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Comparison of Counterimmunoelectrophoresis and the Capsular Reaction Test for Typing of Pneumococci

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The capsular reaction test is recommended as the method of choice for typing of pneumococci. Counterimmunoelectrophoresis also works in most cases, but has the following definite drawbacks. (i) The polysaccharide type antigens of types 7F, 7A, 14, 33F, 33A, and 37 are neutral and therefore do not form precipitates when conventional buffers are used. (ii) Counterimmunoelectrophoresis does not permit differentiation between types within groups since many factor sera do not contain sufficient amounts of precipitating antibodies. Omniserum contains low-titered antibodies against cell wall antigens and also reacts with thioglycolate-containing broth.

Although first described in 1902 by Neufeld, the capsular reaction test (Neufeld test) was not introduced for typing of pneumococci until 1931 (3). When serum therapy of serious pneumococcal infections came into use in Denmark in the late thirties, pneumococcal isolates were typed, and the production of therapeutic sera was controlled by means of this test. After serum therapy was abandoned, Statens Seruminstitut continued to produce diagnostic pneumococcal typing antisera, the titers and specificity of which are still controlled by the capsular reaction test (9).

Lately, typing of pneumococci isolated from serious infections has become a necessary prerequisite for usage of the newly introduced 14-valent pneumococcal vaccine (4, 6, 9, 11). Apparently, the technique of counterimmunoelectrophoresis (CIE) has been adapted for this purpose by many laboratories, presumably because this technique already was in use for other purposes and possibly also because of lack of experience with the capsular reaction test.

Anhalt and Yu (2) described a CIE performed with conventional buffers that fails to detect the neutral polysaccharides of the frequently disease-causing pneumococcal types 7F and 14. In addition, the type 37 polysaccharide might also be neutral because it has been shown to consist nearly exclusively of glucose subunits (8).

The aim of the present investigation was to systematically evaluate typing of pneumococci by means of CIE by comparing the results thus obtained with those obtained by the capsular reaction test, the latter serving as the reference test system.

MATERIALS AND METHODS

Bacterial strains. The 83 pneumococcal laboratory strains routinely in use for production of diagnostic pneumococcal typing antisera were employed. In addition, the non-encapsulated (rough) *Streptococcus pneumoniae* strain, ATCC 12213, was used for control and absorption experiments.

Fifty-seven pneumococcal isolates from patients with meningitis, pneumonia, bacteremia or otitis media were examined in Göteborg; they represented the following types or groups: group 6 (13 strains), type 3 (9 strains), group 23 (9 strains), group 19 (8 strains), type 14 (6 strains), group 11 (4 strains), group 15 (2 strains), group 7 (1 strain), group 9 (1 strain), group 12 (1 strain), group 17 (1 strain), group 18 (1 strain), and type 34 (1 strain).

Diagnostic pneumococcal antisera. Four kinds of rabbit antisera were used: (i) omniserum, which gives a capsular reaction with all 83 known pneumococcal types; (ii) nine pooled sera, A to I, each reacting with 7 to 11 types, together covering all 83 types; (iii) 46 type or group sera which react with single types or groups (Table 1); (iv) so-called "factor" sera, rather heavily absorbed sera that allow differentiation between types within groups (Table 1).

These sera were all produced by means of the standard procedure of the WHO Collaborating Centre for Reference and Research on Pneumococci, Statens Seruminstitut, Copenhagen, as described elsewhere (9).

In contrast to the other antisera, factor sera are not commercially available.

An equine anti-type 7 antiserum (horse no. 1074) containing 510 μg of anti-C carbohydrate antibody N per ml and 500 μg of anti-type 7 antibody N per ml was supplied by Michael Heidelberger, New York, N.Y.

Typing of pneumococci. Regardless of which of the following two methods was used, typing was carried out by successively examining the reaction of the strains with omniserum, pooled sera, relevant type or group sera, and relevant factor sera.

The capsular reaction test, which has recently been reviewed by Austrian (4), was carried out as described and depicted earlier (6, 9).

TABLE 1. *Pneumococcal types and groups*

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

[a, c] Eighty-three types altogether. Note that no. 26 and 30 are not in use.

[a, b] Forty-six type or group sera.

CIE was performed as described by Colding and Lind (5) and by Trollfors et al. (12) with either Veronal (VER) (5) or barbital (BAR) (1) buffer or *m*-carboxyphenylboronic acid (CPB) in combination with barbital buffer (CPB-BAR) (1). Electrophoresis was run for 45 min with a voltage gradient of 5 V/cm. Slides were inspected immediately and after 18 h at 4°C.

Immunoelectrophoresis was performed as described by Wadsworth and Hanson (13) with 0.075 M VER, pH 8.6.

Antigens. For CIE typing of pneumococci, supernatants of 16- to 18-h-old serum broth cultures (37°C) were used undiluted and diluted 1:10, 1:100, 1:200, and 1:400 with saline. Controls were run with uninoculated serum broth.

Pneumococcal polysaccharide antigens isolated by precipitation with the cationic detergent Cetavlon as described by Kaijser (7) were used for immunoelectrophoretic characterization of the antigens.

