Indian J Med Res 130, December 2009, pp 765-771

Epidemiology of group A streptococcal pharyngitis & impetigo: A cross-sectional & follow up study in a rural community of northern India

R. Kumar, H. Vohra[*], A. Chakraborty[*], Y. P. Sharma[**], S. Bandhopadhya, V. Dhanda, V. Sagar[*], M. Sharma[†] B. Shah[†] & N. K. Ganguly[†,‡]

School of Public Health, Deparments of [*]Experimental Medicine & Biotechnology & [**]Cardiology Postgraduate Institute of Medical Education & Research, Chandigarh, & [†]Indian Council of Medical Research, New Delhi, India

Received August 14, 2008

***Background & objectives*: Group A streptococcus (GAS) causes a wide array of human diseases. Epidemiological picture of streptococcal infection in India is not complete. Hence, disease burden due to GAS in 5-15 yr old school children in northern India was studied and *emm* typing of GAS isolates was carried out to help in designing prevention strategies.**

***Methods*: A cross-sectional survey was carried out among 4249 school children (5-15 yr) from Raipur Rani Block of Panchkula district in Haryana during 2000-2002; 334 children were followed up fortnightly for one year. Standard clinical and microbiological procedures were used for collection of swabs from throat and skin and confirmation of GAS and its *emm* types.**

***Results*: Of the 4249 children studied, 658 (15.5%) had pharyngitis; 579 of them could be swabbed, of which 2.8 per cent had GAS. From 3591 children without pharyngitis, 3385 who could be swabbed, GAS was found in 1.3 per cent of them. Impetigo was rare (0.7%), but 7.1 per cent (2/28) children had GAS. In the followup study, 17.4 per cent (776/4447 child-contacts) had pharyngitis, 761 could be swabbed and 2.4 per cent had GAS; among those without pharyngitis, 2016 swabs could be taken and GAS was found in 1.3 per cent; whereas only 2.6 per cent (2/75) of skin sores had GAS. Three children had GAS pharyngitis twice during follow up. Fourteen different GAS *emm* types were found. *emm* 71, 77 and 81 constituted 69 per cent of the pharyngeal isolates. GAS pharyngitis and impetigo were more common in winters and summers respectively.**

***Interpretation & conclusions*: In north India, pharyngitis was more common than impetigo. Most prevalent *emm* types of GAS in this region differ from those included in M protein-based vaccines.**

Key words Group A streptococcus (GAS) - *emm* types - impetigo - pharyngitis - prevalence

Streptococcus pyogenes, *i.e.* Lancefield group 'A' beta haemolytic Streptococcus (GAS) is capable of causing a wide variety of diseases, such as scarlet fever, pharyngitis, impetigo, acute rheumatic fever (ARF),

[‡] *Present address*: Translation Health Science, Technology Institute, National Institute of Immunology, Aruna Asaf Ali Marg New Delhi 110 067, India

rheumatic heart disease (RHD), glomerulonephritis, necrotizing fascitis and toxic shock syndrome, *etc*. The global burden of group A streptococcal diseases is an estimated 616 million GAS pharyngitis cases per year[1]. Epidemiological data from developing countries are scarce. Prevalence of GAS pharyngitis and carriage in asymptomatic children in different countries varies from 9 to 34.1 per cent[2-6]. In India, prevalence of GAS pharyngitis ranged from 4.2 to 13.7 per cent[7-11]. The incidence of GAS culture positive pharyngitis among 5-15 yr old urban slum children near Chandigarh in northern India is 0.95 episodes per child per year, as compared to 0.13 episodes per child year in urban Melbourne[11,12]. It is estimated that 0.3 per cent of the streptococcal sore throat episodes in endemic situation and 3 per cent during epidemics develop into ARF[13,14]; and 60 per cent of the ARF episodes may progress to damage the heart valves causing RHD. Although ARF/RHD has declined in many parts of the world, but continues to be a major public health problem causing significant cardiovascular morbidity and mortality in India[15].

Strain prevalence rather than the innate virulence potential is considered to be a major factor for the observed increase in serious group A streptococcal infections[16]. The epidemiological picture of streptococcal infections in India is quite different from that of the developed countries, suggesting that may be some serotypes are much more common than others within a population in different geographical locations[17]. In the absence of GAS type distribution data, true propensity of any M type to cause a specific clinical manifestation still remains controversial. Moreover, the eventual introduction of vaccines, especially those based on multiple M protein protective epitopes, requires better understanding of group A streptococcal pharyngitis. Hence, we studied GAS sore throat and skin sores in 5-15 yr old school children, and carried out *emm* typing of the GAS isolates.

