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Simple latex agglutination method for typing pneumococci

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Serotyping of *Streptococcus pneumoniae* was important before the introduction of antibiotics when intravenous administration of type specific antisera was the only treatment available for invasive pneumococcal infections. Now that pneumococcal vaccines are available, the typing of pneumococci has reassumed importance, especially as these vaccines contain only a limited number of polysaccharides.

Since its description by Neufield in 1902,[1] the capsule "swelling" (Quellung) reaction has been regarded as the standard method of typing pneumococci. It is, however, somewhat labour intensive as a routine test. We describe a simple slide agglutination method for typing pneumococci by means of specific antibody adsorbed to latex particles.

Material and methods

Five hundred consecutive strains of pneumococci isolated from clinical specimens were collected. Each isolate was confirmed as *S pneumoniae* on the basis of colonial morphology and sensitivity to optochin.

A pure culture of each isolate was obtained on a blood agar plate from which a broth culture in Todd and Hewitt broth was made (overnight incubation at 37°C). The organism on the plate was then serotyped by staphylococcal coagglutination according to the method described by Kronvall,[2] and the broth culture was used for serotyping by a latex agglutination method. The method of Severin[3] was used with several modifications. The antiserum (Statens Seruminstitut, Copenhagen) was diluted 1/20 with sodium glycine buffer (pH 8·2). Equal volumes of the diluted antiserum and latex suspension (polystyrene latex particles—0·81 μm in diameter, Difco) were mixed and incubated in a water bath for two hours at 37°C. An equal volume of 0·1% albumin solution in sodium glycine buffer was then added as a preservative. The latex agents were stored at 4°C. The reagents for both typing methods have to be brought to room temperature before performance of the tests.

In the latex test one drop of sensitised latex particles was added to a drop of the broth culture of the organism on a black tile. The drops were mixed for two minutes and examined for agglutination. In positive cases a distinct agglutination formed. When the suspension remained milky the test was negative. Each strain was tested against a range of pneumococcal antisera to determine the monotype (nine types) or serogroup (13 groups). The types or groups were chosen to be the same as the antigen types included in the 14 and 23 valent pneumococcal vaccines. One hundred strains were referred to another laboratory for serotyping by counterimmunoelectrophoresis (CIE).

Results

Of the five hundred isolates collected, 488 were tested. Most of the strains (390 strains, 80%) were isolated from sputum culture. Thirty two strains (6%) were isolated from blood or cerebrospinal fluid and the remainder from eye, ear, and peritoneal swabs.

The table shows the results of the serotyping. The

Table *Serotype distribution of 488 strains of pneumococci relative to coverage of appropriate vaccines*

Serogroup or serotype vaccine related		
14-valent vaccine	*23-valent vaccine*	*No (%)*
1	1	1 (0·2)
2	2	0 (0·0)
3	3	48 (9·8)
4	4	5 (1·0)
	5	2 (0·4)
6 (6A)	6 (6B)	47 (9·6)
7 (7F)	7 (7F)	5 (1·0)
8	8	22 (4·5)
9 (9N)	9 (9N, 9V)	57 (11·7)
	10 (10A)	6 (1·2)
	11 (11A)	23 (4·7)
12 (12F)	12 (12F)	0 (0·0)
14	14	26 (5·3)
	15 (15B)	14 (2·9)
	17 (17F)	26 (5·3)
18 (18C)	18 (18C)	12 (2·5)
19 (19F)	19 (19A, 19F)	46 (9·4)
	20	8 (1·6)
	22 (22F)	17 (3·5)
23 (23F)	23 (23F)	62 (12·7)
25		0 (0·0)
	33 (33F)	11 (2·3)
Other types		50 (10·2)
Total		488

Accepted for publication 14 July 1987

coagglutination and latex agglutination methods gave results that were in complete agreement. The 100 strains referred to another laboratory for serotyping by CIE gave similar results to our own with the exception of types 7, 14, and 33 which could not be typed.

Ninety per cent of the pneumococci were constituents of the 23 valent vaccine and 67% of the 14 valent vaccine.

Discussion

Our results indicate that the results of the latex agglutination test are in complete agreement with the staphylococcal coagglutination test. The results of the 100 strains referred to another laboratory for serotyping by CIE (performed in Veronal buffer at pH 8·4) were the same as our own except for types 7, 14, and 33 which could not be identified. The antigenic polysaccharides 7, 14, and 33 are basic and need a special and complicated buffer to make them move.[4]

The latex reagents are easy to prepare and use very small amounts of antiserum. One ml of neat antiserum makes 60 ml of stabilised latex reagent ready for use, and is adequate for about 3000 tests. Stored at 4°C, the reagents remain stable for prolonged periods. The optimal dilution of antiserum (1/20) was determined by preliminary experiments. Todd and Hewitt broth was used for the broth culture because it was found to give a high concentration of free antigen. In most cases a positive reaction occurred within seconds. With the nine pools and type 3 reagents, however, it took a little longer—over a minute before agglutination was fully developed.

The capsule-"swelling" reaction has been used since the early 1900's as the standard test for serotyping *S pneumoniae*,[1] but it is a labour intensive method and uses a lot of antiserum. CIE can be used to detect pneumococcal antigens in clinical specimens[5] and to serotype pneumococci, but the method is elaborate, requires expensive equipment, and uses a lot of antiserum. Coagglutination by means of specific antibody adsorbed to protein A-containing staphylococci has been used successfully by Kronvall to type pneumococci.[3] Although the test is rapid and easy to perform, the preparation of the reagents is not straightforward. Polyvalent counterimmunoelectrophoresis has been used to serotype pneumococci from plate cultures.[6] Although only two reagents are needed, this method is time consuming and requires special equipment. Moreover, some strains need to be tested many times before a definite serotype can be ascertained. Latex agglutination has been used to detect pneumococcal antigens from body fluids.[7] It has also been used for serological grouping of meningococci and encapsulated strains of *Haemophilus influenzae*.[8]

Ninety per cent of our pneumococcal isolates were constituents of the 23 valent vaccine and 68% of the 14 valent vaccine. Type 1, which is one of the commonest types isolated elsewhere,[9] was rarely found in our survey—only one being isolated. The other noteworthy finding was the absence of types 2, 12, and 25. Type 25 was one of the types in the 14 valent vaccine but was not included in the 23 valent vaccine.

Cross infection with *S pneumoniae* which was acquired in hospital, has been documented recently,[10] and if this is shown regularly, pneumococcal typing will be helpful in investigation of the possible sources ("disseminators") of the organism and may change our management of patients with pneumococcal infections.

There is a need for continuing surveillance of pathogenic pneumococcal serotypes in the United Kingdom. The latex agglutination method we describe is easy to perform; the reagents are simple to prepare, last for a long time, and it is economical in the use of antisera. It would be a convenient way for a busy routine laboratory to type pneumococcal isolates.

We thank Mr P Boreland from the Waveney Hospital Laboratories, Ballymena, for serotyping the 100 pneumococcal strains by CIE.

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