Indian J Med Res 139, March 2014, pp 438-445

Antibiotic susceptibilities, streptococcal pyrogenic exotoxin gene profiles among clinical isolates of group C or G *Streptococcus dysgalactiae* subsp. *equisimilis* & of group G *S. anginosus* group at a tertiary care centre

Bijayini Behera[*,1], Purva Mathur[*], Nidhi Bhardwaj[**], Neetu Jain[*], M.C. Misra[***], Arti Kapil[#] & Sarman Singh[##]

Departments of [*]Microbiology, [**]Laboratory Medicine, [***]Surgical Disciplines, JPNA Trauma Centre, AIIMS & Departments of [#]Microbiology & [##]Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, India

Received January 2, 2012

***Background & objectives*: Group C and group G streptococci (together GCGS) are often regarded as commensal bacteria and their role in streptococcal disease burden is under-recognized. While reports of recovery of GCGS from normally sterile body sites are increasing, their resistance to macrolides, fluoroquinolone further warrants all invasive β haemolytic streptococci to be identified to the species level and accurately tested for antimicrobial susceptibility. This study was aimed to determine the prevalence, clinical profile, antimicrobial susceptibility and streptococcal pyrogenic exotoxin gene profile (*speA, speB, speC, speF, smeZ, speI, speM, speG, speH* and *ssa*) of GCGS obtained over a period of two years at a tertiary care centre from north India.**

***Methods*: The clinical samples were processed as per standard microbiological techniques. β-haemolytic streptococci (BHS) were characterized and grouped. Antimicrobial susceptibility of GCGS was performed using disk diffusion method. All GCGS were characterized for the presence of streptococcal pyrogenic exotoxins (spe) and spe genes were amplified by PCR method.**

***Results*: GCGS (23 GGS, 2GCS) comprised 16 per cent of β haemolytic streptococci (25/142 βHS, 16%) isolated over the study period. Of the 25 GCGS, 22 (88%) were recovered from pus, two (8%) from respiratory tract, whereas one isolate was recovered from blood of a fatal case of septicaemia. Of the total 23 GGS isolates, 18 (78%) were identified as *Streptococcus dysgalactiae* subsp *equisimilis* (SDSE, large-colony phenotype), five (21%) were *Streptococcus anginosus* group (SAG, small-colony phenotype). The two GCS were identified as SDSE. All GCGS isolates were susceptible to penicillin, vancomycin, and linezolid. Tetracycline resistance was noted in 50 per cent of SDSE isolates. The rates of macrolide and fluoroquinolone resistance in SDSE were low. Twelve of the 20 SDSE isolates were positive for one or more *spe* genes, with five of the SDSE isolates simultaneously carrying *speA+ speB+ smeZ+ speF* or *speB+ smeZ+speF, speI+speM+speG+speH* or, *speI+spe M+speH* or *speA+ speB+ speC+ smeZ+ speF*. One notable finding was the presence of *spe B* in four of the five isolates of the *Streptococcus anginosus* group. No isolate was positive for *ssa*.**

***Interpretation & conclusions*: Our study showed no association between GCGS isolates harbouring streptococcal pyrogenic exotoxins and disease severity. This might be attributed to the small sample size of *spe*-positive isolates.**

Key words β-haemolytic streptococci - groups C and G streptococci - streptococcal pyrogenic exotoxins - superantigens - *Streptococcus dysgalactiae* subsp. *equisimilis* - *S. anginosus* group

[1]*Present address*: Department of Microbiology, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India

Beta-haemolytic streptococci (βHS) of Lancefield groups C and G (together GCGS) are emerging as pathogens of increasing interest world-over[1]. Traditionally regarded as commensals, the spectrum of human infections caused by GCGS appears to be ever increasing[1]. While at one end, reports of the recovery of GCGS from various invasive infections like bacteraemia, endocarditis, meningitis, arthritis, osteomyelitis, pneumonia are on rise; a few recent studies have also reported higher GCGS throat colonization rates relative to group A *Streptococcus* (*Streptococcus pyogenes*, GAS)[1-3].

