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Survival of *Streptococcus pneumoniae* in Sputum from Patients with Pneumonia

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The isolation rate of *Streptococcus pneumoniae* in sputum cultures from patients with pneumococcal pneumonia is low. An investigation was made to determine whether this low yield might be due to loss of pneumococci and/or overgrowth by pharyngeal flora before the specimen is plated. Pneumococcal survival times and pharyngeal overgrowth at 4°C and at room temperature were determined in sputum obtained from 42 patients with pneumococcal pneumonia. It was found that pneumococci survived for long periods in sputum—2.2 ± 1.4 days at room temperature and 9.5 ± 3.6 days at 4°C. Overgrowth by pharyngeal flora occurred in only 6 of 42 specimens kept at 4°C and in 31 of 42 specimens kept at room temperature. The low yield of *S. pneumoniae* in sputum from patients with pneumococcal pneumonia is not explained by decreased viability of the organism.

The diagnosis of pneumococcal pneumonia often rests on clinical suspicion and Gram stain evidence for the presence of pneumococci in sputum. Sputum culture reports, which should provide the needed corroborative data, frequently indicate recovery of only normal flora. Several studies have documented that approximately 55% of patients with bacteremic pneumococcal pneumonia have *Streptococcus pneumonia* isolated from sputum (4, 9). It has been suggested that this poor recovery rate may be related to decreased viability of the pneumococcus and/or overgrowth by pharyngeal flora when plating of the specimen is delayed (4). The purpose of this study was to determine whether immediate culturing of sputum from patients with pneumonia would increase the yield of *S. pneumoniae*. The length of time that *S. pneumoniae* could be recovered from sputum kept at 4°C and at room temperature was determined.

MATERIALS AND METHODS

During the 12 months from February 1976 to June 1976 and from October 1976 to April 1977, 70 adult patients at the Cincinnati Veterans Administration Hospital and the Cincinnati General Hospital were referred for study by the house staff as soon as they were admitted. Of this group, 42 patients had clinical symptoms suggesting pneumonia, roentgenographic evidence of pulmonary infiltrates, and *S. pneumoniae* isolated from sputum. Patients who had only bronchitis or who appeared to have merely pharyngeal colonization with *S. pneumoniae* were excluded. Blood cultures were performed on all patients and grew *S. pneumoniae* in four.

Within 0.5 h of referral, sputum was obtained by expectoration from 30 patients, nasotracheal aspiration from 10 patients, and transtracheal aspiration from 2 patients. The subsequent laboratory procedures were performed by one person (S.G.W.). The specimen was divided immediately into two aliquots—one aliquot was kept at room temperature, and the other was kept at 4°C for the remainder of the study. Gram stains performed on each aliquot were compared with regard to numbers of epithelial cells and neutrophils and types of bacteria. There were no differences found between the two aliquots from each specimen. Immediately and every 12 to 18 h thereafter until the specimen was depleted, a 0.3-cm loopful of each aliquot was streaked onto sheep blood agar (5% sheep blood in Trypticase soy agar) (Baltimore Biological Laboratory [BBL], Cockeysville, Md.) by the quadrant isolation technique (8). The plates were incubated in an 8% CO_2 atmosphere at 35°C for 18 to 24 h. Plates were observed with a ×3.5 hand lens to allow assessment of colony morphology, and two colonies from each plate were selected for further identification. A total of 8 specimens were found to have mucoid colony types; 32 specimens contained characteristic umbilicated colony types. In two specimens the colony morphology was indistinguishable from that of alpha-hemolytic streptococci.

Isolates were identified as pneumococci if they fulfilled the following criteria: (i) alpha-hemolytic colonies on sheep blood agar; (ii) 18-mm or larger zone size around an ethylhydrocupreine hydrochloride (Optochin) disk (BBL); (iii) bile solubility; and (iv) positive quellung reaction using Omniserum (Staten Seruminstitut, Copenhagen, Denmark) (2). All other species isolated were identified by standard techniques. All isolates were graded as to abundance by the following

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criteria: abundant, detectable growth in quadrants 3 and/or 4; moderate, detectable growth in only quadrants 1 and/or 2; rare, detectable growth in less than one-half of quadrant 1; and very rare, less than 20 detectable colonies.

