Rev. Inst. Med. trop. São Paulo
24(5):277-281, *setembro-outubro*, 1982

UDC 616.981.214

SEROLOGICAL CHARACTERIZATION OF GROUP A STREPTOCOCCI IN RIO DE JANEIRO, BRASIL

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SUMMARY

A total of 164 strains of group A streptococci from skin lesions, throat and nose were serologically classified. The streptococci were first classified according to T-typing patterns and ability to produce the serum opacity factor. The percentage of T-typable strains reached 96%. Strains with the T-patterns 3/13/B3264 and 8/25/Imp.19 were common among isolates from skin lesions. Few (30%) of the group A streptococci could be M-typed with the existing antisera. Newly recognized M-types predominated among strains from skin infections.

INTRODUCTION

The definitive serological classification of strains of group A streptococci is of fundamental value in studies of the epidemiology of streptococcal diseases. Streptococcal epidemiology makes it mandatory that M-antigens of **S. pyogenes** be identified [16]. Classification by T-agglutination reactions is also useful [19].

In earlier studies from Brazil, strains of group A streptococci were classified by T-agglutination. T-typing was performed in a survey among rural school children [14] and in a further study of streptococcal strains isolated from 1966 until 1970 [15]. T-typing has also been carried out on strains isolated from patients with acute glomerulonephritis, mainly related to pyoderma or impetigo, in whom the predominant strains were T 14/49, the Red Lake strain [9]. Information on the distribution of M types is restricted to a few strains from the rural school children, in whom only 4 of 38 (11%) strains examined were M typable [14].

We report here studies on the identification of M- and T-antigens among throat and skin lesion strains of streptococci isolated in the northern urban of Rio de Janeiro from cases of pharyngitis and uncomplicated impetigo respectively. Production of the serum opacity factor by the streptococcal isolates was also assayed.

MATERIALS AND METHODS

Streptococcal strains — One hundred and sixty four strains of **S. pyogenes** were isolated from September 1978 until September 1980 from clinical specimens, predominantly throat and skin lesions. The details on body sites of isolation of the streptococcal strains, and age and sex distribution of individuals investigated have been previously reported [1]. Stock cultures were stored in sheep blood in the lyophylized state.

Media — Blood agar plates for isolation of colonies of beta-hemolytic streptococci and broth for bacterial growth have been described elsewhere [1].

Sera — Rabbits were immunized in Rio de Janeiro with vaccine strains of Lancefield groups A, B, C and G provided by Lewis W. Wannamaker, University of Minnesota, Minnea-

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Typing sera were from the Centers for Disease Control. The standard set of T-agglutination antisera included the following pools and individuals antisera: "T" pool: 1, 3, 13, B3264; "U" pool: 2, 4, 6, 28; "W" pool: 5, 11, 12, 22, 27, 44; "X" pool: 8, 14, 25, Imp 19; "Y" pool: 22, 23; "Z" pool: 9, 18.

M-antisera included antisera for M-types 1-6, 8, 11, 12, 14, 15, 17-19, 23-27, 29-33, 36-43, 46, 47, 49, 51-57, 60, 65, and provisional types 68-74.

Grouping of streptococci — Lancefield grouping was performed by using the capillary precipitin test [8] with streptococcal antigen extracted by the nitrous acid method described by EL KHOLY et al. [5]. One hundred and one strains were also grouped after extraction of the carbohydrate by hot hydrochloric acid [8].

Typing of streptococci — T-typing was performed by slide agglutination [19]. M-typing by precipitation was performed in agar gel by the double — diffusion method [13] and by the capillary precipitin test [16].

Serum opacity reaction — All streptococcal strains were tested for the production of serum opacity factor by the slide method of MAXTED et al. [11] with hot-acid extracts or culture supernatants.