In addition to being classified by Lancefield group carbohydrate, GCGS are also distinguished morphologically on the basis of whether they form large or small colonies on sheep blood agar plates[4]. GCGS large-colony phenotypes are usually associated with human infection and are classified in the same subspecies, *Streptococcus dysgalactiae* subsp. *equisimilis* subsp. nov (SDSE)[5]. After being considered non pathogenic for many years, SDSE is now recognized as an important bacterial pathogen. The clinical spectrum of diseases caused by this species closely resembles *S. pyogenes* infections, including the occurrence of post streptococcal sequelae[6]. Small colony forming species are placed in the *S. anginosus* group (SAG, formerly known as *S. milleri*) and are less common causes of invasive infections, compared to SDSE. The SAG comprises three species *S. anginosus*, *S. intermedius,* and *S. constellatus* of which two subspecies, *S. constellatus* subsp. *constellatus* and *S. constellatus* subsp. *pharyngis*, are further distinguished[7]. Streptococci of the anginosus group can reside commensally in various mucosal sites, such as the intestinal and the genital tracts, in addition to the human oral cavity, but are reported to cause pharyngitis, bacteraemia, and serious purulent infections in the deep neck and soft tissue and in internal organs such as the brain, lung, and liver[8,9]. The differences of habitat and pathogenicity among distinct species emphasize the interest of the distinction of *S. anginosus* from *S. constellatus* among groups C and G isolates of the SAG.

Although GCGS isolates remain almost uniformly susceptible to penicillin and other β-lactam agents, there are recent reports of isolates with a slightly increased penicillin minimum inhibitory concentration (MIC) of 0.25 μg/ml[10]. Reports of macrolide and fluoroquinolone resistance in GCGS further warrants all invasive βHS to be identified to the species level and accurately tested for antimicrobial susceptibility[10].

Several streptococcal pyrogenic exotoxins (Spe) have been shown to play major roles in invasive GAS infections, some of these effectors belong to the family of superantigens (SAgs)[11]. Due to their designation as minor human pathogens, the virulence attributes of human GCGS are less extensively studied than those of GAS. A few studies which have examined the presence of the GAS-associated virulence repertoire in human GCGS have substantially aided in understanding the potential role of these SAgs in the pathogenesis of GCS and GGS[12,13]. However, very few studies have looked into the role of various Spe in the pathogenesis of SDSE infections.

This study was aimed to describe the clinical and molecular characteristics of GCGS obtained over a period of two years at a tertiary care hospital of north India.

Material & Methods

During a two year period (from January 2009 to December 2010), various clinical samples including pus, blood, wound, endotracheal aspirates and throat swab. Out of 25 GCGS isolates during the study 22 (85%) were recovered from pus and wound, two (11.5%) were recovered from respiratory tract (endotracheal aspirate and throat swab) and one isolate was recovered from blood of a fatal case of septicemia were received from outpatients and admitted patients in the clinical Microbiology laboratories of the 190-bedded, level-1 Trauma Centre of the All India Institute of Medical Sciences (AIIMS) and the 2000 bedded AIIMS hospital, New Delhi, India. The clinical samples were processed according to standard microbiological techniques[14]. βHS were initially identified by their ability to lyse red cells on sheep blood agar plates (BioMe´rieux, France). βHS were further characterized by Gram stain, a negative catalase reaction, zone diameter around bacitracin disk (0.04 units) and colony size (large colony vs small colony βHS). Small colonies were defined as having "sand-like" morphology, with a colony diameter of less than 0.5 mm. Large colonies were defined as having a diameter of ≥1.0 mm. Two clinical isolates were recovered from a private hospital in south India which were sent to us for characterization. The study was approved by the institute's ethical committee.

All the isolates (n=142) were identified by the Vitek -2 system, using GP-61 cards. (BioMerieux Vitek, Hazelwood, Mo, France). Grouping was performed by the agglutination kit (Histrep™, Hi Media laboratories, Mumbai, India) according to the manufacturer's instructions.

