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Serotyping of *Streptococcus pneumoniae* by Coagglutination with 12 Pooled Antisera

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We report on the performance of a recently introduced commercial chessboard method using 12 antisera, in comparison with that of the 55-antiserum panel used in determining the serogroups and types (SGTs) of *Streptococcus pneumoniae*, both of which were carried out by a coagglutination technique. Of a total of 150 strains of *S. pneumoniae* studied, 135 (90%) belonged to the SGTs represented in the 23-valent pneumococcal vaccine; of these, 130 (96.3%) were identified as the same SGTs by both typing methods. The remaining five strains showed cross-reactivity with more than two pools by the chessboard method, but could be assigned to a single SGT by the Quellung test. The 96.3% concordance of the chessboard method suggests it can be adopted for determination of the SGTs of *S. pneumoniae* in laboratories.

Streptococcus pneumoniae is a common etiological agent of invasive diseases such as meningitis, septicemia, and pneumonia. There are 90 serotypes of *S. pneumoniae* (1), of which only a limited number cause most invasive disease in any given region (2). The currently available 23-valent pneumococcal vaccine (8) is comprised of about 90% of the types of *S. pneumoniae* isolated from invasive diseases. Serotyping of *S. pneumoniae* is important, because there have been changes in the rank order of types isolated from invasive diseases over a period of time in a single locality. This is important with reference to vaccine formulations (9, 11). Differences in the types that are isolated from different age groups (2) and regional differences in distribution of types have also been reported (9, 11). The Quellung reaction has been the "gold standard" method for typing of *S. pneumoniae* (5). The conventional method of serotyping *S. pneumoniae* uses pneumococcal typing antisera obtained from Statens Seruminstitut, Copenhagen, Denmark. It is comprised of nine pools, designated A to I, and 46 individual group- or type-specific antisera represented in the pools; thus a total of 55 antisera are required. The new chessboard system described consists of 12 pooled antisera designated A to H (except G and I) and P to T (G and I are not included in the pools, because they consist of nonvaccine serogroups or types [SGTs]) which comprise most of the SGTs that are included in the presently available 23-valent pneumococcal vaccine. Since the serotyping procedure using the 55-antiserum panel (3, 4) has been simplified by utilizing the coagglutination (COA) method, we report an assessment of the chessboard serotyping system by using the COA method.

S. pneumoniae cultures from blood, cerebrospinal fluid, and other body fluids of patients admitted to Christian Medical College (Vellore, Tamil Nadu, India) during a period of 6 months (from July 1996 to February 1997) as part of an ongoing Invasive Bacterial Infection Surveillance (IBIS) study and from other IBIS centers (Delhi and Nagpur) and the IBIS networking center (Pondicherry) were used in the study.

The 12 pools (A through H and P to T) of antisera (Table 1) and the 55-antiserum panel comprised of 9 pools (A through H and I) and the individual 46 SGTs were obtained from Statens Seruminstitut, Copenhagen, Denmark. Cowan 1 staphylococcal cells used for sensitizing the antisera were prepared in the Department of Microbiology at Christian Medical College and Hospital, as described elsewhere (4). The COA reagents were prepared by sensitizing 10% of the staphylococcal cells with each of the 12 pooled antisera, and each was checked by using a known SGT isolate included in that particular pool for a positive reaction. Normal rabbit antiserum was used as the negative control.

The bacterial isolates to be typed were subcultured onto Trypticase soy agar (Difco Laboratories, Detroit, Mich.) with 5% sheep blood and incubated in 5 to 10% CO_2. Freshly isolated colonies were used for testing. The colonies were emulsified in normal physiological saline and the opacity was adjusted to MacFarland opacity 1. The serotyping was carried out by a rapid slide agglutination method (4). In a ceramic ring slide, 25 μl of the COA reagent was mixed with 25 μl of the cell suspension. The slide was gently rocked for 2 to 3 min and observed for clumping and clearance, the presence of which was taken as a positive reading. The pools in which the positive results were first noted were taken as the final reading.

The 12 pools were tested simultaneously and the two pools which gave positive results were used to identify the SGT of the strain with the help of the chart. For example, a strain that gave a positive result with pools A and P was identified as belonging to serotype 1. For comparison, all isolates were also tested in the nine pools A to I, and further typing was with antisera from the pool that gave the positive reaction. All tests were carried out independently and interpreted by two persons with neither observer being aware of the results of the other. The Neufeld Quellung test was done according to standard procedures (5). The objective of the Quellung test was to establish unequivo-

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TABLE 1. Pneumococcal types and groups reacting with the 12 pooled antisera[a]

Pool	SGT in pool:					Nonvaccine types or groups
	P	Q	R	S	T	
A	1	18	4	5	2	
B	19	6	3	8		
C	7				20	24, 31, and 40
D			9		11	16, 36, and 37
E			12	10	33	21 and 39
F				17	22	27, 32, and 41
H	14	23		15		13 and 28
G						29, 34, 35, 42, and 47
I						25, 38, 43, 44, 45, 46, and 48

[a] Note that G and I are counted separately from the other 12 antisera (see text for details).

cally the SGTs of those strains that were found to belong to either pool G or pool I by the chessboard method, which could identify only the pools and not the types or those which gave cross-reactions in more than two pools.