Material & Methods

Of the 257 villages of Raipur Rani Block in Panchkula district of Haryana in northern India, 25 were selected randomly. A total of 4249 school children in the 5-15 yr age group, from 34 government schools (22 primary, one middle, and 11 high schools) located in the sampled villages, were included in this cross-sectional survey done from November 2000 to January 2002. In addition, from three schools, a cohort of 334 children were also enrolled and followed up for one year. Only one child dropped out during the tenth follow up month. The parents or guardians of school children were informed about the purpose of this study and their written consent was taken. The study was approved by the institute ethics committee.

A pre-tested structured clinical performa was used to record the symptoms and signs and physical examination findings[18]. The throat swabs (peritonsillar and posterior wall of pharynx) and skin swabs (upper surface of skin) were taken once from all children by trained medical officer or a public health nurse. Children in the follow up study were examined every fortnight. At every examination, swabs were taken from those who had pharyngitis or skin lesion. At every second fortnightly follow up, swabs were also taken from those who were not having sore throat or skin lesion. Throat and skin swabs were also taken from family members of a child who was found to have GAS infection or carriage.

Children having any symptoms and signs of pharyngitis on the day of examination or within previous two weeks before the examination were considered as cases of pharyngitis. Children, who had any of the symptoms/signs of pharyngitis and also GAS in culture, were labelled as cases of GAS pharyngitis, and those who did not have any symptoms/signs of pharyngitis on the day of examination or in previous 15 days but had GAS in cultures were considered to be GAS carriers.

Children with skin sores on the day of examination were taken as cases of impetigo and those showing GAS on culturing were labelled as GAS skin sore cases. Children without any skin sores were swabbed from wrist, back of neck and ankles and those showing GAS were labelled as skin carriers.

Pharyngitis patients were given paracetamol and anti-histaminics, if needed and those with skin infection were given betadine and Whitfield's ointment for bacterial and fungal infections respectively. All those showing GAS in the cultures were given a course of oral penicillin (for < 20 kg body weight, 125 mg 4 times daily, and for ≥20 kg body weight, 250 mg 4 times daily for 10 days).

Throat and skin swabs were transported on the same day at room temperature to the laboratory of Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Sciences, Chandigarh. Swabs were inoculated on Columbia blood agar media (Hi-Media, Mumbai) containing 5-7 per cent defibrinated sheep blood, incubated for 24 h

at 37°C under 5-10 per cent CO_2 atmosphere to check the presence of beta-haemolytic streptococci (BHS). The bacitracin sensitivity test[19], using bacitracin disks (Hi-Media, Mumbai), was carried out on the selected streptococci and only sensitive isolates were further examined for Lancefield's grouping by Lancefield's Hot HCl extraction method[20]. Group A specific antigen was confirmed by latex agglutination test with positive and negative controls (Difco, Becton Dickinson, USA). Agglutination in one minute was considered positive for group A streptococci. The process was repeated again on the negative samples after antigen extraction before labelling them as negative. After October 2001, grouping for C and G was also performed using the group C and G specific antigen by latex agglutination test, using similar procedure as was done for group A. group A, C and G streptococcal isolates were preserved in 15-20 per cent glycerol stocks at -70°C.

Of the 112 preserved GAS samples, 71 were *emm* typed after reconfirmation with Streptex kit (Murex Biotech Ltd, UK). Genomic DNA was isolated using modified sodium dodesyl sulphate (SDS) phenol method[21]. Briefly, bacterial cells were harvested from 1.5 ml saturated culture of GAS in Todd – Hewitt broth (THB) and resuspended in 567 µl of Tris-EDTA (TE) buffer (*p*H 7.8). Following addition of 30 µl of SDS and 3 µl proteinase K (20 mg/ml) to a final concentration 100 µg/ml, the cells were incubated at 37°C for 1 h then lysed with 5M NaCl and 10 per cent cetyl trimethyl ammonium bromide (CTAB)/NaCl mixture at 65°C for 10 min. DNA was extracted with chloroform – isomyl alcohol followed by phenol chloroform–isomyl alcohol and precipitated with 0.6 volume of isopropyl alcohol. DNA pellet was washed with 70 per cent ethanol, dried and dissolved in 30 µl of TE buffer. Following this, DNA was quantitated and analyzed by running it on 0.8 per cent agarose gel. The DNA was stored at -20° C for further use.

