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Serotyping of *Streptococcus pneumoniae* Strains by Coagglutination and Counterimmunoelectrophoresis

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A total of 217 *Streptococcus pneumoniae* strains were serotyped by coagglutination (COA) and counterimmunoelectrophoresis (CIE). With all strains tested, there was full agreement between results obtained by COA and CIE, except for strains belonging to serotypes 7, 14, 33, and 37, which could not be typed by CIE. These strains were serotyped by passive immunodiffusion, results of which were in full agreement with those obtained by COA. Besides having the advantage of identifying strains belonging to all serotypes, COA was also more rapid and economical than CIE.

On the basis of the composition of the capsular polysaccharide, *Streptococcus pneumoniae* strains can be divided into 83 antigenic types (3). Serotyping of pneumococci is of epidemiological interest and offers information about the coverage of pneumococcal infections by the available pneumococcal polysaccharide vaccines. The reference method for serotyping pneumococci is the capsular swelling test (1, 4), a method requiring considerable expertise. Therefore, many laboratories use counterimmunoelectrophoresis (CIE) (4), which is easier to interpret. CIE is usually performed in Veronal buffer at pH 8.2 to 8.6, in which capsular polysaccharides of types 7F, 7A, 14, 33F, 33A, and 37 are neutrally charged and thus cannot be identified (4, 7).

A slide agglutination method, coagglutination (COA), for typing pneumococci by the use of specific antibodies adsorbed to protein A-containing *Staphylococcus aureus* strains has also been described (5).

The aim of this study was to compare CIE and COA for serotyping *S. pneumoniae* with respect to concordance, rapidity, and costs.

Most pneumococcal strains used for this study were clinical isolates from blood or cerebrospinal fluid isolated at the Departments of Clinical Bacteriology of universities at Gothenburg, Umeå, and Malmö, Sweden. The strains had been kept lyophilized after isolation. Sixteen strains of uncommon serotypes were obtained from Statens Seruminstitut, Copenhagen, Denmark. Altogether 217 strains were tested, 150 of which were previously typed and, if relevant, subtyped at the Statens Seruminstitut by the capsular swelling reaction (6). Since the pneumococcal strains were partly selected for the purpose of this study, the serotype distribution reported here must not be considered representative for the region.

Diagnostic antisera were obtained from the Statens Seruminstitut. All strains were tested both by CIE and COA against nine pools of antisera (A through I), together containing antibodies to all 83 pneumococcal types. Thereafter, the pneumococci were tested against relevant type- or group-specific antisera, except antisera against the uncommon types 13, 16, 24, 36, 43, 44, 45, 46, and 48. Preliminary tests with antisera in various dilutions in phosphate-buffered saline (PBS) showed that all antisera could be diluted 1:2 without loss of sensitivity for CIE. For COA, it was possible to dilute the pooled antisera 1:10 and the group- and type-specific antisera 1:20 without loss of sensitivity.

CIE was performed in Veronal buffer as previously described (2) with minor modifications. Briefly, a 1:5 solution of Veronal buffer (pH 8.6, ionic strength 0.1; LKB Produkter AB, Stockholm, Sweden) in distilled water was used to prepare a 1% agarose gel (Seakem ME, Marine Colloids Div., Rockland, Maine). Glass slides (8 by 8 cm) were coated with 15 ml of agarose and allowed to harden for approximately 15 min. A 40-well pattern consisting of three parallel columns of 7, 7, and 6 paired wells, respectively, was then punched. The wells had a volume of about 12 μl each, and the spaces between the wells in each column was 4 mm and between each column 10 mm. A 10-μl amount of a 1:2 dilution of pneumococcal antisera in PBS was placed in each well on the anodic side of the pattern. Approximately 10 colonies of each pneumococcal strain to be tested were obtained from blood agar plates, diluted in 0.5 ml of PBS, and incubated for 2 to 6 h; 10 μl was then placed

in the wells on the cathodic side of the pattern. The plates were placed on a plastic surface previously cooled to 0°C, and a current of 15 V/cm was applied for 15 min, using full-strength buffer in the electrophoretic vessel. Plates were then removed and read immediately without staining.

