Epidemiol. Infect. (2000), **124**, 47–51. Printed in the United Kingdom

Isolation rates of *Streptococcus pyogenes* in patients with acute pharyngotonsillitis and among healthy school children in Iran

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(*Accepted 29 July 1999*)

SUMMARY

We examined three populations from the Tehran region and the North part of Iran (Gilan), in all more than 5000 individuals, for carriage of *Streptococcus pyogenes* (group A streptococci; GAS). Children or adults with acute pharyngotonsillitis and healthy school children harboured GAS in 34·1, 20·0 and 21·0%, respectively. Typing of 421 randomly selected isolates showed a predominance of M-types M4, M5, M11, M12, as well as the provisional type 4245; however, many of the isolates were T and M non-typable. Forty-three percent of all strains were opacity factor (OF) negative. The type distribution differed markedly from that reported in 1973–4, when M types 1 and 12 were predominant.

INTRODUCTION

The group A streptococcus (GAS) is a major human pathogen causing pharyngotonsillitis and impetigo as well as the late complications rheumatic fever and acute glomerulonephritis. GAS may also give rise to more invasive, sometimes life-threatening disease, such as endometritis, erysipelas, pneumonia, septicaemia, meningitis and a toxic shock syndrome [1, 2]. Though reappearing in recent years in the US [3], rheumatic fever constitutes a leading cause of acquired heart valvular disease in the developing world. Similarly, acute poststreptococcal glomerulonephritis, often epidemic, is of particular concern in developing areas [4]. From a number of countries, however, there is a lack of epidemiological data on the occurrence of GAS and streptococcal disease.

The aims of the present report were to study isolation rates and type distribution of GAS from some regions of Iran.

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PATIENTS AND METHODS

Study population

Throat swabs were taken during December 1995–March 1997 from 5152 persons with acute pharyngotonsillitis or without apparent throat symptoms. These individuals were from the South and Central Tehran or from three separate cities (Lahijan, Fuman and Rasht) within the province of Gilan in North Iran. From each region three different groups were investigated:

Group I. Adult patients with acute pharyngotonsillitis.,

Group II. Children (age 1–15 years) with acute pharyngotonsillitis.

Group III. Healthy school children without ongoing symptoms of throat infection; however, many of these children had experienced acute pharyngotonsillitis within some months before obtaining the sample, and, following records by the school nurse, most of them had not been treated with antibiotics.

https://doi.org/10.1017/S0950268899003088

Table 1. *Occurrence of group A streptococci in throat specimens*

	Adult patients			Children with pharyngitis			Children without pharyngitis		
Origin	No	GAS	(%)	No	GAS	(%)	No	GAS	(%)
Central Tehran 1995–7	466	64	13·7	360	86	23·9	436	60	13·7
South Tehran 1995–7	406	150	36·9	314	164	52·1	686	186	27·1
Gilan 1995–7	982	162	16·5	919	275	30·0	981	188	19·2
Total	1854	376	20·0	1413	482	34·1	1885	404	21·4
Central Tehran 1991–2*	125	24	19·2	175	41	23·4	135	15	11·1

* Not included in the calculation of total carrier rates but used for comparison.

