Comparison of Conventional Susceptibility Tests with Direct Detection of Penicillin-Binding Protein 2a in Borderline Oxacillin-Resistant Strains of *Staphylococcus aureus*

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Six selected strains of *Staphylococcus aureus* classified as borderline oxacillin-resistant, according to standard disk diffusion and microdilution susceptibility test methods, and seven methicillin-resistant and seven methicillin-susceptible control strains were examined for the presence of penicillin-binding protein 2a (PBP 2a) by fluorography and immunoblotting and for DNA hybridization with a *mec*-specific probe in a dot blot assay. Oxacillin agar screen tests with and without NaCl supplementation were also performed with all strains. PBP 2a was detected both by fluorography and by immunoblotting in all seven methicillin-resistant control strains and in none of the susceptible controls. PBP 2a was detected in two borderline strains. Results of agar screen tests performed without NaCl supplementation were completely concordant with susceptibility determined by PBP 2a and *mec* detection methods. Agar screening with NaCl supplementation was less accurate. These findings were confirmed with 20 additional borderline strains. Direct detection methods for the presence of PBP 2a or *mec*, the gene encoding it, allow accurate and definitive classification of borderline strains. Further efforts to develop a rapid, clinically useful, antibody detection system for PBP 2a are warranted.

Accurate methods to detect methicillin resistance among strains of staphylococci are essential to ensure the institution of appropriate antimicrobial therapy for infections caused by these organisms. The reliability of susceptibility tests for staphylococci has been a problem because many factors, including inoculum size, bacterial growth phase, incubation time, temperature, pH, and NaCl concentration, are known to influence phenotypic expression of methicillin resistance (12, 22). Typically, methicillin resistance is heterogeneous (2, 12, 16, 22)—only a few cells within the total population of cells (e.g., 1 of 10⁶) express the trait.

Methicillin resistance is mediated by the penicillin-binding protein (PBP) 2a (4, 10, 11, 13). PBP 2a and the gene encoding it have been found in all methicillin-resistant strains of staphylococci reported to date and only in these strains. PBP 2a binds beta-lactam antibiotics relatively poorly and probably confers resistance because it can substitute for the other normal PBPs, which are bound and inhibited at much lower drug concentrations. Resistance is heterogeneous because factors besides PBP 2a influence the degree to which it is expressed (3, 12, 19).

Test methods that have been recommended for methicillin susceptibility testing utilize conditions that enhance phenotypic expression of resistance (e.g., addition of NaCl to the medium, lower temperature of incubation, and higher inoculum) (7, 20, 21, 25). Despite the overall reliability of these test methods, some strains are difficult to classify. A strain can appear susceptible by one method and borderline or resistant by another. Furthermore, the conditions that are used to enhance expression of methicillin resistance also can cause the susceptibility test results for susceptible strains to shift toward or above the breakpoint for resistance (18).

Strains for which test results border on or just exceed the breakpoint for susceptibility (e.g., the oxacillin disk test zone of 11 to 13 mm or oxacillin MICs in the range of 1 to 4 µg/ml) have been termed borderline or low-level resistant. The mechanisms responsible for low-level resistance are poorly understood. Very heterogeneous, PBP 2a-producing methicillin-resistant strains may show only low-level resistance even with modified test conditions.

Low-level resistance also may be due to mechanisms other than PBP 2a production. Strains with low-level resistance may produce large amounts of β-lactamase, which enables them to hydrolyze penicillinase-stable antibiotics (17). Some strains with low-level resistance, however, produce little or no β-lactamase. Modifications affecting the binding of antibiotic to the normal PBPs have been proposed as one mechanism, but there may be others (5, 26).

In vitro susceptibility data, experimental data from animal studies, and some clinical data indicate that low-level resistance in strains not producing PBP 2a probably is not important clinically and that beta-lactam antibiotics are effective for infections caused by these strains (5, 15, 24).

Because all known isolates of methicillin-resistant staphylococci produce PBP 2a, methods to detect this PBP could be used to determine resistance and might be more clinically reliable than standard test methods. Furthermore, identification of PBP 2a in strains with low-level resistance might help distinguish those infections requiring vancomycin treatment from those likely to respond to beta-lactam drugs. The purpose of this study was to compare the accuracy of standard susceptibility tests in classifying selected susceptible, borderline, and methicillin-resistant strains of *Staphylococcus aureus* to the accuracy of tests based on identification of PBP 2a by radiolabeling and immunoblotting. The accuracy of PBP 2a detection was confirmed with a dot blot hybridization assay for *mec*, the gene encoding PBP 2a.

