or both. The selective approach uses chromogenic media with different inhibitors to enhance detection.[5-9] Studies comparing these procedures have reported variation in performance, workload, and turnaround time. A new selective medium, oxacillin resistance screening agar base (Oxoid Limited, Basingstroke, England), recently became available and has been the subject of investigations.[10,11] This novel medium contains aniline blue to demonstrate mannitol fermentation, has a higher NaCl concentration, and is supplemented with oxacillin and polymyxin B.

Resistance to methicillin in staphylococci is defined by the presence of the *mec*A gene. This gene encodes the production of a modified penicillin binding protein, known as PBP2', and confers resistance to penicillinase-stable penicillins.[12] At the phenotypic level, using a lower temperature, prolonged incubation, or both; a higher inoculum density; and a higher salt concentration in culture media enhances the demonstration of resistance. These modifications, especially when combined, may produce false resistance in strains of *S. aureus* that do not carry the *mec*A gene.[13,14] Tests to discriminate resistant from susceptible staphylococci in isolated colonies have been standardized by the National Committee for Clinical Laboratory Standards in both qualitative (agar diffusion) and quantitative (agar or broth dilution) formats.[12]

The goal of this study was to evaluate three different types of media (including selective and nonselective) in the detection of MRSA from clinical specimens of patients in an intensive care unit (ICU).

METHODS

From September to November 2001, 224 specimens were obtained from the nares of patients in the ICU of

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Hospital Mãe de Deus, Porto Alegre, Brazil, as part of the routine surveillance of MRSA. They were plated on Stuart's transport media (CB Products, São Paulo, Brazil). The incidence of MRSA in this hospital is approximately 4.5 cases per 1,000 admissions.

Specimens were initially inoculated on a nonselective medium, blood agar (trypticase soy agar with 5% sheep blood; Biolab-Mérieux, Rio de Janeiro, Brazil). Selective media were further inoculated: mannitol salt (7% salt concentration) agar containing 2 µg/mL of oxacillin (MSAO) (Biobras, Montes Claros, Brazil) and oxacillin resistance screening agar base (ORSAB). In ORSAB, mannitol fermentation is demonstrated by aniline blue in staphylococci and inhibition of organisms other than MRSA is due to oxacillin (2.0 µg/mL), polymyxin B (50,000 IU/L), and 5.5% NaCl concentration. The order in which selective media were plated changed each week to minimize any order effect.

Plates were incubated at 35°C and inspected at 24 (MSAO and ORSAB) and 48 (blood agar, MSAO, and ORSAB) hours. Colonies with typical morphology on blood agar and mannitol-fermenting colonies on MSAO (yellow colonies) and ORSAB (deep blue colonies) were tested for coagulase production using the tube test.[15] Coagulase-producing isolates were identified as *S. aureus* and were further submitted to susceptibility tests using agar diffusion.[12]

For cases in which discrepant results were obtained (defined as a resistant isolate originating from only one or two media), a polymerase chain reaction (PCR) procedure was used to verify the presence of the *mec*A gene. Primers (*mec*A1: 5'-TGG CTA TCG TGT CAC AAT CG; and *mec*A2: 5'-CTG GAA CTT GTT GAG CAG AG) amplified a 310-bp segment of the gene that was visualized after electrophoresis on 2% agarose containing ethidium bromide.[16] Besides PCR for the *mec*A gene, we tested the presence of PBP2' in isolates with discrepant results. For this purpose, two rapid slide latex agglutination assays that detect the PBP2' of MRSA were employed, Slidex MRSA Detection (bioMérieux, Marcy l'Etoile, France) and the Oxoid PBP2' Latex Agglutination Test (Oxoid Limited).

McNemar's chi-square was used to compare two paired proportions.

RESULTS

MRSA was detected in 32 (14.3%) of 224 specimens. After 48 hours of incubation, mannitol-fermenting colonies were found in 43 and 45 specimens in MSAO and ORSAB, respectively. Coagulase production was verified in 31 (72.1%) of 43 in MSAO and in 28 (62.2%) of 45 in ORSAB. All coagulase-positive isolates originated from both media showing resistance to oxacillin. Overall, the number of confirmed MRSA carriers varied according to the medium (17, 31, and 28 for blood agar, MSAO, and ORSAB, respectively). In 24 hours of incubation, MSAO and ORSAB detected 22 MRSA carriers each. The difference in detection was not significant comparing MSAO with ORSAB ($P > .5$); however, it was significant for MSAO compared with blood agar ($P \leq .01$) and for ORSAB compared with blood agar ($P < .01$). Four MRSA carriers were detected exclusively on MSAO.

