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Six Newly Recognized Types of *Streptococcus pneumoniae*

JØRGEN HENRICHSEN*

World Health Organization Collaborating Centre for Reference and Research on Pneumococci, Statens Seruminstitut, Copenhagen, Denmark

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The serological properties of six new pneumococcal capsular types are described. A table listing all 90 pneumococcal types and their cross-reactions is included.

When *Streptococcus pneumoniae* type 47A was described in 1972 (7), the number of known pneumococcal capsular types totaled 83 (see also reference 6). In 1985, Austrian and coworkers described a new type: 16A (1).

In 1979, a worldwide pneumococcal typing surveillance study, aimed mainly at typing isolates from blood and cerebrospinal fluid, was initiated under the auspices of the World Health Organization. Since then, more than 25,000 strains, most from normally sterile body sites, have been typed in Copenhagen, Denmark (2, 9). Typing was performed according to a procedure described previously (6). During the course of this study, six new types, 10B, 10C, 11D, 12B, 25A, and 33D, with which this report deals, were detected by the procedure described by Mørch (8) based on the dictum: "the serological identity of two types can only be proved by means of cross-adsorption of the mutual immune sera" (4). Antisera to these strains were prepared in two or more rabbits according to the standard immunization procedures of the World Health Organization Reference Centre (6), and antibodies to them are now included in Omniserum, in the diagnostic serum pools D, E, and I, and in antisera to groups 10, 11, 12, 25 (previously type 25), and 33, made at Statens Seruminstitut, Copenhagen, Denmark (3, 6).

Antigenic formulas (4), an expression used throughout this paper, represent arbitrary designations of cross-reactions as seen by the capular reaction (4–6). Mørch (8, p. 98) wrote: "Antigen a signifies the factor characteristic of the individual type or common to the types of a group. The letters b, c, d and so on indicate the additional partial antigens, which in certain cases may have developed more strongly than the antigen particular to the type". For instance, the two factor sera, 6b and 6c, necessary to distinguish type 6A from type 6B, are of course made by reciprocal absorption which takes experience because there is only a relatively narrow interval between incomplete absorption of the unwanted reaction and complete absorption of all type-specific antibodies, including heterologous antibodies.

The reason for including Tables 1 to 5, therefore, is that the antigenic similarities and differences shown represent the only way of demonstrating that the six new types are, in fact, different from the ones to which they are most closely related.

Seven strains of *S. pneumoniae* type 10B were isolated from 1982 to 1991, one from cerebrospinal fluid in Sri Lanka (1983) and the others from blood in Australia (1991), Belgium (two strains in 1986), Denmark (two strains in 1982 and 1988), and The Netherlands (1983).

Two strains of type 10C have been isolated, one from cere-

TABLE 1. Capsular titers of unabsorbed and reciprocally absorbed rabbit antisera to types in pneumococcal group 10

Antiserum	Titer to type:			
	10F	10A	10B	10C
10F	128	4	8	64
10F absorbed with 10A	64	Neg[a]	8	64
10F absorbed with 10B	64	Neg	Neg	32
10F absorbed with 10C	16	Neg	4	Neg
10A	16	64	32	32
10A absorbed with 10F	Neg	32	32	4
10A absorbed with 10B	Neg	8	Neg	4
10A absorbed with 10C	Neg	32	16	Neg
10B	32	64	128	64
10B absorbed with 10F	Neg	8	64	Neg
10B absorbed with 10A	8	Neg	16	Neg
10B absorbed with 10C	16	16	64	Neg
10B absorbed with 10F, 10A, plus 10C	Neg	Neg	16	Neg
10C	64	32	32	128
10C absorbed with 10F	Neg	4	2	8
10C absorbed with 10A	32	Neg	Neg	32
10C absorbed with 10B	16	2	Neg	16
10C absorbed with 10F, 10A, plus 10B	Neg	Neg	Neg	8

[a] Neg, negative.

* Mailing address: The Streptococcus Department, Statens Seruminstitut, Artillerivej 5, DK-2300 Copenhagen S, Denmark. Phone: 45 32 68 36 00. Fax: 45 32 68 38 65.

