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Coagglutination and Counterimmunoelectrophoresis for Detection of Pneumococcal Antigens in the Sputum of Pneumonia Patients

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Coagglutination was compared with counterimmunoelectrophoresis (CIE) for sensitivity and specificity in the detection of pneumococcal antigens in sputum. Initial sputum samples from patients with pneumococcal pneumonia (<12 h of antibiotic therapy) were positive for antigens in 37 of 44 cases (84%) by either test. There was a decline in the number of positive results with sputum samples obtained during continuing antibiotic therapy, but the decline was greater with CIE (only 29% of samples were positive at 3 days of therapy) than with coagglutination (61% of samples were positive at 3 days of therapy) ($P < 0.05$). Sputum from 3 of 11 patients (27%) and from 2 of 11 patients (18%) with nonpneumococcal pneumonia was positive for pneumococcal antigens by CIE and coagglutination, respectively, indicating a similar degree of non-specificity. Coagglutination produced the same results as CIE with sputum from patients with chronic bronchitis but without pneumonia; 9 of 23 of these patients were positive. Coagglutination was simpler to perform than CIE and required only a fraction (about 1/30) of the antiserum required for CIE. These advantages, plus the greater sensitivity of coagglutination with sputum samples obtained during antibiotic therapy, suggest that coagglutination is preferable to CIE.

Pneumococcal pneumonia remains a major medical problem, in spite of the availability of specific therapy. Substantial diagnostic problems persist, including the inability to detect pneumococci in sputum samples reliably by culture and the inability to differentiate between true infections and sputum contaminated with normal flora which may include pneumococci. A Gram-stained smear is frequently used to make a presumptive diagnosis of pneumococcal pneumonia on the basis of numerous Gram-positive diplococci in sputum. Cultures of expectorated sputum have been found to be of doubtful value because pneumococcal organisms are often carried in the nasopharynx (1). The search for a reliable and simple method for identifying pneumococci in clinical samples of patients with pneumococcal disease continues.

In recent years, pneumococcal antigens have been detected in the sputum, urine, and serum of patients with lobar pneumonia by counterimmunoelectrophoresis (CIE) (2, 4, 5). Evidence has accumulated which shows that the presence of pneumococcal antigens in the sputum correlates very closely with the presence of pneumococcal disease (6, 7, 9). This finding, which differs from the experience with Gram stain or with the culture of sputum, provides a new dimension in the laboratory diagnosis of pneumococcal infection.

A rapid slide test based on coagglutination has recently been developed to detect pneumococcal antigens in sputum (3). The present report compares coagglutination and CIE for the detection of pneumococcal antigens in sputum samples. In addition, the reproducibility of the CIE test as performed by two different laboratories was evaluated.

MATERIALS AND METHODS

Clinical samples. Sputum samples were collected from 70 patients with clinical and roentgenographic evidence of bacterial pneumonia who were admitted to the University of Kentucky and the Veterans Administration hospitals in Lexington, Ky. during the years 1976 and 1977. Patients were assigned to one of the following diagnostic groups based on the clinical and bacteriological findings: (i) group I, 14 patients (44 sputum samples) with proven (bacteremic) pneumococcal pneumonia; (ii) group II, 45 patients (110 sputum samples) with probable pneumococcal pneumonia (sterile blood culture but predominance of gram-positive cocci in smears of sputum samples and response within 72 h to narrow-spectrum antibiotic therapy); and (iii) group III, 11 patients (25 sputum samples)

with nonpneumococcal pneumonia (patients were bacteremic with nonpneumococcal organisms).

In addition, patients with chronic bronchitis who did not have pneumonia were studied and grouped according to their sputum cultures, as follows: (i) group IV, 8 patients (8 sputum samples) with chronic bronchitis with pneumococci isolated from sputum; and (ii) group V, 15 patients (15 sputum samples) with chronic bronchitis without pneumococci in the sputum.

A total of 202 samples of sputum were obtained. One or more samples of sputum were obtained in-hospital over a 3-day period from patients with pneumonia. Single samples of sputum were obtained in the out-patient department from patients with bronchitis. Many of the sputum samples included in the present study were previously tested by CIE for pneumococcal antigens at the Veterans Administration Hospital in Lexington, and the results of those studies have been published elsewhere (5). The sputum samples were stored at −20°C, coded, and shipped frozen in Dry Ice to the Naval Health Research Center in San Diego, Calif. for coagglutination and CIE studies at that facility. The samples were tested in blinded fashion at the Naval Health Research Center, and the results were returned to the Veterans Administration laboratory in Lexington for decoding and analysis.

