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Serotypes of *Streptococcus pyogenes* Isolated from Healthy School Children in Kangwon-do

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Seventy-seven Streptococcus pyogenes strains isolated of children of three elementary schools located in Kangwon-do in spring, 1992 were serotyped with M, opacity factor (OF) and T typing antisera. In the M/OF typing results, M-78 (46.8%) and M-28 (22.1%) were most frequently encountered, while M-4 (6.5%), M-12 (5.2%), M-3 (1.3%), M-5 (1.3%) and M-6 (1.3%) were rarely observed. Twelve strains (15.6%) were not typable with M or OF typing system. In the T typing results, T-11 (35.1%) and T-28 (27.3%) were most common. We were able to identify 77.9% of S. pyogenes strains by T typing, 94.8% with T typing and OF typing. With the addition of M typing, 97.4% were typable. Through the serotypings, we could know the basic distribution of serotypes of S. pyogenes of healthy children which could be comparable to those of rheumatic fever, poststreptococcal glomerulonephritis and other severe streptococcal disease.

Key Words : *Streptococcus pyogenes, M typing, Opacity factor, T typing*

INTRODUCTION

After World War II, the cases of rheumatic fever (RF) dramatically decreased due to benzathine penicillin G treatment, improved socioeconomic status and personal hygiene, etc (Bisno, 1990 ; Kaplan and Johnson, 1992). But in the 1980s several outbreaks of RF took place in the USA and other developed countries. These outbreaks had characteristics of which patients were mostly middle-class whites (Bisno, 1990 ; Schwartz et al., 1987). The etiologic *Streptococcus pyogenes* strains appeared very mucoid and were serotyped as M types, 1, 3, or 18 (Bisno, 1990 ; Kaplan et al., 1989). To investigate the outbreaks of RF, poststreptococcal glomerulonephritis (PSGN), or other severe infections such as toxic streptococcal syndromes, serotypings are essential tools. Most previous serotyping data have been drawn from the RF or PSGN patients, and the basic serological data from healthy children are scarce. These basic data of healthy children will give clinicians or epidemiologists valuable information if compared with those of bona fide streptococcal infections (Johnson et al., 1992). Serotyping data of *S. pyogenes* of the school children -such as M type, opacity factor(OF) and T type-have not been reporded in Korea yet. In this study we determined the serotypes of the strains isolated from the healthy children with T, OF, and M typing systems. These serotyping data would enable us to know the geographical distribution of the strains and to step an epidemiological approach to the *S. pyogenes* infections.

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MATERIALS AND METHODS

Children

The children of three elementary schools and their kindergartens located in rural areas of Kang-won-do which is mountainous and remote with low socioeconomic status were subject to this study from March to April, 1992. The schools were distanced about 8 Km from each other. The numbers of boys and girls were 288 and 229 in Kirin, 73 and 69 in Hanam and 85 and 54 in Sangnam elementary school respectively. The proportion of each age group (7-13 years old) was similar and they did not have symptoms or signs of pharyngitis.

Bacteriology

S. pyogenes strains were isolated from the throats of children and identified with Bacitracin disk (0.04 U) and latex agglutination test (Seroiden Streptokit, Eiken Chemical Co., Tokyo, Japan).

Serotypings

Seventy-seven strains of 79 *S. pyogenes* isolated from the children were serotyped by D.R. Johnson at the WHO Collaborating Center for Reference and Research on Streptococci (Minnesota University, Minneapolis, Minn., USA) with T typing, serum opacity reaction (SOR), OF and M typing systems. The following M typing sera were used : types 1-6, 8, 12, 14, 15, 17-19, 23-26, 29-33, 36-41, 43, 47, 49, 51-53, and 55-57. OF antisera used included types 2, 4, 9, 11, 22, 25, 28, 48, 49, 58-64, 66, 68, 73, 75-78, and 81. Thus a total of 57 M/OF typing sera were available. For T typing all of the recognized antisera were used (Kaplan and Johnson, 1992). The indicated M serotype refers to either M protein and/or OF (Johnson et al., 1992 ; Kaplan and Johnson, 1992). For instance, M-2 means either M-2 or OF-2. The T typing was undertaken by slide agglutination after trypsinization of strains, M typing by Ouchterlony double immunodiffusion after hot acid extraction, and SOR and OF typing by microwell technique (Johnson and Kaplan, 1988).

Antistreptolysin O test

Sera taken from sixty-five children carrying *S. pyogenes* were tested for antistreptolysin O (ASO) titers with ASO TITER reagent (Nissui, Tokyo, Japan) which is based on the Rantz-Randall method. A titer of above or equal to 250 Todd unit (TU)/mL was interpreted as positive.

