Methicillin-Resistant \textit{Staphylococcus aureus} Susceptibility Testing by an Automated System, Autobac I

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Received for publication 22 November 1977

The Autobac I system was used to evaluate the antibiotic susceptibility pattern of methicillin-resistant \textit{Staphylococcus aureus} isolates. The results of the Autobac I were compared with the results of the disk diffusion method. The disk diffusion susceptibility pattern showed resistance to methicillin/oxacillin, penicillin, erythromycin, clindamycin, and kanamycin. All isolates were susceptible to cephalothin, chloramphenicol, tetracycline, and gentamicin. There was at least 96% agreement using the Autobac I system with all antibiotics except methicillin and clindamycin. Seventy percent of 57 isolates were recorded as susceptible to methicillin, whereas 9% had an intermediate susceptibility. With clindamycin, 14% were recorded as susceptible and 7% were recorded as intermediate. Upon prolonged incubation of the Autobac I cuvette, the agreement between the two methods was 44% for methicillin and 93% for clindamycin. Changes in the environmental conditions, such as use of 5% sodium chloride broth and a 32°C incubation temperature, did not increase the detection of methicillin-resistant isolates by the Autobac I system.

When penicillinase-resistant, semisynthetic penicillins came into general use in the early 1960s, naturally occurring strains of \textit{Staphylococcus aureus} resistant to these agents were found (12, 13, 18). Such strains became of epidemiological importance in England, Europe, and Ethiopia (18). However, the expected increased prevalence of these organisms in the United States did not appear. Several small outbreaks have been reported in hospitals in the United States, the first being at Boston City Hospital in 1967–1968 (2). Subsequent outbreaks (14, 17) and sporadic cases (5, 6) have been reported.

Resistance to methicillin/oxacillin in \textit{S. aureus} is poorly understood. It is known that antibiotic inactivation by enzymes is not a mechanism for resistance. There are, however, several factors that influence the in vitro determination of methicillin-resistance. These include the incubation temperature, the incubation time, the inoculum size, and the salt concentration of the test media (1, 11, 19, 21). Because of these factors it was predicted that the determination of methicillin resistance by the automated system Autobac I might not give valid results (22).

Recently, increased numbers of methicillin-resistant \textit{S. aureus} organisms have been isolated from our in-patient population. These isolates were examined with the Autobac I system, and the results were compared with other antibiotic susceptibility testing methods.

**MATERIALS AND METHODS**

**Organisms.** All \textit{S. aureus} test organisms were clinical isolates from the microbiology laboratory at Jackson Memorial Hospital. The organisms were identified by standard biochemical procedures (15) and screened for methicillin susceptibility by the disk diffusion method. Organisms were maintained on Trypticase agar slants at room temperature.

**Susceptibility test methods.** The Autobac I testing was performed according to the manufacturer's instructions. The susceptibility procedures outlined in the original evaluation of the Autobac I system were followed (22). A light-scattering index (LSI) of <0.5 indicates resistance, whereas an LSI from 0.50 to 0.59 indicates intermediate susceptibility. Values >0.60 indicate antibiotic susceptibility. The penicillinase-resistant class disk supplied by the Pfizer Diagnostic Co. for testing in the Autobac I system is a 5-μg methicillin disk.

The disk diffusion method based on the Bauer-Kirby procedure (4, 16) was used. Mueller-Hinton agar base was used in all procedures. A 1.0-μg oxacillin disk (Baltimore Biological Laboratories) was used in the disk diffusion procedure instead of a methicillin disk because of its prolonged stability on storage (9).

The agar dilution method as described by Ericsson and Sherris was used (10). Mueller-Hinton agar plates were prepared and used within 24 h. All antibiotics were supplied as standard powders from the respective pharmaceutical companies. A final concentration of
5% NaCl was used for testing the susceptibility of the isolates to the penicillinase-resistant class antibiotics.

