Evaluation of the AutoMicrobic System for Detection of Resistance of \textit{Staphylococcus aureus} to Methicillin

BERT F. WOOLFREY,* RICHARD T. LALLY, AND MARY N. EDERER

Clinical Microbiology Section, Department of Anatomic and Clinical Pathology, St. Paul-Ramsey Medical Center, St. Paul, Minnesota 55101

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The AutoMicrobic system (AMS) (Vitek Systems, Inc., Hazelwood, Mo.) was tested for its ability to determine oxacillin and gentamicin susceptibility of 98 known oxacillin-susceptible and 103 known oxacillin-resistant \textit{Staphylococcus aureus} isolates. AMS and reference oxacillin susceptibility results were in agreement for all 95 (100%) oxacillin-susceptible isolates. In contrast, only 23 (22.3%) of the 103 known oxacillin-resistant isolates were correctly reported. For the known oxacillin-resistant isolates, 65 received AMS reports at 3 to 4 h, with only 9% being correct, whereas 38 were reported at 5 to 6 h, with 47% being correct. The reliability of AMS gentamicin susceptibility results was evaluated by testing the 198 \textit{S. aureus} isolates in parallel with MIC-2000 broth dilution tests. AMS gentamicin susceptibility results were found to be reliable and essentially identical to MIC-2000 results. The possibility of improving AMS oxacillin resistance detection by using gentamicin resistance as a linked screening marker for oxacillin resistance was evaluated with data from the parallel AMS and MIC-2000 gentamicin susceptibility tests and from data accrued on recent clinical laboratory isolates. By these two approaches, respective sensitivities of 97 and 99.8%, and specificity of 72%, were found for detection of oxacillin-resistant isolates by using gentamicin resistance as a marker.

MATERIALS AND METHODS

Experimental design. Two hundred \textit{S. aureus} stock clinical isolates were tested for susceptibility to oxacillin and gentamicin by the AMS system. The test sample was chosen so as to be comprised of approximately equal numbers of oxacillin-susceptible and oxacillin-resistant isolates. Discrepancies in AMS results and known oxacillin susceptibility were referred by retesting with the standardized disk agar diffusion test. Concurrent with the AMS oxacillin and gentamicin susceptibility tests, all isolates were tested for susceptibility to gentamicin by a reference broth microdilution procedure. The resultant data were tabulated and analyzed by standard statistical methods.

Microorganisms. Two hundred recent \textit{S. aureus} clinical isolates were selected, on the basis of their known susceptibility to oxacillin, from the St. Paul-Ramsey Medical Center Clinical Microbiology Laboratory stock culture collection.

Each isolate had previously been well characterized for oxacillin susceptibility by the standardized disk agar diffusion test and by the agar dilution procedure, as described below. Isolates were retrieved from frozen stock cultures by two 24-h passages on sheep blood agar plates incubated in air at 35°C. Material selected from several well-isolated colonies on the second stock retrieval plate was used as a common source for preparation of inoculum suspensions for the AMS and broth microdilution tests.

AMS tests. AMS Gram-Positive Susceptibility Cards were used in conjunction with the most recently available AMS microprocessor program, AMSP 12.ROB, for determination of oxacillin and gentamicin susceptibility. The AMS Gram-Positive Susceptibility Cards provide a single concentration of oxacillin (2.0 \text{µg/ml}) and three concentrations of gentamicin (0.5, 4.0, and 16.0 \text{µg/ml}) as a basis for determining susceptibility. The AMS program provides two susceptibility designations for oxacillin, susceptible (≤2 \text{µg/ml}) and resistant (>2 \text{µg/ml}), and four susceptibility categories for gentamicin: very susceptible (<0.5 \text{µg/ml}), moderately susceptible (1 to 4 \text{µg/ml}), moderately resistant (8 to 16 \text{µg/ml}), and very resistant (≥16 \text{µg/ml}). By using materials collected from discrete colonies on the second stock retrieval blood agar plate, inoculum suspensions were prepared, and AMS Gram-Positive Susceptibility Cards were inoculated and processed by the automated AMS system according to the instructions of the manufacturer. Inoculum suspensions were prepared and tested were processed in batches of 10 to 20 to ensure uniformity of initial processing time. Quality control was performed daily with \textit{S. aureus} ATCC 29213 and \textit{Streptococcus faecalis} ATCC 29212 obtained fresh weekly from subculture of frozen stock strains.

