Comparison of Tests for Detection of Methicillin-Resistant
Staphylococcus aureus in a Clinical Microbiology Laboratory

S. ÜNAL,1,2* K. WERNER,3 P. DEGIROLAMI,2,3 F. BARSANTI,4 AND G. ELIOPOULOS1,2*

Departments of Medicine,1 Pathology,3 and Pharmacy,4 New England Deaconess Hospital, Boston, Massachusetts 02215, and Harvard Medical School, Boston, Massachusetts 02115

Received 28 June 1993/Returned for modification 14 September 1993/Accepted 16 November 1993

By microdilution testing, 186 of 1,450 clinical isolates of Staphylococcus aureus were preliminarily classified as oxacillin resistant (MIC ≥ 4 μg/ml); 15 of these isolates gave conflicting results by alternative methods and were studied further. Only 2 of these (MIC > 4 μg/ml) were mecA positive; 13 were inhibited by oxacillin at 4 μg/ml. Significant numbers of S. aureus strains classified as resistant with an oxacillin MIC of 4 μg/ml may prove susceptible by other methods.

Methicillin-resistant strains of Staphylococcus aureus pose an important problem in hospitals, nursing homes, and other health care settings. Serious infections due to these organisms currently necessitate use of non-β-lactam antimicrobial therapy, options for which are quite limited (4). For this reason, accurate detection of methicillin or oxacillin resistance among staphylococci is of great importance, and several techniques have been applied to this task, including microdilution broth testing in medium supplemented with sodium chloride, agar plate screening, and disk diffusion tests (8, 9). Such tests, while generally straightforward, do not always yield fully reliable results. As a result, in our laboratory, strains which appear resistant on initial microdilution broth testing are subjected to a confirmatory test by an alternative method.

The overwhelming majority of methicillin-resistant strains of S. aureus carry the meca gene, which encodes production of the low-affinity penicillin-binding protein PBP 2a, which is responsible for phenotypic expression of methicillin resistance (1). Although low levels of resistance to methicillin in meca-negative strains of S. aureus can arise because of hyperproduction of β-lactamase (6), production of normal penicillin-binding proteins with altered binding capacity (14), or other as yet unidentified factors (2), it has not been determined that alternatives to β-lactam therapy are necessary in the treatment of infections caused by such organisms. The meca gene is not found in methicillin-susceptible isolates of staphylococci (1). Thus, detection of the meca gene by DNA hybridization techniques and detection by PCR have been investigated as potentially sensitive methods for detection of methicillin-resistant strains (1, 3, 7, 11–13, 15, 16). At present, such methods have not been widely adopted for use in clinical laboratories. In the present study, we used the PCR with primers specific for meca and a rapid cell lysis procedure to examine strains of S. aureus which were initially suspected to be oxacillin resistant on the basis of microdilution broth testing but which gave discordant results by a second testing method.

(This paper was presented in part at the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Anaheim, Calif., 11 to 14 October 1992 [15a].)

Between May 1991 and January 1992, a total of 1,450 strains of S. aureus isolated from various specimens were subjected to susceptibility testing in the clinical microbiology laboratory of the New England Deaconess Hospital. Susceptibility to oxacillin was initially determined by a microdilution method in medium supplemented with 2% sodium chloride (Microscan Gram Positive MIC Type 6 panel; Baxter Diagnostic, Inc., Deerfield, Ill.). Testing was performed according to the manufacturer's instructions. One hundred eighty-six isolates with oxacillin MICs of ≥4 μg/ml were preliminarily classified as oxacillin resistant. Between May and September 1991, such isolates were then examined by a disk diffusion test using oxacillin (1 μg) disks (8); between October 1991 and January 1992, the agar plate screening test with Mueller-Hinton agar supplemented with 4% sodium chloride and containing oxacillin (6 μg/ml) was used as the confirmatory test (9). For agar plate screening, three to five colonies from an overnight plate culture were suspended in tryptic soy broth to match a 0.5 McFarland standard. The plates were spot inoculated and incubated at 35°C for 24 h. Colonies showing atypical morphology or very poor growth were subcultured to verify purity. Fifteen of these 186 isolates, all from different patients, gave conflicting results with the two tests and were the subject of this study. For these strains, microdilution testing was repeated (in each case giving results identical to the first determination of the oxacillin MIC) and the complementary confirmatory test was performed so that each strain was tested by all three methods; MICs of oxacillin were also determined by the microdilution broth technique (10). Strains were stored on tryptic soy agar slants at 4°C and frozen at −70°C. Bacterial DNA was obtained by the rapid cell lysis method described by Ünal et al. (16). Primers for detection of the meca gene by PCR were derived from regions of the meca gene which exhibited at least 50% G+C content in order to avoid nonspecific annealing to the A+T-rich regions of the gene (5). Regions complementary to the primers were located within the meca gene open reading frame and separated by 1.1 kb. Primer 1, which reflected nucleotides 466 to 489, was 5'-GACCgAACAAtgGaaatttgCC-3'; primer 2, the reverse complement of nucleotides 1549 to 1573, was 5'-CACCttGTggTACcGATACgC-3' (16). PCR was performed in a DNA Thermal Cycler with a Gene Amp kit according to the manufacturer's recommendations (Perkin Elmer Cetus, Norwalk, Conn.). We used a thermal step program which included the following parameters: denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and primer extension at 72°C for 2 min, with a total of 30 cycles.

