Indian J Med Res 143, May 2016, pp 659-662
DOI:10.4103/0971-5916.187116

Correspondence

M types & toxin gene profile of group A streptococci isolated from children in Dibrugarh district of Assam, India

Sir,

Streptococcus pyogenes is one among the more than 100 known species of the genus *Streptococcus*[1] and invasive group A streptococci (GAS) infection is associated with mortality and morbidity globally[2]. Streptococcal M protein, pyrogenic exotoxins (SPE), streptococcal mitogenic exotoxin Z (SMEZ) and streptococcal superantigen (SSA) are among the virulence determinants of GAS[3]. Invasiveness of the GAS has been associated with the presence of these virulence factors[3]. It is necessary to know the molecular epidemiology of a bacterial strain to plan any preventive strategy. The M protein is encoded by the *emm* gene. This is one of the candidate genes that have been studied for vaccine development though its success as a vaccine candidate has been questioned due to the variation of circulating strains in different geographical regions[4]. New *emm* types are being continuously added to the Centers for Disease Control and Prevention (CDC) database[5]. The production of the toxin enzymes has been shown to vary between different *emm* types[6]. Studies have documented geographical variation of the *emm* types as well as toxin gene prevalence among the isolates of streptococci[7-11]. A higher incidence of SPE A, B and F has been found among GAS isolates from rheumatic fever/rheumatic heart disease (RF/RHD) cases as compared to pharyngeal isolates from India[8]. In the present study we tried to evaluate the frequency of nine toxin genes namely *spe A*, *B, C, F, G, H, J*, streptococcal mitogenic exotoxin Z (*smeZ*) and streptococcal superantigen (*ssa*) as well as *emm* types among the GAS isolates from throats of healthy school children and from their skin lesions from the northeastern region of India.

The GAS isolates (n=14) obtained during a previous study[12] were used. Isolates were stored in 15 per cent glycerol stock at -70°C. Identification was done by Gram stain, catalase test and bacitracin (0.04 units) susceptibility test as mentioned previously[12]. The isolates were Lancefield serogrouped using HiStrep Latex test kit (Hi-Media, Mumbai) according to the manufacturer's instructions[12].

For the isolation of bacterial genomic DNA, a loopful of colony was inoculated into 1.5 ml Todd Hewitt broth (Hi-Media, Mumbai) with added yeast extract and incubated overnight at 37°C. Bacterial cells were harvested by centrifugation and DNA was recovered using Gene JET Genomic DNA Purification kit (Fermentas, Thermo Scientific, Lithuania) according to the manufacturer's instruction. DNA was stored at -20 °C for later use.

M (*emm*) typing was carried out as per protocol described by CDC[13] with modification in the thermal profile for carrying out PCR. *Emm* primer 1: 5'-TATTCGCTTAGAAAATTAA-3' and primer 2: 5'-GCAAGTTCTTCAGCTTGTTT-3' available at CDC site (*http://www.cdc.gov/streplab/protocol-emm-type.html*) were taken for M typing. Ten pmol of each primer was used in 50 µl PCR reaction mixture (2x PCR master mix, Promega, USA) to amplify the *emm* gene. The PCR reaction was done in a thermal cycler (Veriti 96 well thermal cycler, Applied Biosystems-Life Technologies Corporation), and the thermal profile used for the PCR was as follows: initial denaturation at 94°C for 10 min, 30 cycles of denaturation at 94°C for one min, primer annealing at 50°C for one min, extension at 72°C for 90 sec and final extension for seven min at 72°C. PCR product (5 µl) was mixed with 1 µl of 6x gel loading dye (Promega, USA), and subjected to electrophoresis on 1.5 per cent agarose gel to confirm the PCR amplification. The amplified product was purified using Roche DNA Purification Kit (Roche Diagnostics GmbH Mannheim, Germany) following which commercial sequencing was done

(Xcelris Labs Limited, Ahmadabad, India). Sequences obtained were assembled and aligned using CodonCode Aligner software (*http://www.codoncode.com/aboutus.htm*). The resultant emm gene sequence was initially searched for homology by BLAST (Basic Local Alignment Search Tool) search analysis (*https://blast.ncbi.nlm.nih.gov/Blast*) and sequences were finally submitted to CDC website (*cdc.gov/ncidod/biotech/strep/strepblast.htm*) for allotment of *emm* type.