emm gene PCR was done following standardized conditions as described earlier[22]. Chemicals from Roche Applied Sciences, USA, were used for PCR. PCR products were checked on 0.8 per cent gel, their yield was analyzed and products were then purified by QIAGEN PCR purification kit USA. PCR product (15-45 ng) was sequenced using ABI Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA). The unincorporated dye, unused primers and dNTPs were removed by ethanol precipitation by following protocol provided by the manufacturer. To the dried pellet, template suppression reagent (TSR) was added and after denaturation, product was loaded on an ABI 377 Automated Sequencer as per manufacturer's instructions (Applied Biosystems, USA). The *emm* gene sequence was initially searched for homology by BLAST (Basic Length Alignment Search Tool) search analysis (*http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi*) but to be specific the sequences were also submitted to CDC website (*http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm*) that designates the types and subtypes by comparing the pair wise nucleotide homology for first 160 bases of the hyper-variable region. Strains showing ≥ 95 per cent sequence homology having maximum alignment with the reference strain in the CDC Gene Bank database were selected and designated particular parental *emm* type.

EPI-6 (Epi Info Version 6) software was used for data analysis. Chi square test was applied to test significant differences. $P<0.05$ was considered statistically significant.

Results

Of the 4249 school children studied, 2235 (52.6%) were boys whereas amongst the 334 children enrolled in the follow up study, boys constituted 53.6 per cent (179). Of the total, 658 (15.5%) children reported symptoms/signs of pharyngitis. Of these 658 children with pharyngitis, 579 consented for throat swabs, among them BHS was found in 149 (25.7%) and GAS was found in 16 (2.8%) children. From 3591 children who did not have pharyngitis, 3385 consented for sampling. Among them BHS and GAS were found in 15.4 per cent (520) and 1.3 per cent (44) respectively (Table I). In the fortnightly followup of 334 children, a total of 4447 child contacts were made and among these, 776 (17.4%) had pharyngitis. A total of 761 swabs were collected and BHS and GAS isolation was found to be 9.2 per cent (70) and 2.4 per cent (18) respectively. In every second fortnightly followup, among child-contacts who did not have pharyngitis, 2016 were swabbed. Among these, BHS and GAS were isolated from 5.5 per cent (112) and 1.3 per cent (26) respectively (Table II). GAS isolation from pharyngitis cases was significantly ($P<0.05$) higher compared to those without pharyngitis. In winter months, the presence of GAS pharyngitis was significantly ($P<0.001$) higher than the summer months.

Of the 44 children who had GAS infection or carriage in the follow up study, families of only 14 children could be contacted and only 42 members of

Table I. Pharyngeal revalence of beta haemolytic streptococci (BHS) and group A streptococci (GAS) among 5-15 yr old school children in cross sectional study

Season	No. of children examined	Symptoms/signs of pharyngitis on the day of swabbing or in prior two weeks							
		Yes				No			
		Number (%)	Throat swabs	BHS	GAS	Number	Throat swabs	BHS	GAS
Winter	2291	435	358	126	10	1865	1694	423	36
Summer	1958	223	221	23	6	1735	1691	97	8
Total	4249	658 (15.5)	579 (88.0)	149 (25.7)	16 (2.8)	3591 (84.5)	3385 (94.3)	520 (15.4)	44 (1.3)

Winter-October to March: Summer-April to September

Table II. Pharyngeal prevalence of beta haemolytic streptococci (BHS) and group A streptococci (GAS) among 5-15 yr old school children in a one year follow up study

Season	No. of children examined	Symptoms/signs of pharyngitis on the day of swabbing or in prior two weeks							
		Yes				No			
		Number	Throat swabs	BHS	GAS	Number	Throat swabs*	BHS	GAS
Winter	2399	560	554	63	15	1839	1052	85	18
Summer	2048	216	207	7	3	1832	964	27	8
Total	4447	776 (17.4%)	761 (98.0%)	70 (9.2%)	18 (2.4%)	3671 (82.6%)	2016 (54.9%)	112 (5.5%)	26 (1.3%)

Winter-October to March: Summer-April to September, *from every second fortnightly follow-up examination

these families could be swabbed; nine of these had GAS in their throat swabs.