Antimicrobial susceptibility testing of GCGS was performed using disk-diffusion methods on sheep blood agar plates according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[15]. The following antibiotics were tested by the disc diffusion method: Penicillin G (10 units), ampicillin (10 µg), amoxicillin / clavulanic acid (20/10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), tetracycline (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), clindamycin (2 µg), vancomycin (30 µg), teicoplanin (30 µg), and linezolid (30 µg) (BBL™ BD, USA). The minimum inhibitory concentrations (MICs) of Penicillin G, ampicillin, ceftriaxone, ciprofloxacin, levofloxacin, tetracycline, erythromycin, clindamycin, vancomycin and linezolide were determined by E-test BioMerieux, France according to the manufacturer's instructions. The inhibition zone diameters and MIC breakpoints were adopted according to CLSI guidelines for βHS[15]. Since CLSI does not provide breakpoints for ciprofloxacin, the levofloxacin/ofloxacin breakpoints were taken for interpretation. *S. pneumoniae* ATCC 49619 was taken as control for all antimicrobial susceptibility testing methods.

All GCGS were characterized for the presence of streptococcal pyrogenic exotoxins (Spe). For this, template DNAs from GCS and GGS was extracted by using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Primers used for the amplification of (*speA, speB, speC, speF, smeZ, speI, speM, speG, speH* and *ssa*) genes are shown in Table I[16-21]. *S. pyogenes* ATCC strains 12351 was used as positive control for *speA, speB, speC, speF and smeZ,* and *S. pyogenes* ATCC 12344, 700294 and 51500 were used as positive controls for *speA, speC,* and *ssa*, respectively.

Results

A total of 142 isolates of βHS were recovered from various clinical samples (n=13,203) received in the microbiology laboratories of Trauma Centre and AIIMS hospital. Of these 142 isolates, GAS comprised 109 (77%) isolates. Five isolates (3.5%) belonged to group B and two (1.4%) to group F. Twenty (n=20) β-haemolytic streptococcal isolates were of large colony phenotype and five were of small colony phenotype. All the 20 large colony phenotype BHS isolates were unequivocally identified as *S. dysgalactiae* subsp. *equisimilis* by the Vitek 2 system with 99%

Table I. Primers and PCR conditions for amplification of superantigens genes

Gene	Primer direction	Primers sequence	Annealing temperature (°C)	Amplicon size (bp)
speA[16]	Forward Reverse	CCAAGCCAACTTCACAGATC CCCTTCATGATTTGTTACCCC	60	309
speB[17]	Forward Reverse	GTGGAGTCTCTGACGGCTTC GTGTTTTCGGCACAAAAGGT	50	170
speC[18]	Forward Reverse	GAT TTC TAC TTA TTT CAC C AAA TAT CTG ATC TAG TCC C	47	584
ssa[16]	Forward Reverse	TGAGGTAATTGGGGAGATGA CTAATTCTTGAACAGTGACTTG	53	621
speF[18]	Forward Reverse	TAC TTG GAT CAA GAC G GTA ATT AAT GGT GTA GCC	45	782
smeZ[19]	Forward Reverse	CAGATATAGTAATTGATTTTA AGCTAGAACCAGAAGAATAT	45	399
speI[20]	Forward Reverse	ATC TTT CAT GGG TAC G TTT CAT GTT TAT TGC C	47	678
speM[20]	Forward Reverse	GCTCTATACACTACTGAGAGTGTC CATATCAATCGTTTCATTATCTG	55	612
speG[21]	Forward Reverse	AGA AAC TTA TTT GCC C TAG TAG CAA GGA AAA GG	48	115
speH[21]	Forward Reverse	AGA TTG GAT ATC ACA GG CTA TTC TCT CGT TAT TGG	49	416

Superscript numerals denote reference numbers

confidence. Eighteen of the 20 *S. dysgalactiae* subsp. *equisimilis* isolates belonged to group G, while two belonged to group C. Five small colony phenotype β-haemolytic streptococci were identified as belonging to "*Streptococcus anginosus* group" by the Vitek system with >95% confidence. All isolates belonged to Lancefield group G (Table II). Of the 25 GCGS (23 GGS, 2 GCS) 22 (85%) were recovered from pus, two (11.5%) from respiratory tract (endotracheal aspirate and throat swab), whereas one isolate was recovered from blood of a fatal case of septicaemia.

