Pour-Plate Method for the Detection of Coagulase Production by Staphylococcus aureus

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A pour-plate method is described for the detection of coagulase production by Staphylococcus aureus. Either Brain Heart Infusion agar or yeast extract-Trypticase soy agar containing swine plasma was the best medium for the detection of coagulase by this method. The advantages of this method and its potential utilization in the clinical laboratory, in food microbiology, and in specialized studies with S. aureus are discussed.

The production of coagulase is accepted generally as the most reliable test for the identification of Staphylococcus aureus. Two common methods for its detection are the slide test and the tube test. The former, which tests for the production of bound coagulase (clumping factor), correlates poorly with coagulase production by the tube method and is not recommended for routine diagnostic work (4, 6). Penfold (8) and Reid and Jackson (10) introduced an alternate method of coagulase testing whereby staphylococci are inoculated onto the surface of a medium containing plasma. Although the surface inoculation method has not gained wide acceptance, Lack and Wailling (5) found it a satisfactory substitute for the tube test. In this paper we describe a variation of the surface inoculation method in which a pour-plate method is utilized to detect coagulase production. This method has potential uses in medical and food microbiology and in genetic studies with S. aureus.

MATERIALS AND METHODS

Cultures. The cultures included 18 propagating strains of the International Series of Staphylococcal Bacteriophages, 8 other strains of S. aureus, and 20 strains of Staphylococcus epidermidis from the collection of one of us (J.N.B.).

Media. The following Difco broth media, to which 3% agar (Difco) was added, were used: Brain Heart Infusion (BHI agar), nutrient broth (NB agar), synthetic broth AOAC (AOAC agar), Mueller-Hinton medium (MH agar), and Trypticase soy broth (BBL) with 0.3% yeast extract (YETS agar). To all media, 0.1% (vol/vol) polyoxyethylene sorbitan monoooleate (Tween 80; Matheson Coleman and Bell, Norwood, Ohio) was added.

Plasmas. Human plasma was obtained from blood bank specimens and pooled. Rabbit and bovine plasmas containing 10% (vol/vol) sodium citrate were obtained from Pel-Freeze Biologicals, Inc., Rogers, Ark. Fresh swine blood collected with 10% (vol/vol) sodium citrate was obtained from the slaughter house and centrifuged at 650 × g for 10 min to remove the blood cells. All plasmas were sterilized by filtration through 0.2 mm filters (Sefar, Millipore, Millford, Mass.). Plasmas were dispensed in 50-ml amounts and stored at −20°C. Just prior to use, a sufficient quantity of plasma was thawed and added to the nutrient medium to a final concentration of 20% (vol/vol). When certain lots of plasma were thawed, they exhibited an intense turbidity which disappeared upon warming the plasma in a water bath at 37°C. For the success of the pour-plate method, it is essential that the plasma be clear.

Method. For the pour-plate method of coagulase testing, molten agar medium containing plasma (plasma agar) was inoculated, and 8 to 10 ml was poured into a petri dish. After incubation at 37°C for 18–24 h, coagulase-producing colonies were detected by the presence of dense halos of precipitated fibrin around them (Fig. 1). The addition of Tween 80 to the nutrient medium aided in its clarification and facilitated the pouring of agar plates with small volumes of medium.

To determine the best medium and plasma for the detection of coagulase production by the pour-plate method and whether coagulase-negative colonies of staphylococci could be distinguished from coagulase-positive colonies, 26 cultures of S. aureus and 20 cultures of S. epidermidis were grown in YETS broth at 37°C for 18 to 24 h. Dilutions of these cultures were made in distilled water so that approximately 2 × 10^8 to 2 × 10^9 colony-forming units (CFU) was inoculated into 8 ml of molten agar medium containing 2 ml of plasma and poured into petri dishes. The nutrient media used were YETS agar, BHI agar, NB agar, AOAC agar, and MH agar and either bovine, human, rabbit, or swine plasma. After incubation at 37°C for 18 to 24 h, zones of precipitated fibrin
produced in each medium by colonies of *S. aureus* were compared, or the presence or absence of fibrin halos around colonies of *S. epidermidis* was recorded.