**Antibiotic-resistant population.** The antibiotic-resistant *S. aureus* population was determined by using plates containing 4 μg of oxacillin per ml. The isolates were first adjusted to an inoculum of approximately 10^6 organisms/ml, and then serial 10-fold dilutions of the Autobac broth were made. Mueller-Hinton plates supplemented with 5% NaCl were used to determine the number of viable organisms in the broth. A 0.1-ml sample of each dilution was streaked to both an antibiotic-free control plate and a plate containing 4 μg of oxacillin per ml. After 48 h of incubation, the percentage of antibiotic-resistant organisms was determined by comparing the number of organisms on the two plates.

**Bacteriophage typing.** The method described by Blair and Williams was used to determine bacteriophage types (7). The bacteriophages used in the routine typing included: 29, 52, 52A, 79, 80 (group I); 3A, 3C, 55, 71 (group II); 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85 (group III); and 81, 94, 95, 96 (miscellaneous). All bacteriophages were obtained from the Clinical Bacteriology Branch, Center for Disease Control, Atlanta, Ga.

**RESULTS**

The percent agreement between the disk diffusion method and the Autobac I system (Table 1) was greater than 96% for isolates that were susceptible to the antimicrobial agents by the disk diffusion method. Only one of the 57 isolates tested was resistant to cephalothin by the disk diffusion method; this organism was susceptible in the Autobac I system. Two isolates susceptible to gentamicin by the disk diffusion method were not susceptible by the Autobac I system. With the exception of methicillin and clindamycin, isolates that were resistant by the disk diffusion method showed >98% agreement with the Autobac I system. Seventy percent were recorded as susceptible to methicillin, whereas 9% were initially susceptible in the Autobac I system. With clindamycin, 14% were susceptible, and 7% were intermediate/susceptible with Autobac I. Upon prolonged incubation of the Autobac I cuvettes (>5 h), all isolates originally determined to be intermediate/susceptible or with an LSI <0.75 for methicillin and clindamycin eventually were recorded as resistant. The susceptibility pattern for the other antibiotics in the cuvette did not change from the original reading. With prolonged incubation the agreement between the two methods was 44% for methicillin and 93% for clindamycin.

Factors known to alter the in vitro determination of methicillin resistance include temperature, NaCl concentration, inoculum size, and incubation time. As demonstrated, the incubation time, if prolonged, will increase the number of methicillin-resistant isolates. However, the majority of isolates were recorded as susceptible, even after prolonged incubation in the Autobac I system. Other in vitro parameters were examined to determine if changes in the Autobac I test procedure would increase the reliability of detection of these resistant isolates.

To determine the effect of salt concentration in the Autobac I system, a 5% final concentration of NaCl was added to the inoculum broth. The increased salt concentration did not affect methicillin susceptibility (Fig. 1). The organisms included in this test ranged from those that had a high degree of susceptibility to methicillin to those that had some degree of methicillin resistance and gave an LSI from 0.8 to 0.55. The 5% salt concentration caused a slight decrease in the LSI of highly susceptible isolates and an increase in the LSI of more resistant organisms. The high salt concentration markedly decreased the activity of gentamicin. All isolates that were initially susceptible were recorded as resistant after incubation in the 5% NaCl broth. The LSI

![Table 1. Comparison of Autobac I and disk agar diffusion results](http://aac.asm.org/)

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Disk result&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Agreement (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Very major</th>
<th>Major</th>
<th>Minor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalothin</td>
<td>S</td>
<td>98.2</td>
<td>1.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>S</td>
<td>100</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>R</td>
<td>79</td>
<td>14.0</td>
<td>0.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>R</td>
<td>98.2</td>
<td>0.0</td>
<td>0.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>S</td>
<td>96.4</td>
<td>0.0</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>R</td>
<td>98.2</td>
<td>1.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Methicillin</td>
<td>R</td>
<td>21</td>
<td>70.0</td>
<td>0.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Penicillin</td>
<td>R</td>
<td>100</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>S</td>
<td>100</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Fifty-seven *S. aureus* isolates were tested.
<sup>b</sup> S, Susceptible; I, intermediate; R, resistant.
<sup>c</sup> Very major, Susceptible by Autobac 1, resistant by disk diffusion; major, resistant by Autobac 1, susceptible by disk diffusion; minor, intermediate by only one of the two methods (Autobac 1 or disk diffusion).
for the other antibiotics examined was not affected by the increased salt concentration.