RESULTS

The distribution of M types of 77 *S. pyogenes* strains isolated from the children of three schools is shown in Table 1. In Kirin elementary school, M-78 was the most common type (58%, 29/50) and M-28 the next most common one (18%, 9/50). In Hanam elementary school M-78 (40%, 6/15) and M-4 (27%, 4/15) were frequently encountered. M-28 was predominant (58.3%, 7/12) in Sangnam elementary school. M types 12, 3, 5, and 6 were rarely encountered and M non-typable strains were 12 (15.6%). The relationship of M type and horse serum opalascence is also presented in Table 1. All strains of M types 78, 28, 4, and 3 were SOR positive while all of M types 12, 5, and 6 were SOR negative. SOR positivity of M non-typable strains

Table 1. The results of M typing and serum opacity reaction (SOR) of *S. pyogenes*

Children at schools		M types								
		78	28	4	12	3	5	6	NT[a]	Total
Kirin	No.	29	9	1	1	1	1		8	50
	%	58.0	18.0	2.0	2.0	2.0	2.0		16.0	100
Hanam	No.	6	1	4	2			1	1	15
	%	40.0	6.7	26.7	13.3			6.7	6.7	100
Sangnam	No.	1	7		1				3	12
	%	8.3	58.3		8.3				25.0	100
Total	No.	36	17	5	4	1	1	1	12	77
	%	46.8	22.1	6.5	5.2	1.3	1.3	1.3	15.6	100
SOR(+)	No.	36	17	5	0	1	0	0	8	67
	%	100	100	100	0	100	0	0	66.7	87.0

[a] Non-typable.

Table 2. The relation between M types and T types of *S. pyogenes*

T types	M types									
	78	28	4	12	3	5	6	NT[a]	Total	%
11	23							4	27	35.1
28		17						4	21	27.3
4			5						5	6.5
12				2				1	3	3.9
3					1			1	2	2.6
5/27/44						1			1	1.3
6							1		1	1.3
NT[a]	13			2				2	17	22.1
Total	36	17	5	4	1	1	1	12	77	100
%	46.8	22.1	6.5	5.2	1.3	1.3	1.3	15.6	100	

[a] Non-typable.

Table 3. Distribution of ASO titers according to M types of *S. pyogenes*

ASO titer (TU/mL)	M types								
	78	28	4	12	3	5	6	NT[a]	Total
12			1						1
50	1								1
100		2							2
125								2	2
166	2	2	1		1			3	9
250	4	1					1	2	8
333	3	2						1	6
500			1						1
625	2	3		3				1	9
833	12	2	2			1		2	19
1250	4								4
≥2500	2	1							3
Total	30	13	5	3	1	1	1	11	65
% of ASO test positive[b]	90.0	69.2	60.0	100	0	100	100	54.5	76.9

[a] Non-typable.
[b] A titer of above or equal to 250 TU/mL.

was 66.7% (8/12). Overall 87% (67/77) of the strains were SOR positive.

The results of M types and T types were compared in Table 2. Twenty-three of 36 M-78 strains were identified as T-11, while 23 of 27 T-11 strains were M-78. This finding suggests that the M/OF typing and T typing systems could be supplementary each other. Rarely encountered M types were relatively compatible with each T types. Among the seventeen T non-typable strains, 13 strains were identified as M-78 and 2 as M-12. Twelve M non-typable strains revealed as 4 T-11, 4 T-28, 1 T-12 and 1 T-3, respectively.

The relation between each M type and ASO titer of the children carrying the organism is shown in Table 3. The ASO positivity (90%) of children carrying M-78 was statistically significantly higher than that (69%) of M-28 carriers(t-test, $p<0.05$). As other M types were few in number, the relation between each M type and ASO titer could not be determined. In total 77% (50/77) of the children carrying *S. pyogenes* were ASO positive.

DISCUSSION

In the USA, several outbreaks of RF developed from asymptomatic or mild symptomatic pharyngitis patients (Kaplan et al., 1989 ; Veasy et al., 1987) and toxic streptococcal syndromes (Bartter, 1988) have been reported recently. In Korea an outbreak of food-borne streptococcal pharyngitis occurred among the students in Sangju girls' high school in 1992 and its causative organism was identified as serotype T-11 *S. pyogenes* (Communicable Disease Monthly Report, 1992). Because *S. pyogenes* pharyngitis continues to be a major public health problem in many developing nations (Berrios et al., 1986), these toxigenic or virulent strains of *S. pyogenes* should be well studied especially with serological typings. Identification of *S. pyogenes* based on specific M protein type is essential in studies of the pathogenesis and epidemiology of streptococcal infections (Breese, 1978). Since 1964 there have been attempts to carry out an international survey of the distribution of *S. pyogenes* serotypes (Parker, 1967), but unfortunately there have been no data on M or T types in Korea yet.

