Performance of Eight Methods, Including Two New Rapid Methods, for Detection of Oxacillin Resistance in a Challenge Set of Staphylococcus aureus Organisms

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Using a set of 55 Staphylococcus aureus challenge organisms, we evaluated six routine methods (broth microdilution, disk diffusion, oxacillin agar screen, MicroScan conventional panels, MicroScan rapid panels, and Vitek cards) currently used in many clinical laboratories and two new rapid methods, Velogene and the MRSA-Screen, that require less than a day to determine the susceptibility of S. aureus to oxacillin. The methods were evaluated by using the presence of the mecA gene, as detected by PCR, as the “gold standard.” The strains included 19 mecA-positive heterogeneously resistant strains of expression class 1 or 2 (demonstrating oxacillin MICs of 4 to \(>16 \mu g/ml\)) and 36 mecA-negative strains. The oxacillin MICs of the latter strains were 0.25 to 4 \(\mu g/ml\) when tested by broth microdilution with 2% NaCl-supplemented cation-adjusted Mueller-Hinton broth as specified by the NCCLS. However, when tested by agar dilution with 4% salt (the conditions used in the oxacillin agar screen method), the oxacillin MICs of 16 of the mecA-negative strains increased to 4 to 8 \(\mu g/ml\). On initial testing, the percentages of correct results (% sensitivity/% specificity) were as follows: broth microdilution, 100/100; Velogene, 100/100; Vitek, 95/97; oxacillin agar screen, 90/92; disk diffusion, 100/89; MicroScan rapid panels, 90/86; MRSA-Screen, 90/100; and MicroScan conventional, 74/97. The MRSA-Screen sensitivity improved to 100% if agglutination reactions were read at 15 min. Repeat testing improved the performance of some but not all of the systems.

The oxacillin agar screen test has been used for many years to aid in the identification of oxacillin-resistant staphylococci. Recently, this test was shown to be ineffective for coagulase-negative staphylococci (20), and the NCCLS no longer recommends its use for these organisms. In a previous study, the inoculation methods for the oxacillin agar screen test were more clearly defined (19a), and recommendations were made to use a 1-\(\mu l\) loop or an expressed swab inoculated into an area of 10 to 15 mm in diameter. In that study, a challenge group of Staphylococcus aureus isolates was tested, including several borderline oxacillin-resistant mecA-positive strains (\(n=19\)) that would be difficult to detect and mecA-negative strains (\(n=36\)) that might be falsely detected as resistant. Since several lots of oxacillin screen agar lacked specificity, the issue of the reliability of all methods for detection of oxacillin resistance was raised. Thus, using that same group of challenge organisms, we chose to examine the accuracy of the other routine methods for detecting oxacillin resistance. These methods were broth microdilution, disk diffusion, oxacillin agar screen, MicroScan rapid panels, and Vitek. We also evaluated two new rapid methods, Velogene, a cycling probe assay, and MRSA-Screen, a latex agglutination method that detects the presence of PBP-2a (also known as PBP-2’), the penicillin-binding protein (PBP) responsible for the most common form of oxacillin resistance in staphylococci. In addition, the performance of the quality control strain, S. au-

reus ATCC 43300, was evaluated by these methods. Some researchers have believed this strain to be inadequate for use as a positive (resistant) control strain in tests for detection of oxacillin resistance (13).

The 19 mecA-positive strains tested were previously determined to be in expression class 1 or 2 (i.e., very heteroresistant). The 36 mecA-negative strains fell into two groups, including 16 strains for which the oxacillin agar dilution MICs were \(\geq 4 \mu g/ml\) when tested with 4% salt (6), but \(<2 \mu g/ml\) with 2% salt, and 20 strains for which the oxacillin MICs were 1 to 2 \(\mu g/ml\) with 4% salt. Five strains tested in the previous study whose resistance mechanism was likely to be due to PBPs with altered affinity to oxacillin (the “MOD” type) (21) were not included in this testing. Four quality control strains were also tested: S. aureus ATCC 43300 (heterogeneous oxacillin resistance), S. aureus ATCC 33591 (homogeneous oxacillin resistance), S. aureus ATCC 29213 (oxacillin susceptible), and S. aureus ATCC 25923 (oxacillin susceptible). The strains were streaked twice from freezer storage before use in the study. Initial testing was done on 3 different days, with all methods tested simultaneously. All inocula were prepared directly from growth on blood agar plates (TSA II; BBL/BD Bioscience, Sparks, Md.).