Agar dilution tests. Agar dilution tests for oxacillin were performed according to National Committee for Clinical Laboratory Standards guidelines (20) with Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) dilution plates prepared in the clinical microbiology laboratory.
Oxacillin concentrations ranged from 1.0 to 32 \( \mu \)g/ml in twofold dilution steps. Isolates were removed from stock culture by two consecutive 24-h passages on sheep blood agar plates which were incubated in air at 35°C. Each inoculum suspension was prepared by picking three to five colonies from the second stock retrieval plate to a tube containing 5 ml of cation-supplemented Mueller-Hinton broth, with further dilution to achieve an inoculum density of 10\(^7\) CFU/ml. From such inoculum suspensions, dilution plates were inoculated with 10\(^4\) CFU of each isolate by using a replicator device. Inoculated plates were incubated in air at both 30 and 35°C for 18 to 24 h before the initial interpretation and were incubated for an additional 18 to 24 h for a second interpretation. Oxacillin susceptibility was defined as growth at \( \leq 2 \) \( \mu \)g/ml, and resistance was defined as growth at >2 \( \mu \)g/ml. Quality control was performed daily with \( S. aureus \) ATCC 29213 and \( S. faecalis \) ATCC 29212 obtained fresh weekly from subculture of frozen stock strains.

**Disk agar diffusion tests.** Disk agar diffusion tests for oxacillin susceptibility were performed according to National Committee for Clinical Laboratory Standards (19) with Mueller-Hinton agar (BBL) and 1-\( \mu \)g oxacillin disks (General Diagnostics, Warner-Lambert Co., Santurce, Puerto Rico). Inhibition zone diameter breakpoints used to define susceptibility and resistance were, respectively, \( \geq 13 \) and \( \leq 10 \) mm. All isolates were tested at the time of isolation in the clinical microbiology laboratory. Results which were not in agreement with the agar dilution test results were retested by incubation in air at both 30 and 35°C and interpreted at 24 and 48 h. Quality control was performed daily with a fresh subculture of \( S. aureus \) ATCC 25923 obtained from weekly culture Bactrol disks (Difco Laboratories, Detroit, Mich.).

**Broth microdilution tests.** We prepared microdilution test panels in the clinical microbiology laboratory by using the MIC-2000 (Dynatech, Alexandria, Va.) system as previously described (30). Panels were prepared so that gentamicin concentrations differed by 1-\( \mu \)g/ml increments ranging from 1 to 16 \( \mu \)g/ml. Inoculum suspensions were prepared by picking several discrete colonies from the second stock retrieval blood agar plate to 5 ml of cation-adjusted (Ca\(^{2+} = \) 5.5 \( \pm 0.2 \) mg/dl; Mg\(^{2+} = 2.5 \) \( \pm 0.2 \) mg/dl) Mueller-Hinton broth, which was then incubated for 3 to 5 h, adjusted to the density of a 0.5 McFarland standard, and further diluted 1:10 with Mueller-Hinton broth. Panels were inoculated with the MIC-2000 inoculating apparatus and were incubated at 35°C in air for 18 h before interpretation. MIC was defined as the minimum concentration of gentamicin which produced no visual turbidity, no clusters or clumps, and no visual opacity >1 mm in diameter. Quality control was performed daily with \( S. aureus \) ATCC 29213 and \( S. faecalis \) ATCC 29212 obtained fresh from frozen stock subculture.

**RESULTS**

Table 1 summarizes the number and correctness of AMS oxacillin susceptibility reports in relation to time of reporting with reference designations established by agreement between the two reference tests. Only 23 (22.3%) of the 103 oxacillin-resistant isolates were correctly reported. Whereas all 95 (100%) of the oxacillin-susceptible isolates were correctly reported, 80 of the 175 (45.7%) AMS reports of oxacillin susceptibility were false. For the known oxacillin-resistant isolates, 65 reports occurred at 3 to 4 h, with only 5 (7.7%) being correct. The remaining 38 known oxacillin-resistant isolates received reports at 5 to 6 h, with only 18 (47%) being correct.

<table>
<thead>
<tr>
<th>Reference designation (no. of isolates)</th>
<th>AMS designation (no. of isolates reported)</th>
<th>No. of isolates reported at following time:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 h</td>
</tr>
<tr>
<td>Resistant (103)</td>
<td>Resistant (23)</td>
<td>2</td>
</tr>
<tr>
<td>Susceptible (95)</td>
<td>Susceptible (80)</td>
<td>5</td>
</tr>
</tbody>
</table>

AMS and MIC-2000 gentamicin susceptibility interpretations were compared for the known oxacillin-susceptible and resistant \( S. aureus \) isolates. For the known oxacillin-susceptible isolates, AMS reported 89 (94%) as being susceptible to gentamicin and 6 (6%) as being resistant. Except for one discrepancy, AMS results agreed completely with MIC-2000 results. Both AMS and MIC-2000 reported 98 (97%) of known oxacillin-resistant isolates to be resistant to gentamicin and 3 (3%) to be susceptible. It is interesting to note that all five of the oxacillin-resistant and gentamicin-susceptible isolates corresponded to the five isolates which, on reference testing, were interpreted as oxacillin susceptible by the disk agar diffusion test at both 35 and 30°C at 24 h but judged to be resistant on 48-h interpretation and by agar dilution testing.

