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Capillary Precipitin Typing of *Streptococcus pneumoniae*

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The Neufeld test is presently the method of choice for typing *Streptococcus pneumoniae*. Although the test is reliable and relatively easy to perform, a simpler test, not requiring microscopic examination, would facilitate large-scale testing. A capillary precipitin test has been designed and tested for its usefulness in typing pneumococci. The type-specific carbohydrate antigens were obtained from broth culture supernatants. The antigens were reacted with type-specific antisera in glass capillary pipettes. Results from 82 reference antigens and 166 antigens from diagnostic pneumococcal strains showed that the reactions were specific, and the results agreed with Neufeld test results. These results indicate that the precipitin test is as specific as the Neufeld test. The test is easy to perform, requires small amounts of antiserum, and can be completed in a short period of time.

Streptococcus pneumoniae (pneumococcus) continues to be a leading cause of morbidity and mortality in the populations of the world (17). The pneumococcus can cause pneumonia, meningitis, otitis media, endocarditis, peritonitis, and arthritis (2). The organism is the most common cause of community-acquired bacterial pneumonia, which is the fifth-most-frequent cause of death in the United States (3). On the basis of limited observations, the annual incidence of pneumococcal pneumonia in the United States has been estimated to be 400,000 to 500,000 cases (16). The pneumococcus is the second-most-frequent cause of bacterial meningitis in the United States (3). Otitis media caused by this organism is believed to result in some hearing impairment in many children, particularly those from socioeconomically deprived segments of the population (3).

The recent licensure of pneumococcal vaccines for use in the United States has increased the demand for large-scale serological typing of pneumococci to determine the most common types in a given population. Although other methods of pneumococcal typing have been reported (6, 7, 13), the Neufeld test or quellung reaction (8, 11) remains the method of choice. The Neufeld test is reliable and relatively easy to perform, but typing large numbers of bacterial strains by this method is time consuming.

In this study we show that polysaccharide antigen elaborated into the medium during the growth of pneumococci can be used to react against group- and type-specific antisera to type pneumococcal strains in a capillary precipitin test; we also compare this test with the Neufeld test.

MATERIALS AND METHODS

Bacterial strains. A total of 166 diagnostic isolates submitted to the Center for Disease Control (CDC) Streptococcus Laboratory for typing were used in the study. A total of 83 pneumococcal reference strains were obtained from Jorgen Henrichsen, Pneumococcus Department, Statens Seruminstitute, Copenhagen, Denmark. The reference strains were kept stored in rabbit blood at −70°C until used.

Isolation of type-specific antigen. The bacteria were cultured in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) under CO_2 at 37°C for 16 h. Amounts of 2 ml of the bacterial suspension were transferred to disposable glass culture tubes (12 by 75 mm; Corning Glass Works, Corning, N.Y.). The culture tubes were placed in a water bath at 100°C for 5 min and then cooled in tap water. The tubes were centrifuged in a clinical centrifuge (International Equipment Co., Div. Damon Corp., Needham Heights, Mass.) at maximum speed for 5 min. The supernatant containing the type-specific antigen was kept, and the sediment was discarded. Several strains were cultured in Trypticase soy broth to determine whether antigen would be produced in this medium also.

Rabbit sera. The group- and type-specific antisera against *S. pneumoniae* used in the study were the standard CDC antisera used for the Neufeld reaction in the Streptococcus Reference Laboratory at CDC. The sera were obtained from the Biological Products Division of CDC.

Capillary pipettes. The capillary pipettes, approximately 14 cm in length, were made from Neutraglas tubing (N-51A glass; Kimble Glass Co., Vineland, N.J.). The outside diameter varied from 0.7 to 1.0 mm, and the wall was 0.2 mm thick. The pipettes were steam sterilized, dried, and stored in test tubes until used.

Procedure of capillary pipette typing. Except for minor modifications, the procedure used for typing *S. pneumoniae* was the same as that described by

Swift et al. (15) for M-typing group A streptococci. The capillary pipettes were dipped into the antiserum until a column about 15 mm long was drawn in by capillary action. The pipettes were wiped off with clean tissue paper and dipped into the pneumococcal antigen solution until a similar volume had been taken up. They were inverted until a column of air was present at each end and were inserted vertically in the appropriate plasticine squares (15) in the holders so that the serum was on the top of the column. Similar pipettes were set up with each serum to be tested.

Readings were made with the naked eye immediately after the antigen was mixed with antiserum and again within 30 min of reaction time. The precipitate could be easily seen if the rack was held against a black background with a tungsten light bulb or with a fluorescent light.