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MATERIALS AND METHODS

Bacterial strains. Twenty strains of *S. aureus*—seven methicillin-resistant controls, seven methicillin-susceptible controls, and six borderline controls—were selected from a sample of clinical isolates from the clinical microbiology laboratory of Thomas Jefferson University, Philadelphia, Pa. The six borderline strains were selected because they had been among the most troublesome to classify as either susceptible or resistant of more than 88 borderline strains examined (14). After these strains were tested for methicillin resistance by microdilution and disk diffusion tests, they were forwarded to the investigators in San Francisco, who were blinded to the results of microdilution and disk susceptibility tests and strain classification. Two laboratory strains with known resistance (heterogeneously resistant strain 67-O and sensitive strain 209P) served as reference positive and negative controls.

Twenty additional, previously characterized, borderline strains (14) were examined to validate the results obtained with the strains used in the blinded studies.

Antibiotics. Sterile standard powders of oxacillin, nafcillin, clavulanic acid, 2-(2'-carboxyphenyl)-benzyl-6-aminopenicillanic acid (CBAP), and 6-aminopenicillanic acid were used. Antibiotic solutions were prepared in advance and stored at -70°C for less than 2 months before use.

β -Lactamase determination. β -Lactamase production was identified by using nitrocefin disks (BBL Microbiology System, Cockeysville, Md.).

Microdilution and disk susceptibility tests. Microdilution MICs of oxacillin were performed in cation-supplemented Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) containing 2% NaCl inoculated with 5×10^5 CFU/ml (20, 25). Results were recorded 24 h after incubation at 35°C .

Disk diffusion tests were performed by using 1 μg of oxacillin disks and amoxicillin-clavulanic acid (Augmentin) disks placed on Mueller-Hinton agar without NaCl supplementation (17, 21). Results were recorded 24 h after incubation at 35°C .

Strains with an oxacillin disk test zone diameter of ≤ 12 mm and an oxacillin MIC of ≥ 8 $\mu\text{g}/\text{ml}$ were classified as methicillin resistant. Strains with a disk test zone diameter of ≤ 12 mm plus an oxacillin MIC of ≤ 4 $\mu\text{g}/\text{ml}$ were classified as borderline. Strains with an oxacillin disk test zone diameter of ≥ 13 mm and an oxacillin MIC of < 1.0 $\mu\text{g}/\text{ml}$ were classified as susceptible.

Agar screen susceptibility tests. Agar screening was performed with oxacillin by using the method of Thornsberry and McDougal (20, 25), except that Mueller-Hinton agar both with and without 4% NaCl supplementation was used. Either 10^4 or 10^7 CFU (7) was spot inoculated onto Mueller-Hinton agar containing 6 μg of oxacillin per ml. Plates were read at 24 and 48 h of incubation at 35°C . Agar screens were considered positive for resistance when any colonies were apparent.

PBP 2a fluorography. PBP 2a was assayed in whole cells. Cells were grown in 10 ml of Trypticase soy broth (BBL) with 4% NaCl in a shaking incubator at 37°C . Exponentially growing cells were harvested at an A_{570} of 0.9. Clavulanic acid (to inhibit β -lactamase and to saturate PBP 2) and CBAP (to induce PBP 2a) at final concentrations of 100 $\mu\text{g}/\text{ml}$ were added to 1-ml samples of cells, which then were incubated for 30 min at 37°C . Cells were collected by centrifugation at $5,000 \times g$ for 5 min. The pellet was resuspended in 15 μl of 50 mM Tris-HCl–10 mM MgCl_2 (pH 7.6) containing 100 μg of lysostaphin, 5 μg of DNase, and 5

μg of RNase per ml. To label PBP 2a specifically, cells were incubated for 10 min in 10 μg of nafcillin per ml and then in 20 μg of ^3H -penicillin per ml for 30 min at 37°C (6). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10% acrylamide–0.26% bisacrylamide) with a minigel apparatus (Bio-Rad Laboratories, Richmond, Calif.). Gels were stained with Coomassie blue. Radiolabeled proteins were detected by fluorography with prefogged Kodak X-Omat film.