**DISCUSSION**

The results of our investigation indicate that AMS, in its present stage of development, does not reliably detect oxacillin resistance in \( S. aureus \). This was particularly manifest in the finding of 77.7% false-susceptible reports for isolates known to be resistant to oxacillin. Of special interest was the finding that, for known oxacillin-resistant isolates, 92.3% of reports generated at an early time period of 3 to 4 h were given false-susceptible designations, whereas 52.6% were false susceptible reports at a later reporting period. This suggests a possible improvement in AMS oxacillin susceptibility results if reporting times could be purposefully delayed. Other rapid susceptibility test systems have also been found to have difficulty in detecting oxacillin resistance in \( S. aureus \) (2, 5, 6). This appears to be a common problem for systems which use the measurement of growth kinetics over short periods of time. Such an approach may not permit the detection of \( S. aureus \) isolates which have a low level of heteroresistance. This type of resistance, first described by Knox (18) in 1961, is thought to be due to the presence of microorganisms having various degrees of resistance in a particular \( S. aureus \) strain. Such strains, particularly those with very small proportions of resistant microorganisms, might be readily missed by susceptibility systems that use small volume, low CFU per milliliter inocula, or short incubation periods. Thornsberry and McDougal (28) recently reported accurate microdilution test results with cation-standardized Mueller-Hinton broth with 2% NaCl and modifications in inoculum preparation; however, reliable test interpretation could be made only after 24 h of incubation. Accurate, reliable use of AMS for detection of oxacillin-resistant \( S. aureus \) has been reported for a series of \( S. aureus \) strains from various regions in the United States (27); however, endemic strains of \( S. aureus \) may vary widely with respect to the heterogeneity of oxacillin resistance in each population. The presence of low-level heteroresistance...
among strains in our test population may account in part for the insensitivity of AMS in detecting oxacillin resistance.

The gentamicin susceptibility data was generated to determine whether AMS could reliably measure gentamicin susceptibility for S. aureus and to assess whether gentamicin resistance might be used as a marker to screen for oxacillin-resistant isolates missed by AMS. We found AMS gentamicin susceptibility results to be essentially identical to those produced by the MIC-2000 microdilution reference test. For the 95 known oxacillin-susceptible stock isolates, 6% were found to be gentamicin resistant. For the 103 known oxacillin-resistant isolates, 3% were found to be gentamicin susceptible. This translates to a 6% false-positive and a 3% false-negative rate if gentamicin resistance were used as a marker for detecting oxacillin resistance. A strong degree of correlation between methicillin (or oxacillin) resistance and gentamicin resistance has been reported by some investigators (7, 12, 22, 24); however, methicillin (or oxacillin) susceptibility has also been reported for gentamicin-resistant strains (10, 29). As a check on the results derived from our stock cultures, which represented a random collection of clinical isolates, we surveyed disk agar diffusion test data for 634 consecutive first S. aureus isolates from patients at the St. Paul-Ramsey Medical Center during the past year. Of these, 520 (82%) were susceptible to both oxacillin and gentamicin, 81 (12.8%) were resistant to both oxacillin and gentamicin, 32 (5%) were susceptible to oxacillin but resistant to gentamicin (28% false oxacillin resistance rate if gentamicin resistance is used as a linked marker), and 1 (0.2%) was resistant to oxacillin but intermediate to gentamicin (0.2% false-negative rate if gentamicin resistance is used as a marker). The findings from this survey of clinical isolates, in conjunction with those derived from the stock culture study, indicate a sensitivity approaching 100% for the use of gentamicin resistance as a screening marker for AMS detection of oxacillin resistance. In our laboratory setting, the use of AMS gentamicin resistance results to screen for oxacillin-resistant S. aureus isolates would necessitate follow-up testing of ca. 18% by a reliable standard susceptibility test.

In summary, our findings indicate that AMS does not reliably detect resistance of S. aureus isolates to oxacillin but is capable of accurately testing for gentamicin susceptibility. The observed decreased incidence of false oxacillin susceptibility results for isolates requiring longer reporting times suggests the possibility of improving AMS results by lengthening reporting periods. The use of gentamicin resistance as a linked marker for oxacillin resistance potentially provides AMS with the ability to screen for oxacillin-resistant S. aureus isolates.

LITERATURE CITED