To compare coagulase production by the pour-plate method and by the surface inoculation method, YETS agar containing either 20% (vol/vol) swine or rabbit plasma was poured into petri dishes which were then dried overnight at 37°C. Twenty-six cultures of *S. aureus* grown for 24 h in YETS broth were diluted 10⁻¹ in distilled water and then streaked onto the surfaces of these plasma agar plates. Molten tubes of plasma agar inoculated with from 10⁴ to 5 × 10⁵ CFU of these same 26 cultures were poured into petri dishes. After incubation at 37°C for 18 to 24 h, zones of precipitated fibrin produced by each culture were compared by the two methods.

The pour-plate method for coagulase testing was compared with a procedure used in our laboratories to isolate coagulase-positive staphylococci from the nose. Nasal swabs from 55 young adults were incubated in BHI broth at 37°C for 2 h. One-tenth milliliter of these cultures was then streaked onto phenol-red-mannitol agar containing 7.5% NaCl (PRMS; Difco) and also inoculated into 8 ml of molten YETS agar containing 2 ml of swine plasma. After incubation at 37°C for 18 to 24 h, either the presence or absence of fibrin halos around colonies in plasma agar or the presence of mannnit-fermenting or mannnit-nonfermenting colonies on the PRMS agar was recorded for each nasal culture. From each of the PRMS plates, subcultures of either a mannnit-fermenting colony or several mannnit-nonfermenting colonies were inoculated directly into molten swine plasma agar. The number of cultures yielding coagulase-producing colonies upon subculture from PRMS into swine plasma agar and the number yielding coagulase-producing colonies from the plasma agar plates used alone for primary isolation was compared. From both sets of plasma agar plates, all colonies identified tentatively as coagulase producers were tested with the tube method to confirm their coagulase reaction.

RESULTS AND DISCUSSION

The detection of coagulase production by the pour-plate method was best with swine plasma in either BHI or YETS agar. To be acceptable for coagulase testing, a plasma must have an adequate amount of coagulase-reacting factor (CRF) and fibrinogen, be relatively free of fibrinolytic activity due to activation of the plasminogen-plasmin system, and be free of significant amounts of inhibitors (11). Plasmas not only from different species of animals but also from different animals of the same species vary in their suitability for coagulase testing (11, 12). To obviate the possibility of the presence of inhibitors or antibodies to coagulase (9, 13) in the plasmas used in our study, either pooled human plasma or several batches of bovine, rabbit, and swine plasmas were used before concluding its suitability for use in the pour-plate method. In NB agar, the zones of precipitated fibrin were poorest, regardless of the plasma used. In either MH or AOAC agar with either rabbit or swine plasma, the zones of precipitated fibrin were not as consistently intense as with either BHI or YETS agar. With bovine or human plasmas, regardless of the nutrient medium, the zones of precipitated fibrin were either faint or not discernible. When rabbit and swine plasma were compared with YETS agar as the nutrient medium, 9 (34.6%) of 26 cultures produced less-intense zones of precipitated fibrin in rabbit plasma agar than in swine plasma agar, and 4 (15.4%) produced undetectable zones of coagulase activity in rabbit plasma agar. We found BHI agar with swine plasma comparable to YETS agar with swine plasma in the consistency and clarity of coagulase reactions in the pour-plate method. Both human and rabbit plasmas have adequate amounts of CRF, fibrinogen, and generally are free of inhibitors, so that either plasma is recommended for the tube test for coagulase in the American Public Health Association manual of diagnostic procedures (2).

However, as demonstrated in our study, we do not recommend human plasma for the pour-plate method of coagulase testing, and we found rabbit plasma markedly inferior to swine plasma. Interestingly, Weiss et al. (Bacteriol. Proc., p. 23, 1972) found swine plasma less variable than rabbit plasma in their tube coagulase tests. Although Gross (3) reported
the use of swine plasma many years ago, its acceptability for coagulase testing has not received much attention. According to Orth et al. (7), plasmin activity is greater in both rabbit and human plasmas than in swine plasma. As a result, staphylokinase and staphyloccocal Müller factor activate the plasminogen-plasmin system, and the plasmin thus formed causes fibrinolysis and false negative reactions when rabbit plasma is used for coagulase testing. Although we have no data to support the conclusions of Orth et al. (7) as to the reason for the inferiority of rabbit and human plasmas in our study, the availability of large volumes of swine plasma from slaughter houses and the superiority of this plasma over the other plasmas we tested make the use of swine plasma in the pour-plate method both economical and practical. Its acceptance for routine coagulase tests should be considered.