Impetigo was not common in cross-sectional study, *i.e.*, only 28 (0.7%) children had skin sores. Of them, two had BHS and both these cases were Group A. None of the children without skin sores had BHS or GAS on the wrist, back of neck or ankles. In the follow up study 84 (1.9%) children had skin sores, but 75 consented for sampling. Among them, BHS and GAS in skin lesions was found in two children (2.6%) each. Presence of BHS on skin without any lesion was 0.09 per cent and that of GAS was 0.04 per cent Table III. Occurrence of skin sores was highest in September.

In cross-sectional study, amongst the 94 pharyngitis cases, two group G streptococci (GGS) isolates were confirmed in November 2001, however, no GCS or GGS was found in 623 children without pharyngitis. In the follow up study, one each of GCS and GGS was isolated in November 2001 and January 2002 respectively from 304 pharyngitis cases. Three GGS isolates were found in 515 children who did not have pharyngitis during December 2001. In the follow up study, a single isolate of GGS was found in December 2001 on the skin among children who did not have any skin sores.

A total of 71 confirmed GAS isolates were *emm* typed (Table IV). Most commonly identified *emm* type was 77 (29.6%) followed by 81 (25.4%) and 11(14.1%). The presence of *emm* type 77 was high (34.9%) in cross-sectional survey as compared to the follow up study (21.4%) and were more common during winter months. The other *emm* types found in pharyngitis cases were- 18, 44, 68, 75, 77, 81, 87, 88, 92, 118, ST854. Three *emm* types (11, 77 and 81) constituted 69 per cent of the pharyngeal isolates. Three GAS isolates identified from skin sores were *emm* type 77 (one each in cross-sectional and follow up study) and 44 from the cross-sectional study (Table IV).

Of the 25 villages surveyed in the cross-sectional study, 43 *emm* types were encountered in 12 villages and 16 of these were from one village. Twelve of the 28 isolates, in the follow up study, were from the same village but belonged to several *emm* types.

During the follow up, three children were found to have GAS in their throat cultures on two occasions each. One child had *emm* 71 on two occasions, once in the presence of pharyngitis and then after a gap of 2 months without pharyngitis. Second child from another village showed different *emm* type causing different clinical status, *i.e.*, type 81 without pharyngitis and 87 with

Table III. Prevalence of beta haemolytic streptococci (BHS) and group A streptococci (GAS) among 5-15 yr old school children with/ without impetigo

Study Type	No. of child examinations	Symptoms/signs of impetigo							
		Yes				No			
		Number (%)	Skin swabs	BHS	GAS	Number	Skin swabs	BHS	GAS
Cross-sectional	4249	28 (0.7)	28 (100)	2 (7.1)	2 (7.1)	4221 (99.3)	4221 (100)	0	0
Follow up	4447	84 (1.9)	75 (89.3)	2 (2.6)	2 (2.6)	4363 (98.1)	2312* (53.0)	2 (0.09)	1 (0.04)

*from every second fortnightly followup examination

Table IV. Distribution of emm types of group A beta haemolytic streptococci isolated from throat and skin sores from school children in 5-15 yr age group

emm type	Total	Cross-sectional study	Follow up study
Pharynx			
77	19	14	5
81	18	11	7
11	10	8	2
71	4	0	4
69	3	3	0
18	2	2	0
75-1	2	1	1
118	2	1	1
44	2	0	2
ST854	2	0	2
68	1	0	1
87	1	0	1
88	1	0	1
92	1	1	0
Skin			
77	2	1	1
44	1	1	0
Total	71	43	28

pharyngitis at an interval of 6 months. Again from the same village another child without pharyngitis showed two different *emm* types, (81 and 44) at different times at an interval of 8 months.

Discussion

During the one year follow up, GAS was isolated from 12.3 per cent children at least once and a second time from three children indicating high endemicity in the rural community of Haryana. Only a small proportion of children experienced a repeated GAS episode which is similar to the observations made in another study[23]. The GAS isolation rate of 0.12 per child per year was lower than the urban slums of Chandigarh[11] but was similar to that in urban Melbourne[12]. The pharyngeal GAS isolation was common during the winter, an observation similar to that made a few decades ago in a community near Varanasi in northern India[7]. The GAS impetigo is more common (6.9 per 100 child visits) in the tropical climate of south India and in the aboriginal communities of northern territories of Australia[24] compared to present study.

