

Comparison of M-Serotypes of *Streptococcus pyogenes* Isolated from Healthy Elementary School Children in Two Rural Areas

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Serotypings have been used as markers for epidemiological surveys of Streptococcus pyogenes infections. Seventy-seven strains of S. pyogenes isolated from the throats of elementary school children in Kangwon Province in Korea in March and April 1992 were serotyped with M and / or opacity factor (OF) antisera. Sixty-eight strains of S. pyogenes from healthy school children in Chungnam Province in March 1993 were also serotyped and the distribution of serotype was compared with the isolates from those living in Kangwon Province. The distributions of M types were quite different between the two areas. M-78 (46.8 %) and M-28 (22.1 %) were most frequently encountered in Kangwon Province, while M-5 (20.6 %), M-12 (16.2 %), M-3 (13.2 %), M-1 (11.8 %) and M-62 (11.8 %) were frequently isolated in Chungnam Province. Eighty-seven percent of strains in Kangwon produced OF while 33.2 % of those in Chungnam produced OF ($P < 0.0001$). The difference in the distribution of serotypes and of OF production in the isolates from the children in the two provinces may be responsible for differences in the epidemiology of group A streptococcal infections and their sequelae.

Key Words: *Streptococcus pyogenes*, M types, OF production

INTRODUCTION

To investigate the outbreaks of rheumatic fever (RF), poststreptococcal glomerulonephritis (PSGN), or other severe manifestations of group A streptococcal

infections such as toxic streptococcal syndrome, serotypings of the etiologic strains are essential for knowing which strains cause specific diseases and the epidemiological pattern of the pathogens (Johnson and Kaplan, 1988; Markowitz and Kaplan, 1989; Schwartz et al., 1990; Majeed et al., 1992). Most previous serotyping data originated from studies of RF or PSGN patients. Epidemiological data from healthy children is less available, although such studies have been previously carried out (Johnson et al., 1992). Data from healthy children would be valuable in

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interpreting the serotyping data from serious group A streptococcal infections and their complications, as they could reflect the prevalence of various serotypes within the community. In this study we compared the distributions of serotypes of the strains isolated from healthy Korean children between two geographically separated rural areas.

MATERIALS AND METHODS

Throat cultures were performed in March 1993 in an elementary school located in Nonsan of Chungnam Province. This is a flat, semiclosed community. The area is warmer, about three times larger, and there is greater interaction with other areas than Kangwon Province, of which serotyping data was previously reported (Kim et al., 1994). Nine-hundred and eighty school children ages from 7 to 12 were included in Chungnam. None were suspected as having *S. pyogenes* pharyngitis. The throat culture was taken with a wood cotton swab, then directly inoculated onto sheep blood agar plate, which was incubated overnight at 37°C. Small colonies showing beta hemolysis were identified with Bacitracin disc (0.04 U, Becton Dickinson, Cockeysville, MD, USA) and latex agglutination (Strep LA, Denka Seiken, Tokyo). Sixty-eight isolates of *S. pyogenes* were serotyped at the WHO Collaborating Center for Reference and Research on Streptococci (University of Minnesota, Minneapolis, MN, USA) with M and opacity factor (OF) antisera. These antisera are not commercially available. M typing was undertaken by Ouchterlony double immunodiffusion after hot acid extraction of the strain. Serum opacity reaction (SOR) was screened. SOR was compared to that of Kangwon by chi-square test. OF typing was done by inhibition of opacity with OF antisera in the microwell (Johnson and Kaplan, 1988).

RESULTS

One hundred and eighty-seven (19.1 %) yielded BHS. Of the isolates, 141 (75.4 %) were identified as *S. pyogenes*, 24 (12.8 %) group G, 4 (2.1 %) group B, 2(1.1 %) group C and 16 (8.6 %) non group A, B, C, G(kim et al., 1993). There were significant differences in the prevalent serotypes of *S. pyogenes* between Chungnam and Kangwon Provinces (Fig. 1). M-78 (46.8 %) and M-28 (22.1 %) were predominant in the Kangwon Province strains. Only one strain of M

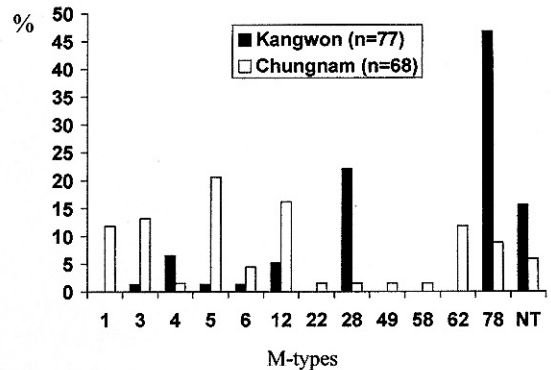


Fig. 1. Distribution of M-types of *S. pyogenes*.

type 3, 5 and 6 was isolated each. The non-M-typable strains were 15.6 %. In Chungnam Province, by contrast, the frequency of several M types were not markedly different. M-5 (20.6 %) was the most frequent and M-12 (16.2 %), M-3 (13.2 %), M-1 (11.8 %), and M-78 (8.8 %) were commonly encountered. The non-M-typable strains were 5.9 %. OF production was observed in 87 % of the strains in Kangwon compared to 33.8 % in Chungnam (Fig. 2, $P < 0.0001$).