TABLE 2. Capsular titers of unabsorbed and reciprocally absorbed rabbit antisera to types in pneumococcal group 11

Antiserum	Titer to type:				
	11F	11A	11B	11C	11D
11F	64	32	8	Neg[a]	32
11F absorbed with 11A	16	Neg	8	Neg	8
11F absorbed with 11D	8	Neg	4	Neg	Neg
11A	32	64	Neg	2	64
11A absorbed with 11F	Neg	16	Neg	Neg	8
11A absorbed with 11D	Neg	2	Neg	Neg	Neg
11D	128	64	Neg	Neg	128
11D absorbed with 11F	Neg	Neg	Neg	Neg	Neg
11D absorbed with 11A	Neg	Neg	Neg	Neg	Neg
11D absorbed with 11F plus 11A	Neg	Neg	Neg	Neg	Neg

[a] Neg, negative.

TABLE 3. Capsular titers of unabsorbed and reciprocally absorbed rabbit antisera to types in pneumococcal group 12

Antiserum	Titer to type:		
	12F	12A	12B
12F	128	8	16
12F absorbed with 12A	32	Neg[a]	8
12F absorbed with 12B	32	Neg	Neg
12A	2	256	32
12A absorbed with 12F	Neg	128	16
12A absorbed with 12B	Neg	128	Neg
12B	128	256	256
12B absorbed with 12F	Neg	8	8
12B absorbed with 12A	8	Neg	16
12B absorbed with 12F plus 12A	Neg	Neg	4

[a] Neg, negative.

brospinal fluid in China in 1987 and the other from blood in Norway in 1983.

One strain of type 11D was isolated from blood in a Danish regional hospital in 1986.

Also from blood, a pneumococcus type 12B was isolated in 1981 in The Gambia.

Two strains of type 25A were isolated, one from cerebrospinal fluid in Singapore (1981) and the other from blood in The Netherlands (1986); they have been referred to previously (10).

Finally, three strains of type 33D were isolated in New Delhi, India, in 1979, all from cases of meningitis (in males aged 8, 12, and 24 years).

All strains were typical pneumococci: gram-positive, lanceolate diplococci often occurring in short chains and growing with typical glistening, dome-shaped colonies showing signs of central autolysis with time. The isolates were optochin sensitive and bile soluble and reacted with anti-C polysaccharide antiserum (11). Their abilities to ferment carbohydrates were consistent with the identification of *S. pneumoniae*. Virulence in mice was not examined because it does not correlate with virulence in humans, and mice are used rarely today to isolate pneumococci from respiratory secretions.

Pneumococci of both types 10B and 10C reacted with diagnostic pneumococcal antiserum pool E, group 10 antiserum, and both factor sera 10b and 10c (Statens Seruminstitut); therefore, they were neither type 10F nor type 10A. Results of immunization and cross-absorptions, carried out as described elsewhere (6, 8), are shown in Table 1. Their antigenic formulas are as follows: for 10B, 10a, 10b, 10c, 10d, 10e; and for 10C, 10a, 10b, 10c, 10f.

TABLE 4. Capsular titers of unabsorbed and reciprocally absorbed rabbit antisera to pneumococcal types 25F, 25A, and 38

Antiserum	Titer to type:		
	25F	25A	38
25F	128	32	2
25F absorbed with 25A	64	Neg[a]	Neg
25F absorbed with 38	128	32	Neg
25A	64	256	128
25A absorbed with 25F	Neg	64	16
25A absorbed with 38	8	128	Neg
25A absorbed with 25F plus 38	Neg	32	Neg
38	16	16	128
38 absorbed with 25F	Neg	16	64
38 absorbed with 25A	Neg	Neg	64

[a] Neg, negative.

TABLE 5. Capsular titers of unabsorbed and reciprocally absorbed rabbit antisera to types in pneumococcal groups 6 and 33

Antiserum	Titer to type:			
	6A	6B	33B	33D
6A	256	64	Neg[a]	8
6A absorbed with 6B	64	Neg	Neg	Neg
6A absorbed with 33D	128	64	Neg	Neg
6B	8	128	Neg	2
6B absorbed with 6A	Neg	64	Neg	Neg
6B absorbed with 33D	4	128	Neg	Neg
33B	Neg	Neg	256	128
33B absorbed with 33D	Neg	Neg	16	Neg
33D	4	8	128	256
33D absorbed with 6A	Neg	Neg	128	256
33D absorbed with 6B	Neg	Neg	128	128
33D absorbed with 6A plus 6B	Neg	Neg	128	128
33D absorbed with 33B	2	4	Neg	16
33D absorbed with 6A, 6B, plus 33B	Neg	Neg	Neg	8

[a] Neg, negative.