Of the total 23 GGS isolates (large-colony phenotype), eighteen (78%) were identified as SDSE, five (small-colony phenotype) (21%) were identified as SAG. The two GCS (large-colony phenotype) were identified as SDSE. The clinical and microbiological details are shown in Table II.

All the 25 GCGS isolates were sensitive to penicillin, ampicillin, vancomycin and linezolid. The antimicrobial susceptibility of SDSE is summarized in Table III.

Twelve of the 20 SDSE isolates screened during this study were positive for one or more *spe* genes, with five of the SDSE isolates simultaneously carrying *spe A+ speB+ sme + speF* or *speB+ smeZ+speF, spe I+speM+speG+speH* or, *speI+ speM+ speH* or *spe A+ spe B+ spe C+ sme Z+ spe F*. One notable finding was the presence of *speB* in four of the five isolates of the *Streptococcus anginosus* group. No isolate was positive for *ssa* (Table IV).

Table II. Clinical and microbiological characteristics of SDSE *Streptococcus dysgalactiae* subsp *equisimilis* isolates

Patient No.	Age (yr)/ gender	Diagnosis	Isolation sites	Lancefield group antigen	Outcome
1	30/M	Septicaemia	Blood and pus	G	Expired
2	16/M	RTA, cellulitis	Pus	G	Recovered
3	19/M	RTA, cellulitis	Pus	G	Recovered
4	30/M	Surgical wound infection; degloving injury	Wound swab	C	Recovered
5	28/M	Non-healing ulcer, lower extremity	Pus	G	Recovered
6	12/M	Purulent discharge from previous pigtail catheter site.	Pus	G	Recovered
7	30/M	RTA, ventilator-associated pneumonia	Endotracheal aspirate	G	Recovered
8	23/M	Post-laparotomy ventral hernia	Pus	G	Recovered
9	20/F	RTA, Post-surgical infection	Pus	C	Recovered
10	12/F	Ulcer, soft palate	Pus	G	Recovered
11	15/F	Acute tonsillo-pharyngitis	Throat swab	G	Recovered
12	31/F	Non-healing ulcer	Pus	G	Recovered
13	17/M	RTA, wound-Infection	Wound swab	G	Recovered
14	45/M	Post amputation raw area	Pus	G	Recovered
15	25/M	Non-healing ulcer	Pus	G	Recovered
16	44/M	RTA, post-surgical wound	Pus	G	Recovered
17	37/M	RTA, post-surgical wound	Pus	G	Recovered
18	29/M	RTA, post-surgical wound	Pus	G	Recovered
19	28/M	RTA, post-surgical wound	Pus	G	Recovered
20	45/F	Abdominal abscess	Pus	G	Recovered

RTA, road traffic accident

Table III. Antibiotic susceptibility of *S. dysgalactiae* subsp. *equisimilis* (SDSE) isolates (n=20) in the study

Antibiotic	CLSI MIC breakpoints (μg/ml)			MIC range of clinical isolates (μg/ml)	Susceptible N (%)	Intermediate N (%)	Resistant N (%)
	Resistance	Intermediate	Susceptible				
Ampicillin	-	-	≤0.25	0.016 - 0.125	20 (100)	0	0
Penicillin	-	-	≤0.12	0.008 - 0.10	20 (100)	0	0
Erythromycin	≥1	0.5	≤0.25	0.023 - > 256	18 (92)	0	2 (8)
Clindamycin	≥1	0.5	≤0.25	0.023- 0.5	19 (96)	1 (4)	0
Vancomycin	-	-	≤1	0.19 - 1	20 (100)	0	0
Linezolid	-	-	≤2	0.25 - 1	20 (100)	0	0
Tetracycline	≥8	4	≤2	0.19 - 48	10 (50)	3 (15)	7 (35)
Ciprofloxacin*	≥8	4	≤2	0.25 - >32	16 (80)	4 (20)	0
Levofloxacin	≥8	4	≤2	0.125 - > 32	20 (100)	0	0
Ceftriaxone	-	-	≤0.5	0.016 - 3	20 (100)	0	0