The 14 purified polysaccharide antigens contained in the pneumococcal vaccine Pneumovax provided by Merck Sharp & Dohme were used in concentrations of 1, 0.1, 0.01, and 0.001 μg/ml to assess the sensitivity of antigen detection by CIE.

RESULTS

Typing by means of CIE. Using omniserum, the nine pooled sera, and the type or group sera, all 83 pneumococcal types except type 7A could be correctly typed or grouped by means of CIE. Well-defined precipitates were formed with the supernatants diluted 1:10 or more. However, typing of 7F, 14, 33F, 33A, and 37 required the use of CPB buffer (Table 2). Even with this buffer types 7A and 37 formed weak and rather ill-defined precipitates which were only seen with undiluted supernatants. Type 7A reacted neither with pool C serum nor with group 7 serum.

The precipitates formed between omniserum and types 7A, 33F, 33A, and 37 as well as between pool D serum and type 37 with VER or BAR buffers were not specific and appeared only with undiluted supernatants [in Table 2 they are listed as (+)]. They were probably due to antibodies directed against cell wall antigens since they were seen as two lines which could both be removed by absorption with the rough strain of *Pneumococcus*, ATCC 12213. Similar precipitates were formed between the equine anti-type 7 antiserum with a high content of anti-C car-

TABLE 2. *Reactions of those pneumococcal types which are difficult or impossible to type by means of CIE*[a]

Type	Omniserum		Pooled sera A-I		Type or group sera	
	VER/BAR	CPB-BAR	VER/BAR	CPB-BAR	VER/BAR	CPB-BAR
7F	−	+	−	+ (C)	−	+
7A	(+)	+	−	−	−	−
14	−	+	−	+ (H)	−	+
33F	(+)	+	−	+ (E)	−	+
33A	(+)	+	−	+ (E)	−	+
37	(+)	+	(+) (D)	+ (D)	−	+

[a] + indicates formation of a precipitate with Omniserum, the corresponding serum pool (designated by a capital and entered in parentheses), or the corresponding type or group serum. (+) indicates formation of unspecific precipitate(s) (see text).

bohydrate antibodies and types 7A, 33F, 33A, and 37. Furthermore, the non-encapsulated strain ATCC 12213 reacted in the same way as these types with both the equine anti-type 7 antiserum and omniserum before—but not after—absorption with strain ATCC 12213.

Neither serum broth nor trypsin broth gave rise to precipitates with the diagnostic pneumococcal antisera. However, omniserum and the pooled sera E and G formed precipitates with an uninoculated thioglycolate-containing medium on a simple broth base.

Of the 56 factor sera currently in use at Statens Seruminstitut, 17 do not give rise to the formation of precipitates in CIE. As a result, differentiation between types within the following eight groups, comprising a total of 28 types, cannot be carried out by means of CIE: 7, 11, 15, 18, 23, 24, 32, and 35.

Immunoelectrophoresis of capsular antigens isolated from types 7F, 7A, 33F, 33A, and 37 showed that these polysaccharides are neutral or maybe slightly positively charged; not very well-defined precipitates were seen between the central well containing antiserum and the holes containing antigen, with a suggestion of a disposition towards the cathode.

Sensitivity of the CIE technique. By using the 14 purified pneumococcal capsular polysaccharides present in the currently available vaccine, it was found that the CIE technique allowed the detection of 0.1 μg of most of the types per ml. Type 19F, however, could be detected in a concentration as low as 0.01 μg/ml. Types 7F and 14, for which the CPB buffer had to be used, were only detectable in concentrations of ≥1.0 μg/ml.

Typing of clinical isolates by means of CIE directly upon isolation did not give results that differed from those obtained with the standard laboratory strains.

DISCUSSION

In addition to pneumococcal type 7F and 14 polysaccharides, which are known to be neutral (2), types 7A, 33F, 33A, and 37 were found in the present study to be neutral (or maybe slightly positively charged). Therefore, these types could only be correctly identified by CIE with CPB buffer. For some unknown reason type 7A, even with this buffer, only reacted with omniserum.

With conventional buffers the CIE technique, therefore, fails to detect a total of six pneumococcal polysaccharide type antigens. Interpretation of the capsular reaction test obviously requires some experience, but the test is easy to perform and rapid and requires only minute amounts of antisera. For these reasons we advise the use of the capsular reaction test for typing of pneumococci.

The CIE technique, furthermore, cannot be used for the differentiation between types within groups because nearly a third of the necessary factor sera do not contain sufficient amounts of precipitating antibody.

The finding that omniserum contains antibodies against pneumococcal cell wall antigens with lower titers than the type-specific antibodies is not surprising. Even when great care is taken to make vaccines from exponential-phase cultures, a certain—albeit low—admixture of lysed cells is to be anticipated. Small amounts of cell wall antigens will induce the formation of antibodies directed against these antigens. Such antibodies will be recovered in omniserum in the same or higher titers as in the pools out of which the omniserum is made, in contrast to the type-specific antibodies that will be present in omniserum with a titer about half of that seen in the pools (10).

The sensitivity of the CIE technique found in the present study compares well with that reported by Anhalt et al. (1).

It is a finding of some practical importance that omniserum forms a precipitate with thioglycolate-containing broth.

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