Rapid Identification of *Staphylococcus aureus* by Using Lysostaphin Sensitivity

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Lysostaphin sensitivity was evaluated as a rapid screening test to differentiate *Staphylococcus aureus* from other species of staphylococci and micrococi. A total of 168 strains of staphylococci, 108 of which were *S. aureus*, were cultured overnight in brain heart infusion broth. Gram stains were performed before and after a 1:10 dilution of the culture was exposed to 2 μg of lysostaphin per ml at 37°C for 30 min. A reduction of 90% or greater in the number of organisms seen on comparison of the pre- and posttreatment Gram stains was considered a "positive" test result and was found in 106 of 108 *S. aureus* strains; 60 of 60 non-*S. aureus* staphylococci had a negative test result, showing no difference between the pre- and posttreatment Gram stains. Identical results were obtained using commercial blood culture media in place of brain heart infusion broth. Also studied prospectively were 100 blood or broth cultures which the clinical microbiology laboratory identified as containing gram-positive cocci suggestive of staphylococci. All 33 cultures later found to contain *S. aureus* gave positive test results; 67 of 67 non-*S. aureus* staphylococci, micrococi, and streptococci were negative.

In 1960, Schindler and Schuhardt described an extracellular substance produced by the organism *Staphylococcus staphylospiticus* which caused the lysis of other species of staphylococci (16). This substance, lysostaphin, is a mixture of proteins, the most important of which is a lytic peptidase active against the peptidoglycan portion of the staphyloccocal cell wall (2, 9). The cell walls of *Staphylococcus aureus* and *Staphylococcus epidermidis* differ in the relative amounts of glycine and serine present in the interpeptide bridging pentapeptides (4, 5, 19, 22), and since lysostaphin's peptidase acts on these bridging pentapeptides, this difference between the cell wall structure of *S. aureus* and *S. epidermidis* results in different sensitivities to lysostaphin (7, 21). We have used this property of the staphylococcal cell wall as the basis of a screening test to rapidly differentiate *S. aureus* from other species of gram-positive cocci in blood or broth cultures of body fluids.

**MATERIALS AND METHODS**

Organisms. Organisms tested consisted of 108 laboratory strains of *S. aureus*, 56 strains of *S. epidermidis*, 2 of *Staphylococcus saprophyticus*, and 2 of other species of micrococi. All 168 strains, which were of human origin, had been identified by standard clinical laboratory methods (10) and had been maintained on brain heart infusion slants at 20°C.

Lysostaphin. Lysostaphin (Sigma Chemical Co., St. Louis, Mo.; lot 18C-0426) with specific activity of 287 U/mg, was stored lyophilized at −20°C until reconstituted in phosphate-buffered saline (pH 7.3). Thereafter, aliquots were kept at −70°C for prolonged periods and at −20°C for less than 30 days.

Test procedure. Strains to be tested were inoculated in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) and incubated at 37°C for 18 h. A 1:10 dilution of the broth culture was made in brain heart infusion broth and contained approximately 2 × 10⁶ colony-forming units per ml. Two drops of the sample specimen was removed for Gram stain preparation. Lysostaphin was added to the remaining specimen at a final concentration of 2 μg/ml (0.574 U/ml). The specimen was incubated on a tilting mixer for 30 min at 37°C, and a posttreatment Gram stain was then prepared. The pre- and posttreatment Gram stains were viewed with the oil immersion objective, and the number of organisms per field in five random fields was counted. A 90% or greater reduction in the number of organisms visualized between the pre- and posttreatment slides was defined as a positive test. Each test during this study was performed by an observer who did not know the species of organism being tested.

Simulated blood culture. Blood cultures from hospitalized patients submitted to our clinical microbiology laboratory and subsequently determined on day 10 to be bacteriologically sterile were used for this series of experiments. Vacutainer blood culture bottles (Becton-Dickinson, Fort Rutherford, N.J.) were used for all cultures. The blood culture bottles were inoculated with the organism to be tested, incubated at 37°C for 18 h, and then studied as described above.

Clinical specimens. To determine the usefulness of this technique on clinical specimens, we studied
prospectively 100 blood or broth cultures submitted to the Clinical Microbiology Laboratory, Ann Arbor Veterans Administration Medical Center, from May to December 1979, which were found to contain gram-positive cocci. These specimens consisted of blood cultures (70) and broth cultures of cerebrospinal fluid (11), intravenous catheter tips (11), pleural fluid (4), joint fluid (1), peritoneal fluid (1), and skin bleb fluid (2). After the laboratory identified a culture as containing gram-positive cocci, 1 ml of the specimen was removed, diluted 1:10 in brain heart infusion broth, and tested for lysostaphin sensitivity as described.

RESULTS

Of the 108 S. aureus strains, 106 gave a positive test result (Fig. 1); of the 60 non-S. aureus staphylococci, all 60 gave a negative test result (Fig. 2 and Table 1). The two S. aureus strains giving a false-negative result were isolated from patients with endocarditis; by tube dilution methodology, both strains demonstrated very high lysostaphin minimum inhibitory concentrations of 16 and 128 µg/ml, respectively. Overall these results correspond to a test sensitivity of 98% and a specificity of 100%.