* Corresponding author. Mailing address: Department of Medicine, New England Deaconess Hospital, 185 Pilgrim Rd., Boston, MA 02215. Phone: (617) 632-8586. Fax: (617) 632-7442.
† Present address: Hacettepe University School of Medicine, Ankara, Turkey.
TABLE 1. Results of conventional susceptibility tests and detection of mecA by PCR for 15 isolates of S. aureus

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Isolate source</th>
<th>Oxacillin MIC* (µg/ml)</th>
<th>Oxacillin screen*</th>
<th>Oxacillin KB inhibition zone diam (mm)</th>
<th>mecA PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Foot ulcer</td>
<td>4</td>
<td>S</td>
<td>14</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>Sputum</td>
<td>4</td>
<td>S</td>
<td>15</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>Wound</td>
<td>4</td>
<td>S</td>
<td>15</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Foot ulcer</td>
<td>&gt;4</td>
<td>R</td>
<td>13</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>Sputum</td>
<td>4</td>
<td>S</td>
<td>13</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Bone marrow</td>
<td>4</td>
<td>S</td>
<td>15</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>Leg ulcer</td>
<td>4</td>
<td>S</td>
<td>13</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>Foot ulcer</td>
<td>4</td>
<td>S</td>
<td>17</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>Blood</td>
<td>4</td>
<td>S</td>
<td>14</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>Ear</td>
<td>4</td>
<td>S</td>
<td>14</td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td>Foot ulcer</td>
<td>4</td>
<td>S</td>
<td>12</td>
<td>Negative</td>
</tr>
<tr>
<td>12</td>
<td>Foot ulcer</td>
<td>4</td>
<td>S</td>
<td>14</td>
<td>Negative</td>
</tr>
<tr>
<td>13</td>
<td>Blood and sternal bone</td>
<td>4</td>
<td>S</td>
<td>15</td>
<td>Negative</td>
</tr>
<tr>
<td>14</td>
<td>Foot ulcer</td>
<td>4</td>
<td>S</td>
<td>13</td>
<td>Negative</td>
</tr>
<tr>
<td>15</td>
<td>Foot ulcer</td>
<td>&gt;4</td>
<td>R</td>
<td>14</td>
<td>Positive</td>
</tr>
</tbody>
</table>

* MIC determined by microdilution broth testing.
* Oxacillin agar plate screen test (Mueller-Hinton agar supplemented with 4% NaCl and oxacillin [6 µg/ml]). S, susceptible; R, resistant.
* Kirby-Bauer (KB) disk diffusion test with oxacillin disk (1 µg).

Ten-microliter samples of the PCR mixture were analyzed by agarose gel electrophoresis. A positive result was indicated by the presence of a 1.1-kb amplified DNA fragment. Such amplified fragments of the PCR product were shown to be part of the mecA gene in our previous study (16).

Microdilution broth testing yielded MICs of >4 µg/ml for two strains and a MIC of 4 µg/ml for the remaining 13 isolates of S. aureus (Table 1). All but one of these strains fell within the oxacillin-susceptible range by disk diffusion testing with zones of inhibition ≥13 mm in diameter (9). Thirteen strains proved susceptible to 6 µg of oxacillin per ml by the agar plate screening method, and two were resistant. When these two strains were studied by the microdilution broth method, actual oxacillin MICs were 64 and 128 µg/ml; for the 13 isolates which proved susceptible by agar plate screening, the MICs were found to be 4 µg of oxacillin per ml. All 15 isolates were examined for the presence of the mecA gene by PCR. Only two strains were positive by this technique, and these were the isolates which were resistant by agar plate screening and for which the MICs were >4 µg/ml. For the 13 strains with oxacillin MICs of 4 µg/ml, repeat susceptibility testing in the presence of 16 µg of sulbactam per ml reduced the MICs of oxacillin to less than 1 µg/ml; sulbactam failed to lower the MICs of the two mecA-positive strains below 16 µg/ml. This result supports the hypothesis that the relative resistance to oxacillin among those strains inhibited by oxacillin at 4 µg/ml was due to hyperproduction of β-lactamase. It has been proposed, however, that β-lactamase inhibitor–β-lactam combinations may occasionally exert synergistic activity against staphylococci by other mechanisms because such effects can also be demonstrated against non-β-lactamase-producing isolates (2). Because all of the isolates we studied were β-lactama-mase-producing strains, we could not further explore this possibility. It can be assumed that the prevalence of β-lactamase-hyperproducing or other borderline oxacillin-susceptible strains of S. aureus in a specific institutional setting will influence the frequency of discordant results obtained by these susceptibility testing methods.

When current criteria of the National Committee for Clinical Laboratory Standards were used, neither microdilution broth testing in sodium chloride-supplemented medium nor oxacillin disk diffusion testing accurately identified mecA-positive strains of S. aureus. Reliance on the microdilution results alone would have precluded β-lactam therapy for 13 patients whose isolates were mecA negative. While such false-resistant strains accounted for 13 of 15 instances of discordant oxacillin susceptibility test results, they represent <1% of the total isolates tested in this period, a proportion which seems acceptable given the limitations of current methods. The disk diffusion method did not identify the two truly oxacillin-resistant isolates. The insensitivity of this method in identifying mecA-positive strains has been noted by others (2). While there was complete concordance in our study between PCR results and those of the agar plate screen, Gerberding et al. (3) have shown the latter technique to be less than fully sensitive in identifying mecA-positive borderline methicillin-resistant strains when agar is supplemented with 4% sodium chloride.

Unless it can be shown that therapy of infection due to certain subgroups of mecA-negative strains of S. aureus with low levels of resistance to oxacillin requires alternatives to β-lactam antibiotics, residual difficulties in the identification of truly methicillin-resistant isolates by classical microbiologic methods warrant continued effort to adapt methods for detection of the mecA gene or its product, PBPs 2a, for use in clinical microbiology laboratories.

This work was supported in part by a grant from Eli Lilly and Company to S. Ünal.

REFERENCES