Preparation of sputum samples. At the Naval Health Research Center, sputum samples were digested with an equal volume of Sputolysin (Calbiochem, San Diego, Calif.), and a sample was clarified by centrifugation. The clarified sample was then either heated for 5 min at 65°C or placed in a boiling water bath for 30 to 60 s before testing by coagglutination. The remainder of the digested sputum was used for CIE.

Coagglutination. This procedure was carried out as previously described (3). A drop of the test sample was placed on each of two marked areas of a microscope slide. A drop (0.025 ml) of staphylococci sensitized with polyvalent pneumococcal antiserum (Omniserum; Statens Seruminstitut, Copenhagen, Denmark) was placed on one test area, and a drop (0.025 ml) of staphylococci sensitized with normal rabbit sera was placed on the other test area. Sputum and staphylococci were mixed rapidly with an applicator stick and then tilted to and fro to continue mixing for 1 to 5 min. Most positive sputum samples showed agglutination within 2 min. However, tests were not considered negative until after 5 min of observation. All observations were made with a stereoscope.

CIE. CIE was performed with a minor modification of the procedure described by Leach and Coonrod (5). Briefly, the test was performed with 1% agarose in 0.075 M barbital buffer (pH 8.6) with 0.1 M barbital buffer (pH 8.6) in the electrophoresis reservoirs. A 12-ml amount of agarose was used to cover a lantern slide (80 × 120 mm). All samples were tested against Omniserum. Electrophoresis was performed with 15 mA for each lantern slide for 1 h. Slides were read immediately with indirect lighting and a hand lens (3×).

Cultures. Pneumococci were isolated on 5% sheep blood agar in 5% CO_2. Identification was based on the inhibition of growth of 15 or more mm around an optochin disk (Difco Laboratories, Detroit, Mich.) on incubation in room air.

RESULTS

The coagglutination and CIE results for initial sputum samples of patients with pneumonia are listed in Table 1. Because antibiotic therapy can influence antigen detection (5), only patients who had received less than 12 h of antibiotic therapy when the initial sputum samples were obtained are included in Table 1. A majority of these patients had detectable pneumococcal antigens in the sputum. Of the 44 patients with proven or probable pneumococcal pneumonia, 35 were positive for pneumococcal antigens by both CIE and coagglutination; 2 were positive by CIE only (antigen types 3 and 4); and 2 were positive by coagglutination only (isolates were available and were types 6 and 9). Thus, CIE and coagglutination had similar levels of sensitivity.

From the 44 patients listed in Table 1 in groups I and II, pneumococci were isolated from the sputum of 20 (2 in group I and 18 in group II). Of the 20 with pneumococci isolated from sputum, 19 had positive tests for antigens (18 positive with both tests and 1 positive by coagglutination only). Of the 24 without pneumococci in the sputum, 20 had positive tests for antigens (by coagglutination or CIE).

As indicated in Table 1, sputum samples from three patients with nonpneumococcal pneumonia were positive for pneumococcal antigens by CIE, and sputum samples from two patients were positive by coagglutination. One of these three patients was bacteremic with peptostreptococcus and had pneumococci in the sputum.

TABLE 1. *Detection of pneumococcal antigens in initial sputum samples collected from patients with pneumonia or bronchitis*

Group	No. of patients	No. (%) positive[a] by:	
		Coagglutination	CIE
Pneumonia			
Proven pneumococcal	10	9 (90)	8 (80)
Probable pneumococcal	34	28 (82)	29 (85)
Proven nonpneumococcal	11	2 (18)	3 (27)
Bronchitis			
With pneumococci isolated from sputum	8	6 (75)	6 (75)
Without pneumococci in sputum	15	3 (20)	3 (20)

[a] Samples were collected at 0 to 12 h of antibiotic therapy.

The other two patients were bacteremic with *Staphylococcus aureus* and *Escherichia coli*, and neither had pneumococci isolated from the sputum.

In the patients with chronic bronchitis (Table 1) there was a strong correlation between the presence of pneumococcal antigens and a positive culture for pneumococci.