Preparation of antibody to PBP 2a. Polyclonal anti-PBP 2a antibody was raised in rabbits immunized with SDS-PAGE gel-purified protein from cell membranes of strain 67-O. Membranes were prepared from logarithmic-phase cells of strain 67-O grown in Trypticase soy broth containing 4% NaCl. Cells were harvested at a density of 2×10^8 to 5×10^8 CFU/ml by centrifugation at $10,000 \times g$ for 10 min at 4°C and washed in 100 ml of 50 mM Tris-HCl (pH 7.5)–5 mM MgCl_2 –1 M NaCl. Cells were resuspended in the same buffer containing 10 μg of DNase and 10 μg of RNase per ml and were mechanically disrupted with 100- μm -diameter glass beads. Beads were washed with 50 mM Tris-HCl–5 mM MgCl_2 –0.145 M NaCl. Supernatants were pooled and centrifuged at $10,000 \times g$ for 10 min. The supernatant was then centrifuged at $100,000 \times g$ for 30 min at 4°C . The membrane pellet was resuspended in 50 mM potassium phosphate, pH 7.2.

Membranes were then subjected to affinity chromatography in order to remove the high-affinity PBPs. Affinity chromatography was performed with 6-aminopenicillanic acid coupled to a Sepharose-4B affinity column (Pharmacia, Piscataway, N.J.) according to the method of Wyke (27). The column was equilibrated, and the membranes were diluted 1:1 with 50 mM potassium phosphate–1 M NaCl–0.1% Triton X-100 buffer (pH 7.2). Membranes were added to the column, and the flowthrough fraction was collected and subjected to SDS-PAGE. The protein band corresponding to PBP 2a was identified in the gel by Coomassie blue staining. The band was sliced from the gel and emulsified in saline. This preparation was used to immunize rabbits. Antibodies were purified from immune serum with a Sepharose-4B–protein A column (Pharmacia), according to the instructions of the manufacturer.

PBP 2a immunoblotting. Cell membranes were prepared as described above from cultures grown in 100 μg of CBAP per ml. Samples were subjected to SDS-PAGE in a minigel apparatus and transferred to nitrocellulose paper at 115 V for 1 h at 4°C . The nitrocellulose was blocked with 10% human serum and 5% nonfat dry milk in 10 mM Tris-HCl–150 mM NaCl (pH 8.0) buffer plus 0.05% Tween 20 (TBST) overnight at 4°C . Anti-PBP 2a antibody was diluted 1:1,000 in TBST and added to the nitrocellulose for 10 min. Goat anti-rabbit immunoglobulin G alkaline phosphatase-conjugated antibody (Proteoblot; Promega, Madison, Wis.) was added for 10 min. The paper was then washed three times for 10 min each time with TBST. Substrate buffer (100 mM Tris-HCl [pH 9.5], 100 mM NaCl, 5 mM MgCl_2) plus nitroblue tetrazolium plus 5-bromo-4-chloro-3,3'-indolylphosphate (Promega) was added for 10 min and poured off, and the reaction was stopped with 20 mM Tris-HCl–5 mM EDTA (pH 8.0). Specificity of PBP 2a antibody was confirmed by fluorography of radiolabeled membrane blots, use of preimmune antiserum controls, and use of control membranes from methicillin-susceptible strains, which lack PBP 2a.

Dot blot DNA hybridization. The whole-cell lysate method of de Lencastre et al. (8), with modifications, was used to detect *mec*-specific DNA. Cells from a 0.5-ml culture grown

TABLE 1. Results of susceptibility tests and PBP 2a and DNA detection with the 20 strains in the blinded study by disk and microdilution methods

Strain ^a	Zone diam (mm) and classification with disk ^b		OX MIC (μg/ml)	Growth determined by agar screen ^c				Detection of ^d		DNA
	OX	AMOX-CLAV		+NaCl		−NaCl		PBP 2a		
				Lo	Hi	Lo	Hi	FLU	BLOT	
Resistant										
21	0 R	10 R	>16 R	++	++	++	++	+	+	+
29	0 R	12 R	16 R	++	++	++	++	+	+	+
58	0 R	12 R	8 R	++	++	++	++	+	+	+
79	0 R	20 S	>16 R	++	++	++	++	+	+	+
94	0 R	13 R	>16 R	++	++	++	++	+	+	+
100	0 R	12 R	>16 R	++	++	++	++	+	+	+
130	0 R	11 R	16 R	++	++	++	++	+	+	+
Susceptible										
34 ⁱ	21 S	40 S	≤0.1 S	--	--	--	--	−	−	−
43	14 S	29 S	≤0.1 S	--	++	--	--	−	−	−
102 [−]	20 S	40 S	ND ^e	--	--	--	--	−	−	−
103	13 S	24 S	0.2 S	--	--	--	--	−	−	−
115	15 S	25 S	0.2 S	--	--	--	--	−	−	−
131 [−]	18 S	38 S	ND	--	--	--	--	−	−	−
139	14 S	22 S	0.5 S	--	--	--	--	−	−	−
Borderline										
11	12 B	23 S	0.2 S	--	--	--	--	−	−	−
13	0 R	20 S	4 B	++	++	++	++	+	+	+
32	0 R	20 S	1 B	--	++	++	++	+	+	+
30 ⁱ	9 R	21 S	0.5 S	--	--	--	--	−	−	−
35 ⁱ	10 R	21 S	1 B	--	--	--	--	−	--	−
77	12 B	29 S	0.2 S	--	--	--	--	−	−	−

