Antiserum Agar Method for Identification of Smith Type Exopolysaccharides in Clinical Isolates of Staphylococcus aureus

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We used an antiserum agar method to identify clinical Staphylococcus aureus strains producing an exopolysaccharide antigenically identical to the S. aureus Smith diffuse strain. S. aureus blood isolates were obtained from 137 patients, and three additional isolates were obtained from bone debridement. The 140 patients were clinically divided into the following groups: 1, endocarditis (7 patients); 2, pneumonia, empyema, or both (33 patients); 3, intravascular device (34 patients); 4, superficial or wound infection or both (35 patients); 5, deep tissue infections (18 patients); and 6, unknown bacteremias (13 patients). Ninety (64.3%) of the total 140 S. aureus isolates were found to produce precipitin halos on the antiserum agar. The percentage was greatest in the isolates from the endocarditis group (100%) and least in deep tissue infections (55.5%). The presence of clinical S. aureus strains producing exopolysaccharides antigenically identical to the Smith diffuse strain exopolysaccharide appears to be a common phenomenon.

Although the encapsulated Staphylococcus aureus Smith diffuse strain was isolated in 1930, the production of exopolysaccharides by clinical S. aureus isolates is controversial (7, 12). Marme et al. (8–10) used electron microscopy to demonstrate extensive exopolysaccharide deposition surrounding S. aureus infections of pacemaker leads, peritoneal dialysis catheters, and intravenous devices. The techniques used for screening S. aureus isolates for exopolysaccharide production previously depended upon the serum-soft agar technique (14, 15) and immunoelectrophoresis (6). We (13) recently reported an antiserum agar method for use in detecting exopolysaccharide production by S. aureus. Similar antiserum agar methods are now employed for serogrouping Neisseria meningitidis and other encapsulated organisms (1, 2). In the present investigation we used the antiserum agar method to screen clinical isolates of S. aureus for the production of an exopolysaccharide antigenically identical to the exopolysaccharide by the S. aureus Smith diffuse strain.

MATERIALS AND METHODS

Isolates of bacteria. Clinical isolates of S. aureus were collected from specimens submitted to the Clinical Microbiology Laboratories of the Erie County Medical Center and Veterans Administration Medical Center from January 1982 through August 1984. The majority of the isolates were obtained from blood cultures of separate patients. The remaining isolates were obtained from bone cultures collected at surgery. The identity of S. aureus was based on routine laboratory procedures and a tube coagulase test. After the identification of S. aureus in a clinical specimen, a subculture was made to Columbia agar (Difco Laboratories, Detroit, Mich.) supplemented with 1.0% yeast extract (Difco).

Patient clinical categorization. After a review of their clinical records, patients were divided into seven major disease categories. Endocarditis (group 1) was based on the following criteria: bacteremia and pulmonary infiltrates in abusers of intravenous drugs, bacteremia with the appearance of valvular insufficiency or peripheral manifestations of endocarditis, and bacteremia and positive valve cultures at surgery. Pneumonia or empyema or both (group 2) was diagnosed when a S. aureus bacteremia was associated with a simultaneous culture of S. aureus from pleural fluid or radiographic and clinical evidence of pulmonary infection. Intravascular catheter or access device infections (group 3) were diagnosed in patients with a S. aureus bacteremia and an intravascular device in which no other focus of infection was identified and there was evidence of catheter infection either by broth culture of the catheter or inflammation at the catheter site. Superficial or wound infections or both (group 4) were infections of surgical wounds or skin (cellulitis, decubitus ulcers, furuncles, etc.) with an associated S. aureus bacteremia. A deep tissue infection (group 5) was diagnosed when the primary focus of the S. aureus could be related to deep visceral or extensive infection. S. aureus bacteremia in which a primary focus could not be identified was classified as unknown (group 6).

