Comparison of Conventional Susceptibility Testing, Penicillin-Binding Protein 2a Latex Agglutination Testing, and *mecA* Real-Time PCR for Detection of Oxacillin Resistance in *Staphylococcus aureus* and Coagulase-Negative *Staphylococcus*

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Penicillin-binding protein (PBP) 2a latex agglutination was compared with conventional susceptibility testing and *mecA* real-time PCR for the detection of oxacillin resistance in *Staphylococcus aureus*. Inoculum volume and induction with oxacillin were PBP 2a testing variables. For coagulase-negative *Staphylococcus*, an increased inoculum volume of 10 μ l greatly reduced the number of isolates requiring induction.

Staphylococcus aureus and coagulase-negative *Staphylococcus* (CoNS) are common isolates from positive blood and sterile body fluids (1). At our institution, oxacillin resistance in these organisms is relatively high; 47% of *S. aureus* and 71% of CoNS isolates are resistant to oxacillin by conventional (phenotype-based) antimicrobial susceptibility testing (AST) (2004 antibiogram; University of North Carolina Hospitals, McLendon Clinical Laboratories). The *mecA* product, penicillin-binding protein (PBP) 2a, mediates oxacillin resistance. PBP 2a has a lower affinity for beta-lactam antibiotics than do endogenous PBPs of staphylococci, thus allowing peptidoglycan synthesis in the presence of lethal doses of beta-lactams. Due to high rates of oxacillin resistance, empirical vancomycin therapy is rampant. To reduce the unnecessary use of vancomycin, the clinical microbiology laboratory must be able to provide accurate oxacillin susceptibility results with rapid turnaround times. However, the heteroresistant nature of oxacillin resistance in staphylococcal isolates can make detection problematic (3). Disk diffusion and oxacillin screening plate results are occasionally discordant for *S. aureus* isolates, and resistance detection in CoNS at our institution relies solely on disk diffusion. To improve the performance of phenotype-based susceptibility tests, we sought to evaluate the use of PBP 2a detection in staphylococcal isolates by using *mecA* real-time PCR as the reference method (11). In addition, the data in the literature vary regarding the necessities of inducing CoNS with oxacillin and of increasing the inoculum volume for the detection of PBP 2a (6–10, 13, 20, 21, 23). This study aims to determine a standard protocol for PBP 2a detection in CoNS that minimizes the time to detection of oxacillin resistance.

(A preliminary report of this work has been presented previously [M. B. Miller, H. Meyer, E. Rogers, and P. H. Gilligan, Abstr. 104th Gen. Meet. Am. Soc. Microbiol., abstr. C-119, 2004].)

Retrospective analysis of significant levels of *S. aureus* and CoNS isolates from blood and sterile body fluids was evaluated by multiple methods for the detection of oxacillin resistance. In our laboratory, oxacillin resistance in *S. aureus* is detected phenotypically by the use of Kirby-Bauer disk diffusion in conjunction with an oxacillin screening agar plate containing 6 μ g ml⁻¹ oxacillin, whereas oxacillin resistance in CoNS is detected by Kirby-Bauer disk diffusion only. At the time of this study, disk diffusion data were obtained using oxacillin as opposed to cefoxitin to predict oxacillin resistance in *Staphylococcus* (5, 12, 17). However, isolates that demonstrated discrepant susceptibility results were subsequently tested for cefoxitin susceptibility. Isolates were identified to the genus level with Gram staining and examination of catalase production and colony morphology. Species identification was performed using a tube coagulase test or the BactiStaph latex reagent (Remel, Lenexa, KS) for *S. aureus* ($n = 50$) and the bioMérieux APIStaph kit (Marcy l'Etoile, France) for CoNS ($n = 100$). CoNS species identified included *Staphylococcus epidermidis* ($n = 70$), *S. hominis* ($n = 8$), species with no identification ($n = 8$), *S. haemolyticus* ($n = 6$), *S. lugdunensis* ($n = 3$), *S. capitis* ($n = 3$), and *S. xylosus* ($n = 2$). The manufacturer's package insert instructions were followed for the PBP 2a latex agglutination test (Slidex methicillin-resistant *S. aureus* detection kit; bioMérieux, Marcy l'Etoile, France) with the exceptions of those pertaining to inoculum volume and oxacillin induction. Uninduced inocula of 5 μ l and 10 μ l from a 5% sheep blood agar (SBA) plate, as well as 5 μ l and 10 μ l of inoculum that had been grown overnight on an SBA plate containing a 1- μ g oxacillin disk (Becton Dickinson, Sparks, MD) were used for PBP 2a detection. Colonies were taken directly from an SBA plate, and volumes were measured using 5 loopfuls of a 1- μ l calibrated loop or a 10- μ l calibrated loop. Cell lysates for PCR were prepared as previously described (14). Real-time PCR was performed on a Roche LightCycler (Roche Diagnostics, Indianapolis, IN) with previously published primers (14) and on a Roche LightCycler FastStart DNA Master SYBR green I kit (Roche Diagnostics, Indianapolis, IN) for amplicon detection.