The temperature in the Autobac I incubator was lowered to determine if temperatures <36°C altered the results. When susceptibility tests were run at 32°C, there was no increased detection of methicillin-resistant organisms. The lower temperature did increase the normal 3-h incubation time beyond 5 h and up to 8 h.

Several susceptible isolates consistently had a high LSI (>0.7), whereas others had a lower LSI (<0.7). When these isolates were classified with respect to their origin in the hospital and phage susceptibility, certain patterns were seen. All organisms isolated from the burn unit had a high LSI and a nonreactive phage type (Table 2). With two exceptions, organisms isolated from the surgical intensive care unit had a lower LSI and phage type 47/54/75/83A/+. Two organisms from the surgical intensive care unit that did not have a low LSI were the first initial methicillin-resistant S. aureus strains to be isolated at Jackson Memorial Hospital. Organisms with a phage type similar to that found in the surgical intensive care unit but from other hospital locations had both low and high LSIs.

It has been reported that cultures of methicillin-resistant S. aureus represent a heterogeneous population of susceptible and resistant cells (8, 19, 20). The results of this study suggest that organisms with a lower LSI have a greater population of methicillin-resistant cells in the inoculum. The inoculum delivered to each cuvette cell in the Autobac system was 10⁶ to 2 × 10⁶ organisms/ml. The antibiotic-resistant population was quantitated using isolates with different LSIs (Table 3). The number of resistant organisms in the population appeared independent of the LSI. Isolates with a high LSI, indicating susceptibility to the antibiotic, often had varying proportions of resistant colonies. Likewise, organisms with a low LSI, indicating resistance, frequently had smaller percentages of resistant organisms. The colonies on the drug plate were consistently smaller than the colonies found on the antibiotic-free plate with the corresponding inoculum.

**DISCUSSION**

The Autobac I system, a method to measure an early in vitro response to antibiotics, was found to be specific and reproducible by Thornsberry et al. (22). Because of its automation, the system is less subject to certain technical deviations and errors that may be encountered when dilution and diffusion techniques are performed in routine clinical laboratories. One of the potential problems that these authors thought might be encountered with the system was the inability to reliably detect methicillin-resistant S. aureus isolates. Due to the unusual "heteroresistant" nature of methicillin-resistant isolates and the short incubation time in the Autobac I system, organisms may appear susceptible to methicillin.

The minimal inhibitory concentration (MIC) of our methicillin-resistant S. aureus isolates as determined by the agar dilution method was

**TABLE 2. Correlation of Autobac I susceptibility to the phage type and location in the hospital**

<table>
<thead>
<tr>
<th>Phage type</th>
<th>Hospital ward</th>
<th>No. of isolates with LSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonreactive</td>
<td>Burn unit</td>
<td>&lt;0.7 13</td>
</tr>
<tr>
<td>47/54/75/83A/+</td>
<td>SICU*</td>
<td>15 2</td>
</tr>
<tr>
<td>47/54/75/83A/+</td>
<td>Other†</td>
<td>13 9</td>
</tr>
</tbody>
</table>

* SICU, Surgical intensive care unit.
† Patients were never located in the burn unit or the surgical intensive care unit.

**TABLE 3. Methicillin-resistant S. aureus population**

<table>
<thead>
<tr>
<th>Methicillin LSI</th>
<th>Resistant population (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>0.97</td>
<td>41</td>
</tr>
<tr>
<td>0.96</td>
<td>9.5</td>
</tr>
<tr>
<td>0.94</td>
<td>86</td>
</tr>
<tr>
<td>0.78</td>
<td>93</td>
</tr>
<tr>
<td>0.57</td>
<td>43</td>
</tr>
<tr>
<td>0.54</td>
<td>11.8</td>
</tr>
<tr>
<td>0.54</td>
<td>9</td>
</tr>
<tr>
<td>0.54</td>
<td>1.7</td>
</tr>
<tr>
<td>0.44</td>
<td>0.96</td>
</tr>
</tbody>
</table>