When *S. epidermidis* was grown in plasma agar, none of the 20 cultures tested produced zones of precipitated fibrin. In nasal cultures where colonies of *S. epidermidis* were clustered together, faint hazy zones with diffuse edges were observed occasionally. However, these faint zones were easily distinguished from the typical dense zones with definite circumscribed edges produced by coagulase activity around individual colonies of *S. aureus*.

In Table 1 are summarized the results of testing 26 cultures of *S. aureus* by the pour-plate method and by the surface inoculation method with swine or rabbit plasma and YETS agar as the nutrient medium. All of the 26 cultures produced either large or small dense zones of precipitated fibrin by the pour-plate method with swine plasma. When tested by the surface inoculation method with swine plasma, 7 (26.9%) of 26 cultures produced zones of precipitated fibrin which were either faint or not detectable. When rabbit plasma was used with either the pour-plate method or the surface inoculation method, the detection of coagulase activity was variable (Table 1). With the pour-plate method, 18 (61.5%) of 26 cultures produced either large or small dense zones of precipitated fibrin, whereas 10 (38.5%) produced zones which were either faint or not detectable. The results were even more variable with the surface inoculation method. Only 5 (19.2%) of 26 cultures produced either large or small dense zones of precipitated fibrin, whereas 21 (80.8%) produced zones which were either faint or could not be detected. With the pour-plate method we have observed surface colonies with faint or undetectable zones of precipitated fibrin and subsurface colonies in the same plasma agar plate with large dense zones of precipitated fibrin around them. Thus, the pour-plate method appears to be a more-sensitive method for coagulase testing than the surface inoculation method.

When the pour-plate method was compared with the use of PRMS to isolate *S. aureus* from 55 nasal cultures, *S. aureus* was detected in 21 plasma agar cultures, whereas only 18 PRMS cultures yielded detectable *S. aureus*. The three cultures negative for *S. aureus* contained such small numbers of *S. aureus* that they were overgrown or inhibited by other staphylococci present on PRMS plates inoculated with the nasal cultures. The presence of fibrin halos around colonies in the plasma agar cultures correlated perfectly with coagulase production with the tube method. These observations indicate that the pour-plate method is a more sensitive method for isolating *S. aureus* and can be used for the primary isolation, enumeration, and detection of *S. aureus* in a single step. It might be used advantageously in certain instances in the clinical laboratory and has potential uses in detecting *S. aureus* in food.

We have used this pour-plate method in genetic studies with *S. aureus* (Parisi et al., manuscript in preparation). It may prove valuable in other specialized studies with this organism since it is possible to detect a single coagulase-producing colony in a plate containing 8 to 10 ml of plasma agar inoculated with 5 × 10^4 non-coagulase-producing CFU (Fig. 1). For convenience, 8 ml of BHI or YETS agar can be stored in 18-mm screw-capped tubes and then 2 ml of swine plasma can be added to the molten medium just prior to inoculation. This provides the proper amount of plasma in the nutrient medium and insures the proper amount of plasma agar for pouring into a petri dish.

| Type of zone of precipitated fibrin | No. of cultures of *S. aureus* in YETS agar with: |
|-----------------------------------|-----------------------------------------------|
|                                   | Swine plasma | Rabbit plasma |
|                                   | Pour plate | Surface inoculation | Pour plate | Surface inoculation |
| Large dense                       | 23         | 16 | 13 | 4 |
| Small dense                       | 3          | 3  | 3  | 1 |
| Faint                             | 0          | 3  | 6  | 6 |
| None                              | 0          | 4  | 15 | 15 |
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It has been reported that some strains of Streptococcus fecalis, Streptococcus pyogenes, Escherichia coli, Serratia marcescens, and Pseudomonas aeruginosa are able to clot plasma (1, 2). Bayliss and Hall (1) found this reaction occurred only with citrated plasma due to the utilization of citrate by the microorganisms, thereby releasing calcium for normal physiological clotting. Although we have not attempted an exhaustive search for organisms other than S. aureus able to clot plasma, we have not found any that could be mistaken for S. aureus with our pour-plate method. Perhaps the concentration (10% vol/vol) of citrate we use in our plasma precludes its complete utilization by other microorganisms with the capacity to clot citrated plasmas.

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