*Breakpoints for ciprofloxacin have not been provided by CLSI. Therefore, levofloxacin/ofloxacin breakpoints were taken for interpretation

Discussion

The prevalence of invasive (*e.g.* STSS (Streptococcal toxic shock syndrome), necrotizing fasciitis, cellulitis, urosepsis, and pneumonia) and non invasive SDSE infections has increased gradually over the years in India and other Asian countries. In our study, GAS was the predominant βHS, followed by GCGS. In an earlier study from our institute, which examined the prevalence of GGS and GCS in various body samples from outdoor and admitted patients during a five year period, the prevalence of GCGS among βHS was 14.7 per cent. Respiratory tract was the most common source of GGS (92%), followed by soft tissues (5%)[22]. In the present study, the majority of GCGS were recovered from pus samples of post surgical infections in trauma patients.

Among the GCGS, there was four-fold higher isolation of SDSE with respect to SAG. SDSE remains the most common pathogen among human GCGS. Similar observations have been made in a study from Vellore, south India, which found a high incidence of *Streptococcus dysgalactiae* subsp. *equisimilis* (81%) relative to *S. anginosus* (19%) in a 2-year study period (2006-2007)[7].

The usefulness of the Vitek 2 system in species level identification of Group G streptococci has been compared to the molecular gold standard '16S rRNA gene sequencing' by Woo *et al*[23]. In that study, Vitek system (GPI) was able to identify 94 per cent group G streptococcal isolates as SDSE with >95% confidence[23]. In another study[24], pyrosequencing methodology was compared to the biochemical systems Vitek 2[24]. Full accordance between pyrosequencing and Vitek 2 was observed for S. *dysgalactiae* isolates belonging to group G. The streptococcal isolates belonging to anginosus group (*S. anginosus, S constellatus)* was better resolved with the biochemical systems (Vitek 2) in comparison to pyrosequencing[24].

Comprehensive examinations of the GAS-associated virulence repertoire in human GCGS have been attempted by a few investigators[12,13]. Davies *et al*[12] used a group A streptococcal virulence array comprising 60 genes previously described as GAS virulence factors, and another 159 genes predicted to encode for extracellular proteins to examine the extent of their occurrence in a contemporary collection of GCGS isolates, pooled from GAS endemic and non-endemic regions. Between 25 and 50 per cent of the genes represented on the array were present in the GCGS isolates. In relation to the frequency of the known and putative GAS virulence factors, there was no distinction between the isolates whether these were derived from a GAS endemic or a non-endemic region[12]. In another study by Hashikawa *et al*[13], which characterized 12 GCGS isolates (isolated from cases of streptococcal toxic shock syndrome) for 13 Spe (*speA, speB, speC, speF, smeZ, spegg, speH, speI, speJ, speL, mf-2,mf-3, sagA*), only *sagA* gene was found in all of the strains except one. The *speG* gene was detected

Table IV. Exotoxin gene profile of *S. dysgalactiae* subsp. *equisimilis* (SDSE) (n=20)