M typing is limited by the fact that many M serotypes are poorly immunogenic in rabbits. These M antisera are difficult to prepare and expensive (Johnson and Kaplan, 1988). Krumwiede (1954) reported that some *S. pyogenes* formed α-lipoproteinase which was capable of producing opacity in horse serum. A survey of a large number of strains of *S. pyogenes* has found that those strains which carry an easily identifiable M antigen rarely produce the SOR. On the other hand those types that elaborate M antigen poorly or not at all are usually good producers of the SOR (Top and Wannamaker, 1968 ; Widdowson et al., 1970). For these reasons the SOR has been used as a routine serological typing of *S. pyogenes*, especially in the situation of the questionable results of M typing and the same T typing results from different sources (Prakash and Dutta, 1991). The OF production is consistently and exclusively associated with specific M type. OF typing can be used instead of M typing for the 27 currently recognized OF positive strains (Johnson and Kaplan, 1988). OF typing results in a significant saving in time and material, especially in M typing antisera. The OF typing proved to be a valuable epidemiological marker in this study, as most SOR positive strains were confined to specific OF, and most T non-typable strains were identified with the OF test (data not shown). M typing was also found to be useful in the identification of SOR negative strains or OF unavailable ones. Of the M typed strains of this study, M types 3, 5 and 6 are known to be associated with rheumatogenecity (Berrios et al., 1986 ; Bessen et al., 1989), and M types 4 and 12 with nephritogenecity (Berrios et al., 1986 ; Kaplan et al., 1989). We may suggest that the healthy children harboring these rheumatogenic or nephritogenic strains could be administered with oral penicillin prophylactically. M type, 1 or 18 which is known to be mucoid and highly associated with recent RF outbreaks (Kaplan and Johnson, 1989 ; Schwartz et al., 1987) was not present among the strains in this study. Four T-11 strains which showed no precipitation with available M antisera, M-2, 4, 11, 44, 48, 49, 58, 75, 76, or 78 may belong to new M types. The positive rate (87.0%) of SOR in this study is higher than the 52% (448/866) in the USA and the 50% (62/123) in Thailand. The distribution of M types is also quite different from those in the USA and Thailand (Kaplan et al., 1992). These data of M types would furnish epidemiologists or clinicians with the basic distribution of M types in healthy children in Kangwon-do.

Although the final typing is performed with more specific M antisera, there are some reasons for preference of T agglutination to M typing. T typing has been widely used as an screening tool as it is stable and present in almost all streptococci (Breese, 1978). However, it has the drawbacks of cross reaction and lack of correlation with virulence (Prakash and Dutta, 1991). In Japan, there have been numerous reports on the trends of regional and annual distribution of T types of *S. pyogenes*. Although the subjects of reports in the various districts of Japan were different-normal elementary school children in Osaka (Nakajima, et al., 1983), all strains isolated in the clinical laboratory during eight years in Kyoto (Imai, et al., 1987), scarlet fevers in Sapporo (Takizawa, et al., 1987), *S. pyogenes* infections in Kobe (Morikawa and Taba, 1992)-, the distributions of T types were similar one another. In Japan, T-12 was the most common and T-4 and T-1 were also frequently observed. The distribution of T types in Japan is quite different from that in our study. Those frequently isolated T types, 12, 4, and 1 in Japan were very rarely encountered in Korea, while the frequent T types 11 and 28 in Korean children were rare in Japan. The percentage of T typable strains is 77.9% (60/77) which is lower than the range of 89.7% to 97% in Japanese (Imai, et al., 1987 ; Morikawa and Taba, 1992 ; Nakajima et al., 1983 ; Takizawa, et al., 1987). We should consider that T, OF or M typability and SOR positivity depends on the source of strains whether obtained from normal children or from patients with streptococcal infections.

Comparing the ASO positivity between M-78 and M-28 carrying children (Table 3, $p<0.05$), we could infer that M-78 strains may be more streptolysin O producing or be more virulent than M-28 ones.

We were able to classify 77.9% (60/77) of strains with T typing. After OF typing which is easier and more simple to perform than M typing, the identification rate increased to 94.8% (data not shown). Usually M typing is performed on the strains which are difficult to identify with T typing or OF typing. With the addition of M typing, most strains (97.4%, 75/77) were identified. These three typing systems might support one another. Although it is very difficult to draw conclusions with the relatively small numbers of strains, these results indicate that there are two prevalent M types (M-78, 46.8% ; M-28, 22.1%) and T types (T-11, 35.1% ; T-28, 27.3%) in healthy children in Kangwon-do. The more *S. pyogenes* strains of Korean children or patients serotyped, the more toxigenic or common type would be documented and continuous surveillance of *S. pyogenes* infections will be necessary.

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