RESULTS

During the 2-year period of the study, 164 strains of group A streptococci were recovered from patients with pharyngitis and uncomplicated impetigo. Of the 55 (33.5%) strains recovered from the throat and nose, 52 (94.5%) were classified by T-agglutination. The T-types or — patterns of these strains are shown in Table I. Only 12 (21.8%) possessed M antigens that were detectable with the antisera in our possession (See **Materials and Methods**). The M antigens detected were M-4, M-11, M-12, M-32, M-49, M-55, M-60, M-70 and M-74.

A total of 109 (66.5%) strains of group A streptococci from skin lesions were classified serologically as shown in Table I. Two agglutination patterns, 3/13/B3264 and 8/25/Imp 19, together accounted for 30(27.5%) of the strains classified. Seventeen of the 22 strains sharing antigens in the 3/13/B3264 pattern were M-typable. Five of the 8/25/Imp 19 strains proved to be M-typable. Some M types (M-69, M-70, M-72, M-74) proved to be representatives of new or recently described M-serotypes [6]. Ten of the 11 strains that produced the T-6 antigen were M-nontypable strains and there were 18 strains with complex and uncommon T agglutination patterns (Table I).

The results of the serum opacity reactions were in good agreement with those described in other reports [3,11]. However the 4 strains of the M-type 74 and one strain of M-type 60 (T-type 4) did not produce the lipoproteinase (Table I). Two strains of M-type 70 with different T-patterns produced the opacity factor (Table I).

DISCUSSION

In an earlier study from the laboratory in Rio de Janeiro strains from patients with pharyngitis or streptococcal pyoderma were not classified by T-agglutination and M-precipitation reactions [1]. We report here a percentage of T-typable strains of 96%. In Brazil similar percentages have been reported [9,15]. Our report confirms the common occurrence of certain T-agglutination types such as 11, 3/13/B3264 and 8/25/Imp 19 among pyoderma streptococci [2,3,4,7,9]. It is noteworthy that eighteen strains gave a reaction with at least 2 pools (uncommon patterns, Table I).

Many of the 164 strains were nontypable with available M-antisera and remained identified by agglutination alone. Seventy one of the 114 M-nontypable strains were recovered from impetigenous skin lesions: it could be that they do not produce M-antigen or represent new M types. Since 59 of the strains produced the serum opacity factor, the study of the type-specificity of the antibodies to the lipoproteinase could serve as an aid towards identification [11,17]. Type 49 which has been isolated in various parts of the world [10] occurred only once in our study. We also found a type 2 strain with the 8/25/Imp 19 agglutination complex in a skin lesion. Such a strain has been seen in Alabama, USA [4]. Two strains of type M-12, T-12 commonly encountered in cases of pharyngitis [3,18] were seen in our investigation and 6 of the M-60 strains were identified as types T-4. This type 60 has already been encountered in Trinidad [12], Israel [3] and Alabama [4]. We also observed the newly recognized M-types 69, 70, 72 and 74. To

BENCHETRIT, L. C.; FACKLAM, R. R. & TEIXEIRA, L. M. — Serological characterization of Group A streptococci in Rio de Janeiro, Brazil. **Rev. Inst. Med. trop. São Paulo** 24:277-281, 1982.

T A B L E I

Serotypes and serum opacity reaction of 164 strains of group A streptococci recovered from nose and throat and skin lesions