In 13 cases, duplicate sputum samples were processed routinely by the clinical microbiology laboratory. The same media and isolation procedures were employed. All cultures were plated immediately upon arrival in the clinical laboratory. Technologists handled these cultures in the usual manner and did not use a hand lens for assessing colony morphology.

RESULTS

All 42 sputum specimens grew *S. pneumoniae* and at least one other organism on the initial culture. A total of 15 specimens, including the 2 transtracheal aspirates, had one other organism; 15 had two other organisms; and 12 had three or more organisms isolated besides pneumococci. *Neisseria* species, alpha-hemolytic streptococci, and gram-negative bacilli were found in 39, 16, and 15 specimens, respectively. Diphtheroids, *Haemophilus influenzae, Staphylococcus aureus,* beta-hemolytic streptococci, and yeast were each found in fewer than three specimens. In all of the 42 specimens, pneumococci were present in abundant quantities. In 18 specimens, pneumococci were the only organisms present in abundance; in 24 samples, pneumococci and one or two other organisms were present in abundance.

Sputum specimens kept at room temperature yielded pneumococci for as long as 6 days (mean, 2.2 ± 1.4) (Fig. 1). No loss of organisms occurred in the first 12 h after procurement of the sputum. In samples kept at 4°C, there was no decrease in the recovery rate of pneumococci until 2 days had elapsed, and then a slow decline in the recovery rate ensued. The mean length of time during which pneumococci were recovered was 9.5 ± 3.6 days, with one sample yielding pneumococci for as long as 27.5 days. In 25 of 39 specimens kept at 4°C, the supply of sputum was depleted at a time when the last sampling still grew *S. pneumoniae.*

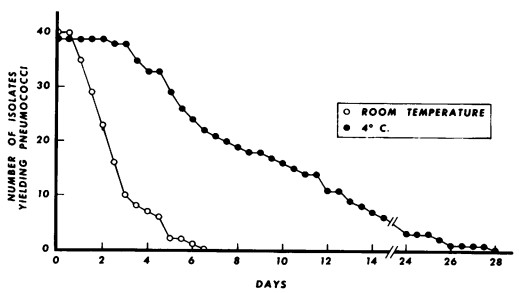

FIG. 1. *Number of sputum specimens from which S. pneumoniae was recovered. Specimens were cultured every 12 to 18 h until depleted.*

Not only were pneumococci recoverable from sputum for long periods of time, but they persisted in abundance during most of that time period (Fig. 2). The mean length of time, during which pneumococci persisted in abundant quantities was 1.9 ± 1.3 days in sputum kept at room temperature and 7.2 ± 4.8 days in sputum kept at 4°C.

At 4°C, only six specimens showed enhanced growth of pharyngeal flora with a concomitant decline in the abundance of pneumococci (Fig. 3). *Neisseria* species and diphtheroids were responsible for overgrowth in two specimens each; *Escherichia coli* and *S. aureus* overgrew pneumococci in one sputum each. The normal pharyngeal flora declined at a rate similar to that shown by *S. pneumoniae.* At room temperature, significant masking of pneumococcal colonies by pharyngeal flora occurred, beginning 12 h after the specimen was taken. Of the 40 specimens kept at room temperature, 31 showed enhanced growth of pharyngeal flora after a mean time interval of 1.8 ± 1.0 days. The organisms responsible were *Neisseria* species in 15 specimens and gram-negative bacilli (Proteus species, *E. coli, Serratia marcescens, Klebsiella pneumoniae*) in 12 specimens. Alpha-hemolytic streptococci, although initially present in 16 specimens, overgrew pneumococci in only 1.

