

# Value of Sputum Culture in Diagnosis of Pneumococcal Pneumonia

W. LAWRENCE DREW

Department of Clinical Pathology and Laboratory Medicine, Mount Zion Hospital and Medical Center,  
San Francisco, California 94115

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In our laboratory, culture of sputum was extremely useful in diagnosing the etiology of pneumococcal pneumonia. Of 31 consecutive patients with bacteremic pneumococcal pneumonia, 29 (94%) had *Streptococcus pneumoniae* cultured from sputum. Recovery of pneumococci in culture was enhanced by anaerobic incubation as well as by a plate bile test and an optochin disk on a primary blood agar plate.

Utilization of a smear and culture of sputum to establish the etiology of bacterial pneumonia has fallen into disrepute. Three reports have indicated that only one-half of patients with bacteremic pneumococcal pneumonia have pneumococci recovered from sputum (1, 5, 9). Buoyed by two recent studies (12, 13) from a Veterans Administration Hospital and an Indian health service indicating that pneumococci were present in the sputum of 75 to 97% of patients with pneumococcal pneumonia, we have reviewed our experience in an urban community hospital.

## MATERIALS AND METHODS

**Patient selection.** The medical records of all patients with bacteremia due to *Streptococcus pneumoniae* in a 36-month period (April 1973 to March 1976) were reviewed retrospectively. Only patients with the following criteria were included in this study: (i) pneumococcal bacteremia; (ii) radiographical evidence of pneumonia; and (iii) sputum obtained prior to antibiotic therapy.

In a second phase of the study, the medical records of all patients who had a sputum culture positive for *S. pneumoniae* in March 1976 were reviewed. Patients were designated as having pneumonia if they had symptoms and signs of acute pneumonia as well as radiographical evidence of pulmonary infiltrates.

Sputum was examined in the clinical microbiology laboratory by a direct Gram stain of the specimen and by culture at 35°C on the following media: (i) 5% sheep blood agar (SBA) and 5% chocolate agar in 5% CO<sub>2</sub> in air; (ii) 5% SBA under anaerobic condition (GasPak jars; Baltimore Biological Laboratory, Division of Bioquest, Cockeysville, Md.); and (iii) MacConkey medium in air.

Quantitation of bacterial growth was based on the results of classical streaking on the original plates as follows: 1+, light growth in the primary streaking zone only; 2+, heavy growth in the primary

streaking zone only; 3+, growth in the primary and secondary streaking zones; and 4+, growth in the primary, secondary, and tertiary streaking zones.

To facilitate recognition of the presence of *S. pneumoniae*, an optochin disk was placed directly on the primary streaking area of the 5% SBA plate incubated in CO<sub>2</sub>; similarly a Bacitracin disk (10 U) was placed on the chocolate agar plate to aid detection of *Haemophilus* species (7).

A plate bile test was performed on all specimens by adding a drop of 2% bile to an area where lysis of discrete alpha-hemolytic colonies could be observed on the SBA plate. In addition, one to two individual alpha-hemolytic colonies were subcultured to 5% SBA plates for performance of the optochin test as recommended (2), whether or not they grossly resembled typical pneumococcal colonies.

Blood culture procedures varied during this study period but always included a two-bottle system (one incubated vented, one nonvented) and routine "blind" subcultures at 3 and 7 days of incubation.

All isolates from both sputum and blood were finally identified as *S. pneumoniae* by optochin susceptibility with pure cultures (2) and a zone of inhibition  $\geq 15$  mm.

Gram stains of sputum were assessed for leukocytes, epithelial cells, and bacteria on the following scale: 1+, 1 per oil immersion field; 2+, 1 to 5 per oil immersion field; 3+, 5 to 30 per oil immersion field; and 4+, 30 per oil immersion field.

## RESULTS

Thirty-one patients met the criteria for bacteremic pneumococcal pneumonia; of these 29 (94%) had one or more sputum cultures positive for *S. pneumoniae*. The growth of pneumococci was 3+ or greater in 22 of the sputa (76%). The general quality of the sputum specimens may be assessed by noting that epithelial cells were present in 1+ or less amounts in 83%, whereas leukocytes were present in 3+ or greater

amounts in 72%. In one-half of these specimens the bacteria seen on Gram stain were sufficiently suggestive of pneumococci that the technologist made a note of this in the comment section of the report and/or phoned the result to the physician.

In the second phase of this study the overall rate of sputum cultures positive for *S. pneumoniae* in a 1-month period was 20 of 165 (12%); 2 of these were from bacteremic patients. Of the remaining patients with pneumococci in their sputum, 12 had pneumonia by the criteria set forth above and 6 had bronchitis.

### DISCUSSION

Several studies have shown that sputum cultures are frequently negative in patients with bacteremic pneumococcal pneumonia (Table 1). However, a recent report (12) from an Indian health service had results closer to those of this study. Using a stereo-dissecting microscope to locate suspected pneumococcal colonies, 75% of their bacteremic patients had pneumococci in sputum on routine culture, and the yield was increased to 97% by injecting sputum intraperitoneally in mice.

Neither mouse inoculation nor sputum digestion nor a stereomicroscope was used in our laboratory. The only other methodological differences from the previous studies were (1, 5, 9, 12, 13) that: (i) an optochin disk was placed on the primary culture plate; (ii) a bile test was done on the primary culture plate; and (iii) sputum was cultured anaerobically on blood agar plates.