Type strain and production of antisera. The S. aureus Smith diffuse strain and the nonencapsulated variant Smith compact strain were obtained from M. Ann Melly, Vanderbilt University, Nashville, Tenn. Antiserum to the Smith diffuse strain was produced in rabbits (11). The immunization schedule consisted of three series of 3 successive days of intravenous injections (10⁶ to 10⁹ CFU) of Formalin-killed whole bacteria into the marginal ear veins of rabbits. Each series was separated from the previous by an interval of 5 days. The rabbits were bled 7 days after the last inoculation.

Preparation of antiserum agar plates. The Smith diffuse antiserum was heat inactivated at 60°C for 30 min and filter sterilized. Columbia broth (Difco) was supplemented with 0.5% agarose (Litex, Denmark), 1.0% yeast extract (Difco), and 7.5% sodium chloride and then autoclaved and allowed to cool in a water bath to 56°C. The heat-inactivated sterile antiserum was mixed with the supplemented Columbia agar for a final concentration of 5.0% and poured into sterile Falcon petri dishes (35 by 10 mm; Becton Dickinson Labware, Oxnard, Calif.). The plates were stored for up to 14 days at 4°C in a closed, humidified container until used.

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The S. aureus isolates to be tested were inoculated onto the agar plates with one line slightly embedded into the medium. Control S. aureus and compact strains were included with each series. The plates were then incubated in a closed humidified container at 37°C overnight. They were further incubated for an additional 24 to 48 h at 4°C in a closed humidified container and examined at each interval with indirect light against a dark background. The majority of the isolates were able to be transferred to antiserum agar within two to three subcultures from the original laboratory specimen.

**RESULTS**

**Patient groups.** S. aureus endocarditis (group 1) was diagnosed in seven patients. Pneumonia or empyema or both (group 2) were found in 33 patients. Empyema was specifically present in 5 of the 33 patients in which the diagnosis was sought by pleural fluid culture. S. aureus bacteremia was related to an infected intravascular catheter or access device or both (group 3) in 34 patients. Superficial or wound infections (group 4) were responsible for 35 episodes of bacteremia. An infected surgical wound was identified in 17 of the patients, whereas the remainder of the 35 patients had a variety of infections including cellulitis, furuncles, decubitus ulcers, and leg ulcers. A deep tissue infection (group 5) was identified in 18 patients. A further breakdown of the specific sites of deep tissue infection showed the following involvement: central nervous system (2 patients), urinary tract (3 patients), osteomyelitis (5 patients), ilioptosous abscess (1 patient), ischemic bowel (1 patient), prosthetic joint (1 patient), pericarditis (1 patient), septic joint (1 patient), septic thrombophlebitis (1 patient), subepithelial abscess (1 patient), and thigh abscess (1 patient). The source of the S. aureus bacteremia was unknown (group 6) in 13 patients. A total of 140 S. aureus isolates were tested by antiserum agar. All but three of the isolates were obtained from blood cultures.

**Identification of precipitin halos on Smith antiserum agar.**

<table>
<thead>
<tr>
<th>Clinical group no.</th>
<th>Disease</th>
<th>Total no.</th>
<th>No. positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Endocarditis</td>
<td>7</td>
<td>7 (100)</td>
</tr>
<tr>
<td>2</td>
<td>Pneumonia or empyema or both</td>
<td>33</td>
<td>22 (66.7)</td>
</tr>
<tr>
<td>3</td>
<td>Intravascular catheter or access device or both</td>
<td>34</td>
<td>21 (61.8)</td>
</tr>
<tr>
<td>4</td>
<td>Superficial or wound infection or both</td>
<td>35</td>
<td>21 (60.0)</td>
</tr>
<tr>
<td>5</td>
<td>Deep tissue infection</td>
<td>18</td>
<td>10 (55.6)</td>
</tr>
<tr>
<td>6</td>
<td>Unknown</td>
<td>13</td>
<td>9 (69.2)</td>
</tr>
</tbody>
</table>