The methods tested included the NCCLS reference broth microdilution method (13) with plates prepared with Difco Mueller-Hinton broth (BD Bioscience) adjusted for cation content, the NCCLS reference disk diffusion method (14) with Mueller-Hinton II agar (BBL/BD Bioscience), the oxacillin agar screen method (BBL), MicroScan conventional panels (Pos Combo 10 panels; Dade Behring, Inc./MicroScan, Inc.,
TABLE 1. Results of testing of a challenge group of 55 *S. aureus* isolates by seven methods for the detection of oxacillin resistance as shown by the number (percentage) of strains that gave correct results for each method using presence of *mecA* gene as the reference

<table>
<thead>
<tr>
<th>Organism group (n)</th>
<th>Test*</th>
<th>No. (%) of correct results as determined by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Broth microdilution</td>
</tr>
<tr>
<td>mecA positive (19)</td>
<td>First</td>
<td>19 (100)</td>
</tr>
<tr>
<td></td>
<td>Repeat</td>
<td>18 (95)</td>
</tr>
<tr>
<td>mecA negative (36)</td>
<td>First</td>
<td>36 (100)</td>
</tr>
<tr>
<td></td>
<td>Repeat</td>
<td>32 (89)</td>
</tr>
</tbody>
</table>

* Repeat, repeat testing was done only on strains with discrepant results. The results reported include discrepant strains that were repeated in duplicate and considered correct only if both repeat results were correct.

* For mecA-positive strains, results in the “F” or “R” category were considered correct.

West Sacramento, Calif.), MicroScan rapid panels (Rapid Pos MIC 1), Vitek (GPS-107; bioMérieux Inc., Hazelwood, Mo.), Velogene (Alexon-Trend, Inc., Ramsey, Minn.), and MRSA-Screen (Denka Seiken Co., Ltd., Tokyo, Japan). All testing was done and results were read according to NCCLS or the manufacturer’s recommendations. Inoculum suspensions for all of the tests were prepared from plates subcultured from the same plate. The same 0.5 McFarland suspension was used (with an appropriate dilution) to inoculate broth microdilution, disk diffusion, and oxacillin salt-agar screen plates. Separate 0.5 McFarland suspensions were prepared for Vitek, Velogene, MRSA-Screen, and MicroScan. The inoculation of the oxacillin salt-agar screen test was done by using a 1-μl loopful of a 0.5 McFarland suspension spread in an area of 10 to 15 mm in diameter (19a). For strains whose initial testing results were discrepant based on the mecA results, testing was repeated in duplicate on a second day. For analysis of repeat testing, correct results were assigned only if both repeat values were correct.

The results of initial and repeat testing are given in Table 1 as the number and percentage of correct results by group as determined by the presence or absence of the mecA gene. Agreement with the mecA results for broth microdilution, MicroScan, and Vitek was based on breakpoints of ≤2 μg/ml for susceptility (i.e., mecA negative) and ≥4 μg/ml for resistance (i.e., mecA positive). Disk diffusion results were considered correct for resistant strains if an intermediate or resistant category were considered correct. The agar screen method was considered correct for resistance if any growth of >1 colony was seen. The MRSA-Screen was read at 3 min (according to the manufacturer’s recommendation), 6 min, and 15 min (J. Vuopio-Varkila, J. Swenson, G. Killgore, B. Hill, S. McAllister, and F. Tenover, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 874, 1999).

For all methods except MicroScan conventional panels, the sensitivity (percentage of mecA-positive strains correctly identified) was ≥90%. The MicroScan conventional method failed to identify 5 of the 19 mecA-positive strains as resistant on both the initial and repeat testing. These five strains were among the most heteroresistant strains tested, all belonging to expression class 1. To see whether manual reading of the plates would increase the sensitivity with these five strains, we tested them again in duplicate on the MicroScan conventional panels. The machine reading again missed all five of the duplicate testings. With the manual reading, one isolate was correctly read as resistant in both repeat tests; four isolates remained incorrect for one or both of the repeat tests. All of these five strains were correctly classified by the MicroScan rapid method; with the latter method, two different strains were incorrectly identified as susceptible, but both were correctly identified as resistant when the test was repeated. On initial testing, two mecA-positive strains failed to grow on the agar screen; only one was positive when the test was repeated. With the MRSA-Screen, for the two mecA-producing strains that were negative after the recommended 3-min agglutination period, one strain was found to be positive at 3 min; the other strain was found to be positive after a 6-min agglutination period.

For broth microdilution, Velogene, and MRSA-Screen, the specificity (i.e., the percentage of mecA-negative strains correctly identified) was 100%. The specificity for the disk diffusion and MicroScan rapid methods was <90% for both initial and repeat testing, i.e., several strains were categorized as falsely resistant (for disk diffusion, three of the four specificity errors were minor, i.e., susceptible strains were categorized as intermediate).

