JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1975, p. 453-455
Vol. 2, No. 5

Printed in U.S.A.

Methods to Improve Detection of Pneumococci in Respiratory Secretions

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Received for publication 18 June 1975

Simple methods to enhance the detection of pneumococci in respiratory secretions are needed. Sheep blood agar containing 5 μg of gentamicin per ml was more often positive (89%) than either standard sheep blood agar (54%) or mouse inoculation (65%) in recovering pneumococci from 62 adult and pediatric patients. In adults, the direct quellung test on sputum smear was a rapid, sensitive method for predicting subsequent pneumococcal isolation by culture (19 of 20 patients, 95%). The quellung test and gentamicin plate show improved sensitivity over current techniques for pneumococcal detection and can be recommended for general use.

Accurate and rapid identification of pneumococci in clinical specimens from patients with acute lower respiratory tract infection is important for selection of appropriate antimicrobial therapy (14). However, techniques for pneumococcal detection now in general use are not entirely satisfactory. The Gram stain lacks specificity due to the close morphological resemblance of pneumococci to other streptococci (1, 10, 13). Culture on sheep blood agar lacks sensitivity (4, 7, 8, 10, 11, 17), and mouse inoculation, although having recognized sensitivity (7, 8, 11, 17), is expensive and cumbersome for most clinical laboratories.

This study compares the two standard isolation techniques mentioned with the direct quellung test on sputum and a new selective culture technique, sheep blood agar containing gentamicin (5).

MATERIALS AND METHODS

Thirty-two adult and 30 pediatric patients with acute lower respiratory tract infection were referred for study by the house staff from December 1973 to March 1974. All patients had either acute pneumonia or acute bronchitis based on physical exam and chest X-ray.

Expectorated sputum was obtained by the house staff on all adult patients prior to antibiotic therapy. Smears were made immediately for Gram stain and the quellung test. The quellung test was done as previously described (13). A drop of omniserum containing antibody against all 82 pneumococcal types (Staten Seruminstitut, Copenhagen, Denmark) was placed on the smear. A drop or two of methylene blue was placed on the underside of a cover glass, which was positioned over the smear. This preparation was then immediately viewed under a light microscope with low illumination. Pneumococci were identified by a dark precipitin line completely encircling the capsule of the diplococcus. Gram stains of sputum smears were done by standard methods. All sputa tested had polymorphonuclear leukocytes and were without epithelial cells.

Sputum samples from adults and throat swabs from children were immediately cultured on the following media: (i) plain sheep blood agar (5% sheep blood in Trypticase soy agar); (ii) sheep blood agar containing 5 μg of gentamicin sulfate per ml (Schering Corp., Bloomfield, N.J.); and (iii) Avery's broth (6) for subsequent mouse intraperitoneal inoculation. All cultures were handled by a single technician. The agar plates were incubated at 37 C in candle extinction jars for 18 to 24 h. Avery's broth was incubated at 37 C for 2 h, and then 0.5 ml was inoculated intraperitoneally in male adult white mice. Mice that did not die within 72 h of inoculation were sacrificed. Peritoneal fluid and heart blood from sacrificed and dying mice were inoculated onto plain sheep blood agar. This agar was incubated for 18 to 24 h at 37 C in a candle extinction jar. Pneumococci on agar plates were identified by colonial morphology and confirmed by quellung reaction with omniserum. Pneumococci were typed by a combinatorial pool method (9), using nine antisera pools plus monospecific antisera. The differences in results among culture techniques were analyzed using McNemar's test for significance of changes, with Yates' correction (16).

RESULTS

Sixty-two patients had the three culture techniques performed. Thirty-seven had *Streptococcus pneumoniae* isolated by one or more techniques. Of these 37 positive isolates, the gentamicin plate detected 33 (89%), plain sheep blood agar detected 20 (54%), and mouse inoculation detected 24 (65%) (Table 1). The gentamicin plate was therefore significantly more

sensitive than either the sheep blood agar plate ($P < 0.01$) or mouse inoculation ($P < 0.05$). Fourteen pneumococcal isolates detected by the gentamicin plate were not identified on plain sheep blood agar. Only one isolate that was detected by sheep blood agar was not detected by the gentamicin plate. Similarly, 12 isolates detected on the gentamicin plate were not isolated by mouse inoculation, whereas mouse inoculation isolated three strains not grown on the gentamicin plate. The results with sheep blood agar and mouse inoculation were almost equivalent (7 versus 11 differences, respectively; $P = 0.51$). Five of the mice inoculated with specimens that grew pneumococci by the other methods failed to yield pneumococci. At the time of death in these animals, there was heavy overgrowth of fecal flora in the peritoneal fluid and heart blood, which presumably interfered with the recovery of pneumococci.

TABLE 1. *Comparison of pneumococcal culture methods on respiratory secretions from 32 adult and 30 pediatric patients*

Positive culture	No. of patients		% Positive from total
	Adult	Pediatric	
By any method	23	14	100
Gentamicin plate	20	13	89
Mouse inoculation	16	8	65
Sheep blood agar	13	7	54

Twenty-seven of the above patients also had quellung tests performed on sputa. Nineteen of 20 patients (95%) who had pneumococci isolated from sputum had a positive quellung test. One of six patients with negative sputum cultures had a positive quellung test. Of the 20 patients with positive quellung tests, the gentamicin plate was positive in 17 (85%), mouse inoculation was positive in 14 (70%), and plain sheep blood agar was positive in 13 (65%).