Table 1 summarizes the evaluation of these isolates for the presence of precipitin halos on the Smith antiserum agar. Of the 140 isolates examined, 90 (64.3%) were found to produce precipitin halos after 48 h of incubation at 4°C. When the results were evaluated after 24 h of incubation, the number of isolates producing halos was slightly less at 82 (58.6%). Prolonged incubation beyond 48 h led to a loss of distinction of some of the halos and confusion in their interpretation. The control variant, S. aureus Smith compact strain, remained without halos throughout the 4°C incubation. The percentage of strains in each clinical group producing a halo ranged from 55.6% in deep tissue infections (group 5) to 100% in endocarditis (group 1). These two groups represented only a small proportion (25 patients) of the total 140 patients (17.9%). The three groups in which the largest number of patients (total 102) were found varied from 60.0% in superficial or wound infections (group 4) through 61.8% in intravascular catheters or access device infections (group 3) to 66.7% in pneumonia or empyema (group 2).

**DISCUSSION**

The S. aureus Smith diffuse strain was isolated from a patient with osteomyelitis in 1930 and was not described until 1956 (12). The strain attracted laboratory attention because of its unique capacity for virulence after mouse intraperitoneal inoculation (5). Koenig (7) later isolated an avirulent variant (Smith compact strain) and demonstrated that the virulence of the Smith diffuse strain was related to the presence of an exopolysaccharide that the variant lacked.

Although these observations on the virulence of the two Smith strains were exciting, the clinical significance of exopolysaccharide production by S. aureus remains controversial. Early investigations into exopolysaccharide produced by S. aureus was based on the serum-soft agar technique described by Finkelstein and Sulken (3). Their basal medium consisted of 0.15% agar with 1.0% normal serum. Strains producing an exopolysaccharide when suspended in this medium produced a characteristic diffuse colonial morphology as compared with their nonproducing variant's compact colonial morphology. Yoshida (14) found that specific antiserum in the serum-soft agar technique would convert a diffuse colonial morphology to compact and therefore allow the demonstration of different antigenic types of exopolysaccharides. Although the exact mechanism responsible for the differences in the colonial morphology of exopolysaccharide producing S. aureus on serum-soft agar is not certain, an antigen-antibody precipitin reaction is not involved (3).

Yoshida (14) used serum-soft agar to examine 103 clinical S. aureus isolates. He found that 19 (18.4%) strains exhibited a diffuse type growth; 14 of these strains were antigenically identical to the Smith diffuse exopolysaccharide. In a further study with medium with a pH of 6.0, Yoshida et al. (15) determined that 82 of 91 (90.1%) isolates now exhibited diffuse growth. When Yoshida et al. (16) used a fluorescent antibody technique, they found that 125 (76.6%) of 163 isolates produced exopolysaccharide. A Smith diffuse antigenic type was detected in 54.4% of the isolates.

More recently, Karakawa and Vann (6) used immunoelectrophoresis to screen crude antigens prepared from S. aureus cultures. They formulated a provisional classification of eight different antigenic types, with type 8 the most frequently encountered (4). Strain Smith diffuse was not included among their antigenic types; however their type 2 was reported to cross-react with Smith diffuse.

We (13) previously investigated an antiserum agar technique for the detection of exopolysaccharide production from the Smith diffuse strain. Whereas colonies of the Smith diffuse strain produced a clearly visible precipitin halo, the variant, Smith compact strain, lacked these halos. For the present study, we modified the technique by using enriched agar to enhance exopolysaccharide production and also decreased the agarose content to 0.5% to allow improved diffusion into the medium. The sodium chloride content was maintained at 7.5% to minimize any possible interference from protein A production. The variant, Smith compact
strain, when cultured on this medium again lacked a surrounding precipitin halo.

Our investigation was limited to the detection of exopolysaccharides antigenically identical to the well-known S. aureus Smith diffuse strain. We found that 64.3% of 140 clinical S. aureus isolates produced an antigenically identical exopolysaccharide. The addition of antiserum agar to other antigenically diverse S. aureus exopolysaccharides may further increase the percentage of clinical exopolysaccharide producing S. aureus detected. Our results with the antiserum agar method with antiserum to the Smith diffuse strain suggest that the production of exopolysaccharides by clinical strains of S. aureus is a common phenomenon. Further investigations may determine the utility of S. aureus typing systems based upon the antigenic type of exopolysaccharide.

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LITERATURE CITED