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Bacteriologic Characteristics and Serotypings of *Streptococcus Pyogenes* Isolated from Throats of School Children

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Abstract

To determine the carrier rate of beta-hemolytic streptococci (BHS), throat cultures were taken from healthy elementary school children in four separate areas of Korea from 1992 to 1995, including Inje, Nonsan, Seoul and Chinju. The strains of *Streptococcus pyogenes* had been serotyped with anti-T, -OF and -M sera. The isolation rates of BHS and *S. pyogenes* ranged from 14.1-32.4% and 10.9-18.5% respectively. More than half of the carriers showed heavy growth of BHS. M78 (48.6%) and M28 (22.2%) were most common in Inje, M12 (23.6%) and M5 (20.3%) in Nonsan, M12 (48.8%) and M5 (14.6%) in Seoul, and M12 (26.3%) and M22 (14.5%) in Chinju, respectively. About 15% of school children were positive for *S. pyogenes* in throat cultures, and the distribution of serotypes varied according to geographical regions.

Key Words: Throat culture, beta-hemolytic streptococci, *Streptococcus pyogenes*, serotype

INTRODUCTION

The major important bacteria in acute pharyngotonsillitis is *Streptococcus pyogenes*. If the organism is found, two possibilities exist: The patient either has *S. pyogenes* tonsillitis, or is a carrier concomitantly suffering from tonsillitis of a different etiology.[1] Streptococcal pharyngitis is quite common in school children. The carriers of *S. pyogenes* can spread the bacteria to other children via aerosol. The reason is unknown why some have *S. pyogenes* without disease in their throats, and others suffer not only pharyngitis but also rheumatic fever (RF) or poststreptococcal glomerulonephritis (PSGN). Previous exposure to the strain or host factor such as human leukocyte antigen may have a role to some extent.[2] Although RF or PSGN have become rare in developed countries, as well as in Korea, these sequelae are still important public health problems in developing countries.[3,4] The recent recurrence of RF and the emergence of severe invasive disease such as necrotizing fasciitis or streptococcal toxic shock-like syndrome (TSLS) in developed countries require the continuous surveillance of streptococcal infection and microbiologic and epidemiological assessment of *S. pyogenes*.[5,6] Group C or group G beta-hemolytic streptococci (BHS) rarely cause pharyngitis and group C can induce TSLS.[7] Since the streptococcal carriers could be the source of infection, the distribution of serotypes in school children provides an epidemiological background of streptococcal disease.[8] Throat cultures were taken to investigate the carrier rate of BHS. Bacteriologic characteristics were analyzed by identification and counting of colonies. Serotyping was performed to compare the distributions of serotypes in geographically-different areas.

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MATERIALS AND METHODS

Bacterial isolation

Bacteria were isolated from school children in four areas of Korea, including Inje in February 1992, Nonsan in April 1993, Seoul in December 1993 and Chinju in May 1995. The approximate distance between each of the two regions is about 200-300 km. Inje is a very mountainous, isolated village; Nonsan

Table 1. Isolation Rate of BHS in Throats by School Grade and Gender

		Inje (1992, Feb)		Nonsan (1993, Apr)		Seoul (1993, Dec)		Chinju (1995, May)	
		Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)
School grade	1	101	11.9	163	8.6	76	11.8	71	38.0
	2	90	17.8	158	21.5	94	8.5	75	29.3
	3	129	13.2	161	14.3	121	21.5	86	33.7
	4	108	19.4	164	33.0	94	12.8	92	19.6
	5	133	20.3	176	22.0	109	22.0	72	38.9
	6	126	2.4	158	14.6	96	25.0	80	37.5
Gender	M	389	14.7	504	19.6	324	18.6	245	31.0
	F	298	13.1	406	18.6	266	16.5	231	33.8
Total		687	14.1	980	19.1	590	17.5	476	32.4

is located in typical agricultural farmland; Seoul is the capital of Korea; and Chinju is a small urban city. The author visited the schools and took throat cultures after a brief history taking and physical examination. Both tonsils were vigorously swabbed with a cotton swab after illumination with a pen light. Each throat swab was immediately plated onto a 5% sheep blood agar plate (BAP) and incubated for 18 to 24 hours at 37°C. Beta hemolytic colonies on BAP were identified with bacitracin disk and latex agglutination test (A Strep AD, Denka Seiken, Tokyo, Japan). The degree of growth was expressed as 1+ to 4+ according to the number of colony-forming units (CFU), as described in Table 3.