Carriers of GAS are an important source of infection and represent a pool which may cause active throat infections. A rise in the titre of antibodies can differentiate infection and carriage but due to operational difficulties serology could not be done. Throat carriage of GAS was 1.3 per cent in the present study which was lesser compared to other studies from India and abroad[2,3,5,8]. Throat carriage of GAS was found to be 3.7 per cent in Aboriginal Australians[24]. Studies in India showed GAS carriage of 2.3 to 20 per cent[8,25,26].

Asymptomatic spread of *Streptococcus pyogenes* has also been reported among family members. All family members of the children enrolled in this study could not be contacted due to operational difficulties, however, limited study among a few families from whom GAS was isolated from throat, indicates occurrence of transmission within the family members, a finding similar to that reported from a study from Melbourne[12].

The presence of group C and G streptococci which are genetically close relatives of GAS, was found to be low. Traditionally GCS and GGS were thought to be the commensals, however, recently these have

been considered capable of eliciting a range of clinical manifestations just as GAS[27]. It has been hypothesized that non GAS infections may be the driving forces behind ARF in the aboriginal communities of Australia[28]. These organisms may have acquired known GAS virulence factors through genetic transfers[29].

Accurate identification and typing of GAS is an essential part of epidemiological and pathogenetic studies of streptococcal diseases. The M proteins of GAS are the major virulence factors which are the main target in vaccine development. Serological methods were not able to type a significant proportion of the GAS isolates, hence, *emm* gene (encoding M protein) sequencing based on 5` region is becoming a universal method of choice for characterization of GAS. There are currently around 180 *emm* sequence types and 800 *emm* subtypes described but new types and subtypes are still being identified regularly in different geographical regions[30]. As an individual GAS serotype enters and leaves a community fairly quickly, inter-site and temporal variations in *emm* types are common[31]. Even strain variations have been noted within M types[32,33]. Replacement of the traditionally described rheumatogenic M types with non rheumatogenic types has been observed in acute pharyngitis cases[34]. A few unusual serotypes have been reported that have the potential for infecting both throat and skin sites[31]. Of the 59 *emm* types isolated from south India, 11, 82, 105, 108, 100 and 112 accounted for 33.5 per cent of the isolates and most predominant *emm* types in pharyngitis were 11, 110, 82 and 108[35]. In present study, *emm* 11, 77 and 81 were the most common emm types showing high heterogeneity among *emm* types isolated from these two regions of India. These types differ from those reported from across the world[36-38]. Of the six M types most commonly seen in United States (*emm* 1, 3, 4, 11, 12 and 28), only *emm* 11 was found to be prevalent in the northern as well in the southern region of India[35].

Till date, no effective vaccine is available against streptococcal diseases, though significant advances have been made on M protein epitope based vaccines. However, these vaccines require multiple amino terminal epitopes that are prevalent in a community so as to provide complete protection. With a few exceptions, the GAS types isolated in India are generally different from the ones taken into consideration while developing multivalent vaccines in USA and Australia. Therefore, the efficacy of currently available vaccines based on N terminal variable region sequence of M proteins found in the western world will be doubtful in India.

To conclude, in the rural communities of Haryana in northern India, pharyngitis was found to be more common than impetigo. The most prevalent GAS *emm* types found in this region differ from those included in the multivalent vaccine under clinical trials, limiting the effectiveness of the proposed M protein-based multivalent GAS vaccines in India. Hence, epidemiological surveillance needs to be continued for GAS, and if possible for GCS and GGS also, in different geographic areas to have adequate information on the trends of *emm* type distribution for selection of appropriate vaccine strategy.

Acknowledgment

This study was a part of Indian Council of Medical Research (ICMR) Jai Vigyan Mission Mode Project on “Community Control of RF/RHD in India”. Authors acknowledge the financial and technical support of ICMR for this project.

