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## Group A Streptococcal Serotypes Isolated from Healthy Schoolchildren In Iran

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**Abstract** Serotypes of group A streptococci are still a major cause of pharyngitis and some post-infectious sequelae such as rheumatic fever. As part of the worldwide effort to clarify the epidemiological pattern of group A streptococci in different countries, the present study was conducted to assess the prevalence of *Streptococcus pyogenes* serotypes in Iran. A total of 1588 throat swabs were taken from healthy school children in the city of Gorgan during February and March 1999. Of those isolates, 175 resulted positive for group A streptococci. The distribution pattern was similar for girls and boys, with 10.8% and 11.2%, respectively. Urban school children showed a higher rate of colonization compared to those in rural areas. Serotyping was performed on 65 of the positive isolates using standard techniques, and only 21 (32%) were M-type isolates. Their profiles fell into four types with M1 predominating, which could reflect the presence of rheumatic fever in the region. However, when isolates were challenged for T-antigen types, nearly all were positive (94%). The pattern of T types was diverse (18 types), with the most common T types being T1 (26%), TB3264 (15%), TB\1-19 & B\25\1-19 (9.2%)

and T2 & 2\28 (7.7%). When isolates were tested for opacity factor, only 23 (35%) were positive while 34 (52%) responded to the serum opacity reaction test. Although the number of isolates in this study was not sufficient to make any epidemiological conclusions, the scarcity of serotyping studies in Iran could render these data useful for future attempts to develop a streptococcal vaccine.

### Introduction

Lancefield group A streptococci (GAS) constitute the major cause of a variety of clinical syndromes ranging from pharyngitis and impetigo to more invasive infections such as toxic shock-like syndrome and necrotizing fasciitis [1, 2, 3, 4]. The prevalence of GAS in the upper respiratory tract of healthy schoolchildren varies considerably (10–35%) depending on environmental and social factors [5, 6]. Epidemiological studies have classically focused on serological markers such as anti-phagocytic M proteins, which are encoded by *emm* gene(s). However, many GAS isolates are non-typeable using the currently defined M antisera [7, 8]; thus, other factors such as T proteins [9] and streptococcal serum opacity factor (OF), an enzyme that reacts with and causes opacity in human sera, are used for the classification of non-M-typeable isolates [10].

Differentiation of GAS isolates into OF+ and OF– could also provide valuable clues as to the potential pathogenicity of strains and their association with suppurative and non-suppurative infections [10]. Studies on the distribution of GAS serotypes in the Western Hemisphere along with those from Southeast Asia [8, 11] and the Persian Gulf region [6, 12] show some variability, especially regarding the M-typeability of the GAS strains. The study presented here aimed to clarify the present pattern of GAS serotypes in part of Iran, in accordance with the international challenge to develop a potent multivalent vaccine against the most frequent pathogenic

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types [13, 14, 15], and to understand more about the epidemiology of streptococcal infections in this country.

## Materials and Methods

### Bacterial Isolates

A total of 1588 swab samples were taken from the throat of healthy children (age range, 6–13 years) attending primary school in the city of Gorgan and its surrounding districts during February and March 1999. Children with nasopharyngeal infections were excluded. The throat swabs were inoculated onto plates containing 5% sheep blood, then incubated in 5% CO<sub>2</sub> at 35°C for 24 h. Pinpoint beta-hemolytic colonies were Gram stained. Gram-positive cocci displaying no catalase activity but resistance to sulfamethoxazole and trimethoprim and sensitivity to 0.04 U of bacitracin were confirmed as GAS.

### Serotyping of the Isolates

The confirmed GAS isolates were fixed on sterile Wattman number 1 filter papers wrapped in aluminum foil and shipped to the World Health Organization Collaborating Center for Reference and Research on Streptococci at the University of Minnesota, USA, for serotyping. Serological typing for T proteins was conducted using slide agglutination tests. Polyvalent antisera were applied first followed by monovalent antisera [16]. Determination of M proteins and testing for serum opacity factor and serum opacity reaction were conducted as described previously [16]. M protein gene (*emm*) typing of some non-M-typeable strains was carried out using non-radioactive oligonucleotide probes [17].

## Results

### Distribution of GAS Isolates

Of the 1588 throat samples taken, 175 isolates were confirmed as *Streptococcus pyogenes*, accounting for 11% of the total. Boys and girls had a similar distribution pattern with 11.2% (92 of 821 throat swabs cultured) and 10.8% (83 of 767 throat swabs cultured), respectively. A significantly higher ( $P < 0.005$ ) prevalence of GAS was found among children attending city schools (13.7%, 113 of 834) compared to those in rural areas (8.1%, 61 of 754).

Table 1 summarizes the distribution pattern of GAS among the different age groups. Similar prevalence rates were obtained for the different age groups, with the exception of the 6–7 and 12–13-year-old children; these two groups showed nearly half and double the rates of the other groups, respectively. However, since the numbers of isolates in these two groups were limited, a conclusion regarding the statistical significance of the results could not be made.