Individual PCRs were performed to check the presence of streptococcal pyrogenic exotoxin A (*speA*), B(*speB)*, C(*speC)*, F (*speF*) G(*speG), H(speH), J(speJ), smeZ* and *ssa* in all the streptococcal isolates with the reported primers[7]. PCRs were performed 30 cycles each consisting of an initial denaturation at 96°C for five min, denaturation at 96°C for 50 sec, annealing at 44°C for 65 sec, elongation at 72°C for 70 sec and final elongation at 72°C for five min. PCR products were resolved on a 2 per cent (w/v) agarose gel. *Staphylococcus aureus* ATCC 29213 was taken as negative control.

Table I shows the *emm* types of GAS isolates. A total of five different *emm* types were identified among the 14 isolates. The most common *emm* type was 123.0. Five different toxin gene profiles were observed among the 14 isolates. The genes *speB and G*, were detected in all the isolates, *speF* in 13*, smeZ* in 12, *spe H* in three, *ssa* in one whereas *speA, speC, speJ* were detected in none of the isolates. All isolates belonging to *emm* 123.0 except one had carried *speB, speF, speG, smeZ* genes. A similar pattern of toxin gene profile was observed among isolates belonging to both *emm* 131.0 (one isolate) and *emm* 91.0 while isolate belonging to *emm* 42.0 had a similar pattern with that of *emm* 123.0. The skin isolate belonged to 71.0 *emm* type and its toxin gene profile was different from the throat isolates.

Table I. *emm* types and toxin gene profile of GAS isolates from throat and skin of schoolchildren in Dibrugarh district of Assam, India

S. No.	Isolate ID	*emm* type	Toxin gene profile
1	S-1	*emm*71.0	*speB, speF, speG, speH*
2	T-6777	*emm*123.0	*speB, speF, speG, smeZ*
3	T-4733	*emm*123.0	*speB, speF, speG, smeZ*
4	T-6376	*emm*123.0	*speB, speF, speG, smeZ*
5	T-482	*emm*123.0	*speB, speF, speG, smeZ*
6	T-6571	*emm*123.0	*speB, speF, speG, smeZ*
7	T-315	*emm*123.0	*speB, speF, speG, smeZ*
8	T-300	*emm*123.0	*speB, speF, speG,*
9	T- 363	*emm*123.0	*speB, speF, speG, smeZ*
10	T- 360	*emm*123.0	*speB, speF, speG, smeZ*
11	T-303	*emm*91.0	*speB, speF, speG, speH, smeZ*
12	T-338	*emm*131.0	*speB, speF, speG, speH, smeZ*
13	T-6281	*emm* 131.0	*speB, speG, ssa, smeZ*
14	T-6475	*emm*42.0	*speB, speF, speG, smeZ*

A large scale study done in India which included ten centres including Dibrugarh, reported the prevalence of rheumatic heart disease to vary from 0.13 to 1.5/1000 (overall 0.9/1000) in the 5 to 14 yr age group[14]. The M protein has been implicated in the pathogenesis of rheumatc fever/ rheumatic heart disease, a post-streptoccal sequelae, due to its molecular mimicry with host proteins[2]. There seems to be variation of *emm* type distribution and toxin gene profile within the country[8-11,15-20]. Much of the existing data are from northern and southern part of India and no such data are available from northeastern part of India. The present study though includes small number of isolates, provides baseline data for future reference.

M types 1, 3, 5, 6, 14, 18, 19, 24, 27, and 29 are considered rheumatogenic while 1, 2, 4, 12, 15, 18, 25, 42, 49, 55, 56, 57, 59, 60, 61 are known to have nephritogenic potential[3]. The *emm* profile of the study isolates was found to be different from that reported from other parts of the country (Table II). In the present study, one of the isolates belonged to *emm* type 42.0 which is known to be nephritogenic.