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S. aureus isolates ($n = 50$) had 100% correlation between PBP 2a detection and *mecA* PCR when 5 μ l of uninduced inoculum was used in the PBP 2a assay. *S. aureus* isolates used in this study included 56% ($n = 28$) that were oxacillin resistant. In addition, the PBP 2a and *mecA* results were identical to phenotypic AST data from these isolates. Although a relatively small number of *S. aureus* isolates were characterized, these data are consistent with previous reports for the use of PBP 2a in *S. aureus* (2, 11, 15, 16, 18, 19, 22). None of the *S. aureus* isolates tested required increased inoculum volume or induction by oxacillin for the detection of PBP 2a. However, *S. aureus* strains requiring oxacillin-cefoxitin induction for PBP 2a detection have been reported (15, 16, 20).

Although the CoNS isolates ($n = 100$) also demonstrated 100% correlation between PBP 2a and *mecA* testing, 5 μ l of uninduced inoculum in the PBP 2a assay detected only 42% ($n = 30$) of *mecA*-positive isolates ($n = 71$). For PBP 2a detection in CoNS, the induced 5- μ l inoculum performed optimally and demonstrated 100% sensitivity and specificity relative to PCR. However, increasing the uninduced inoculum to 10 μ l allowed for the detection of 97% ($n = 69$) of *mecA*-positive isolates by the PBP 2a test. Only 3% ($n = 2$) of *mecA*-positive isolates required induction for detection of PBP 2a by latex agglutination.

The use of 10- μ l inocula of CoNS did not result in false positives, and therefore, these inocula could be used to detect PBP 2a in CoNS without induction. Thus, for detection of *mecA*-mediated oxacillin resistance in CoNS, the laboratory could use a 10- μ l inoculum and have positive results within 20 min of obtaining an isolate. However, negative results obtained upon initial testing should be followed up with overnight oxacillin induction to prevent false negatives. It should be noted that the use of 10- μ l inocula of *S. aureus* resulted in a 14% false-positivity rate due to increased interpretation variability, and this method is not recommended for *S. aureus*.

There were three very major errors and six major errors in our conventional AST reporting (oxacillin disk diffusion) for CoNS when using PBP 2a/*mecA* testing as the reference method. These data did not show a correlation between species of CoNS and the reporting of very major or major errors in conventional AST. Subsequent testing by cefoxitin disk diffusion showed that eight reporting errors would have been avoided by the use of cefoxitin to predict oxacillin resistance. One very major error still occurred by cefoxitin disk diffusion; resistance in this isolate was detected in the PBP 2a agglutination assay using an uninduced 10- μ l inoculum.

Although the use of cefoxitin disk diffusion increases the accuracy of *S. aureus* and CoNS conventional AST, testing still requires 24 h after colony formation. PBP 2a latex agglutination can be performed in 20 min with minimal technical training. The use of PBP 2a detection for staphylococci allows for accurate same-day results for all *S. aureus* and oxacillin-resistant CoNS isolates. This can be achieved by increasing the inoculum size to 10 μ l for CoNS isolates and limiting induction-dependent testing to PBP 2a-negative CoNS isolates. A 3% false-negative rate for PBP 2a detection in CoNS without induction precludes the use of this test as a "rapid" means for oxacillin resistance detection in CoNS, as overnight incubation is required for the induction step. However, simply placing an oxacillin disk on subculture medium from positive blood cul-

tures with a Gram stain consistent with the presence of staphylococci would eliminate the delay associated with induction testing (8). In addition, several reports have demonstrated the use of the PBP 2a latex agglutination method directly from positive blood cultures; however, the sensitivities and specificities of these procedures vary significantly (4; T. Yamazumi, I. Furuta, T. Maeno, Y. Tsubakimoto, and M. A. Pfaller, Abstr. 102nd Gen. Meet. Am. Soc. Microbiol., abstr. C-99, 2002; L. A. Bassiwa and D. Craft, Abstr. 103rd Gen. Meet. Am. Soc. Microbiol., abstr. C-86, 2003). Decreasing the turnaround time for the detection of oxacillin resistance in critical cultures such as those for blood and sterile fluids will allow physicians to initiate or change a patient's antimicrobial regimen more appropriately and perhaps prevent the overuse of vancomycin.

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