Each of these procedures may have acted as better screening devices since alpha-hemolytic colonies, not morphologically typical of pneumococci, may be seen to be inhibited by optochin or lysed by bile, even in mixed culture. Moreover, pneumococci grow better anaerobically than in CO<sub>2</sub> in air, and they are more easily recognized (7a). Some strains may even be non-aerotolerant on initial isolation, although they grow aerobically when subcultured (7a). Sputum cultures were incubated anaerobically solely to enhance the growth of pneumococci and not to recover anaerobes.

The plate bile solubility test was used only as a screening device; however, as with the optochin screening test, it provided a means of studying many colonies, even those not typical

TABLE 1. Recovery of pneumococci in sputum of patients with bacteremic pneumococcal pneumonia

Study	No. of patients	Sputum cultures positive for pneumococci (%)	Culture method <sup>a</sup>	Method of recognition of possible pneumococci	Identification method
1. Rathbun and Govani (9)	69	55 (83) <sup>b</sup>	1. Blood agar; thio-glycolate broth with subculture 2. Mouse inoculation	Colony morphology	Optochin sensitivity
2. Fiala (5)	25	56	1. 5% sheep blood agar in air and CO <sub>2</sub> 2. Mouse inoculation (in most cases)		Quellung reaction
3. Barrett-O'Connor (1)	33	48	1. Blood agar, anaerobically	Colony morphology	Optochin sensitivity
4. Tempest et al. (12)	40	75 (95) <sup>a</sup>	1. Blood and chocolate agar 2. Mouse inoculation	Colony morphology, using stereo-dissecting microscope	Quellung reaction
5. Drew	31	94	1. 5% sheep blood in CO <sub>2</sub> , anaerobically 2. Optochin disk on original plating	Optochin sensitivity; bile solubility	Optochin sensitivity

<sup>a</sup> Atmosphere of incubation was not reported in studies 1 and 4.

<sup>b</sup> Figures in parentheses indicate percentage of sputa positive for pneumococci when mouse inoculation was used.

of pneumococci. Optochin susceptibility was used for definitive identification of pneumococci in addition to its use as a screening device. Definitive testing was performed as recommended (2) with a requirement for 15 mm of inhibition to identify a pneumococcus and exclude nonpneumococcal alpha-hemolytic streptococci. In our screening procedure, the same 15-mm zone of inhibition was required for presumptive evidence of pneumococcal presence. With this finding and evidence of bile solubility, a preliminary report was sent to the physician suggesting that pneumococci had been isolated.

The precise contribution of each of these three procedures to an enhanced recovery of pneumococci is difficult to estimate since they were initiated simultaneously. Howden (7a), however, doubled the recovery of pneumococci from respiratory specimens by the single measure of anaerobic incubation. In our study this procedure seemed to be the most important, although the screening optochin test was positive in 76% of the sputa and the plate bile test was positive in all.

The poor results of sputum culture reported in previous studies (1, 9) may well be due to the fact that only colonies grossly resembling pneumococci were further tested by the optochin disk method, whereas our screening devices are apt to identify the presence of pneumococci, even when their colonial appearance is not typical.

In addition to the useful results of sputum culture in this study, the stained sputum smear was an extremely helpful and rapid diagnostic aid. In 20% of the sputa from bacteremic patients the only bacteria seen were gram-positive diplococci, and, in each of these instances, a notation was made by the technologist that they resembled pneumococci. In 1976, the smear was considered sufficiently suggestive to warrant calling the result to the physician on 70% of the sputa which were subsequently positive for pneumococci on culture. In contrast, there was no instance in which a Gram stain was called to the physician as positive for pneumococci when subsequently the culture proved negative.

The smear and also the culture were equally helpful in a different sense by indicating which bacteria were not the etiological agents of the pneumonia. In only 2 of 29 pneumococcus-positive specimens were staphylococci mentioned in a Gram-stain report; *S. aureus* was only grown from four (14%) specimens, never in greater than 2+ amounts. Similarly, gram-negative rod-resembling coliforms were recovered only

three times from pneumococcus-positive specimens, and only in 1+ or less amounts. Even in specimens contaminated with saliva, it has frequently been possible to examine microscopically a purulent area and satisfy oneself that coliform bacilli (e.g., *Klebsiella*) or staphylococci are not etiological agents of the pneumonia. This observation is extremely important, even if one cannot be positive of the presence of pneumococci, since it may eliminate the necessity for antibiotics other than penicillin.

Transtracheal aspiration has been recommended to obtain meaningful specimens from the lower respiratory tract. Complications such as hemoptysis (8), subcutaneous (8) and mediastinal emphysema, and anterior cervical infection (4) may follow this procedure; fatal hemorrhage (11) and cardio-respiratory arrest (11) have been reported. Moreover, in two recent studies (3, 13) sputum culture has been shown to be as sensitive as transtracheal aspiration culture for diagnosis of pneumococcal pneumonia, although additional bacteria are more apt to grow from a sputum specimen.

In the second phase of this study, the rate of isolation of pneumococci from all sputa was calculated to determine whether these cultural modifications had rendered sputum culture too sensitive, i.e., that too many sputa were now positive for pneumococci. The validity of any laboratory test should be assessed in terms of sensitivity and specificity. In this study, the sensitivity of the sputum culture was 94%: i.e., that percentage of patients with bacteremic pneumococcal pneumonia who had a positive sputum culture. As a measure of specificity, sputum cultures were falsely positive in 6 of 165 sputum specimens: i.e., only 4% of patients with this organism in their sputum did not have pneumonia.

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