Among the three reference methods tested (broth microdilution, disk diffusion, and oxacillin agar screen), the oxacillin agar screen test has been evaluated the most thoroughly. In studies performed since 1990 that used the presence of the mecA gene as the gold standard (1, 2, 4, 5, 9, 10, 11, 12, 15, 16, 18, 19, 23, 24, 26), the sensitivity of the agar screen test for the detection of resistant strains was excellent. However, two reports noted that when very heteroresistant strains were tested, the sensitivity decreased (2, 16). Conversely, the specificity among susceptible strains tested was very good unless strains with borderline MICs were included (9, 12). Fewer studies have compared the detection of resistance by disk diffusion to the presence of mecA (2, 8, 11, 15, 16), but two studies (2, 8) that included very heterogeneous strains found sensitivities of detection as low as 61% (2).

For the two automated commercial methods tested, MicroScan and Vitek, there have been very few evaluations performed since 1995. Since software changes frequently for these two systems and several different cards or panels are available, it is difficult to make comparisons of recent data to those that were generated in the past. A 1997 study that evaluated 355 *S. aureus* isolates (65 with the mecA gene) by both conventional and rapid MicroScan panels found 100% sensitivity and 92% specificity for both (3). Two very recent studies found 98% (26) and 100% (7) sensitivity by using the Vitek GPS-106 or Vitek...
GPS-SV cards, respectively (7), although other studies have reported decreased sensitivity and specificity if "troublesome" (4) or borderline strains are tested (9). Ribeiro et al. found that errors with the Vitek GPS-SA or GPS-BS cards were resolved when the GPS-101 cards were used (17).

There have been many recent evaluations of the MRSA-Screen latex agglutination test, most reporting a sensitivity of detection of resistant strains of ≥97% (2, 7, 12, 22, 24, 25, 26). In one recent report, however, when the agglutination reaction was read at 3 min as the manufacturer currently recommends, the sensitivity of detection among 51 mecA-positive strains was only 76% (J. Vuopio-Varkila, J. Swenson, G. Killgore, B. Hill, S. McAllister, and F. Tenover, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 874, 1999). Reading the reaction at 15 min, however, increased the detection rate to 100% in this study. Others have found that if strains are induced prior to testing (25), a larger inoculum is used (12), or the agglutination time is increased (24), the sensitivity of detection is increased without sacrificing specificity. However, one recent study showed increased sensitivity by increasing the agglutination time but decreased specificity if agglutination time was increased to ≥15 min (26). These authors recommend that any strain showing agglutination after 10 min be tested for the mecA gene by using PCR. Although further refinements may be necessary to the MRSA-Screen test, it appears to be a potentially useful test. Only one evaluation of the Velogene test has been published (12), but this method also appears to be a useful procedure, although more technically demanding than the latex agglutination test. Of the two products, currently only the Velogene system has been cleared by the Food and Drug Administration.

Results for S. aureus ATCC 43300 in this study are shown in Table 2; all methods, except for the oxacillin salt-agar screen test, Vitek, and the MRSA-Screen read at 3 min, correctly classified this strain as resistant. In our previous study (19a), the BBL agar screen plate correctly identified ATCC 43300 as resistant; however, the lot used in this study failed to detect the resistance on three of four testing days. In spite of this, the sensitivity of the salt agar screen test in this study was ≥90% (95% after repeat testing, i.e., one resistant strain was incorrectly classified as susceptible). On five testing days, the Vitek GPS-107 card assigned a susceptible MIC (2 μg/ml) to ATCC 43300; however, the sensitivity of detection of the 19 resistant challenge strains with this Vitek card was ≥95%, incorrectly classifying one resistant strain as susceptible (strain 3, a very heteroresistant mecA-positive strain). Both the agar screen and the Vitek methods failed to detect resistance in strain 3 on both initial and repeat testing. This strain also required 15 min of rotation with the MRSA-Screen (as opposed to 3 min as the manufacturer recommended) before being read as positive. With the specific lots of agar screen plates used in the previous study that were able to detect ATCC 43300, strain 3 was classified as resistant. If the sensitivity of the Vitek had allowed the detection of ATCC 43300, it may have also classified strain 3 as resistant. However, although the use of S. aureus ATCC 43300 is recommended for quality control of the BBL salt agar screen plates, it is not recommended for Vitek. It appears, however, that bioMérieux is aware of a potential problem with heteroresistant strains. In February 2001, it issued a notice to Vitek users that strains of S. aureus demonstrating oxacillin MICs of 2 μg/ml by Vitek may have MICs of 4 μg/ml when tested by a reference method and recommended that these strains be confirmed by using a reference method until the software is updated (S. L. Perreand, Product Notice, 15 February 2001 [bioMérieux, Inc.]).

As shown in this and other studies, no phenotypic system, including broth microdilution, is completely reliable for the detection of oxacillin resistance in S. aureus (19). The best approach would be to have several methods available for confirmation of results should resistance be suspected. With the availability of two systems that detect either genes (Velogene) or gene products (MRSA-Screen), increased accuracy of detection is possible. With both phenotypic and genotypic tests, however, the use of adequate quality control strains is critical.

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