JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1985, p. 490–492
0095-1137/85/040490-03$02.00/0

Vol. 21, No. 4

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

TIMOTHY E. WEST,* MARCIA E. WEST, AND JOSEPH M. MYLOTTE

Department of Medicine, Division of Infectious Diseases, State University of New York at Buffalo, Buffalo, New York 14215

Received 14 September 1984/Accepted 24 December 1984

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). Marrie et al. (8–10) used electron microscopy to demonstrate extensive exopolysaccharide deposition surrounding *S. aureus* infections of pacemaker leads, peritoneal dialysis catheters, and intravascular 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 identification 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 (10^8 to 10^9 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.

* Corresponding author.

TABLE 1. Antiserum agar identification of Smith type exopolysaccharides from clinical isolates of *S. aureus* after 48 h of incubation at 4°C

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

The *S. aureus* isolates to be tested were inoculated onto the agar plates with one line slightly embedded into the medium. Control Smith diffuse 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), iliopsoas abscess (1 patient), ischemic bowel (1 patient), prosthetic joint (1 patient), pericarditis (1 patient), septic joint (1 patient), septic thrombophlebitis (1 patient), subphrenic 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 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 exopolysaccaride. 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.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant 1R23AI18654 from the National Institutes of Health.

LITERATURE CITED

1. **Ashton, F. E., B. B. Diena, and C. E. Frasch.** 1979. Evaluation of the antiserum agar method for the serogroup identification of *Neisseria meningitidis*. Can. J. Microbiol. **25**:784–787.
2. **Bradshaw, M. W., R. Schneerson, J. C. Parke, and J. B. Robbins.** 1971. Bacterial antigens cross-reactive with the capsular polysaccharide of *Haemophilus influenzae* type b. Lancet **i**:1095–1096.
3. **Finkelstein, R. A., and S. E. Sulken.** 1958. Characteristics of coagulase-positive and coagulase-negative staphylococci in serum-soft agar. J. Bacteriol. **75**:339–344.
4. **Fournier, J. M., W. F. Vann, and W. W. Karakawa.** 1984. Purification and characterization of *Staphylococcus aureus* type 8 capsular polysaccharide. Infect. Immun. **45**:87–93.
5. **Hunt, G. A., and A. J. Moses.** 1958. Acute infection of mice with Smith strain of *Staphylococcus aureus*. Science **128**:1574–1575.
6. **Karakawa, W. W., and W. F. Vann.** 1982. Capsular polysaccharides of *Staphylococcus aureus*, p. 285–293. *In* J. B. Robbins, J. C. Hill, and J. C. Sadoff (ed.), Seminars in infectious disease, vol. 4. Bacterial vaccines. Thieme Stratton, Inc., New York.
7. **Koenig, M. G.** 1962. Factors relating to the virulence of staphylococci. I. Comparative studies on two colonial variants. Yale J. Biol. Med. **34**:537–559.
8. **Marrie, T. J., and J. W. Costerton.** 1984. Scanning and transmission electron microscopy of in situ bacterial colonization of intravenous and intraarterial catheters. J. Clin. Microbiol. **19**:687–693.
9. **Marrie, T. J., J. Nelligan, and J. W. Costerton.** 1982. A scanning and transmission electron microscopic study of an infected endocardial pacemaker lead. Circulation **66**:1339–1341.
10. **Marrie, T. J., M. A. Noble, and J. W. Costerton.** 1983. Examination of the morphology of bacteria adhering to peritoneal dialysis catheters by scanning and transmission electron microscopy. J. Clin. Microbiol. **18**:1388–1398.
11. **Oeding, P.** 1957. Agglutinability of pyogenic staphylococci at various conditions. Acta Pathol. Microbiol. Scand. **41**:310–324.
12. **Smith, J. M., and R. J. Dubos.** 1956. The behavior of virulent and avirulent staphylococci in the tissues of normal mice. J. Exp. Med. **103**:87–107.
13. **West, T. E., and M. A. Apicella.** 1984. Detection of encapsulation in *Staphylococcus aureus* by use of antiserum agar. J. Clin. Microbiol. **20**:141–144.
14. **Yoshida, K.** 1971. Demonstration of serologically different capsular types among strains of *Staphylococcus aureus* by the serum-soft agar technique. Infect. Immun. **3**:535–539.
15. **Yoshida, K., A. Nakamura, T. Ohtomo, and S. Iwami.** 1974. Detection of capsular antigen production in unencapsulated strains of *Staphylococcus aureus*. Infect. Immun. **9**:620–623.
16. **Yoshida, K., M. Takahashi, T. Ohtomo, Y. Minegishi, Y. Ichiman, and K. Haga.** 1979. Application of fluorescent antibody for detecting capsular substances in *Staphylococcus aureus*. J. Appl. Bacteriol. **46**:147–152.