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Comparison of Three Methods for Grouping Streptococci

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Two new methods for serological grouping of beta-hemolytic streptococci, the nitrous acid extraction procedure of El Kholy et al. and the slide agglutination method of Christensen et al., were compared with the Lancefield hot-hydrochloric acid extraction method in classifying 92 strains of groups A, B, C, and G. The nitrous acid extraction method was easily performed, specific, and sensitive when highly potent antisera were used. For the Christensen method these highly potent antisera had to be diluted to avoid cross-reactions between groups A and C and groups B and G, respectively. A few strains, most of them group B, could not be grouped by the latter method. Using these three grouping methods, two sets of commercial sera were compared with the more potent sera supplied by R. C. Lancefield. The low antibody content of these commercial sera, especially anti-group B and G sera, contributed to the inferior results obtained in some of the grouping reactions.

The precise group classification of beta-hemolytic streptococci is based on serological methods. The classic method is the hot-hydrochloric acid extraction technique first described by Lancefield (11). The aim of this study was to compare two new grouping methods with the Lancefield hot-hydrochloric acid extraction method. One of them was nitrous acid extraction, first described by Swanson et al. (15) for release of the group-specific carbohydrate from cell walls of streptococci for electron microscopy and adapted by El Kholy et al. (6) for serological grouping in capillary tubes. The second one is a simple slide agglutination technique described by Christensen et al. (1), which takes advantage of the unique property of protein A of *Staphylococcus aureus* in binding the Fc portion of immunoglobulin G, leaving the Fab fragment free for the specific antigen.

In addition, commercial sera for grouping streptococci from two different sources were compared with reference sera prepared by R. C. Lancefield in all three test systems. The studies were limited to group A, B, C, and G streptococci, which are those most commonly encountered and identified by serological means in routine laboratories.

MATERIALS AND METHODS

Bacterial strains. Streptococci of groups A, C, and G were taken from the collection of the State Institute of Hygiene, Warsaw, Poland. Strains of group B streptococci were kindly supplied by J. Jelinkova of the Institute of Microbiology and Hygiene, Prague, Czechoslovakia, and partially derived from the collection of the Institute of Hygiene, University of Köln, Germany, and from R. C. Lancefield of Rockefeller University. *S. aureus* strain Cowan I was obtained from the National Collection of Type Cultures, Colindale, England (no. NCTC 8530) and prepared for testing according to the method described by Kronvall (10), as originally used for typing pneumococci and subsequently adapted by Christensen et al. (1) for grouping streptococci.

Sera. Immune sera containing antibodies against C polysaccharide of streptococcal groups A, B, C, and G were generously given by R. C. Lancefield or obtained from two commercial sources, referred to as I and II.

Grouping of streptococci. Extracts were prepared by the methods described by Lancefield (11) and El Kholy et al. (6). Because of the weakness of some of the commercial sera used, precipitation was carried out as a ring test (17) rather than in capillary tubes (16). The agglutination technique was followed according to the descriptions given by Christensen et al. (1).

Other methods. The rhamnose content of Lancefield and El Kholy extracts was determined by the method of Dische and Shettles (4). Estimation of the amount of precipitating antibody was performed by means of the single reverse radial immunodiffusion technique modified from Harrell and George (8), using Lancefield extracts for group A, B, C, and G streptococci versus all sera used in these investigations. The group A and B extracts used had been prepared from strains known to be devoid of type antigens.

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RESULTS

The three methods were compared first by using highly potent antisera against group A, B, C, and G streptococci (Table 1). Using Lancefield hot-hydrochloric acid extracts, all 92 strains tested could be grouped with these sera. With the exception of several group B strains and one group G strain, all strains could also be classified by the El Kholy and Christensen techniques using these reference grouping sera supplied by Lancefield. In the Christensen technique, sensitization of the suspension of Cowan I staphylococcal cells with undiluted sera from Lancefield led to cross-reactions between strains of group A and C and group B and G, whereas none of the sera gave cross-reactions when examined with extracts prepared by the Lancefield or El Kholy method. Appropriate dilution of the sensitizing sera for the co-agglutination technique produced specific positive reactions with all streptococcal strains except those few that gave no reactions at all. For example, the dilution of the group A antiserum supplied by Lancefield to 1:16 completely abolished cross-reactions and, even when diluted to 1:64, still gave strong specific reactions. The possibility was considered that the cross-reactions seen with undiluted sera were due to some surface antigen, e.g., a T protein, common to strains from different groups. Although the strains for producing Lancefield's group C and G antisera have the same type 25 T antigen, no crosses occurred between those two groups. Also, a group G strain with the type 4 T antigen did not react with serum produced with a group A strain containing the type 4 T antigen in the Christensen method.