The toxin gene profile among the GAS isolates in the present study also differed to some extent from that reported from other parts of the country. In contrast to an Indian study[10], *speA* and *speC* were not detected among our isolates. *SpeB* which is a highly conserved gene, was detected in 100 per cent of the study isolates and similar finding was also observed in other geographic region[21]. SpeB antigen of *Streptococcus* has been proposed to have a causal role in glomerulonephritis. From different geographical regions of the country this gene was reported in 33.3[9] to 97 per cent[8,20] of the GAS isolates. In the present study *speG* was present in all the isolates while it was found in 86 per cent of the invasive isolates from north and south India[11]. In our study *speF* was found in 93 per cent isolates while in

Table II. *emm* distribution pattern among GAS isolates from different regions of India and the present study

Study site	North India Dhanda *et al*[9]	North India Sagar *et al*[18]	South India Menon *et al*[19]	South India Sindhulina *et al*[17]	North India Kumar *et al*[16]	Present study (Northeast India)
No. of isolates typed for *emm*	50 isolates from throat	46 throat and 48 skin isolates	25 throat, 2 blood, 8 pus, 1 urine	11 throat isolates	68 throat and 3 skin isolates	13 throat and 1 skin isolates
No. of *emm* types	20	37	13	10	14	5
Relation between type and isolation site	Throat: *emm* 74, 87, 3, 109, 11, 81, 71, 49, 68, 102.1, 86, 77, 60, 28, 89, 75, 2.1, 65, 93, 42	Throat: *emm* 1-2.2, 11.1, 12.13, 18.12, 25.2, 33.0, 43.3, 49.4, 67.0, 68.0, 69.1, 74.0, 75.0, 77.0, 80.0, 81.2, 87.0, 90-2, 93.0, 112.2, 118.0, NT Skin: *emm* 1-2.2, 11.1, 18.12, 28.5, 44.0, 49.4, 55.0, 70.0, 71.0, 75.0, 77.0, 80.0, 81.1, 81.2, 82.1, 86.2, 92.0, 100.1, 102.2, 103.0, 104.0, 106.0, 118.0, 112.2, ST1731.1, ST2861 UK.1, NT (non-typeable)	Throat: *emm* 92, 81, 70, 25, 12, 66, 75, 77/22L, ST 2905, ST 3365 Blood: *emm* 92,23 Urine: *emm* 66 Pus: *emm* 64/14, 23, 49, ST3, 365	Throat: *emm* 1-2.2, 55, 57, 71, 74, 82.1, 95, 112.2, st 11014, st2002.1	Throat: *emm* 69, 71, 75-1, 77, 81, 11, 18, 44, 68, 87, 888, 92, 118, ST 854 Skin: *emm* 71, 44	Throat: *emm* 42.0, 91.0, 123.0, 131.0 Skin: *emm* 71.0

another Indian study its prevalence was 88 per cent[8]; *speJ* was not detected in any of our isolates which was in conformity with an Indian study[15]. Haggar *et al*[11] reported detection of *speJ* in 28 per cent of invasive isolates.

Thus, there exist different M types in this part of the country with a variation in the toxin gene profile. Continuous surveillance for monitoring of prevalence of M types would be helpful for early identification of emergence of new strains.

Acknowledgment

The authors acknowledge the financial support from Indian Council of Medical Research (ICMR), New Delhi.

Conflicts of Interest: None.

Utpala Devi, Prasanta Kumar Borah, Vinita Malik, Pratap Parida & Jagadish Mahanta*
Regional Medical Research Centre,
NE Region (ICMR),
Post Box-105, Dibrugarh 786 001, Assam, India
**For correspondence*:
jmahanta@gmail.com