TABLE
RESULTS FOR METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG THE 224 SPECIMENS PLATED ON BLOOD AGAR, MANNITOL SALT AGAR CONTAINING 2 µG/ML OF OXACILLIN, AND OXACILLIN RESISTANCE SCREENING AGAR BASE

Blood Agar	MSAO	ORSAB	No. of Occurrences	PCR Results for the *mec*A Gene	Assay for PBP2'* Product 1	Assay for PBP2'* Product 2
+	+	+	16	NT	NT	NT
+	-	-	0	NA	NA	NA
-	+	-	4	+	+	+
-	-	+	0	NA	NA	NA
+	+	-	0	NA	NA	NA
+	-	+	1	+	+	+
-	+	+	11	+	+	+
-	-	-	192	NA	NA	NA

MSAO = mannitol salt agar containing 2 µg/mL of oxacillin; ORSAB = oxacillin resistance screening agar base; PCR = polymerase chain reaction; PBP2' = modified penicillin binding protein; NT = not tested; NA = not applicable.
*Product 1 = Slidex MRSA Detection (bioMérieux, Marcy l'Etoile, France); Product 2 = Oxoid PBP2' Latex Agglutination Test (Oxoid Limited, Basingstroke, England).

In 16 cases, a discrepant result was obtained (defined as a resistant isolate originating from only one or two media types). Because all discrepant isolates originating from each medium were independently tested, 28 isolates were submitted to PCR for the *mec*A gene and to assays for PBP2' detection (Table). Results were positive in all cases, indicating that no false-positive resistance occurred in phenotypic tests.

DISCUSSION

MRSA infections have important impact. Practices to control this agent include the detection of carriers. Laboratory methods to recognize MRSA carriers vary in performance, turnaround time, workload, and cost.

In this study, the two selective media employed showed similar results in terms of MRSA detection, in accordance with previous investigations.[10,11] Although MSAO detected more patients colonized with MRSA (31 vs 28), the difference was not significant. However, ORSAB missed 4 MRSA carriers in this study. Results indicate that an incubation period of 48 hours is necessary because both MSAO and ORSAB detected only 22 patients colonized with MRSA after 24 hours of incubation. It is unknown whether an incubation period of 72 hours would further increase MRSA detection, but any such benefit would have to be balanced against the drawback of a further increase in turnaround time. Both types of screening media need a supplementary test to confirm MRSA from mannitol-fermenting isolates. According to our results, a coagulase test was sufficient to confirm MRSA, as all mannitol-fermenting isolates from both

media that were coagulase positive were resistant to oxacillin on agar diffusion test.

The detection of MRSA in clinical laboratories is influenced by experimental conditions that increase the expression of the *mec*A gene. However, false resistance to oxacillin in *S. aureus* may be produced in *mec*A-negative strains with experimental conditions such as briefer or prolonged incubation, a higher inoculum density, and a higher salt concentration in culture media, especially when these are combined.[13,14] The screening approach uses some of these conditions to increase MRSA detection (in our study, 7.5% and 5.5% NaCl concentrations in MSAO and ORSAB, respectively, and 48 hours of incubation for both media). To investigate the possibility of false resistance, we included a nonselective medium in the protocol and submitted all isolates with a discrepant result to PCR for the *mec*A gene and assays for PBP2' detection. Because all discrepant isolates, individually tested, were *mec*A gene positive for PCR and for PBP2', we concluded that neither MSAO nor ORSAB produced false resistance in *S. aureus*. Although the blood agar medium was used in this study to monitor the possibility of false resistance, its use is not justified, alone or in combination with a selective medium, for the detection of MRSA carriers.

Although the genetic diversity of MRSA in our institution was not studied, results from an international survey indicate that the Brazilian clone has a large capacity for geographic expansion and persistence.[17] This observation suggests that results of the current study are likely valid for institutions located in other countries, especially those in Latin America.

Laboratories need an effective and practical way to detect MRSA carriers. Enrichment broths may increase MRSA detection, but they tend to increase workload and time for detection.[5,7,9,11] The best approach for each institution must be determined after consultation between the laboratory director and infection control practitioners. ORSAB constitutes a practical alternative for isolation of MRSA from the nares of hospitalized patients, contributing to control of dissemination of this agent.

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