References

1. Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. *Lancet Infect Dis* 2005; *5*: 685-94.
2. Jasir A, Noorani A, Mirsalehian A, Schalen C. Isolation rates of *Streptococcus pyogenes* in patients with acute pharyngotonsillitis and among healthy school children in Iran. *Epidemiol Infect* 2000; *124*: 47-51.
3. Durmaz R, Durmaz B, Bayraktar M, Ozerol IH, Kalcioglu MT, Aktas E, *et al*. Prevalence of group A streptococcal carriers in asymptomatic children and clonal relatedness among isolates in Malatya, Turkey. *J Clin Microbiol* 2003; *41*: 5285-7.
4. Ahmed J, Zaman MM, Keramat Ali SM. Identification of serogroups of beta hemolytic streptococci in children with tonsillo-pharyngitis. *Bangladesh Med Res Counc Bull* 2003; *29*: 113-7.
5. Mzoughi R, Bouallegue O, Selmi H, Ben Said H, Essoussi AS, Jeddi M. Group A streptococci in children with acute pharyngitis in Sousse, Tunisia. *East Mediterr Health J* 2004; *10* : 488-93.
6. Campbell J, Sow S, Diallo S, Keita M, Page C, Tamboura B, *et al*. The epidemiology of Group A Streptococcus among children in Bamako, Mali. In: Program and abstracts of the XVIth Lancefield International Symposium on Streptococci and Streptococcal Diseases, held on Palm Cove, Cairns, Australia; 25-29 Sep 2005. p.105.
7. Sarkar S, Biswas R, Gaur SD, Sen PC, Reddy DC. A study on sore throat and β hemolytic streptococcal pharyngitis among rural school children in Varanasi, with reference to age and season. *Indian J Public Health* 1988; *32* : 190-8.
8. Koshi G, Benjamin V. Surveillance of streptococcal infections in children in a south Indian community - a pilot survey. *Indian J Med Res* 1977; *66* : 379-88.
9. Rajkumar S, Krishnamurthy R. Isolation of group A β–hemolytic streptococci in the tonsillopharynx of school children in Madras city and correlation with their clinical features. *Jpn J Infect Dis* 2001; *54*: 137-9.