Neufeld test. The Neufeld test (capsular precipitation) was performed essentially as described by Lund (8). Cultures were grown in Todd-Hewitt broth overnight under CO_2 at 37°C. A loopful of the liquid culture was spread on a glass slide. A loopful of antiserum was added to the culture and allowed to stand for approximately 1 min. Then a loopful of 0.3% aqueous methylene blue was added to each culture-antiserum mixture, and a cover slip was placed on the mixture. The preparations were examined under a microscope with an oil immersion lens. Occasionally, pneumococci were washed from agar slants with sterile saline and typed as previously described.

RESULTS

Capillary precipitin reaction. Culture supernatants (antigens) from 83 pneumococcal types (Table 1) were tested against group- or type-specific antisera in capillary pipettes. Table 2 shows the precipitin results of the 83 antigens against each of 30 CDC group or type antisera. All antigens except subtypes 7B, 7C, 23A, and 23B yielded a precipitate with homologous group or type antisera. However, the latter results were probably due to the antisera used and not to the precipitin test (see Neufeld test results below). Antisera to groups 15, 24, and 28 and to type 25 yielded cross-reactions with heterologous antigen. All of the cross-reactions except that occurring with antigen 42 against antiserum 29 could be explained on the basis of the antigenic make-up of the immunizing pneumococci used in the preparation of antisera.

Neufeld test. Neufeld tests were performed on organisms representing the 83 pneumococcal types and the antisera mentioned above. With only one exception the capsular swelling results were the same as the results from the precipitin test. Antiserum type 29 did not yield a positive Neufeld reaction with type 42 pneumococcus. All of the other cross-reactions were the same in both tests.

Typing of diagnostic isolates by the capillary precipitin test and the Neufeld test. A total of 166 isolates of *S. pneumoniae* were recently received in the Streptococcus Laboratory at CDC for typing. All but 11 of these clincial isolates were confirmed as *S. pneumoniae* by the quellung reaction. The strains that failed to react in the quellung test were con-

TABLE 1. *Correlation of CDC group and type antisera to Danish and American pneumococcal type designations*

CDC group and type antisera	Pneumococcal type designation[a]	
	Danish	American
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6A, 6B	6, 26
7	7A, 7B, 7C, 7F	7, 48, 50, 51
8	8	8
9	9N, 9A, 9L, 9V	9, 33, 49, 68
10	10F, 10A	10, 34
11	11F, 11A, 11B, 11C	11, 43, 76, 53
12	12F, 12A	12, 83
13	13	13
14	14	14
15	15F, 15A, 15B, 15C	15, 30, 54, 77
16	16	16
17	17F, 17A	17, 78
18	18F, 18A, 18B, 18C	18, 44, 55, 56
19	19F, 19A, 19B, 19C	19, 57, 58, 59
20	20	20
21	21	21
22	22F, 22A	22, 63
23	23F, 23A, 23B	23, 46, 64
24	24F, 24A, 24B	24, 65, 60
25	25	25
27	27	27
28	28F, 28A	28, 79
29	29	29
31	31	31
32	32F, 32A	32, 67
	33F, 33A, 33B, 33C	70, 40, 42, 39
	34	41
	35F, 35A, 35B, 35C	35, (47 = 62), 66, 61
	36	36
	37	37
	38	71
	39	69
	40	45
	41F, 41A	38, 74
	42	80
	43	75
	44	81
	45	72
	46	73
	47F, 47A	52, 84
	48	82

[a] Nomenclature of the Danish and American pneumococcal typing systems is from references 10 and 5, respectively.

TABLE 2. *Capillary precipitin reactions with pneumococcal type-specific antigens and group or type antisera: 30 antisera (15 group and 15 type) versus antigens from 83 pneumococcal types listed in Table 1 (Danish nomenclature)*

CDC group or type antisera	Antigen type[a]		
	Homologous reaction	Reaction expected	Heterologous reaction
1	1	1	
2	2	2	
3	3	3	
4	4	4	
5	5	5	
6	6A, 6B	6A, 6B	
7	7F, 7A	7F, 7A, 7B, 7C	
8	8	8	
9	9A, 9L, 9N, 9V	9A, 9L, 9N, 9V	
10	10F, 10A	10F, 10A	
11	11F, 11A, 11B, 11C	11F, 11A, 11B, 11C	
12	12F, 12A	12F, 12A	
13	13	13	
14	14	14	
15	15F, 15A, 15B, 15C	15F, 15A, 15B, 15C	23A
16	16	16	
17	17F, 17A	17F, 17A	
18	18F, 18A, 18B, 18C	18F, 18A, 18B, 18C	
19	19F, 19A, 19B, 19C	19F, 19A, 19B, 19C	
20	20	20	
21	21	21	
22	22F, 22A	22F, 22A	
23	23F	23F, 23A, 23B	
24	24F, 24A, 24B	24F, 24A, 24B	7B, 7C, 19B, 19C, 40
25	25	25	38
27	27	27	
28	28F, 28A	28F, 28A	23B
29	29	29	35B, 42
31	31	31	
32	32F, 32A	32F, 32A	

[a] Antigen = culture supernatant after 16 h of growth on Todd-Hewitt medium.

firmed as pneumococci by the following criteria: typical colonies on blood-agar, gram-positive cocci, soluble in bile, and susceptible to optochin disk. Two investigators typed the isolates by the capillary precipitin method and two different individuals typed the same isolates by Neufeld test. The test results are presented in Table 3. Table 3 shows that with the 166 isolates typed there was 100% agreement between the capillary precipitin reaction and the Neufeld test. The results also show the type distribution of the isolates.