T-pattern	Serological Classification: M-types		Opacity reaction	Number of strains
	Nose/Throat	Skin		
3	NT(2)*	(0)	—**	2
3/13	(0)	NT(1)	+	1
3/13/B3264	32(3), NT(2)	32(6), 33(1), 39(1), 42(1), 53(3), 69(5), NT(5)	—	27
B3264	(0)	NT(1)	—	1
B3264/9	(0)	NT(1)	—	1
3/13/B3264/9	74(1)	74(3), NT(2)	—	6
3/13/B3264/9	(0)	33(2)	+	2
3/13/B3264/14	(0)	NT(3)	—	3
2	NT(2)	(0)	+	2
4	(0)	60(1)	—	1
4	4(1), 60(1), NT(6)	60(4), NT(5)	+	17
6	(0)	6(1), NT(2)	—	3
6	NT(1)	NT(1)	+	2
6/23	NT(3)	NT(3)	+	6
28	70(1), NT(1)	NT(1)	—	3
28	(0)	70(1)	+	1
5/27/44	NT(2)	70(1), NT(4)	—	7
5/27/44	NT(1)	NT(1)	+	2
11	NT(2)	NT(7)	—	9
11	11(1), NT(2)	11(1), NT(5)	+	9
12	12(2), NT(2)	NT(2)	—	6
5/11/12/27/44/28	(0)	70(1), NT(3)	+	4
14	NT(1)	NT(4)	—	5
14	49(1), NT(2)	NT(1)	+	4
8/25/Imp. 19	55(1), NT(3)	55(1), NT(2)	—	7
8/25/Imp. 19	(0)	2(1), NT(3)	+	4
8/25/Imp. 19/2	(0)	NT(1)	—	1
8/25/Imp. 19/2	NT(3)	NT(1)	+	4
8/25/Imp. 19/4	(0)	NT(1)	+	1
Uncommon patterns				
3/13/B3264/12	(0)	72(1)	—	1
3/13/B3264/11/12/27/44	NT(1)	52(1)	—	2
3/5/11/27	(0)	32(1)	—	1
3/13/B3264/5/11/27	(0)	NT(2)	+	2
3/13/B3264/9/8	(0)	74(1)	—	1
13/B3264/9/8/14	(0)	NT(1)	—	1
B3264/9/14	NT(1)	(0)	—	1
11/12/14	(0)	NT(1)	+	1
5/11/12/8/14	(0)	NT(1)	+	1
5/11/12/27/44/14	(0)	NT(1)	+	1
11/12/27/44/8/25/ /Imp. 19/23	(0)	NT(1)	—	1
11/12/14/22	NT(1)	(0)	+	1
11/12/27/44/8/25/ /Imp. 19/22/23	NT(2)	NT(2)	+	4
NT	(0)	NT(1)	—	1
NT	NT(3)	NT(1)	+	4
Total:	55	109		164

* NT, Not M-typable with available sera (number of strains found)

** +, Opacity reaction positive; —, opacity reaction negative

our knowledge, this is the first report on these types other than their reports from the discoverers in Egypt and England [6].

This first survey on M- and T-types of **S. pyogenes** may not be representative of all types of group A streptococci occurring in Brazil. Our investigation embraced the northern urban area of Rio de Janeiro and provides only preliminary information about the prevalent types. This should enable us to initiate typing of the organism as a means for further studies in streptococcal epidemiology.

RESUMO

Caracterização sorológica de estreptococos do grupo A no Rio de Janeiro, Brasil

Um total de 164 amostras de estreptococos do grupo A isoladas de lesões da pele, garganta e nariz foram classificadas sorologicamente. Os estreptococos foram inicialmente classificados segundo os padrões de tipagem T e a capacidade de produção do fator de opacidade do soro. A porcentagem das amostras tipáveis com os antissoros T foi de 96%. Amostras com os padrões T 3/13/B3264 e 8/25/Imp. 19 foram freqüentes entre aquelas isoladas das lesões da pele. Poucas das amostras (30%) de estreptococos do grupo A puderam ser tipadas com antissoros M existentes. Os tipos M que foram recentemente reconhecidos predominaram entre as amostras de infecções na pele.

ACKNOWLEDGMENTS

This study was supported by grants from the Brazilian National Research Council (40.0559/79, L.C. Benchetrit), CEPG (Federal University, Rio de Janeiro) and Financiadora de Estudos e Projetos (FINEP). We thank Lewis W. Wannamaker and Dwight R. Johnson for supplying their reference strains. L.M. Teixeira is the recipient of a fellowship from the National Research Council (Brasília).

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Recebido para publicação em 29/6/1981.