Detection of Methicillin-Resistant *Staphylococcus aureus* in Clinical Specimens from Cystic Fibrosis Patients by Use of Chromogenic Selective Agar

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We evaluated the use of a chromogenic selective medium (MRSA ID) as a useful tool for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in cystic fibrosis (CF) patient samples. Fifty-four MRSA isolates were detected by MRSA ID, while only 24/54 (44%) (odds ratio [OR], 2.79; 95% confidence interval [CI], 1.63 to 4.76) were detected by conventional methods. A chromogenic selective medium for MRSA detection may improve its surveillance in CF patients.

*S. aureus* is a frequently isolated pathogen and may be cultured early in infancy in cystic fibrosis (CF) patients. The incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) infection in CF patients has increased dramatically (4), and the presence of MRSA among CF patients contributes to lung disease. CF patients with MRSA present a significant increase in hospitalization rates and treatment with oral, inhaled, and intravenous antibiotics (15), a greater decline in lung function, as measured by forced expiratory volume in 1 s (FEV1) (3), a higher risk of failure to recover to baseline after pulmonary exacerbations (16), and an increase in mortality (4).

Cultures of respiratory specimens from CF patients represent a challenge for clinical laboratories due to polymicrobial characteristics, making the detection of specific organisms, such as MRSA, particularly difficult. The control measures that are used to reduce the levels of hospital-acquired infections due to MRSA include an active search through surveillance cultures (13) and the use of selective medium containing antimicrobial agents to improve the sensitivity of detection (7, 12, 14). Previous studies have shown that chromogenic selective agars can increase the detection of MRSA in polymicrobial specimens, such as wound and respiratory (8), stool (9), and nasal and groin (11) swabs. Nevertheless, for the culturing of CF specimens, there is a recommendation for the use of a selective medium, such as mannitol salt agar, but the use of a specific selective medium to detect MRSA is not included so far (5). There is a need to investigate the influence of procedures based on these media in the detection of MRSA in CF patients.

In this study, we aimed to evaluate the use of the MRSA ID agar, a specific and selective medium, to detect MRSA from CF respiratory clinical specimens.

A total of 179 consecutive clinical specimens (128 sputa and 51 oropharynx swabs) from 130 CF patients who were admitted to Hospital de Clínicas do Porto Alegre, the main care center for CF patients in the south of Brazil, between January and April 2011 were included in the study. As the conventional procedure, we considered the media routinely used in the microbiology laboratory (5% sheep blood agar [BAP] and mannitol salt agar). After 24 to 48 h of incubation at 35°C, plates were examined and 2 or 3 potential *S. aureus* colonies from a BAP or mannitol salt plate were selected, submitted to a coagulase test, and screened for cefoxitin susceptibility. The MRSA ID (bioMérieux, Marcy l’Etoile, France) was used as an alternative surveillance culture for MRSA detection. Direct identification of MRSA isolates on this medium is based on the distinctive green coloration of α-glucosidase-producing colonies in the presence of 4 μg/ml of cefoxitin after 24 h to 48 h of incubation at 35°C. Conventional and alternative cultures were made independently and carried out by double-blind analysis. Resistance to methicillin was determined by the Kirby-Bauer disk diffusion method using a 30-μg cefoxitin disk (2). The presence of the *mecA* gene was assessed by PCR according to a protocol previously described (17). *Staphylococcus aureus* ATCC 29213 (methicillin-susceptible *S. aureus* [MSSA]) was used for quality control in the broth microdilution method and for the MRSA ID culture as a negative control, and *S. aureus* ATCC 35591 (MRSA) was used as a positive control.

Statistical analyses were carried out using SPSS for Windows, version 13.0 (SPSS Inc., Chicago, IL). Prevalence ratios (PRs), odds ratios (ORs), and 95% confidence intervals (CIs) were calculated. *P* values were calculated using χ² or Fisher’s exact tests.

A total of 138 (77.1%; 95% CI, 70.4% to 82.6%) *S. aureus* isolates were detected from 179 specimens recovered from 130 patients. Of these, 54/138 (39.1%; 95% CI, 31.4% to 47.4%) were *mecA* positive (from 47 patients). A total of 54 MRSA isolates were detected by MRSA ID, while the conventional cultures detected 24 (44.4%) (OR, 2.79; 95% CI, 1.63 to 4.76) (from 18 patients). Table
shows the detection of MRSA in both conventional and alternative cultures. An increase in the detection of MRSA, along with the occurrence of false-positive results in MRSA ID (a decrease in specificity), was observed after 48 h of incubation. This observation is in agreement with those of other investigators who compared different chromogenic media, including MRSA ID, for MRSA detection (1, 6, 11, 20). It is of note that 6 meca-negative S. aureus isolates (oxacillin and cefoxitin MICs ranging from 0.25 to 1 and 0.25 to 2 μg/ml, respectively) were able to grow on MRSA ID and therefore would be mischaracterized as MRSA (Table 1). Leahy et al. (10) described a significant proportion of borderline oxacillin-resistant Staphylococcus aureus (BORSA) isolates—methicillin resistant and lacking meca—in the CF population. In our study, however, MICs to oxacillin and cefoxitin for those meca-negative isolates that presented growth in MRSA ID plates were at least two dilutions below the breakpoint of resistance that rules out the condition of BORSA.

Table 2 shows the detection of other organisms, including MSSA, in the specimens studied. Overall, it makes evident the limitations of the conventional procedure in detecting MRSA in polymicrobial specimens, considering that 10 MRSA isolates were missed by conventional testing when MRSA and MSSA were coinhabitants in patient samples.

The presence of distinct clones of the same species coexisting in the lungs of CF patients has been described (18, 19). In the first few years of life, MSSA is thought to be the primary invader, MSSA and MRSA can coexist for some time, and care should be taken when analyzing sputum cultures because the growth of one microorganism may camouflage the growth of the other. MSSA and MRSA produce identical colonies in primary medium without antibiotics. So far, recommendations for respiratory CF specimen processing do not include the use of a selective and specific medium for MRSA detection.

In our study, MRSA isolates that were present in 30 clinical specimens (from 29 patients) were not detected by the conventional culture media. Still, only three patients were not receiving antimicrobial therapy with vancomycin (Table 2). Although the treatment of CF patients is considered individually and according to factors other than microbiological features, it is essential that the identification of microorganisms, such as MRSA, be done properly.

This is the first study evaluating the use of a chromogenic medium containing cefoxitin for surveillance in CF patients. Thus, based on our results, we recommend the incorporation of a chromogenic selective medium, such as MRSA ID, to enhance the recovery of MRSA from CF respiratory specimens.

**REFERENCES**

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