Of the total of 150 strains of *S. pneumoniae* studied, the SGTs of 135 strains (90%) were found to fall in the vaccine type category. One hundred thirty of them (96.3%) were identified as the same SGT by both the 55-antiserum panel and the chessboard method. The concordant SGTs were as follows (Table 2): 1 ($n = 39$), 6 ($n = 16$), 7 ($n = 11$), 15 ($n = 10$), 5 and 9 ($n = 8$ each), 14 and 18 ($n = 6$ each), 12 ($n = 4$), and others, which included SGTs 2, 3, 8, 10, 11, 17, 19, 20, 23, and 33 ($n = 22$). Of the remaining 20 strains, 8 could not be serogrouped or typed by the chessboard method, because they belonged to pools G and I, which are not included in the 12 pools. Their serotypes were determined by the 55-antiserum panel typing method as 34 ($n = 3$), 35 ($n = 3$), 25 ($n = 1$), and 45 ($n = 1$).

By the chessboard method, seven strains could be assigned only their pools, not their SGTs, because they also belonged to nonvaccine SGTs. Even in the pools that are included, not all of the SGTs are present; some are omitted, because they are not included in the presently available 23-valent pneumococcal vaccine, such as 24 ($n = 3$), 13 ($n = 2$), 21 ($n = 1$), and 39 ($n = 1$). Five strains gave cross-reactions by the chessboard method as well as by the 55-antiserum panel COA procedure. Perhaps, this could represent a technical error. However, the SGTs, as determined by the Quellung reaction, matched the SGT exhibiting the stronger reactivity. The SGTs as determined by the Quellung reaction are 12 ($n = 2$), 14 ($n = 2$), and 4 ($n = 1$).

There are various techniques described for serotyping of *S. pneumoniae* (6, 10). The Neufeld Quellung test is the recognized "gold standard" method. The high cost and the technical expertise required prohibit the adoption of this method for regular serotyping in the laboratory. The COA method has been standardized as an alternative method of serotyping (3, 4). Even by this method, the serogrouping or typing of *S. pneumoniae* by using the presently available panel of 55 antisera has its limitations, because it is a time-consuming procedure requiring expensive antisera. A newly described method consisting of 12 pooled antisera (7) was tested for its performance in comparison with the presently used panel of 55 antisera by the COA technique. To our knowledge, the 12 pooled antisera have not been evaluated by COA. Of the 150 strains tested, 135 belonged to the vaccine type category. One hundred thirty (96.3%) among them gave the same result by both of the typing procedures. As far as the 130 strains are concerned, the chessboard method was able to identify all of the strains that are included in the presently available 23-valent pneumococcal vaccine. The volume of COA reagent (i.e., Cowan 1 staphylococcal cells need to be prepared) will be less for the pool of 12 antisera than for the panel of 55 antisera. Identification of the SGTs of the strains in question is comparatively quicker and easier in the chessboard method, because it is done by a one-step procedure of referring to the chart (Table 1), which saves time, while in the typing method employing the 55-antiserum panel, all of the pools should be tried first, and then the individual types should be tested for the identification of the SGT of a strain. The introduction of this new system for the regular serogrouping or typing of *S. pneumoniae* in the laboratory must be done with the consideration of the prevalence of the SGTs of the organism in a particular region, because it does not identify the nonvaccine SGT. In a region in which there is a considerable heterogeneity in the prevalence of such nonvaccine SGTs, the conventional 55-antiserum panel for serogrouping or typing should be used for the confirmation of those strains that may not be confirmed by the chessboard method. However, the incidence of such a diverse group of strains in a particular region, which would make the serogrouping or typing of the isolate difficult, has been very insignificant. In the present study, the number of such strains encountered was only 10% of the total (15 strains). When the test is performed with a large number of strains, this percentage should be taken into consideration. Available data on the prevalence of such nonvaccine SGTs in a particular region should give an idea about implementing this new method for routine purposes in the laboratory. Subtyping of strains belonging to a type is not possible by the chessboard method the way it is in the conventional method. For example, it is possible to identify only group 18 and not its individual subtypes, 18A, 18B, 18C, and 18F. Hence, the concordance of the chessboard method with the conventional method is only to the level of the group. However, with important vaccine types, such as serotype 1, the comparison is directly related to the types.

TABLE 2. Distribution of pneumococcal SGTs

SGT	No. of SGTs		
	Vaccine type		Nonvaccine type
	Concordant	Cross-reacting	
1	39		
6	16		
7	11		
15	10		
5	8		
9	8		
14	6	2	
18	6		
12	4	2	
4		1	
35			3[a]
34			3[a]
45			1[a]
25			1[a]
24			3[b]
13			2[b]
21			1[b]
39			1[b]
Others[c]	22		
Total	130	5	15

[a] Pools G and I (not included in the chessboard system).
[b] Only pools to which these could be assigned by the chessboard system.
[c] Includes SGTs 2, 3, 8, 10, 11, 17, 19, 20, 23, and 33.

The COA method has been described as being a good alternative for serotyping of *S. pneumoniae* (3, 4). The lower cost, simple procedure, and rapidity in obtaining a result by the COA method compared with the higher cost and technical expertise required for performing and interpreting the Quellung test, as found by the testing of 20 strains, support the earlier finding (3, 4). Thus, the chessboard method can be an equally good method to determine the SGTs of *S. pneumoniae*, which are included in the 23-valent pneumococcal vaccine. Strains giving negative results or cross-reactions may be further typed by conventional procedures.

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