Isolate no.	*Gene*									
	speA	*speB*	*speC*	*ssa*	*smeZ*	*speF*	*spe I*	*speM*	*spe G*	*spe H*
1										
2					Positive					
3		Positive			Positive	Positive				
4									Positive	
5										
6					Positive					
7										
8					Positive					
9										
10					Positive					
11										
12										
13					Positive					
14	Positive	Positive			Positive	Positive				
15							Positive	Positive	Positive	Positive
16	Positive	Positive	Positive		Positive	Positive				
17							Positive	Positive		Positive
18										
19										
20					Positive					

in seven *S. dysgalactiae* isolates, whereas none of the other 11 *spe* genes could be detected[13]. Kittang *et al*[25] also concluded that speG^{dys} and other Sags might not play a major role in the pathogenesis of severe human disease caused by SDSE. Kalia and Bessen[26] have described the presence of exotoxins A and C in GGS being identical to *S. pyogenes genes*. A study by Prabu and Menon[27], which looked for the presence of *speA, speB, speC* and *speG* genes encoding SPEs in 131GCS/GGS isolates from both patients as well as asymptomatic carriers, found 12.97 per cent possessed *speG* gene, 4.5 per cent possessed *speC* gene, 2.29 per cent possessed both *speC* and *speG* genes and none of the isolates possessed the *speA* or the *speB* gene. All the 20 isolates positive for *speC/speG* genes were GGS[27]. Another study also supported streptococcal pyrogenic exotoxins gene transfer from GGS to GAS, particularly streptococcal pyrogenic exotoxin G [speG(dys)][28].

In the present study, SDSE isolates harbouring more than one Spe were recovered from non-fatal cases of cellulitis, and had different types of *spe* combinations. Twelve of the 20 SDSE isolates screened during this study were positive for one or more Spe genes. No isolate was positive for *ssa* and *smeZ* was the commonest *spe* detected. It could be due to sequence variations in the priming sequence or may be the strain harboured a Spe that was not included in the screen such as mf-2. Based on the findings of the present study, there are no clear cut relationships between GCGS isolates harbouring streptococcal pyrogenic exotoxins (Spe) and disease severity, although this may be a reflection of the small sample size of spe-positive isolates. Presence of *speB* has not been reported in GGS and SAG. We could not ascertain the specificity of the *speB* PCR by sequencing and that this was a limitation of this study.

According to literature, human GCGS isolates remain almost uniformly susceptible to penicillin and other β-lactam agents, and penicillin is still considered the drug of choice[1]. Isolates with a slightly increased MIC (0.12 and of 0.25 µg/ml) for penicillin were recently reported in SAG and SDSE[29,30]. Macrolide resistance in SDSE has been shown to be widespread in many countries, with rates up to 16 per cent in Europe, 19 per cent in the United States and 24 per cent in Hong Kong[29,30]. The prevalence of macrolide resistance in members of SAG is low and limited

to certain geographical regions[29,30]. Tetracycline no longer represents an option for the empirical treatment of SDSE isolates; resistance rates upto 60 per cent and higher have been reported[29,30]. In North America and Europe, the incidence of fluoroquinolone-resistance among βHS (1%) remains low[29,30]. However, there are occasional reports of fluoroquinolone-resistance in members of SAG[29,30]. In our study, all SAG isolates remained susceptible to all the antimicrobials tested. Tetracycline resistance was noted in 50 per cent of SDSE isolates. The rates of macrolide and fluoroquinolone resistance in SDSE were low. Inducible clindamycin resistance was not detected in erythromycin resistant SDSE isolates.

The role of GCGS in streptococcal disease burden is under-recognized by clinicians and microbiologists. Accumulating indications of the considerable clinical relevance of the GSGS and the propensity of these bacteria to develop antimicrobial drug resistance suggest that these may be a group of emerging pathogens that should be monitored[31].

In conclusion, 12 of the 20 SDSE isolates screened during this study were positive for one or more Spe genes. SDSE isolates harbouring more than one Spe were recovered from non-fatal cases of cellulitis. One notable finding was the presence of *speB* in four of the five isolates of the *Streptococcus anginosus* group. However, no association could be found between GCGS isolates harbouring streptococcal pyrogenic exotoxins and disease severity in the present study; this might be attributed to the small sample size of spe-positive isolates.

Acknowledgment

Authors thank Shrimati Neelu, Sweety, Rajrani, Shriyut Trilok, Pawan, Vineeth and Miss Deepali, for technical support. The research project was funded by the Indian Council of Medical Research, New Delhi.