Type 11D reacted with pool D antiserum, anti-group 11 antiserum, and factor sera 11b and 11c. Since it did not react with factor serum 11f, it differed from type 11C. Immunization and cross-absorptions (Table 2) established the antigenic formula of type 11D as 11a, 11b, 11c, 11e.

Type 12B reacted with pool E antiserum and anti-group 12 antiserum as well as with both factor sera 12b and 12c. The antigenic formula 12a, 12b, 12c, 12e was established after immunization and cross-absorptions (Table 3).

Type 25 had been defined previously by the antigenic formula 25a, 25b, and incidentally, type 38 had been defined by the formula 38a, 25b (4). The latter was quite arbitrary; it might just as well have been 25a, 25c, and the type could have been called 25A. The designation was made before the Danish nomenclature evolved as a system different from the American one. The cells of both types 25 and 38 have very small capsules. The same applies to the new type 25A with the antigenic formula 25a, 25c, 38a. The two strains isolated reacted nicely with both pool I and type 25 and 38 antisera. Former type 25 hereafter should be called type 25F (F for first defined member of a group) in accordance with the system of nomenclature (see below). In Table 4, the capsular titers (6) of unabsorbed and reciprocally absorbed antisera in group 25 are given.

The type 33D strains, which at first glance seemed to be type 33B pneumococci cross-reacting with group 6, all reacted with pool E antiserum, anti-group 33 antiserum, and 33f and 6a factor sera (Table 5). Consequently, the antigenic formula of new type 33D is 33a, 33c, 33d, 33f, 6a.

An updated listing of recognized capsular types, last printed in 1978 (6), is given in Table 6 together with the antigenic formula of each type. In 1940, Kauffmann et al. in their treatise "On the Serology of the Pneumococcus-group" wrote that they had found "quite a number of new types which, for practical

TABLE 6. Type designations and antigenic formulas of 90 types of pneumococci[a]

Type	Antigenic formula	Type	Antigenic formula
1	1a	19C	19a, 19c, 19f, 7h
2	2a	20	20a, 20b, 7g
3	3a	21	21a
4	4a	22F	22a, 22b
5	5a	22A	22a, 22c
6A	6a, 6b	23F	23a, 23b, 18b
6B	6a, 6c	23A	23a, 23c, 15a
7F	7a, 7b	23B	23a, 23b, 23d
7A	7a, 7b, 7c	24F	24a, 24b, 24d, 7h
7B	7a, 7d, 7e, 7h	24A	24a, 24c, 24d
7C	7a, 7d, 7f, 7g, 7h	24B	24a, 24b, 24e, 7h
8	8a	25F	25a, 25b
9A	9a, 9c, 9d	25A	25a, 25c, 38a
9L	9a, 9b, 9c, 9f	27	27a, 27b
9N	9a, 9b, 9e	28F	28a, 28b, 16b, 23d
9V	9a, 9c, 9d, 9g	28A	28a, 28c, 23d
10F	10a, 10b	29	29a, 29b, 13b
10A	10a, 10c, 10d	31	31a, 20b
10B	10a, 10b, 10c, 10d, 10e	32F	32a, 27b
10C	10a, 10b, 10c, 10f	32A	32a, 32b, 27b
11F	11a, 11b, 11e, 11g	33F	33a, 33b, 33d
11A	11a, 11c, 11d, 11e	33A	33a, 33b, 33d, 20b
11B	11a, 11b, 11f, 11g	33B	33a, 33c, 33d, 33f
11C	11a, 11b, 11c, 11d, 11f	33C	33a, 33c, 33e
11D	11a, 11b, 11c, 11e	33D	33a, 33c, 33d, 33f, 6a
12F	12a, 12b, 12d	34	34a, 34b
12A	12a, 12c, 12d	35F	35a, 35b, 34b
12B	12a, 12b, 12c, 12e	35A	35a, 35c, 20b
13	13a, 13b	35B	35a, 35c, 29b
14	14a	35C	35a, 35c, 20b, 42a
15F	15a, 15b, 15c, 15f	36	36a, 9e
15A	15a, 15c, 15d, 15g	37	37a
15B	15a, 15b, 15d, 15e, 15h	38	38a, 25b
15C	15a, 15d, 15e	39	39a, 10d
16F	16a, 16b, 11d	40	40a, 7g, 7h
16A	16a, 16c	41F	41a, 41b
17F	17a, 17b	41A	41a
17A	17a, 17c	42	42a, 20b, 35c
18F	18a, 18b, 18c, 18f	43	43a, 43b
18A	18a, 18b, 18d	44	44a, 44b, 12b, 12d
18B	18a, 18b, 18e, 18g	45	45a
18C	18a, 18b, 18c, 18e	46	46a, 12c, 44b
19F	19a, 19b, 19d	47F	47a, 35a, 35b
19A	19a, 19c, 19d	47A	47a, 43b
19B	19a, 19c, 19e, 7h	48	48a