Additional sputum samples were available for studies of antigens from patients with pneumococcal pneumonia who had received more than 12 h of antibiotic therapy. These samples were obtained partly from patients who had provided the initial sputum samples described in Table 1 and partly from additional patients who did not provide earlier samples. Results are presented in Fig. 1. The number of positive results declined as the number of days of antibiotic therapy increased. The decline in positive results was more noticeable with CIE than with coagglutination, and the difference between the two tests at 3 days of therapy was significant ($P < 0.05$). Of the 110 samples of sputum obtained at 1 to 3 days of therapy, only 6 were positive by CIE alone, whereas 23 were positive by coagglutination alone ($P < 0.01$). Thus, coagglutination was more sensitive than CIE in detecting pneumococcal antigens in follow-up samples. Differences in the results of coagglutination and CIE did not appear to be related to pneumococcal serotypes. A total of 20 serotypes were encountered. None was predominant. Type 7 was found in only one case (both coagglutination and CIE were positive), and type 14 was not encountered.

The present studies offered an opportunity to compare results obtained by CIE of sputum in two different laboratories, the Naval Health Research Center in San Diego in the current study and the Veterans Administration Hospital in Lexington, where the samples had been evaluated originally 1 to 3 years earlier. The methods of CIE used in the two institutions were similar, but not identical. Polyvalent pneumococcal antiserum (Omniserum) was used by both laboratories. Concordant results were obtained between the two laboratories with 127 of 154 sputum (82.5%) from cases of proven or probable pneumococcal pneumonia. Of the 90 sputum samples that were positive for antigens at the Naval Health Research Center, 75 were positive and 15 were negative at the Veterans Administration Hospital. Of the 64 sputum samples that were negative for antigens at the Naval Health Research Center, 12 were positive and 52 were negative at the Veterans Administration Hospital.

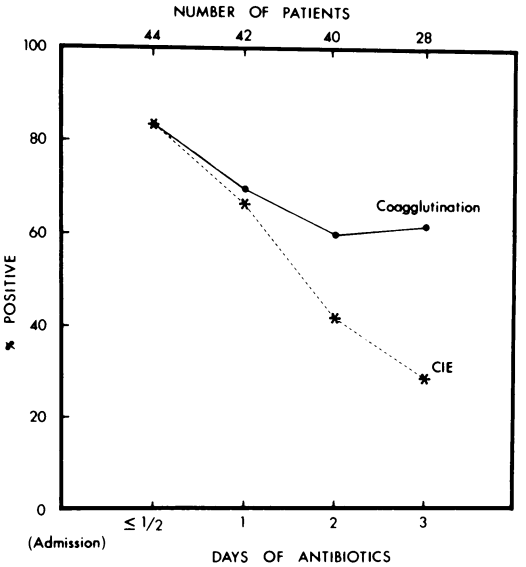

FIG. 1. *Detection of pneumococcal antigens in the sputum of patients with proven or probable pneumococcal pneumonia during antibiotic therapy. The differences between results by coagglutination and by CIE were statistically significant ($P < 0.05$) only at day 3.*

DISCUSSION

These studies confirm the observation (3) that coagglutination can be used to detect bacterial antigens in sputum samples. The results obtained by coagglutination were very similar to the results obtained by CIE. With both tests, pneumococcal antigens were detected in the sputum of a majority of patients with a clinical diagnosis of pneumococcal pneumonia. After the administration of antibiotics, the number of patients with detectable antigens declined. Coagglutination, however, was more sensitive than CIE. Even after 3 days of antibiotic therapy, over 60% of patients with pneumococcal pneumonia had antigens in the sputum detectable by coagglutination. In comparison, CIE was positive in less than 30% of the patients, constituting a significant difference.

These data show that coagglutination can be used very satisfactorily for diagnosis in cases where antibiotic therapy is already under way, whereas even small amounts of antibiotics interfere seriously with the results of cultures (8).

The detection of pneumococcal antigens in the sputum is obviously not totally specific for the presence of pneumococcal pneumonia. Patients with chronic bronchitis but without pneumonia may have a positive test, especially if they harbor pneumococci in the sputum. The overall results of the present study, however, indicate that antigen detection by coagglutination and CIE correlates positively with clinical evidence

of pneumococcal infection.

A potential technical problem in the coagglutination test is that agglutination can occur when staphylococcal particles are exposed to mammalian immunoglobulin G. Heating of sputum or serum has eliminated this reaction with few exceptions (3). Absorption of the test sample with nonsensitized staphylococci will also eliminate this reaction but requires a large amount of particles.

Coagglutination is technically simpler than CIE and requires less laboratory equipment and reagents. A 1-ml amount of Omniserum will make 100 ml of sensitized staphylococcal particles. This provides enough reagent for about 4,000 tests (0.025 ml per test). In contrast, with 1 ml of Omniserum, one can run only about 140 tests by CIE (7 μl per test). The economy of the coagglutination test, plus its greater sensitivity for detecting pneumococcal antigens during the administration of antibiotics, suggest that coagglutination may be preferable to CIE.

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