References

1. Williams GS. Group C and G streptococci infections: emerging challenges. *Clin Lab Sci* 2003; *16* : 209-13.
2. Bramhachari PV, Kaul SY, McMillan DJ, Shaila MS, Karmarkar MG, Sriprakash KS. Disease burden due to *Streptococcus dysgalactiae* subsp. equisimilis (group G and C streptococcus) is higher than that due to *Streptococcus pyogenes* among Mumbai school children. *J Med Microbiol* 2010; *59* : 220-3.
3. McDonald M, Towers RJ, Andrews RM, Carapetis JR, Currie BJ. Epidemiology of *Streptococcus dysgalactiae* subsp. equisimilis in tropical communities, Northern Australia. *Emerg Infect Dis* 2007; *13* : 1694-700.
4. Johnson CC, Tunkel AR. β-hemolytic (groups C and G) streptococci. In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas and Bennett's principles and practices of infectious disease*, 4th ed. New York: Churchill Livingstone; 1995. p. 1852-60.
5. Vandamme P, Pot B, Falsen E, Kersters K, Devriese LA. Taxonomic study of Lancefield streptococcal groups C, G, and L *(Streptococcusdys galactiae)* and proposal of *S. dysgalactiae* subsp. *Equisimilis* subsp. nov. *Int J Syst Bacteriol* 1996; *46* : 774-81.
6. Brandt CM, Spellerberg B. Human infections due to *Streptococcus dysgalactiae* subspecies *equisimilis. Clin Infect Dis* 2009; *49* : 766-72.
7. Reissmann S, Friedrichs C, Rajkumari R, Itzek A, Fulde M, Rodloff AC, *et al*. Contribution of *Streptococcus anginosus* to infections caused by groups C and G streptococci, southern India. *Emerg Infect Dis* 2010; *16* : 656-63.
8. Whiley RA, Beighton D, Winstanley TG, Fraser HY, Hardie JM. *Streptococcus intermedius, Streptococcus constellatus*, and *Streptococcus anginosus* (the *Streptococcus milleri* group): association with different body sites and clinical infections. *J Clin Microbiol* 1992; *30* : 243-4.
9. Ruoff KL. *Streptococcus anginosus* ("*Streptococcus milleri*"): the unrecognized pathogen. *Clin Microbiol Rev* 1988; *1* : 102-8.
10. Biedenbach DJ, Toleman MA, Walsh TR, Jones RN. Characterization of fluoroquinolone-resistant β -hemolytic *Streptococcus* spp. isolated in North America and Europe including the first report of fluoroquinolone-resistant *Streptococcus dysgalactiae* subspecies *equisimilis:* report from the SENTRY Antimicrobial Surveillance Program (1997-2004). *Diagn Microbiol Infect Dis* 2006; *55* : 119-27.
11. Kotb M. Bacterial pyrogenic exotoxins as superantigens. *Clin Microbiol Rev* 1995; *8* : 411-26.
12. Davies MR, McMillan DJ, Sriprakash KS, Chhatwal GS. Distribution of group A streptococcal virulence genes in group C and G streptococci. *Int Congress Ser* 2006; *1289* : 184-7.
13. Hashikawa S, Iinuma Y, Furushita M, Ohkura T, Nada T, Torii K, *et al*. Characterization of group C and G streptococcal strains that cause streptococcal toxic shock syndrome. *J Clin Microbiol* 2004; *42* : 186-92.
14. Collee JG, Miles RS, Watt B. Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. *Mackie and McCartney practical medical microbiology*, 14th ed. Edinburgh: Churchill Livingstone; 1996. p. 131-49.
15. Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing;* Twenty-first informational supplements. CLSI Document M100-S21, Wayne, PA: CLSI; 2011.
16. Commons R, Rogers S, Gooding T, Danchin M, Carapetis J, Robins-Browne R, *et al*. Superantigen genes in group A streptococcal isolates and their relationship with emm types. *J Med Microbiol* 2008; *57* : 1238-46.
17. Jing HB, Ning BA, Hao HJ, Zheng YL, Chang D, Jiang W, *et al*. Epidemiological analysis of group A streptococci recovered from patients in China. *J Med Microbiol* 2006; *55* : 1101-7.