DISCUSSION

The discovery of pneumococcal polysaccharide antigen in culture supernatant is not new. However, such antigen has not heretofore been used for typing pneumococcal isolates. Panichi (12) in 1907 demonstrated the presence of a specific precipitable substance in the filtrate of bouillon cultures of the pneumococcus. Similar findings were reported by Dochez and Avery (4) who also demonstrated the type-specific substance, derived from the pneumococcus, in the blood and urine of patients suffering from lobar pneumonia and in the blood and urine of rabbits infected with the pneumococcus. Neufeld (11) demonstrated that a capsular substance on the pneumococcus was type specific and that it was responsible for the capsular precipitation or quellung reaction with specific antisera. The latter findings have served as a basis for typing the pneumococcus for nearly a century.

Recent development of pneumococcal vaccines (1, 3, 14), which have proven to be efficacious in the prevention of disease in highly susceptible individuals, has increased the need to know what serological groups and types of immunogens should be included in pneumococcal vaccines. The capillary precipitin test was designed to facilitate more rapid screening of defined populations to determine the most prevalent pneumococcal types.

The capillary precipitin test presented in this

TABLE 3. *Typing results with S. pneumoniae isolates by capillary precipitin and Neufeld tests*

No. of isolates	Group or type[a]	% Agreement between capillary precipitin and Neufeld test results
2	1	100
14	3	100
11	4	100
25	6	100
9	7	100
6	8	100
11	9	100
1	10	100
2	11	100
2	12	100
2	13	100
20	14	100
4	15	100
2	17	100
3	18	100
23	19	100
1	21	100
6	22	100
7	23	100
2	24	100
2	31	100
11	Not groups or types 1–32	100

[a] All isolates were typed with CDC pneumococcal antisera groups or types 1 to 32.

publication is as sensitive and specific as the Neufeld test. It appears that the same antigens are involved in both tests because all reactions, except one, were the same. The one exception was that antiserum 29 yielded a precipitate with antigen 42, whereas the quellung reaction was negative for the that antiserum and antigen. There were cross-reactions in both tests with antisera 15, 24, 25, 28, and 29. However, the antigenic formulas of pneumococci (10) afford an explanation as to why certain cross-reactions might have occurred. Two subtypes of group 15 pneumococci possess a common antigen (15c) with group 23 organisms. Two subtypes of group 24 pneumococci possess a common antigen (7h) with two subtypes of groups 7 and 19 and type 40 organisms. Type 29 pneumococcus possesses a common antigen (29b) with a subtype of group 35. However, there are no known common antigens between pneumococcal types 29 and 42.

With additional absorptions of CDC pneumococcal antisera, it is possible that most of the cross-reaction will be eliminated. The Biological Products Division of CDC is presently attempting to remove the cross-reaction from the sera. However, all cross-reactions may not be eliminated. Lund (9) was unable to obtain type-specific sera for types 29, 35, and 42. Serum 29 reacted with 35B. Serum 35 reacted with types 42 and 47, and serum 42 reacted with types 35A and 35C.

We have evaluated the efficacy of applying the capillary precipitin test to the typing of pneumococci. A total of 166 isolates were typed blindly, and these results were compared with Neufeld test results. There was a 100% agreement between the results obtained by the two tests. Present data indicate that the new typing method will probably continue to yield results identical to those of the Neufeld test. However, the test will be further evaluated with more diagnostic isolates, and these results will be compared with those obtained with the Neufeld test. As soon as antisera against the higher pneumococcal types are available, they will be tested in the capillary precipitin test against the 83 type antigens.

We have developed a pneumococcal typing test that is easy to perform and reproducible and yet is more rapid than existing methods. The antigen is sterile and thus does not present a hazard to the individual performing the test. The antigen is stable even after the bacteria have lost their viability. The results are read macroscopically and do not require an extensively experienced individual to interpret them. Because of the very small quantity of serum required, the cost of typing pneumococci is reduced. We believe that the capillary precipitin test will serve as an effective, economical, epidemiological tool in studying the prevalence of pneumococcal types in populations of the world.

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