The failure of the Christensen method to give positive reactions with three group B strains using highly potent antiserum led to the suspicion that the type polysaccharide antigen capsule might inhibit the antibody from reacting with the group polysaccharide. In fact, one type II strain, which gave no reaction with the Lancefield grouping serum in the co-agglutination method, did react well when an antiserum produced against this strain was used.

TABLE 1. *Comparison of three methods of serological grouping of streptococcal strains: results with highly potent grouping sera*[a]

Reference grouping antiserum[a]	No. of strains tested	No. of strains positive		
		Lancefield extract	El Kholy extract	Christensen method
A	25	25	25	25
B	42	42	41	39
C	16	16	16	16
G	9	9	9	8

[a] Obtained from Rebecca C. Lancefield.

The use of commercial sets of grouping sera (Tables 2 and 3) gave weaker reactions with the strains belonging to groups B and G; only group A and C sera of both producers were generally acceptable in their specificity and sensitivity as compared with the original Lancefield serum.

The estimation of rhamnose in representative extracts prepared according to Lancefield and El Kholy revealed higher contents of this sugar in the preparations obtained by the Lancefield extraction method for all groups of *Streptococcus*. For example, in Lancefield extracts of two strains of group A streptococci the rhamnose contents were 420 and 140 mg/ml using the Dische method (4), whereas from a comparable amount of bacterial cell sediment the nitrous acid extraction of El Kholy gave rhamnose values of 191 and 60 mg/ml, respectively.

A comparison of the amount of precipitating antibody in Lancefield or commercial grouping sera estimated by a modification of the single reverse radial immunodiffusion method of Harrell and George (8) showed considerable differences among the sera from various sources (Table 4) when tested against appropriate dilutions of hydrochloric acid extracts incorporated into agar.

TABLE 2. *Comparison of three methods of serological grouping of streptococcal strains: results with commercial sera I*

Commercial grouping antiserum I	No. of strains tested	No. of strains positive		
		Lancefield extract	El Kholy extract	Christensen method
A	25	23	22	25
B	42	12	13	15
C	16	16	16	15
G	9	4	4	6

TABLE 3. *Comparison of three methods of serological grouping of streptococcal strains: results with commerical sera II*

Commercial grouping antiserum II	No. of strains tested	No. of strains positive		
		Lancefield extract	El Kholy extract	Christensen method
A	25	25	25	25
B	42	33	37	34
C	16	16	16	15
G	9	6	7	6

TABLE 4. *Measurement of precipitating antibodies in tested antisera by single radial immunodiffusion test*

Source of sera	Reactions with group extracts			
	A	B	C	G
Lancefield	12.0[a]	7.2	11.1	11.9
Commercial I	5.4	0[b]	6.0	0
Commercial II	6.2	5.2	5.2	0

[a] Mean transverse diameter of the precipitin disk in millimeters; diameter of wells = 3 mm.
[b] 0 = <4 mm.

DISCUSSION

There are a variety of different methods (1, 3, 5-7, 11-14) available for serological grouping of beta-hemolytic streptococci in diagnostic laboratories. The choice of the best and the most advantageous should be the result of individual evaluation of the cost involved, availability of equipment and reagents, and technical experience of the personnel. Both of the two new techniques evaluated in this study could be recommended for diagnostic purposes. The El Kholy method appeared to be simple and quick. Moreover, the sensitivity of the test was satisfactory even though the amount of carbohydrate extracted was significantly lower than in the standard Lancefield method. It supports the finding of El Kholy et al. (6) that some amount of C-carbohydrate is left after nitrous acid treatment and could be further extracted by the hot-hydrochloric acid method. We confirmed that the method proposed by Christensen et al. is useful especially for routine work. It needs only minute amounts of the sensitizing serum. Indeed, sera containing large amounts of antibody need to be diluted to achieve specificity and can be used in high dilution. The cross-reactions observed in this method with the undiluted highly potent antisera are probably due to the immunological relationship of the respective C-carbohydrates (2, 9) rather than to a common T antigen. The sensitized staphylococcal suspensions can be stored at 4°C for several months without evident loss of activity. It is possible that the suspension of streptococci for grouping by the Christensen system can also be used for T typing of group A strains or typing of group B strains (W. Hryniewicz, unpublished data). The Christensen method may have some limitations for grouping highly encapsulated strains, as indicated by its inability to give the group reactions in three group B strains even when sera from Lancefield were used. Incorporation of type antibodies into the suspension may overcome this drawback.

Our results of testing two commercial sets of grouping sera revealed that, in comparison with the reference Lancefield sera, both gave much weaker reactions, requiring the use of ring tests to obtain satisfactory reactions and leaving many strains, especially of group B, ungrouped. This was the result of low amounts of the precipitating antibody against C-carbohydrate in these sera.

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