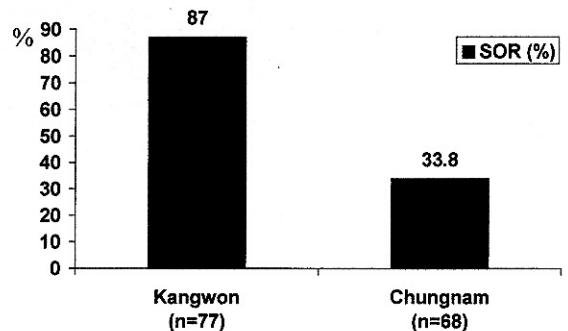


Fig. 2. Positive rate of serum opacity reaction(SOR) of *S. pyogenes*.

DISCUSSION

S. pyogenes pharyngitis and its sequelae continue to be major public health problems in many developing nations (Berrios et al., 1986; Markowitz and Kaplan, 1989). The cases of toxic streptococcal syndrome or necrotizing fasciitis have been increased worldwide (Stevens, 1991). These virulent strains of *S.*

pyogenes should be characterized epidemiologically with serological typings (Parker, 1967; Schwartz et al., 1990). OF typing results are in accordance with M typing ones. OF antisera are easily prepared and very useful instead of M antisera, which are very expensive and hard to obtain. Of the M typed strains in Chungnam, M types 1, 3, 5 and 6 have been known to be associated with rheumatogenicity (Berrios et al., 1986; Bessen et al., 1989; Kaplan and Johnson, 1989; Majeed et al., 1992), and M types 4 and 12 with nephritogenicity (Berrios et al., 1986; Majeed et al., 1992). Children harboring these "rheumatogenic" or "nephritogenic" strains should be closely observed to see whether the sequelae occur, even if they were asymptomatic (Markowitz and Kaplan, 1989; Bisno, 1990; Schwartz et al., 1990). Two M types, M-78 (46.8 %) and M-28 (22.1 %), were predominant in Kangwon that were not related with known virulent serotypes. The percentage of non-M-typable strains in Chungnam was about one-third of that found in children in Kangwon. OF production in strains of these two rural areas was significantly different (Fig. 2). Generally OF positive strains appear to be less virulent than those strains that are not OF producers (Kaplan and Johnson, 1989; Johnson et al., 1992). Although the sources of *S. pyogenes* were different, the distribution of M types in Korean children reported here is different from those of other nations (Takizawa et al., 1987; Johnson et al., 1992; Kaplan et al., 1992; Majeed et al., 1992). Of particular interest was the relatively low percentage of isolates that proved to be non-typable. Recent epidemiological evidence has indicated that a relatively large percentage of isolates from some countries in Asia are non-typable, suggesting that these are previously uncharacterized serotypes (Kaplan et al., 1992). Whether this is the case in Korea could not be determined from this study as either the sample was too small or the study was carried out in a part of the country that has more easily characterized strains.

Although there has been no nationwide survey, the number of RF of PSGN cases in Korea has recently undergone a marked decline. Until 1988, the incidence of RF and acute glomerulonephritis (AGN) were 0.47-1.8 %, 1.59-2.8 % respectively among the inpatients of pediatrics (Cha et al., 1989). The data of throat cultures was not good, because of the improper throat swab technique and the overuse of antibiotics. The isolation rates of *S. pyogenes* from the throats of 1708 patients with respiratory tract infections

and 111 patients with AGN were 2.8 % (Lee et al., 1972) and 15.3 % (Kim et al., 1989), respectively. Although we did not obtain any throat culture data from such patients in the two areas, the environment of these two different rural areas should be considered when attempting to explain the serotype distribution. Kangwon Province has a unique environment situation. It is a very isolated and closed community surrounded by big mountains. The serotyping data of this study in Kangwon included a previous report (Kim et al., 1994). The Chungnam area is a typical Korean agricultural village, where the children have greater contact with others living in nearby areas.

During the study, eight strains in Chungnam produced mucoid colonies on the BAP. Mucoid colony is another characteristic of more virulent strains and have been recovered, for example, during the recent resurgence of acute RF in the USA (Bisno, 1990; Markowitz and Kaplan, 1989). Seven of these mucoid strains were serotyped. One was M-1 and six were M-5.

In conclusion, the distributions of M types of *S. pyogenes* isolated from the throats of healthy elementary school children in the two rural areas were quite different, which suggests different virulence according to the geographical areas.

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