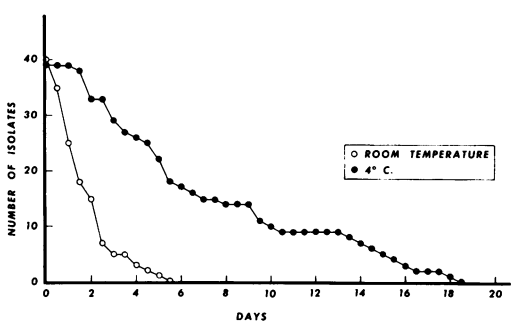

FIG. 2. *Number of sputum specimens that yielded S. pneumoniae in abundant quantities.*

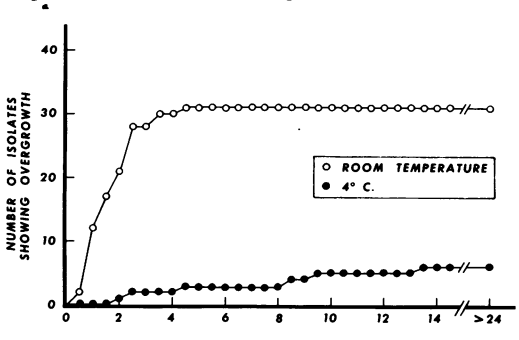

FIG. 3. *Number of sputum specimens showing overgrowth of S. pneumoniae by pharyngeal flora.*

Of the 13 sputum specimens submitted to the clinical laboratory for routine processing, growth of *S. pneumoniae* was reported from only 7 (54%). "Normal throat flora" were reported in the other six specimens. Five of the six specimens in which pneumococci were not found had two or more additional organisms besides pneumococci present in abundant quantities in our study.

DISCUSSION

These studies show that pneumococci can survive for long periods of time in sputum. The organisms may survive for weeks when the sputum is kept at 4°C. Although autolysis readily occurs at room temperature or at 4°C on many different types of media (3), sputum appears to exert a protective effect leading to prolonged viability of the pneumococcus. Blood and other body fluids have been shown to have a similar protective effect (12). Our results are similar to those of Wood (13) and Bordoni-Uffreduzzi (5), who demonstrated that pneumococci can remain viable for many days in dried sputum.

Our data suggest that immediate plating of sputum does not enhance the yield of positive cultures for pneumococci. After 12 h at room temperature, no pneumococci were lost, and only two sputum specimens showed enhanced growth of pharyngeal flora. At 4°C, no loss of organisms occurred until after 1.5 days had elapsed, and pharyngeal flora did not increase in numbers for 2 days. Thus, storage of sputum specimens at 4°C overnight with routine plating the next day will not diminish the yield of pneumococci in the clinical laboratory.

Since pneumococci survive in sputum for long periods of time, the low recovery rate must be related to other factors. Although the numbers are small, the data on the sputum processed in this study and concomitantly by the clinical laboratory in a routine manner suggest that the failure of technologists to recognize pneumococcal colonies is a possible reason for the lower yield. The main difference in our techniques and those used routinely in the clinical laboratory was our use of a hand lens to inspect the plates. Magnification enhances one's ability to pick out the small pneumococcal colonies amid normal pharyngeal flora. The optimal technique, in fact, is the use of a dissecting microscope rather than the hand lens (1).

Selective media, such as sheep blood agar containing 5 μg of gentamicin per ml, have been used to decrease the growth of pharyngeal flora and to increase the yield of pneumococci (6, 7). This method would appear to be useful since we and others (4, 11) have shown that the majority of patients with pneumococcal pneumonia have pharyngeal flora as well as pneumococci isolated in sputum culture. However, this selective medium inhibits the growth of staphylococci, group A streptococci, and some gram-negative bacilli (10). Plain sheep blood agar would have to be used in addition to the selective medium in the clinical laboratory, resulting in an increase in the cost of processing specimens. We reemphasize the value of magnification for recognizing pneumococcal colonies on plain sheep blood agar and feel it is a reasonable alternative to selective media in increasing the yield of *S. pneumoniae* in sputum cultures (1).

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