18. Schmitz FJ, Beyer A, Charpentier E, Normark 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.

19. Darenberg J, Luca-Harari B, Jasir A, Sandgren A, Pettersson H, Schalen C, *et al*. Molecular and clinical characterization of invasive group A streptococcal infection in Sweden. *Clin Infect Dis* 2007; *45* : 450-8.

20. Rivera A, Rebollo M, Miro E, Mateo M, Navarro F, Gurgui M, *et al*. Superantigen gene profile, *emm* type and antibiotic resistance genes among group A streptococcal isolates from Barcelona, Spain. *J Med Microbiol* 2006; *55* : 1115-23.

21. Vlaminckx BJ, Mascini EM, Schellekens J, Schouls LM, Paauw A, Fluit AC, *et al*. Site-specific manifestations of invasive group A streptococcal disease: Type distribution and corresponding patterns of virulence determinants. *J Clin Microbiol* 2003; *41* : 4941-9.

22. Mathur P, Kapil A, Das B. Prevalence of group G & group C streptococci at an Indian tertiary care centre.*Indian J Med Res* 2004; *120* : 199-200.

23. Woo PC, Fung AM, Lau SK, Wong SS, Yuen KY. Group G beta-hemolytic streptococcal bacteremia characterized by 16S ribosomal RNA gene sequencing. *J Clin Microbiol* 2001; *39* : 3147-55.

24. Innings A, Krabbe M, Ullberg M, Herrmann B. Identification of 43 *Streptococcus* species by pyrosequencing analysis of the *rnpB* gene. *J Clin Microbiol* 2005; *43* : 5983-9.

25. Kittang BR, Skrede S, Langeland N, Haanshuus CG, Mylvaganam H. *emm* gene diversity, superantigen gene profiles and presence of *SlaA* among clinical isolates of group A, C and G streptococci from western Norway. *Eur J Clin Microbiol Infect Dis* 2011; *30* : 423-33.

26. Kalia A, Bessen DE. Presence of streptoccal pyrogenic exotoxin A and C genes in human isolates of group G streptococci. *FEMS Microbiol Lett* 2003; *219* : 291-5.

27. Prabu D, Menon T. Genotypic characterization of toxigenic group C and G streptococci isolated in Chennai, South India. *Folia Microbiol (Praha)* 2011; *56* : 345-8.

28. Sachse S, Seidel P, Gerlach D, Günther E, Rödel J, Straube E, *et al*. Superantigen-like gene(s) in human pathogenic *Streptococcus dysgalactiae*, subsp. *equisimilis*: genomic localisation of the gene encoding streptococcal pyrogenic exotoxin G (speG(dys)). *FEMS Immunol Med Microbiol* 2002; *34* : 159-67.

29. Asmah N, Eberspächer B, Regnath T, Arvand M. Prevalence of erythromycin and clindamycin resistance among clinical isolates of the *Streptococcus anginosus* group in Germany. *J Med Microbiol* 2009; *58* : 222-7.

30. Brown DF, Hope R, Livermore DM, Brick G, Broughton K, George RC, *et al*. Non-susceptibility trends among enterococci and non-pneumococcal streptococci from bacteraemias in the UK and Ireland, 2001-06. *J Antimicrob Chemother* 2008; *62* (Suppl 2): 75-85.

31. Takahashi T, Ubukata K, Watanabe H. Invasive infection caused by *Streptococcus dysgalactiae* subsp. *equisimilis*: characteristics of strains and clinical features. *J Infect Chemother* 2011; *17* : 1-10.

Reprint requests: Dr Purva Mathur, Associate Professor, Department of Laboratory Medicine, JPNA Trauma Centre
All India Institute of Medical Sciences, New Delhi 110 029, India
e-mail: purvamathur@yahoo.co.in