Table 2 outlines the test results obtained for the 100 organisms isolated from clinical specimens. These specimens were tested, and results were interpreted 24 to 48 h before final identification of the organism by the microbiology laboratory. Of the 100 clinical isolates, 33 were ultimately identified as S. aureus on the basis of a positive tube coagulase test; all 33 isolates produced a positive lysostaphin test result. Sixty-seven specimens were ultimately found to contain other species of gram-positive cocci, of which 55 were identified as S. epidermidis (coagulase negative, anaerobic dextrose positive), 7 as Micrococcus species (coagulase negative, anaerobic dextrose negative), and 5 as streptococci; all 67 of these specimens were negative by the lysostaphin test. Thus, no false-positive or false-negative results occurred in these 100 specimens.

DISCUSSION

In all hospitals, S. epidermidis is a frequent contaminant of cultures of blood and other body fluids. It is frequently impossible on clinical grounds alone to determine whether gram-positive cocci isolated from these cultures are more likely to be S. aureus or other species of cocci. As a result, antibiotic therapy is frequently initiated or continued until the final laboratory identification is available, a process that may take 24 to 48 h. Thus, a test that could quickly and reliably differentiate between S. aureus and other gram-positive cocci would provide better

FIG. 1. (a) Gram stain of blood culture of S. aureus. (b) Same culture after exposure to lysostaphin at 2 µg/ml; “positive test.” Magnification, 1,000.
of other staphyloccocal species (16). Lysostaphin's most active component is a peptidase, which cleaves the pentapeptide cross-linking bridges of the staphylococcal peptidoglycan (2, 9).

In the 1960s, lysostaphin's potential as an anti-

**TABLE 1. Results of the lysostaphin sensitivity test in 168 strains of staphylococci and micrococci from laboratory collections**

<table>
<thead>
<tr>
<th>Test organism</th>
<th>No. of strains</th>
<th>No. positive</th>
<th>No. negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>108</td>
<td>106</td>
<td>2</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>56</td>
<td>0</td>
<td>56</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Micrococcus species</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

*In broth or simulated blood culture.*

*These two strains of S. aureus had lysostaphin minimum inhibitory concentrations of 16 and 128 μg/ml, respectively.*

**TABLE 2. Results of the lysostaphin sensitivity test for 100 strains of staphylococci, micrococci, and streptococci isolated from clinical specimens**

<table>
<thead>
<tr>
<th>Specimen origin (no.)</th>
<th>S. aureus isolates</th>
<th>Non-S. aureus isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test result</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Blood (70)</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Cerebrospinal fluid (11)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intravenous catheters (11)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Pleural fluid (4)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Joint fluid (1)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Peritoneal fluid (1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Skin bleb fluid (2)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*S. epidermidis* (55), *Micrococcus* species (7), streptococci (5).

**FIG. 2.** (a) Gram stain of blood culture of *S. epidermidis*. (b) Same culture after exposure to lysostaphin at 2 μg/ml; "negative test." Magnification, 1,000.
staphylococcal chemotherapeutic agent was evaluated in mice (15, 18) and dogs (6), and in humans topical lysostaphin was demonstrated to eliminate the nasal carriage of *S. aureus* (13).

Recently, lysostaphin has been evaluated as a means of differentiating various species of staphylococci. Investigations by Schleifer and Kloos (11, 17) and Heddaeus et al. (8) demonstrated considerable strain-to-strain variation in susceptibility of staphylococci to lysostaphin. However, the concentration of lysostaphin used in these studies ranged from 50 to 200 μg/ml, which is 25 to 100 times greater than that used in the present study. It has been shown that 95% of *S. aureus* are sensitive to ≤1.56 μg of lysostaphin per ml, and that 96% of *S. epidermidis* are resistant to 1.56 μg of lysostaphin per ml with a median minimum inhibitory concentration of greater than 100 μg/ml (7, 21). Although Pulverer and Jeljaszewicz found that 56% of 173 strains of *S. epidermidis* were sensitive to <2.5 μg of lysostaphin per ml (14), their studies were done using tube dilution techniques and a 24-h incubation period, conditions markedly different from those used in our test system. It is possible that on rare occasions a strain of *Staphylococcus simulans*, which is coagulase negative and lysostaphin sensitive, could be isolated from a clinical specimen and give a false-positive test result (11).

Lysostaphin resistance was demonstrated in 2 of our 108 laboratory strains of *S. aureus* and is also a potential limiting factor of the test’s usefulness. However, lysostaphin resistance is the result of a mutation in the genome demonstrable only after exposure to lysostaphin; no naturally occurring lysostaphin-resistant strains of *S. aureus* have been reported (20, 22). Resistance to lysostaphin should not be a factor affecting the reliability of this test for new clinical isolates. The lysostaphin sensitivity test as described in this report provides a reliable, rapid method of determining the presence of *S. aureus* in cultures of blood or other body fluids. We have observed no false-positive or false-negative results when testing isolates from clinical specimens, and we believe the procedure deserves consideration for routine clinical laboratory use.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**