10. Gupta R, Prakash K, Kapoor AK. Subclinical group A streptococcal throat infection in school children. *Indian Pediatr* 1992; *29* : 1491-4.
11. Nandi S, Kumar R, Ray P, Vohra H, Ganguly NK. Group A streptococcal sore throat in a periurban population of northern India: a one-year prospective study. *Bull World Health Organ* 2001; *79* : 528-33.
12. Danchin MH, Rogers S, Kelpie L, Selvaraj G, Curtis N, Carlin JB, *et al*. Burden of acute sore throat and group A streptococcal pharyngitis in school-aged children and their families in Australia. *Pediatrics* 2007; *120* : 950-7.
13. Rammelkamp CH, Denny FW, Wannamaker LW. Studies on the epidemiology of rheumatic fever in the Armed Services: In: Thomas L, editor. *Rheumatic fever*. Minneapolis: University of Minnesota Press; 1952. p. 72-89.
14. Siegel AC, Johnson EE, Stollerman GH. Controlled studies of streptococcal pharyngitis in a pediatric population. I. Factors related to the attack rate of rheumatic fever. *N Engl J Med* 1961; *265* : 559-65.
15. Krause RM. A half-century of streptococcal research: then & now. *Indian J Med Res* 2002; *115* : 215-41.
16. Rogers S, Commons R, Danchin MH, Selvaraj G, Kelpie L, Curtis N, *et al*. Strain prevalence, rather than innate virulence potential, is the major factor responsible for an increase in serious group A streptococcus infections. *J Infect Dis* 2007; *195* : 1625-33.
17. Brandt ER, Teh T, Relf WA, Hobb RI, Good MF. Protective and non protective epitopes from amino termini of M proteins from Australian aboriginal isolates and reference strains of Group A streptococci. *Infect Immun* 2000; *68* : 6587-94.
18. Nandi S, Kumar R, Ray P, Vohra H, Ganguly NK. Clinical score card for diagnosis of group A streptococcal sore throat. *Indian J Pediatr* 2002; *69* : 471-5.
19. Maxted WR. The use of bacitracin for identifying group A hemolytic streptococci. *J Clin Pathol* 1953; *6* : 224-6.
20. Lancefield RC. A serological differentiation of human and other groups of hemolytic streptococci. *J Exp Med* 1933; *57* : 571-95.
21. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, *et al*. Preparation of genomic DNA from bacteria. In: *Current Protocols in Mmolecular Biology*, Contents of Supplements, New York: Green Publishing Associates and Wiley Interscience; 1987. p. 2.4.1-2.4.5.
22. Podbielski A, Melzer B, Lutticken R. Application of polymerase chain reaction to study the M protein (-like) gene family in beta hemolytic streptococci. *Med Microbiol Immunol* 1991; *180* : 213-27.
23. St Sauver JL, Weaver AL, Orvidas LJ, Jacobson RM, Jacobsen SJ. Population-based prevalence of repeated group A beta-hemolytic streptococcal pharyngitis episodes. *Mayo Clin Proc* 2006; *81* : 1172-6.
24. McDonald MI, Towers RJ, Andrews RM, Benger N, Currie BJ, Carapetis JR. Low rates of streptococcal pharyngitis and high rates of pyoderma in Australian aboriginal communities where acute rheumatic fever is hyperendemic. *Clin Infect Dis* 2006; *43* : 683-9.
25. Menon T, Shanmugasundaram S, Kumar MP, Kumar CP. Group A streptococcal infections of the pharynx in a rural population in south India. *Indian J Med Res* 2004; *119* (Suppl): 171-3.
26. Lloyd CA, Jacob SE, Menon T. Pharyngeal carriage of group A streptococci in school children in Chennai. *Indian J Med Res*. 2006; *124* : 195-8.
27. Lindbaek M, Høiby EA, Lermark G, Steinsholt IM, Hjortdahl P. Clinical symptoms and signs in sore throat patients with large colony variant beta-haemolytic streptococci groups C or G versus group A. *Br J Gen Pract* 2005; *55* : 615-9.
28. McDonald M, Currie BJ, Carapetis JR. Acute rheumatic fever: a chink in the chain that links the heart to the throat? *Lancet Infect Dis* 2004; *4* : 240-5.
29. Davies MR, McMillan DJ, Sriprakash KS, Chhatwal GS. Distribution of group A streptococcal virulence genes in group C and G streptococci. In: Sriprakash KS, editor. *Streptococci - New Insights into an old enemy*. Netherlands; Elsevier International Congress Series1289, 2006. p. 184-7.
30. Steer CA, Danchin MH, Carapetis JR. Group A streptococcal infections in children. *J Pediatr Child Health* 2007; *43* : 203-13.
31. Anthony BF, Kaplan EL, Wannamaker LW, Chapman SS. The dynamics of streptococcal infections in a defined population of children: serotypes associated with skin and respiratory infections. *Am J Epidemiol* 1976; *104* : 652-66.
32. de Malmanche SA, Martin DR. Protective immunity to the group A Streptococcus may be only strain specific. *Med Microbiol Immunol* 1994; *183* : 299-306.
33. Villaseñor-Sierra A, McShan WM, Salmi D, Kaplan EL, Johnson DR, Stevens DL. Variable susceptibility to opsonophagocytosis of group A streptococcus M-1 strains by human immune sera. *J Infect Dis* 1999; *180*: 1921-8.
34. Shulman ST, Stollerman G, Beall B, Dale JB, Tanz RR. Temporal changes in streptococcal M protein types and the near-disappearance of acute rheumatic fever in the United States. *Clin Infect Dis* 2006; *42* : 441-7.
35. Sharma M, Shah B, Dhaliwal RS, Kumar R, Brahmadathan KN, Vohra H, et al. Heterogeneity of community based pediatric GAS isolates from India: Challenges to the multivalent vaccine approach. In: Sriprakash KS, editor. *Streptococci - New Insights into an old enemy*. Elsevier *International Congress Series1289*, Netherlands: Elsevier; 2006. p. 49-53.
36. Hu MC, Walls MA, Stroop SD, Reddish MA, Beall B, Dale JB. Immunogenicity of a 26-valent group A streptococcal vaccine. *Infect Immun* 2002; *70* : 2171-7.
37. McDonald MI, Towers RJ, Fagan P, Carapetis JR, Currie BJ. Molecular typing of Streptococcus pyogenes from remote Aboriginal communities where rheumatic fever is common and pyoderma is the predominant streptococcal infection. *Epidemiol Infect* 2007; *135* : 1398-405.
38. Erdem G, Mizumoto C, Esaki D, Abe L, Reddy V, Effler PV. Streptococcal *emm* types in Hawaii: A region with high incidence of acute rheumatic fever. *Pediatr Infect Dis J* 2009; *28* : 13-6.

Reprint requests: Dr Rajesh Kumar, Professor & Head, School of Public Health, Postgraduate Institute of Medical Education & Research Chandigarh 160 012, India
e-mail: dr.rajeshkumar@gmail.com