<sup>a</sup> Inoculum approximately 10⁶ to 2 × 10⁶ organisms/ml.
markedly affected by the medium composition and the inoculum concentration. The geometric mean MIC of methicillin was 6.1 µg/ml, using Mueller-Hinton agar and an inoculum size of 10⁵ organisms/ml. Using an inoculum of 10⁶ organisms/ml in the agar dilution method, we obtained a geometric mean MIC of 19.5 µg/ml on Mueller-Hinton medium and 70.6 µg/ml on Mueller-Hinton medium supplemented with 5% NaCl. In the Autobac I system the final concentration of organisms was approximately 10⁶ to 2 \times 10⁶ organisms per cuvette cell. This concentration was one log higher than the inoculum generally used in the agar dilution procedure.

Supplementing the Autobac Eugonic broth media with 5% NaCl did not increase the detection of methicillin resistance. By prolonging the incubation time, there was increased detection of resistant isolates; however, the method was still <50% accurate.

The Autobac I incubator has a preset temperature of 36°C. Several investigators have found that temperatures <35°C increased the detection of methicillin-resistant isolates. When our isolates were tested at 32°C, there was no increased detection of methicillin resistance. The incubation period was generally prolonged for 2 to 3 h because of the slower generation times.

It was interesting to note that within the hospital there were two major groups of organisms based on phage typing and Autobac susceptibility. Organisms isolated from the burn unit were nonreactive to the basic set of phages and always gave high LSI readings. Isolates from the surgical intensive care unit were phage type 47/84/75/83A/+ and consistently gave low LSI readings. Organisms of the latter phage type but from various other areas in the hospital yielded varied LSI values. These results suggested that there was a great deal of heterogeneity in the S. aureus isolates. However, when the resistant population was quantitated, there was no correlation between the number of resistant organisms in the population and the LSI reading. Frequently, organisms with a high LSI reading, which would indicate susceptibility to the antibiotic, had a greater number of resistant organisms in the population than isolates with a low LSI. When these organisms were isolated on the antibiotic-containing plates, the colony sizes were markedly reduced. If individual colonies from the antibiotic-containing plates were retested in the Autobac I, they yielded the same result as the parent population. Resistance was not increased by this selective procedure.

By disk diffusion and Autobac I testing, the isolates were recorded as susceptible to cephalothin. Plorde and Sherris (18) demonstrated that most methicillin-resistant S. aureus isolates were susceptible when cephalothin disks were used in the disk diffusion procedure. Only the cephalaxin disk discriminated between susceptible and heteroresistant S. aureus strains. Our isolates were resistant to cefazolin and cephalaxin when tested by the agar dilution procedure.

The organisms were susceptible to cephalothin (8 µg/ml) and cefamandole (16 µg/ml); however, the MICs were higher than those usually found for methicillin-susceptible strains of S. aureus. In our laboratory the median MICs for cephalothin and cefamandole for methicillin-susceptible S. aureus isolates are 0.4 µg/ml for each antibiotic.

Our results indicate that the Autobac I system does not reliably detect methicillin-resistant organisms. From our evaluation of the Autobac I system, we feel that laboratory personnel must be aware of the antimicrobial susceptibility pattern of methicillin-resistant organisms. It is not uncommon to isolate S. aureus organisms resistant to penicillin/ampicillin; however, it is uncommon to isolate S. aureus organisms that are multiresistant to other antibiotics. Our isolates were resistant to penicillin, erythromycin, clindamycin, kanamycin, and methicillin. Other investigators have reported several different multiresistant patterns (2, 19). Therefore, when an S. aureus isolate yields a multiresistant antibiotic pattern with the Autobac I system, the final report as to the susceptibility to methicillin should be held until it is verified by another accepted procedure.

ACKNOWLEDGMENTS

We thank Virginia Sanchez for technical assistance, and Gary Hancock and Peter B. Smith of the Clinical Bacteriology Branch, Center for Disease Control, Atlanta, Ga., for bacteriophage-typing assistance and strains.

LITERATURE CITED