^a Classification of susceptibility based on the interpretation of microdilution and disk diffusion susceptibility tests. The - superscript indicates β -lactamase-negative strain, and the t superscript indicates that trace production of β -lactamase was detected.

^b OX, oxacillin; AMOX-CLAV, amoxicillin-clavulanic acid; R, resistant; S, susceptible; B, borderline.

^c Agar was supplemented with 4% NaCl or no NaCl (-NaCl) at an inoculum of 10^4 CFU (Lo) or 10^7 CFU (Hi). +, growth; -, no growth. The first and second signs in each pair are the results with a 24- and a 48-h incubation, respectively.

^d Detection of PBP 2a by fluorography (FLU) and immunoblot (BLOT) and detection of *mec*-specific DNA by dot blot assay.

^e ND, not done.

overnight at 37°C in Trypticase soy broth were resuspended in 0.9 ml of 10 mM Tris-HCl-1 mM EDTA buffer (pH 8.0). Fifty microliters of 1,000- μ g/ml lysostaphin was added to a 450- μ l aliquot of cell suspension, which was incubated for 60 min at 37°C. Five microliters of 10% SDS and 2 μ l of 10-mg/ml proteinase K were added, and the sample was reincubated for 2 h at 37°C. Five microliters of 3 M NaOH was added for 10 min of incubation at room temperature. The sample was chilled to 0°C, and 55 μ l of 2 M NH_4 acetate was added. The sample (50 μ l) was spotted onto a nylon membrane that had been soaked in 6 $\times$ SSPE (1 $\times$ SSPE is 0.18 M NaCl, 10 mM NaPO_4 , and 1 mM EDTA [pH 7.7])-0.25% Sarkosyl for 5 min (23). A 96-well dot blot apparatus (Bio-Rad Laboratories) was used.

Membranes were probed at high stringency (23) for *mec*-specific DNA with plasmid pGO164 (a gift of Gordon Archer), which is the 1.1-kb internal *Bgl*II-*Xba*I fragment of the *mec* gene of strain COL cloned into pUC18. The probe was radiolabeled with [³²P]dATP by the random primer method (Amersham Corporation, Arlington Heights, Ill.).

RESULTS

Susceptibility tests. Seven strains were classified as susceptible by the disk and microdilution methods. All were susceptible to both oxacillin and amoxicillin-clavulanic acid by the disk method. The five strains that were tested had

microdilution oxacillin MICs of ≤ 0.5 μ g/ml (MICs were not determined in two β -lactamase-negative control strains) (Table 1).

Seven isolates were classified as resistant by the disk and microdilution methods. These strains showed no zone by oxacillin disk. One strain was susceptible by the amoxicillin-clavulanic acid disk test. These strains had oxacillin MICs of ≥ 8 μ g/ml.

Six strains were classified as borderline. Susceptibility test results were often discordant for this group of strains. Three strains were borderline or low-level resistant according to oxacillin MIC, resistant by oxacillin disk, but susceptible by amoxicillin-clavulanic acid disk test. The other three strains were susceptible according to MIC, borderline or resistant by oxacillin disk, but susceptible by amoxicillin-clavulanic acid disk test. All six borderline strains produced β -lactamase, although in two (strains 30 and 35) only trace activity was apparent.

Agar screen. The seven strains that were classified as resistant by the disk and microdilution methods were also resistant by the oxacillin agar screening performed with NaCl supplementation at low inoculum (i.e., 10^4 CFU) with a 24-h incubation. The seven strains classified as susceptible by microdilution and disk tests were also susceptible by this method.