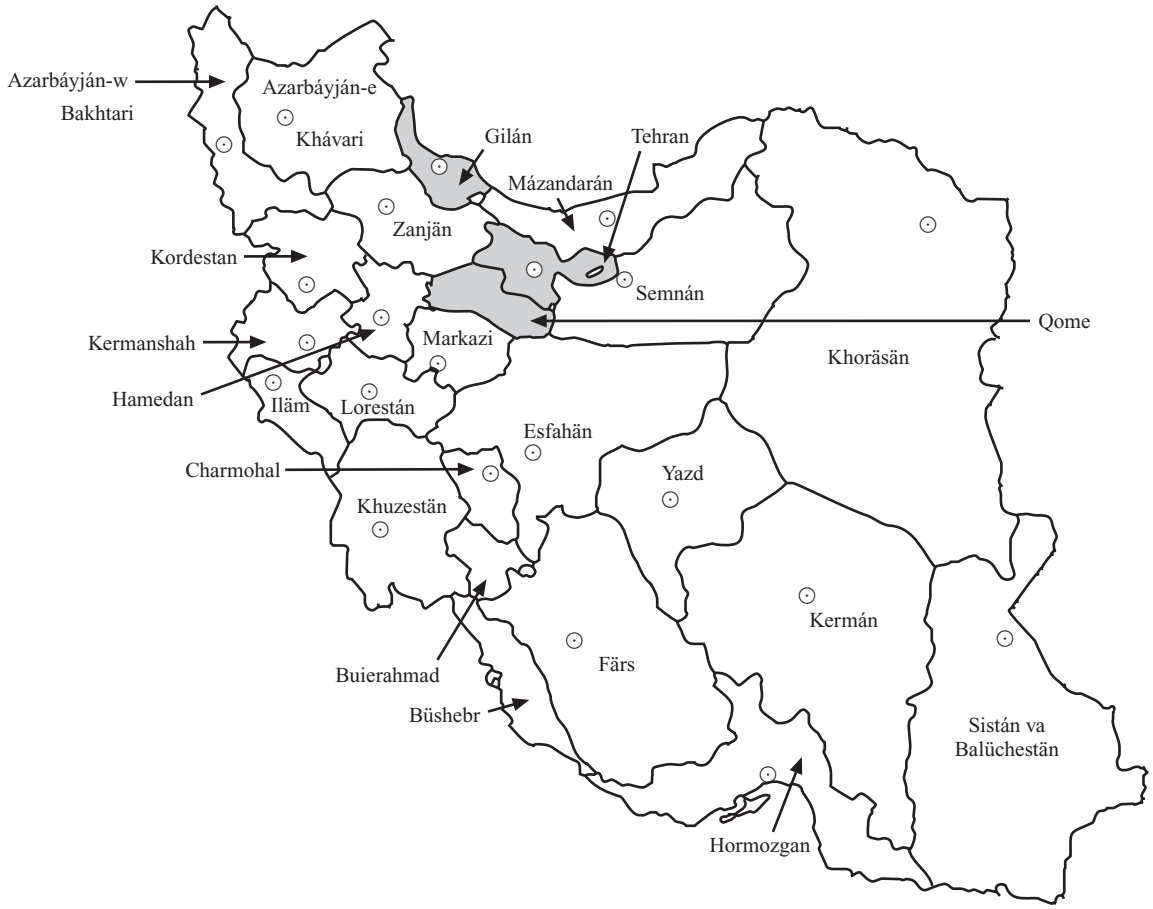

Fig. 1. Map of Iran. Regions where sampling was undertaken are indicated.

In addition, 435 streptococcal strains were obtained from the Pasteur Institute in Tehran. These strains were collected in 1991–2 during an investigation concerning isolation of GAS [5].

Isolation and identification of group A streptococci

From each individual a throat sample was obtained using a cotton tipped swab, which was immediately transferred to the respective laboratory in a Stuart transport medium. The specimen was inoculated onto a 5% horse blood agar plate incubated at 37 °C in 5% CO_2 for 18–24 h. Plates without growth of streptococci were held for an additional 24 h before results were finalized. GAS were identified by colony morphology combined with sensitivity to bacitracin and enhancement of haemolysis by sodium nucleate [6] or, alternatively, the PYR test [7].

Typing procedures

A total of 421 strains, distributed between the three above groups and from the collection from 1991–2, were randomly chosen for T and M typing. T typing

https://doi.org/10.1017/S0950268899003088

was performed by slide agglutination according to Griffith [8] using rabbit antisera from Chemapol (Prague, Czech Republic). Strains were pretreated with trypsin (Sigma, St Louis, MO, USA) 50 g/l at 37 °C for 30 min. Detection of M antigens in Lancefield acid extracts by double diffusion-in-gel and serum opacity-factor (OF) typing by the slide method [9] were performed according to standard procedures at the WHO Streptococcal Reference Unit, Public Health Laboratory Service (PHLS), London, UK.

Statistics

Differences between populations were assessed the χ^2 test using MS dose Quick Stat version 2.6 (Sapio HB). A level of $P < 0{\cdot}01$ was considered significant.

RESULTS

Occurrence of group A streptococci in throat specimens

From three areas, carrier rates of GAS among a total of 3298 children with or without acute pharyngotonsillitis were investigated (Table 1). The total isolation rate of GAS from the children with acute pharyngotonsillitis was 34·1%, compared to 21·4% among those without current throat symptoms. In both cases, the children from South Tehran showed significantly ($P < 0{\cdot}001$) higher isolation rates (52·1 and 27·1%, respectively) than those from Central Tehran or from Gilan. The isolation rates recorded in Gilan and Central Tehran, respectively, did not differ statistically.