DISCUSSION

We have previously demonstrated the value of the quellung test in increasing the specificity of the Gram stain for identifying pneumococci on a sputum smear (13). The present study confirms that the quellung test of sputum smears is a sensitive method of predicting subsequent cultural isolation of *S. pneumoniae*. Since children usually do not produce sputum

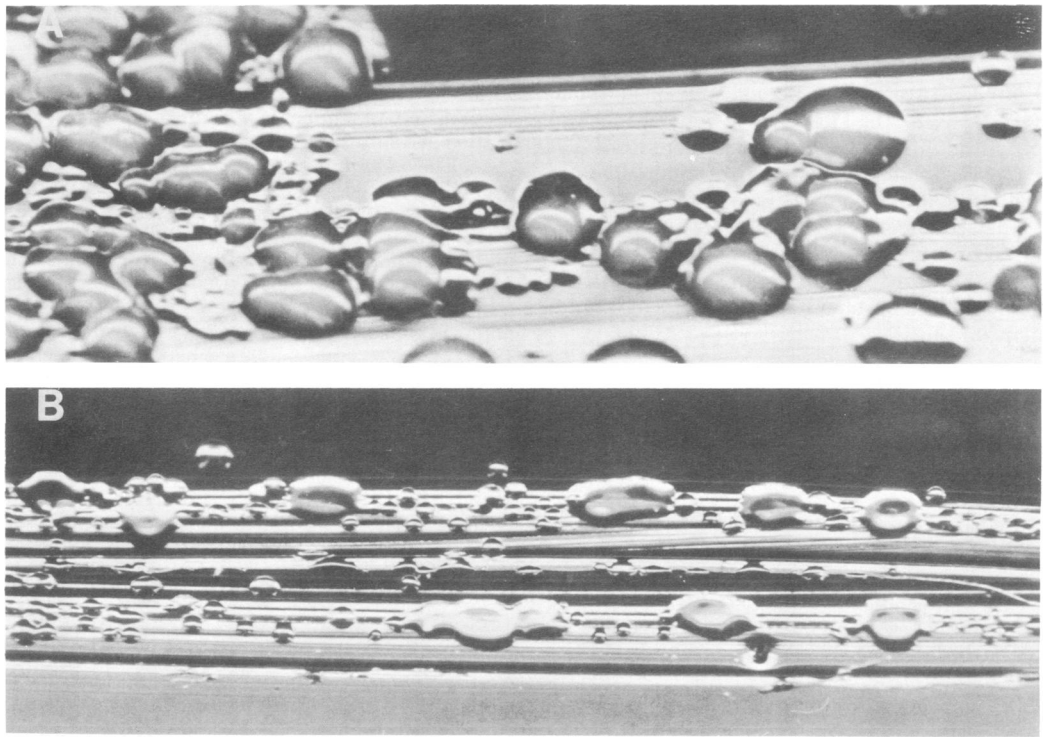

FIG. 1. *(A) Close-up photograph of respiratory secretions cultured on plain sheep blood agar. Heavy growth of mouth flora is present, obscuring the pneumococcal colonies. (B) Close-up photograph of the same specimen cultured on a gentamicin plate. Most of mouth flora is suppressed and fewer colonies are present, allowing easy identification of the concave pneumococcal colonies.*

for examination, the quellung test has limited applicability in the pediatric age group.

The importance and difficulty of detecting pneumococci in clinical specimens has been stressed (4, 12). Standard sputum cultures have failed to yield pneumococci in about one-half of patients with bacteremic pneumococcal pneumonia (4, 7, 15), and several investigators have, therefore, advocated the routine use of mouse inoculation (2, 15, 17). A major problem with the use of plain sheep blood agar for isolating pneumococci from sputum is the usual presence of heavy growth of mouth flora, which makes selection of typical pneumococcal colonies among the other bacterial colonies tedious and difficult (Fig. 1A). The addition of 5 μg of gentamicin per ml to sheep blood agar suppresses growth of many of these interfering pharyngeal bacteria, but not the pneumococcus (3). Therefore, growth on the gentamicin plate is lighter, making selection of the pneumococcal colonies easier and more accurate, since fewer colonies are competing for space and the attention of the technician (Fig. 1B). This study has demonstrated the superior sensitivity of the gentamicin plate over plain sheep blood agar in isolating pneumococci from clinical specimens. Most studies (7, 8, 11, 17) support the superiority of mouse inoculation over sheep blood agar in isolating pneumococci. We were unable to confirm this in the present study. A drawback to mouse inoculation is overgrowth of mouse fecal flora, interfering with interpretation of the test. This is a difficult problem to solve unless dying mice are immediately tested or placed in the refrigerator.

Sputum or pharyngeal culture by any technique does not definitely establish the cause of infection in the lung. However, it was not the intention of this study to establish the etiological diagnosis in these patients, but rather to explore methods to enhance the ability to isolate and detect pneumococci in respiratory secretions. The gentamicin plate and quellung test are simple procedures with improved sensitivity over the standard methods currently used for this purpose.

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