### Serotyping Studies

Of the 65 isolates subjected to serotyping, only 21 (32%) were identified as M types, while 61 (94%) isolates were T types; 23 (35%) were serum OF positive and 34 (52%)

**Table 1** Distribution of isolates positive for group A streptococci among schoolchildren of different ages

Age group (years)	No. tested	No. (%) of GAS isolates
6–7	22	1 (4.5)
7–8	382	40 (10.5)
8–9	414	49 (11.8)
9–10	310	33 (10.6)
10–11	246	26 (10.5)
11–12	193	21 (10.8)
12–13	21	5 (24)
Total	1588	175

**Table 2** Serotypes of 65 group A streptococcal throat isolates

Antigen type	Percent of typeable isolates
M ( $n=21$ )	32
1	26
4	3
12	2
14	2
T ( $n=61$ )	94
1	26
B3264	14
B	7.7
B\1-19 & B\25\1-19	9.2
2 & 2\28	7.7
12\B	4.6
3	3
27	1.5
13\28	4.6
5 & 5\27\44	4.6
4	1.5
8	1.5
OF ( $n=23$ )	35
2	9.2
PT4245	11
77	6.2
25	3.1
4	1.5
22	1.5
44	1.5
75	1.5
SOR ( $n=34$ )	52

OF, opacity factor; SOR, serum opacity reaction

were serum opacity reaction positive (Table 2). A total of four M types were identified (M1, M4, M12 and M14), with M1 being the most common (26%). A diverse range of T patterns was identified (18 types), with the most common being type 1 (26%), type B3264 (14%), type B\1-19 & B\25\1-19 (9.2%) and type B (7.7%). The serum OF-positive isolates fell into eight types, with the most common being PT4245 (11%), type 2 (9.2%) and type 77 (6.2%).

Of the 17 isolates confirmed as M1, 13 (76.5% of the total) were obtained from boys. Clusters of type M1 isolates were also found in urban schools, accounting for 76.5%, while only 23.5% were obtained from children attending rural schools. Five of the non-M-typeable strains were examined for the M protein encoding gene, *emm*, and all were of type stns5.

## Discussion

The overall rate of GAS carriage in this study was 11%, which is at the lower end of the range of internationally reported rates for healthy schoolchildren (10–35%) [6, 18]. The difference in reported rates might be due to environmental as well as socioeconomic conditions of the regions studied. We found no difference in the incidence of throat colonization between boys and girls in our study, but children attending city schools had a considerably higher rate of GAS carriage than those attending rural schools. Crowded conditions in city schools may explain this difference, since schoolchildren in the city of Gorgan are usually densely accommodated, while the rural schools have fewer students in each class.

Ninety-four percent of the GAS strains we isolated were easily T typed, which is well above the findings reported from Kuwait (35%) [12], Malaysia (58%) [11] and Thailand (61%) [8], but in accordance with the figures reported from the USA (93%) [8] and the United Arab Emirates (91%) [6].

Most of the isolates typed for M antigen did not respond to the current array of antisera, and only about one-third ( $n=21$ ) were M typed. This is far behind the 80% M-typeability reported for strains from the USA [8] and the figures reported from countries neighboring Iran, e.g. United Arab Emirates with 76% and Kuwait with 53% typeability [6, 12]. These data suggest that GAS serotypes differ according to geographical region, and a considerable portion of non-typeable strains may represent undefined serotypes of group A streptococci.

Of the 21 isolates for which an M type was obtained in our study, 17 belonged to type M1, which accounts for 26% of the total typed strains. This percentage of M1 carriage is relatively close to the 17% reported for the M1 pattern in the United Arab Emirates [6] but in contrast with the strikingly lower rates reported from Kuwait [12]. It should be noted, however, that epidemiological factors certainly affect the results of serotyping. In one study designed in the USA [19], a dynamic array of predominant M proteins was observed, but samples collected between July and December 1999 were predominantly of the M1 type (92%), while those taken between January and March 2000 were predominantly of the M6 type (84%).

In our study, only 6% of the typed strains recovered from girls were of the M1 serotype, while the incidence among boys was 20%. Children in urban schools had a higher rate of M1 carriage compared to those attending rural schools (20% vs. 6% of the total typed). Since children attending rural schools are usually more socially isolated, this may have, at least in this case, a positive effect on the low rate of GAS carriage and the low incidence of universal M1 type in rural children. If this is true, then modern socialization and the continual growth of cities should lead us to expect a higher prevalence of GAS and the M1 type in Iran in the future. This is sufficient to raise concern about GAS-related diseases,

such as rheumatic fever, which are routinely diagnosed in this region.

A primary study conducted on some of the non-M-typeable strains showed that all of them possessed the *emm* gene(s) that encodes M protein(s). These studies did not include *emm* sequencing, which could have increased the number and percentage of identifiable strains. Future studies using *emm* sequencing would certainly lighten the task of serotyping group A streptococci.

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