Among 1854 adult patients with pharyngotonsillitis, GAS were isolated in 20·0%. Again, the proportion of GAS was higher ($P < 0{\cdot}001$) among the patients from South Tehran than from the other places (Table 1). No significant difference between isolation rates in Gilan and Central Tehran were found.

Type distribution of streptococci

The results of T- and M-typing are summarized in Table 2. Out of the 421 randomly selected strains 182 (43%) were OF^-. Sixty-eight percent of the strains were T-typable; however, a still higher proportion of the strains from Tehran were NT, whereas all strains from the Gilan area were T-typable. Totally, there was a predominance of T-types 1, 4, 5, 6, 9, 11, 12, 13 and B3264. Among the strains from Tehran during 1995–7, however, T types 6, 12 and B3264 predominated, whereas during 1991–2, only T-types 9, 13 and B3264 were prevalent. Of the strains from Gilan, all collected in 1995–7, a majority belonged to T-types 4, 5, 11, 13 and B3264. Within this region, however, differences emerged when comparing the three cities studied; thus, from the city Rasht, most of the strains were of T-types 4 or 13, from Lahijan of T-types 11 or 5, and from Fuman of T-types 13 or B3264.

Among all the 288 T-typable strains, 248 were successfully M- or OF-typed. The most common types identified were M12, M4, M5, M11, M13 and the provisional types PT4245 and PT2110. The latter types occurred in the OF^+ and OF^- T-type B3264 strains, respectively, though PT2110 was also identi-

Table 2. *T. agglutination patterns and M-typing data of clinical strains of group A streptococci in Iran*

T-type	OF	No.	M-type	No.	Origin
1	+	2	59	2	Tehran
1	−	15	1	13	Tehran
1	+	2	68	2	Tehran
4	+	21	4	20	Gilan
5	−	21	5	21	Gilan
6	−	14	6	14	Tehran
9	+	16	9	9	Tehran‡
11	+	23	11	21	Gilan
11	+	4	78	3	Tehran
11	−	3	12	3	Tehran
12	+	2	76	2	Tehran
12	+	3	22	3	Tehran
12	−	40	12	38	Tehran
13	+	38	13	30	Gilan
13	−	3	2110	2	Tehran‡
T25	+	2	25	2	Tehran
28	+	5	28R	3	Tehran
28	+	6	2841	6	Tehran‖
B3264	−	15	2110	13	Tehran‡
B3264	+	37	4245	25	Gilan
28–12	−	4	12	4	Tehran
28–11	−	5	12	4	Tehran
B3264,3,13	−	7	13§	5	Tehran
NT*	+	56	‖	‖	Tehran
NT	−	30	‖	‖	Tehran
Sp†	—	22	‖	‖	Tehran
3Sp	+	25	‖	‖	Tehran
Total		421		248	

* NT, non-typable.
† Sp, spontaneously agglutinating, rough strains.
‡ Strains from 1991–2 (all other isolates were from 1995–7).
§ By exclusion, and awaiting molecular characterization and confirmation.
‖ Data given in Table 3.

https://doi.org/10.1017/S0950268899003088

Table 3. *M-types of non-T-typable and spontaneously agglutinating rough strains of group A streptococci*

M-type	OF	Origin	No.
1	−	Tehran	11
4	+	Tehran	7
6	−	Tehran	4
11	+	Tehran	13
12	−	Tehran	15
22	+	Tehran	4
2841	+	Tehran	5
60	+	Tehran	1
76	+	Tehran	5
78	+	Tehran	6
4245	+	Gilan	3
Total			74

fied in some OF^- T-type 13 strains from 1991–2. The type T13 OF^+ strains from Gilan were not recognized by antisera to M-types 77 or 81 or by any of the sera against OF-positive M-types (antiserum M-type 13 was not available), and were therefore by exclusion considered as belonging to M-type 13 (Table 2).

As shown in Table 3, out of 133 non-T-typable strains, 74 were successfully M- or OF-typed. Among these, some common types, such as M1, M11, M12, and M6 were identified, whereas a few strains belonging to M76, M78 and PT2841 were also found.

