Detection of *Streptococcus pneumoniae* in Lower Respiratory Tract Specimens by Anaerobic Culture Technique

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Received 28 November 1983/Accepted 10 January 1984

The relative efficacy of aerobic and anaerobic culture techniques for the recovery of *Streptococcus pneumoniae* from 1,173 lower respiratory tract specimens was determined. Unlike previous studies, this investigation found no significant difference between the two techniques.

The reliability of routine bacteriological examination of lower respiratory tract specimens in diagnosing acute pneumococcal pneumonia is controversial. However, a number of studies (2, 4, 8) show that anaerobic incubation of respiratory cultures increases the recovery of *Streptococcus pneumoniae*. Several of these studies conclude that enhanced detection of *S. pneumoniae* through anaerobiosis is primarily due to the production of a characteristically large, mucoid colony and suppression of accompanying oral microfloral flora.

This study compared the use of CO₂-enriched aerobic incubation and anaerobic incubation for the recovery of *S. pneumoniae* from lower respiratory tract specimens. The presence or absence of a clinical diagnosis of lower respiratory tract infection was also correlated with the bacteriological findings in culture-positive patients.

A total of 1,173 consecutive respiratory tract specimens, consisting of sputa, bronchial washings, and endotracheal secretions from 722 patients, were cultured in a parallel study over a 6-month period. All specimens were sampled and inoculated directly by streak plate culture technique on duplicate 5% sheep blood agar plates (Carr Scarborough Microbiologics, Inc., Stone Mountain, Ga.) with a 4.0-mm nichrome wire loop. One inoculated blood plate was incubated in a 7% CO₂-enriched aerobic atmosphere with 80% relative humidity. The second inoculated blood plate was incubated in a Bio-Bag Environmental Chamber A anaerobic system (Marion Laboratories, Inc., Kansas City, Mo.). A comparative examination of each duplicate culture was performed by macroscopic examination under oblique light after 24 h of incubation at 35°C. All alpha-hemolytic colonies that were mucoid or had depressed centers as well as 5 to 10 colonies in cultures with a predominance of alpha-hemolytic streptococci were examined by either the Phadebact pneumococcal coagglutination test (Pharmacia Laboratories, Inc., Piscataway, N.J.) or the Optochin disk sensitivity test (Difco Laboratories, Detroit, Mich.). All culture results were confirmed by one of the authors (R. K. Baesman). In addition, a retrospective chart review of all patients with positive pneumococcal cultures was performed by one of the authors (C. L. Strand) to determine the presence or absence of clinical respiratory tract infection. Patients were considered to have a pneumococcal infection if this diagnosis was recorded in the chart or if the patient was febrile (oral temperature, >38°C) and the chest X ray showed a lung infiltrate. The statistical analysis was performed with a nonparametric test for matched pair analysis of discrete data (McNemar test).

*S. pneumoniae* was recovered from 76 (6.5%) of 1,173 cultures and 70 (9.7%) of the total of 722 patients in the study. *S. pneumoniae* was recovered from 70 of the aerobic cultures and 68 of the anaerobic cultures, resulting in percentages of recovery for the aerobic and anaerobic techniques of 92.1 and 89.5%, respectively. This difference was not significant (*P* > 0.05).

The significance of all 76 lower respiratory tract specimens that were positive for *S. pneumoniae* was assessed by a review of the medical records of the patients. Of the 70 patients with positive lower respiratory tract cultures, 41 (58.6%) showed no evidence for a clinical diagnosis of lower respiratory tract pneumococcal infection. For the 29 patients with clinical evidence of lower respiratory tract infection (22 with pneumonia and 7 with acute bronchitis), *S. pneumoniae* was recovered from 32 cultures. The aerobic culture was positive for 31 and the anaerobic culture was positive for 29 of the 32 positive cultures, resulting in sensitivities for the aerobic and anaerobic techniques of 96.9 and 90.6%, respectively. This difference was not significant (*P* > 0.05).

The predictive values of positive cultures for lower respiratory tract pneumococcal infection were 44.3% for the aerobic technique and 42.6% for the anaerobic technique. The predictive values of negative cultures for lower respiratory pneumococcal infection were 99.9% for the aerobic technique and 99.7% for the anaerobic technique.

Lower respiratory tract secretions obtained by expectoration or other means are the most common specimens submitted for the diagnosis of pneumococcal pneumonia. However, the reliability of these cultures in the diagnosis of pneumococcal pneumonia remains controversial. Although some investigators (6, 7) cite *S. pneumoniae* recovery rates from sputum culture of 75 to 97% in pneumococcal pneumonia, others have shown that *S. pneumoniae* is not recovered from sputum or nasopharyngeal culture in 45 to 56% of documented cases of bacteremic pneumococcal pneumonia (1, 3, 5). A major difficulty in examining expectorated or induced sputum for the presence of *S. pneumoniae* is the invariable contamination of the specimen by normal oropharyngeal flora. Such normal flora may inhibit or mask the growth of *S. pneumoniae* colonies. In addition, the isolation of potential pathogens that are unrelated to the illness of the patient can be misleading. For example, in the study of Barrett-Conner (1), a pathogen other than *S. pneumoniae* was isolated from sputum or nasopharyngeal culture from 27% of those patients with a diagnosis of acute pneumococcal pneumonia.

In recent years, at least three studies have shown enhanced recovery of *S. pneumoniae* from respiratory spec-
mens by anaerobic culture techniques (2, 4, 8). Howden (4), in a comparative study of aerobic and anaerobic culture techniques, concluded that 52.3% of the S. pneumoniae respiratory isolates grew only anaerobically upon primary isolation. Wu and colleagues (8) were able to document a recovery rate for S. pneumoniae of 93% with anaerobic culture techniques compared with only 65% with the standard CO2-enriched aerobic culture technique. These investigators suggest that the increased recovery of S. pneumoniae from respiratory tract cultures by anaerobic techniques is the result of several factors. The primary factor appears to be the production of a characteristically large, mucoid colony that makes visual detection easier and aids in distinguishing S. pneumoniae from viridans streptococci and other normal flora. In addition, according to Howden (4) and Wu et al. (8), overnight anaerobic incubation tends to suppress much of the normal flora, which also makes it easier to detect the large mucoid S. pneumoniae colonies. In both of these studies, the authors (4, 8) speculate that some of the S. pneumoniae strains found in the respiratory tract may be non-aerotolerant upon primary isolation.

Unlike previous studies, the current evaluation demonstrated no significant difference in the recovery rate of S. pneumoniae from lower respiratory tract specimens by the aerobic and anaerobic culture techniques. Although the authors are unable to explain the difference between their results and those of previous studies, there is a major difference in the anaerobic technique used. The current study used a Bio-Bag Environmental Chamber A (Marion Laboratories) anaerobic system, whereas previous investigators used either a GasPak jar (BBL Microbiology Systems, Cockeysville, Md.) or an anaerobic jar with an evacuation-replacement technique. Furthermore, the production of large, mucoid pneumococcal colonies was not a consistent finding in the current study, possibly as a result of using the Bio-Bag Chamber. If this is the case, it will be important to determine why the gas environment of the Bio-Bag Chamber does not result in production of large, mucoid pneumococcal colonies.

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