[a] The antigenic formulas represent arbitrary designations of cross-reactions as seen by the capsular reaction (4–6).

reasons, were placed under the types with which they have particularly close antigenic relations" (4). Using group 7 as an example, they include the closely related types designated 7, 7A, 7B, and 7C. Later, an "F" (for first) was added to the first type of each group (5). At that time, one group consisting of types 9L, 9N, and 9V already had been established by Vammen in 1939 (12) (L stands for Lederle, N in all probability stands for Neufeld [although I have no definite proof of this], and V stands for Valdemar, the name of a Danish prince, who, in 1938, succumbed to pneumococcal pneumonia with bacteremia due to this new type). Group 6, consisting of types 6A and 6B, had also been described (4).

Newly recognized types appear, in general, to be infrequent and of low virulence for humans. They are unlikely to necessitate a reformulation of pneumococcal vaccine.

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REFERENCES

1. **Austrian, R., C. Boettger, M. Dole, L. Fairly, and M. Fried.** 1985. *Streptococcus pneumoniae* type 16A, a hitherto undescribed pneumococcal type. J. Clin. Microbiol. **22:**127–128.
2. **Henrichsen, J.** Unpublished data.
3. **Henrichsen, J., and J. B. Robbins.** 1992. Production of monovalent antisera by induction of immunological tolerance for capsular typing of *Streptococcus pneumoniae*. FEMS Microbiol. Lett. **94:**89–94.
4. **Kauffmann, F., E. Mørch, and K. Schmith.** 1940. On the serology of the pneumococcus-group. J. Immunol. **39:**397–426.
5. **Lund, E.** 1970. On the nomenclature of the pneumococcal types. Int. J. Syst. Bacteriol. **20:**321–323.
6. **Lund, E., and J. Henrichsen.** 1978. Laboratory diagnosis, serology and epidemiology of *Streptococcus pneumoniae*, p. 241–262. *In* T. Bergan and J. R. Norris (ed.), Methods in microbiology. Academic Press, London.
7. **Lund, E., A. Munksgaard, and S. M. Steward.** 1972. A new pneumococcus

type. Type 47A. Acta Pathol. Microbiol. Scand. Sect. B **80:**497–500.
8. **Mørch, E.** 1943. Serological studies on the pneumococci, p. 25–43. Oxford University Press, London.
9. **Nielsen, S. V., and J. Henrichsen.** 1992. Capsular types of *Streptococcus pneumoniae* isolated from blood and CSF during 1982–1987. Clin. Infect. Dis. **15:**794–798.
10. **Robbins, J. B., R. Austrian, C.-J. Lee, S. C. Rastogi, G. Schiffman, J. Henrichsen, P. H. Mäkelä, C. V. Broome, R. R. Facklam, R. H. Tiesjema, and J. C. Parke, Jr.** 1983. Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. J. Infect. Dis. **148:**1136–1159.
11. **Sørensen, U. B. S., and J. Henrichsen.** 1987. Cross-reactions between pneumococci and other streptococci due to C polysaccharide and F antigen. J. Clin. Microbiol. **25:**1854–1859.
12. **Vammen, B.** 1939. Serological variants of pneumococcus types 9 and 10. J. Immunol. **37:**359–365.