DISCUSSION

We found high overall isolation rates of GAS from healthy Iranian school children as well as children with acute pharyngotonsillitis, 21·4 and 34·1% respectively. Considerably lower isolation rates among healthy children have been recorded in western countries, for example 10·9% in Denmark [10]. In a Swedish study, carrier rates of 11·3, 5·9 and 0·8% among children 4 years of age, school children and adults, respectively, were found [10]. The present GAS carriage rates among healthy school children, up to 27% in some Iranian districts investigated, were thus high and comparable to those reported from some other countries of similar affluency [12, 13]. It is well known that, except for age, the prevalence of GAS infections is influenced by restricted living conditions, family size and crowding, for example in child day care. Since carriership of GAS is often of long duration, low availability of penicillin and other antibiotics may be of particular importance in promoting spread of GAS in the poor areas.

One difficulty in studies of GAS carriage concerns its definition and distinction against symptomatic infection [14]. Although the healthy population sampled was without actual symptoms, the prevalence of recent throat infection was not known; however, many of the individuals gave a history of throat infection, mostly untreated, within the last few months.

Among the children with acute pharyngotonsillitis, a relatively high isolation rate, 52%, was noted in South Tehran, whereas rates of 24 and 30% were found in the two other areas studied. In Central Tehran, the isolation rates in both healthy and infected children were closely similar during 1991–2 compared to 1995–7. Highly variable isolation rates of GAS in acute pharyngotonsillitis have been reported, for example 35% in 7-year-old children [14] as compared to 4·6% in children below 3 years of age [15]. In an earlier study from Tehran, approximately 22% of children with acute pharyngotonsillitis harboured GAS [16].

In Gilan and Central Tehran 16·5 and 14%, respectively, of adults with acute pharyngotonsillitis harboured GAS as compared to 37% in the South part of Tehran. Obviously, this difference was a reflection of a high background carriage of GAS in South Tehran as opposed to the more wealthy districts of Central Tehran and Gilan. Carrier rates among healthy adults, however, were not investigated. The present overall isolation rate of GAS among adults with acute pharyngotonsillitis, 20%, was in agreement with levels recorded in other studies [17].

In the above-mentioned Iranian study [16], M-types 1 and 12 were predominant among clinical isolates during 1973–4. The present type distribution indicated at least eight different types to be prevalent during 1995–7, though in defined districts a preponderance of one or two types was recorded. As many as 32% of the strains were non-T-typable but more than half of these were successfully M- or OF-typed and found to belong to common types in most cases. Because of non availability of antiserum to M-type 13, we examined the type T13 OF^+ strains with antisera to all OF-positive M-types; the strains were not recognized by any of these antisera and were tentatively classified as M-type 13 isolates (work on molecular typing of these strains is underway). Many strains of the rheumatogenic types M5 and M6 were found; however, T-type B3264 OF^+, PT4245, rarely occurring in Europe, was the commonest type recorded. The combination of T-type B3264 with PT4245, a pro-

https://doi.org/10.1017/S0950268899003088

visional M-type, has not been earlier described. Unexpectedly, nine of the M12 strains were either T28R/T11 or T28R/T12 and thus apparently had two T-antigens; the acquisition of T28 was unusual for M12 strains. Such odd patterns might relate to insufficient trypsinization; however, on increasing the concentration of trypsin these strains still exhibited two T-types.

In conclusion, we found high isolation rates of GAS among patients with acute pharyngotonsillitis as well as children without any actual clinical symptoms. Though carrier rates of similar magnitude have been documented from other areas, we are not aware of any recent epidemiological data from Iran on either streptococcal carriage or the prevalence of rheumatic fever.

ACKNOWLEDGEMENTS

The study was supported by the Erik-Philip Sörensen Foundation and Pharmacia & Upjohn. We wish to thank Dr Bastanhagh for receiving us at the Department of Medical Microbiology, Tehran University of Medical Sciences & Health Services, and Dr Aslani and Mr Shahrokhi for their generosity in giving us the opportunity to study GAS strains collected by them. We are grateful to the staff members of the laboratories of the Imam Khomeini Hospital, the Pars Hospital, the Children Hospital, the Children Medical Center, the Azadi Laboratory, the Bahar Laboratory, the Central Laboratory and the Institute Pasteur for their help in collecting streptococcal strains. Our sincere thanks to Ms Deldari and Ms Makinejad for isolation of clinical streptococcal strains. We are also grateful to the staff of schools in Tehran and Gilan who helped us in this work. Finally we wish to thank Dr A. Efstratiou, Ms A. Tanna and the other staff members of the Public Health Laboratory Service, Streptococcal Unit, Colindale, London for their help and support in the typing work.

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