Serotyping

Serotyping of *S. pyogenes* had been performed at the 'WHO Collaborating Center for Reference and Research on Streptococci (University of Minnesota, Minneapolis, MN, USA)'. T typing was tested by slide agglutination, M typing by double immunodiffusion and opacity factor (OF) typing by horse serum opacity reaction (SOR). The serotype distributions for each area were compared. The indicated M type refers to either M and/or OF protein.

RESULTS

Bacterial isolation

Most of the subjects denied current or recent sore throats. Isolation rates of BHS were 14.1% in Inje, 19.1% in Nonsan, 17.5% in Seoul, and 32.4% in Chinju respectively (Table 1). Group A consisted of 77.1% in Inje, 75.4% in Nonsan, and 73.8% in Seoul, which were significantly higher than 57.1% in Chinju (Table 2). Group G was second most common, ranging from 10.4% to 19.5%. Group B or group C was rare. The children who had less than 10 CFUs (1+) ranged from 9.7% to 25.7% (Table 3). The children who had more than 100 CFUs (4+) ranged from 20.4% to 55.3%.

Table 2. Group (%) of Beta-hemolytic Streptococci

Group	Inje (N=96)	Nonsan (N=187)	Seoul (N=103)	Chinju (N=154)
A	77.1	75.4	73.8	57.1
B	3.1	2.1	1.9	2.6
C	4.2	1.1	3.9	15.6
G	10.4	12.8	17.5	19.5
Non-A,B,C,G	5.2	8.6	2.9	5.2
Total	100	100	100	100

Serotyping

T typing: While T11 (34.7%) and T28 (27.8%) were frequent in Inje, several T types such as T12 (29.3%), T5/27/44 (20.3%), T1 (11.4%) and T3 (11.4%) were relatively common in Nonsan (Table 4). T12 was predominant in Seoul (52.4%) and Chinju (44.7%). T non-typeable strains ranged from 2.4%

Table 3. Degree of Growth (%) of Beta-hemolytic Streptococci

Degree of growth	Inje (N=96)	Nonsan (N=187)	Seoul (N=103)	Chinju (N=154)
1+, <10 CFU	20.4	25.7	9.7	18.2
2+, 10−50 CFU	32.4	21.4	10.7	19.5
3+, 51−100 CFU	26.9	22.5	24.3	35.1
4+, >100 CFU	20.4	30.5	55.3	24.7
Total	100	100	100	100

Table 4. Frequency (%) of Predominant T Types of *S. pyogenes**

T types	Inje (1992, Feb) (N=72)	Nonsan (1993, Apr) (N=123)	Seoul (1993, Dec) (N=82)	Chinju (1995, May) (N=76)
1		11.4	2.4	4.0
3	2.8	11.4	9.8	1.3
4	5.6	1.6	4.9	6.6
5/27/44	1.4	20.3	8.5	
6	1.4	7.3	1.2	7.9
11	34.7	7.3	7.3	6.6
12	4.2	29.3	52.4	44.7
27		8.5		
28	27.8	1.6	1.2	13.2
NT†	22.2	8.1	2.4	11.8

* More than 5% frequency in an area.
† Non-typable.

Table 5. Frequency (%) of M Types of *S. pyogenes**

M types	Inje (1992, Feb) (N=72)	Nonsan (1993, Apr) (N=123)	Seoul (1993, Dec) (N=82)	Chinju (1995, May) (N=76)
1		11.4	1.2	3.9
3	1.4	11.4	4.9	1.3
4	5.6	1.6	1.2	3.9
5	1.4	20.3	14.6	1.3
6	1.4	7.3	1.2	7.9
12	5.6	23.6	48.8	26.3
22		1.6		14.5
28	22.2	2.4		10.5
78	48.6	5.7	4.9	3.9
NT†	13.9	12.2	23.2	23.7

* More than 5% frequency in an area.
† Non-typable.

(Seoul) to 22.2% (Inje).

M typing: The results of M typing were comparable to T typing results. M78 (48.6%) and M28 (22.2%) were most common in Inje, while these types were rare in other areas (Table 5). M12 (23.6%), M5 (20.3%), M1 (11.4%) and M3 (11.4%) were common in Nonsan. M12 was predominant in Seoul (48.8%) and M12 (26.3%), M22 (14.5%) and M28 (10.5%) were frequently isolated in Chinju. M non-typability ranged from 12.2% (Nonsan) to 23.7% (Chinju). SOR was positive in 86.1% in Inje, 24.4% in Nonsan, 15.9% in Seoul, and 54.0% in Chinju.