References

1. Euzéby JP. List of Prokaryotic names with standing in nomenclature-genus *Streptococcus*. Available from: *http://www.bacterio.net*, accessed on December 1, 2014.
2. Wong SSY, Yuen KY. *Streptococcus pyogenes* and re-emergence of scarlet fever as a public health problem. *Emerg Microbes Infect* 2012; *1* : e2.
3. Proft T, Fraser JD. Bacterial superantigen. *Clin Exp Immunol* 2003; *133* : 299-306.
4. Tandon R. Preventing rheumatic fever: M-protein based vaccine. *Indian Heart J* 2014; *66* : 64-7.
5. Centers for Disease Control and Prevention. *Streptococcus pyogenes emm* sequence database. Available from: *http://www.cdc.gov/ncidod/biotech/strep/types_emm103-124.htm*, accessed on December 1, 2014.
6. Cunningham M. Pathogenesis of group A streptococcal infections. *Clin Microbiol Rev* 2000; *13* : 470-511.
7. Schmitz FJ, Beyer A, Charpentier E, Nomark BH, Schade M, Fluit AC, *et al*. Toxin -gene profile heterogeneity among endemic invasive European group A streptococcal isolates. *J Infect Dis* 2003; *188* : 1578-86.
8. Nandi S, Chakraborti A, Bakshi DK, Rani A, Kumar R, Ganguly NK. Association of pyrogenic exotoxin genes with pharyngitis and rheumatic fever/rheumatic heart disease among Indian isolates of *Streptococcus pyogenes*. *Lett Appl Microbiol* 2002; *35* : 237-41.
9. Dhanda V, Vohra H, Kumar R. Group A *Streptococcus* virulence factors genes in north India & their association with *emm* type in pharyngitis. *Indian J Med Res* 2011; *133* : 110-5.
10. Lloyd C, Menon T. *speA* and *spe*C toxin genes among group A *Streptococcus* isolates from schoolchildren in Chennai, India. *J Med Microbiol* 2007; *56* : 1574-5.
11. Haggar A, Nerlich A, Kumar R, Abraham VJ, Brahmadathan KN, Ray P, *et al*. Clinical and microbiologic characteristics of invasive *Streptococcus pyogenes* infections in north and south India. *J Clin Microbiol* 2012; *50* : 1626-31.
12. Devi U, Borah PK, Mahanta J. The prevalence and antimicrobial susceptibility patterns of beta-hemolytic streptococci colonizing the throats of school children in Assam, India. *J Infect Dev Ctries* 2011; *5* : 804-8.
13. Centers for Disease Control and Prevention. Streptococcus Laboratory, Protocol for *emm* typing (*http://www.cdc.gov/streplab/protocol-emm-type.html*), accessed on May 1, 2016.
14. Kumar RK, Tandon R, Rheumatic fever and rheumatic heart disease: The last 50 years. *Indian J Med Res* 2013; *137* : 643-58.
15. Anand TD, Rajesh T, Rajendhran J, Gunasekaran P. Superantigen profiles of *emm* and *emm*-like typeable and nontypeable pharyngeal streptococcal isolates of south India. *Ann Clin Microbiol Antimicrob* 2012; *11* : 3.
16. Kumar R, Vohra H, Chakraborty A, Sharma YP, Bandhopadhya S, Dhanda V, *et al*. Epidemiology of group A streptococcal pharyngitis & impetigo: a cross-sectional & follow up study in a rural community of northern India. *Indian J Med Res* 2009; *130* : 765-71.
17. Sindhulina C, Geethalakshmi S, Thenmozhivalli PR, Jose JM, Brahmadathan KN. Bacteriological and molecular studies of group A streptococcal pharyngitis in a south Indian hospital. *Indian J Med Microbiol* 2008; *26* : 197-8.
18. Sagar V, Kumar R, Ganguly NK, Charaborty A. Comparative analysis of *emm* type pattern of group A streptococcus throat and skin isolates from India and their association with closely related SIC, a streptococcal virulent factor. *BMC Microbiol* 2008; *8* : 150.
19. Menon T, Whatmore AM, Srivani S, Kumar MP, Anbumani N, Rajaji S. EMM types of *Streptococcus pyogenes* in Chennai. *Indian J Med Microbiol* 2001; *19* : 161-2.
20. Mathur P, Bhardwaj N, Mathur K, Behera B, Gupta G, Kapil A, *et al*. Clinical and molecular epidemiology of beta-hemolytic streptococcal infections in India. *J Infect Dev Ctries* 2014; *8* : 297-303.
21. Mengeloglu FZ, Aktas E, Otlu B, Comert F, Kulah C, Tas E, *et al*. Evaluation of *emm* gene types, toxin gene profiles and clonal relatedness of group A streptococci. *Bosn J Basic Med Sci* 2013; *13* : 163-9.