One susceptible strain, 43, appeared resistant at 24 h, when a high inoculum (10^7 CFU) was used in the NaCl-

FIG. 1. Fluorograph of strains producing PBP 2a. Lanes 1 through 13, in order, are whole cell samples of strains 67-O (PBP 2a-producing methicillin-resistant control), 209P (PBP 2a-negative susceptible control), 13, 21, 29, 32, 58, 79, 94, 100, 130, 209P, and 67-O. Control strains were labeled with 20 μ g of 3 H-penicillin per ml without preincubation with CBAP, clavulanic acid, or nafcillin. Arrows indicate the position of PBP 2a.

supplemented agar screen. A second strain, 34, appeared resistant when both high inoculum and a 48-h incubation were used. Both strains were susceptible when agar screening was performed without NaCl supplementation at either inoculum after either a 24- or 48-h incubation.

One of the six borderline strains was classified as oxacillin resistant when agar screening with NaCl was performed at low inoculum after a 24-h incubation. Two strains, 13 and 32, appeared resistant after a 48-h incubation. At the high inoculum, these same two strains were resistant after a 24-h incubation. Five of the six borderline strains appeared resistant when both a high inoculum and a 48-h incubation were used with NaCl-supplemented agar screening.

Two borderline strains, 13 and 32, appeared resistant when agar screening was performed without NaCl supplementation after a 24- or a 48-h incubation. Results were the same for both inocula.

PBP 2a and dot blot assays. The results of PBP 2a fluorography, immunoblotting, and detection of *mec*-specific DNA were identical. The nine strains that showed both radiolabeled PBP 2a and a 78-kDa band corresponding to PBP 2a in immunoblots (Fig. 1 and 2) also hybridized with the *mec* probe in dot blot assays. Seven of the nine PBP 2a-producing strains were the resistant control strains. The other two were the borderline strains, 13 and 32, that were resistant in agar screen tests.

Eleven strains had neither radiolabeled PBP 2a nor the 78-kDa band in immunoblots (Fig. 3 and 4) and did not hybridize with the *mec* probe. These strains included the seven susceptible controls and four borderline strains.

Validation studies. Twenty additional borderline strains were examined by agar screening, fluorography, immuno-

FIG. 2. Immunoblot of strains producing PBP 2a. Lanes 1 through 15, in order, are rainbow molecular weight markers, strains 67-O (methicillin-resistant PBP 2a-producing control), 209P (susceptible, PBP 2a-negative control), 130, 100, 94, 79, 58, 32, 29, 21, 13, 67-O, 209P, and molecular weight markers. Arrows indicate the position of PBP 2a.

FIG. 3. Fluorograph of strains not producing PBP 2a. Lanes 1 through 15, in order, are whole-cell samples of strains 67-O (PBP 2a-producing methicillin-resistant control), 209P (PBP 2a-negative susceptible control), 11, 30, 34, 35, 43, 77, 102, 103, 115, 131, 139, 209P, and 67-O. Control strains were labeled with 20 μ g of 3 H-penicillin without preincubation with CBAP, clavulanic acid, or nafcillin per ml. Arrows indicate the position of PBP 2a.

blotting, and dot blot hybridization for *mec*. Eight strains produced PBP 2a detectable both by fluorography and immunoblotting. These strains also hybridized with the *mec* probe. Twelve strains did not produce PBP 2a detectable by either method and did not hybridize with the probe. On the basis of the classification of borderline strains as susceptible or resistance according to results of assays for PBP 2a and *mec*, the sensitivity and specificity of agar screen tests under various culture conditions ranged from 60 to 100% and 25 to 100%, respectively (Table 2).

DISCUSSION

Detection of PBP 2a by fluorography or immunoblotting was a more definitive method for classifying strains as methicillin-susceptible or -resistant than either the disk diffusion or the microdilution method. PBP 2a detection methods were 100% concordant with results of dot blot hybridization with a *mec*-specific probe, a method which has been shown to be highly accurate in distinguishing susceptible strains from resistant strains (1, 8, 9).

From the set of 20 strains in the blinded study, two of the six borderline strains (strains 13 and 32) that could not be classified by conventional test methods were found to produce PBP 2a and, therefore, could be classified as resistant. The other four borderline strains could be classified as susceptible on the basis of their lack of PBP 2a production. Fluorography and immunoblotting also correctly identified all susceptible and resistant control strains.

The results with agar screen method performed without NaCl were completely concordant with those with the PBP 2a and *mec* detection methods, regardless of inoculum size or duration of incubation (Tables 1 and 2). When agar

FIG. 4. Immunoblot of strains not producing PBP 2a. Lanes 1 through 17, in order, are rainbow molecular weight markers, strains 67-O (methicillin-resistant PBP 2a-producing control), 209P (susceptible, PBP 2a-negative control), 139, 131, 115, 103, 102, 77, 43, 35, 34, 30, 11, 209P, 67-O, and molecular weight markers. Arrows indicate the position of PBP 2a.