DISCUSSION

S. pyogenes is the most frequent cause of bacterial pharyngotonsillitis in school children. The bacteriologic characteristics of throat cultures for Korean school children were investigated. The isolation rates of BHS analyzed by school grade or gender did not show consistent results for each area (Table 1). Group A consisted of about 75% in Inje, Nonsan and Seoul, while a little more than 50% in Chinju. Group B and Group C were quite rare, except in Chinju where the isolation rate of Group C was 15.6%. The isolation rate of group B was very high in Japan, almost equal to that of group A.[9] In a study of carrier rates of BHS among patients in general practice in the USA, group B (3.8%) was more frequent than group A (2.2%).[1] Prakash and Kakshmy reported the isolation rate of group B was less than 4%, but group C or group G was not uncommon.[8] Proportions of each group of BHS might be different by nation, season, or humidity.

Bell and Smith showed that among those children with pharyngitis and positive cultures, 71% had strongly positive cultures of *S. pyogenes*, compared with 10% of those children who were asymptomatic and

had positive cultures of *S. pyogenes*.[10] Breese also emphasized the value of quantitative assessment of colonies on culture to differentiate true infections from carriers.[11] By contrast, more than half of the carriers of BHS had above 3+ in the degree of culture positivity in this study (Table 3). Particularly the children in Seoul showed 55.3% of 4+ in the degree of positivity. The author thought it was not possible to discriminate true infection from carriers by the number of colonies in Korea. And many of the carriers with a high number of colonies possibly suffered from asymptomatic infection. Accurate throat swab technique is mandatory to retrieve precise results of throat culture. Roddey Jr. also insisted that heavy growth of *S. pyogenes* does not necessarily reflect acute infection.[12]

Distribution of T types and M types were different according to areas. In Inje T11 and M78 were predominant, of which serotype was very rare either in other areas in Korea or foreign countries. As Inje is a very closed, remote mountainous area, it was possible to show a very unique distribution of serotypes. While T1 is one of the most common types in Japan (26.6%),[6,9] or United Arab Emirates (17%),[13] it was rare in Korea except in Nonsan (11.5%). T12 was more prevalent in Korea than in Japan. Although M1 or M3 may cause rheumatic fever or severe invasive streptococcal disease, such as necrotizing fasciitis or TSLS,[3,4] these serotypes were not common in Korea. T non-typability was 10.5% in total, while M non-typability was 17.6%. Non-typability was comparable to Japan,[9] the USA[14] or United Arab Emirates,[13] while non-typability was more than 80% in Thailand.[15] Distribution of T types of *S. pyogenes* in India are quite different from ours.[8] The frequent M types from patients with uncomplicated pharyngitis in the USA,[14] such as M types 1, 2, and 4 were very rare in Korea. A serotyping study in Seoul, showed differences between healthy children and pharyngitis.[16] While T12 or T28 was predominant in healthy children, T1, 25 and 4 were common in pharyngitis. Variable distribution of serotypes according to geographic regions suggests a different disease spectrum of streptococcal infection area by area. SOR positivity was very low in Seoul (15.9%) and Nonsan (24.4%). As SOR negative strains tend to be more virulent than SOR positive ones,[14] the strains in Nonsan and Seoul would be more virulent than those in other areas. To develop vaccine, serotyping is the first step in determining which strain is prevalent and a good target for vaccine. The distribution of serotypes of strains isolated from various disease or community needs to be elucidated.

Serial change of serotype distributions in one area could be followed up during a long-term period.[9] The serotyping data of this study would be affected by a different research period for each area. Molecular genetics such as pulsed-field gel electrophoresis (PFGE) could be used for epidemiologic investigation.[17] Beall et al. suggested T typing and *emm* genotyping as effective ways for subgrouping *S. pyogenes*.[18]

The carrier rate of *S. pyogenes* was as high as 18.5% in Chinju. Most of the carriers showed heavy growth of bacteria in their throats. The Inje area showed a peculiar T and M distribution pattern. Relatively virulent strains such as M types 1, 3 and 5 were common in Nonsan. It is necessary to continue surveillance of streptococcal infection or carrier study,[3,4] because *S. pyogenes* is not only ubiquitous and contagious, but it induces various life-threatening invasive diseases.[17]

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