All pneumococcal strains that could not be typed by CIE were tested by passive immunodiffusion (PID) (8), using only antisera against types 7, 14, 33, and 37. PID was performed in low-ash agar (Noble agar; Difco Laboratories, Detroit, Mich.). A 20-ml amount of a 1% solution was layered on a glass slide (8 by 8 cm). A hexagonal pattern with one central well was then cut; 18 μl of undiluted antiserum was placed in the latter, and 18 μl of the pneumococcal suspension was placed in the surrounding wells. Slides were read after 24 and 48 h at room temperature.

A 10% (vol/vol) suspension in PBS of *S. aureus* Cowan 1 that had been treated with formaldehyde and heat was obtained from Pharmacia Diagnostics AB, Uppsala, Sweden. Coating of the staphylococci by pneumococcal antiserum was performed according to the manufacturer's description for the commercially available kit Phadebact. Briefly, 0.5 ml of the staphylococcal suspension was mixed with 50 μl of diluted anti-pneumococcal serum. The pooled antisera A through I were diluted 1:10 and the type- or group-specific antisera were diluted 1:20 before being mixed with the staphylococcal suspension. After mixing for 1 h and centrifugation at 3,000 × *g* for 10 min, the supernatant was discarded. A 0.55-ml amount of a filtered 2% solution of methylene blue was then added to the antibody-coated staphylococci. After renewed mixing and centrifugation, the staphylococci were finally suspended in 2.6 ml of PBS and stabilized for 30 min at room temperature. The COA reaction was performed on paper from the Phadebact kit. Three to five colonies of pneumococci grown on blood agar plates were suspended in 40 μl of the antibody-coated, blue-colored staphylococcal suspension. The agglutination reaction was read after 30 to 60 s.

CIE plus PID and COA were performed by different participants in the study, none of them knowing the results obtained with the other technique nor the results obtained in previous typings. The pneumococcal strains were distributed by a person not participating in the study.

Table 1 shows the numbers of pneumococci of the different serotypes tested and the results obtained by COA and by CIE. There was complete agreement between the results obtained by COA and CIE at pH 8.6, except for the failure of CIE to type any strains belonging to serotypes 7F, 7A, 14, 33F, 33A, or 37. All strains belonging to these serotypes were successfully typed by PID, the results of which agreed with those of COA. There was also complete agreement between the typing results obtained in this study and the results of previous typing by the capsular swelling test.

For several reasons, we chose COA as the preferable method for serotyping *S. pneumoniae*. Strains of all 34 types or groups available could be typed, and the results were in complete agreement with those obtained by CIE or PID and by the capsular swelling reaction. In a previous study, it has also been shown that COA correlates well with the capsular swelling test (5). The major weakness of CIE for detection of

TABLE 1. Number of pneumococcal strains of different serotypes typed by COA and CIE at pH 8.6 or by PID

Test	Serotype	Subtypes included[a]	No. of strains
COA and CIE	1		6
	2		1
	3		19
	4		20
	5		2
	6	A, B	20
	7	B, C	2
	8		10
	9	N, A, V	10
	10	F, A	7
	11	F, A	6
	12	F, A	2
	15	A, C	4
	17	A	3
	18	C	6
	19	F, A	14
	20		3
	21		1
	22	F	5
	23	F	15
	25		10
	28	F	1
	29		1
	31		1
	34		2
	35	F	1
	38		1
	39		1
	40		1
	41	A	1
	42		1
COA and PID	7	F, A	20
	14		15
	33	F, A	4
	37		1

pneumococcal capsular antigen is the inability to detect the neutrally charged capsular antigens of types 7F, 7A, 14, 33F, 33A, and 37 (4, 7), even though it has been reported that CIE in Veronal acetate buffer at pH 4.75 or 5.0 can be used to detect purified capsular antigens of serotypes 7F and 14 (7). Another advantage of COA as compared with CIE is its high sensitivity, which makes it possible to use 10- and 20-fold dilutions of pooled and type-specific antisera, respectively; COA is therefore more economical than CIE. With the dilutions used in this study, 5 μl of type- or group-specific antiserum could be used for 130 COA tests but only for 1 CIE test. For the capsular swelling test, a loopful (about 5 μl) of undiluted antiserum is used (1). COA can be read after 30 to 60 s, whereas CIE takes 15 min, excluding preparation of agar plates and buffers. Also, in contrast to CIE, no specific equipment is required for COA.

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