TABLE 2. Sensitivity and specificity of oxacillin agar screen test for 26 borderline strains, classified as susceptible or resistant on the basis of PBP 2a and *mec*-specific DNA detection methods

Test conditions			Sensitivity (%)	Specificity (%)
Inoculum (CFU)	4% NaCl	Incubation (h)		
10 ⁴	—	24	100	100
10 ⁴	—	48	100	100
10 ⁴	+	24	60	100
10 ⁴	+	48	100	100
10 ⁷	—	24	100	50
10 ⁷	—	48	100	31
10 ⁷	+	24	100	25
10 ⁷	+	48	100	25

screening was performed with 4% NaCl supplementation at low inoculum (10⁴ CFU) with a 24-h incubation, of the 40 strains tested four strains, all of which originally had been classified as borderline, were misidentified as susceptible. When the period of incubation was extended to 48 h, all four became resistant; results did not change for the other strains.

Agar screening performed at the high inoculum either with or without 4% NaCl supplementation lacked specificity. Two susceptible control strains, one after 24 h of incubation and the other after 48 h, were incorrectly classified as resistant with NaCl supplementation. Specificity was 50 and 31% at 24 and 48 h, respectively, for the 26 borderline strains. Omission of NaCl from the agar did not improve specificity at the high inoculum. Thus, the use of high inocula in agar screening cannot be recommended because it lacks specificity when borderline strains are tested.

Conventional methods for identifying methicillin resistance among strains of staphylococci are influenced by numerous factors known to affect the expression of methicillin resistance, which typically is heterogeneous. Selecting the optimal conditions to ensure test sensitivity and specificity has been difficult. The situation is further complicated by the potential for discordance in test results when more than one method is employed. Identifying very heterogeneous, truly methicillin-resistant (i.e., PBP 2a-producing) staphylococci requiring vancomycin therapy when strains demonstrate borderline susceptibility in conventional tests is an even more challenging problem.

Our data indicate that direct detection of PBP 2a allows accurate classification of methicillin susceptibility. As expected, some borderline strains, about a third of those examined, were found to produce PBP 2a and could be reclassified as resistant.

The agar screen is probably the best test currently available to the clinical microbiology laboratory for identifying methicillin-resistant *S. aureus* (1, 7, 25). In this study, it was 100% sensitive and specific at low inoculum at 24 and 48 h of incubation when no NaCl was added to the agar. Use of NaCl supplementation and a low inoculum in the 24-h agar screen test (as is currently recommended by the National Committee for Clinical Laboratory Standards) (20), however, was less accurate, with an overall sensitivity of 77% for detecting resistance among all 40 strains tested and 60% for the borderline group. Sensitivity improved to 100% after 48 h of incubation, without loss of specificity. A 48-h incubation, however, cannot be generally recommended because others have shown that β -lactamase hyperproducers can appear falsely resistant (17).

The direct PBP 2a detection method is similar in concept to methods developed by Archer and Pennell (1) and others (8, 9) who have shown that DNA probes specific for *mec*, which encodes PBP 2a, allow reliable identification of methicillin resistance with staphylococci. Both direct PBP 2a detection and gene probing have the advantage of circumventing the problem of heterogeneous expression of resistance in conventional susceptibility tests.

The fluorographic and immunoblotting techniques used in these studies are not practical outside a research setting. The former is labor intensive and requires use of radiolabeled material. The latter method involved a polyclonal antibody which cross-reacted with membrane proteins other than PBP 2a in immunoblots. However, immunologic detection of PBP 2a by using a monoclonal antibody with an enzyme-linked immunosorbent assay or a similar rapid, convenient, and easily reproducible method might be a realistic future goal. Such a method might be particularly helpful in establishing methicillin resistance or susceptibility for strains with borderline or discordant results with conventional susceptibility test methods.

In the absence of accurate tests based on PBP 2a protein or gene detection, clinical laboratories should consider employing a second reference test for methicillin resistance when serious infections (endocarditis or sepsis) are suspected. An agar screen test would be recommended on the basis of results of direct detection methods. However, whether NaCl supplementation should be used when borderline strains are tested is not clear. Further studies are needed to determine the best method for testing borderline strains in the absence of direct detection methods for PBP 2a or the gene encoding it.

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