emm type diversity of β -haemolytic streptococci recovered in Chennai, India

Beta-haemolytic streptococci (BHS) are widely spread human pathogens, and include group A, G and C streptococci. Group A streptococci (GAS) are mostly found in the throat and on the skin and commonly cause pharyngitis and impetigo, although they are most notorious for causing a broad spectrum of severe invasive disease (Cunningham, 2000). Recent studies have indicated that group C and G streptococci (GCS, GGS) also carry *Streptococcus pyogenes* superantigen genes and have been reported with increasing frequency from a variety of severe infections (Davies *et al.*, 2005; Kalia & Bessen, 2003). The M type specific typing scheme for GAS, based upon the hypervariable region of the *emm* gene, also applies to GCS and GGS (Bisno *et al.*, 1996; Collins *et al.*, 1992).

The objective of this study was to investigate the diversity of BHS in Chennai, south India, by *emm* gene sequencing.

Ninety-five isolates comprising 49 GGS, 34 GAS and 12 GCS from our laboratory collection were *emm* typed/subtyped and T typed according to standard protocols (http://www.cdc.gov/ncidod/biotech/strep/protocol_emm-type.htm). Two isolates were from blood; the remainder were from non-invasive infections. With the exception of eight isolates, all were recovered from individuals within the age range of 2–15 years.

Approval for the research was obtained from the Ethical Committee of the institution.

Forty-nine of the 95 isolates (51.6%) were found to be GGS, 12 (12.6%) were GCS and 34 (35.8%) were grouped as GAS. All 61 GGS/GCS and one GAS isolate (*emm*49.4) were bacitracin-resistant.

Fourteen *emm* types were identified among the 49 GGS isolates, the most common type being *stG485* (12/49) followed by *stGLP1* (6/49) (Table 1). *stG485* was also

the most common type reported from group G bacteraemia in Jerusalem, from toxigenic GGS strains in Japan and from GCS strains in Portugal, and was found from a GAS strain in Hungary (Cohen-Poradosu *et al.*, 2004; Hashikawa *et al.*, 2004; Pinho *et al.*, 2006; Krucsó *et al.*, 2005). *stG6792* and *stGLP1* each represented 25% of the pyoderma isolates, and have also been reported from Portugal (Pinho *et al.*, 2006). Four of our isolates were *stC5345*, a type originally reported from invasive isolates in Argentina (Lopardo *et al.*, 2005); other GGS *emm* types in our study included *stC36*, *stG2078*, *stC1400*, *stG4831*, *stG643*, *stG10* and *stG2574*, which have been previously reported from invasive infections (Horii *et al.*, 2006; Cohen-Poradosu *et al.*, 2004; Pinho *et al.*, 2006; Lopardo *et al.*, 2005).

The reported prevalence of GCS/GGS carriage and disease incidence varies greatly worldwide. Most studies originate from temporal climate regions of the northern hemisphere and limited data are available from tropical regions (McDonald *et al.*, 2007).

Twelve GCS were isolated, representing five different *emm* types; the most prevalent were *stG245.0* (4/12, 33.33%) and *stC1400* (4/12, 33.33%). Subtype *stC1400.0* was found in both GGS and GCS (Table 1). One type found among the GCS, *stCK401*, was originally found in a GAS isolate recovered in the Northern Territory of Australia (see http://www.cdc.gov/ncidod/biotech/strep/types_emm103-124.htm).

We found two new *emm* sequence types among the GGS and GCS in this study, *stG866*, found in two GGS isolates associated with pharyngotonsillitis, and *stC922.0*, from a GCS isolate associated with pharyngitis.

Thirty-four GAS isolates could be typed as 22 distinct *emm* types, reflecting high strain diversity (22/34, 64.7%). The most

common *emm* type was *emm49*, accounting for four isolates and two different subtypes, followed by *emm82* and *emm74*. We found two non-invasive isolates each of *emm121*, *emm118*, *emm71* and *emm105*. Two independent type *emm109* invasive isolates were also identified. Other *emm* types were represented by only one isolate each. We had a few types rarely or never reported in the USA, which included *emm8*, *emm55*, *emm95*, *emm71*, *emm74*, *emm104*, *emm105*, *emm106*, *emm109* and *st2460* (Shulman *et al.*, 2004). In contrast, six of these types (*emm55*, *emm71*, *emm74*, *emm104*, *emm105* and *emm106*) were observed among a recent collection of non-invasive isolates recovered in western Nepal (Sakota *et al.*, 2006). Five of these types (*emm8*, *emm74*, *emm95*, *emm106* and *emm109*) were found during a carriage study in Ethiopia (Abdissa *et al.*, 2006), and four of these types (*emm55*, *emm71*, *emm74* and *emm95*) were found during invasive GAS surveillance in Israel (Moses *et al.*, 2003). We also found, similar to previous observations among isolates recovered in Nepal, that M types did not have the characteristic T type associations observed in the USA and western Europe (Johnson *et al.*, 2006). These unusual associations (for the USA and western Europe) included T12/27/*emm49*, TB3264/*emm82*, T14/27/Imp19/*emm28*, T2/*emm75* and T8/*emm87*. The T12/*emm49*, TB3264/*emm82* and T2/*emm75* associations were also observed among the isolates recovered in Nepal, and further genetic characterization revealed the Nepalese isolates to be of completely different clonal lineages to the corresponding *emm28*, *emm49*, *emm82*, *emm75* and *emm87* isolates recovered in the USA (Sakota *et al.*, 2006).

The prevalence of *emm*-typable GGS/GCS in this region could reflect a high association of these strains with non-invasive infections, and could also conceivably reflect significant association

Table 1. *emm* subtypes and groups of BHS recovered in Chennai

<i>emm</i> subtype (T type if group A)	No. of isolates
GAS	
<i>emm49.0</i> (T14, T27/12)	2
<i>emm49.4</i> (T27/12, T12)	2
<i>emm74.0</i> (T3/13/B3264/5/27/44; T3/13/B3264; B3264)	3
<i>emm82.1</i> (T14; T3/13/B3264)	2
<i>emm82.0</i> (TB3264)	1
<i>emm71.0</i> (T1/3/13/B3264; 13/B3264)	2
<i>emm118.0</i> (T27/12; T-nontypeable)	2
<i>emm109.0</i> (T6)	1
<i>emm109.1</i> (T6)	1
<i>emm121.0</i> (T-nontypeable {2})	2
<i>emm105.0</i> (TB3264; T-nontypeable)	2
<i>st2460.1</i> (T3/13/B3264)	1
<i>emm8.0</i> (T-nontypeable)	1
<i>emm28.5</i> (T27/14/Imp19)	1
<i>emm55.0</i> (T-nontypeable)	1
<i>emm58.8</i> (T-nontypeable)	1
<i>emm66.0</i> (T4)	1
<i>emm75.1</i> (T2)	1
<i>emm77.0</i> (T-nontypeable)	1
<i>emm87.0</i> (T8)	1
<i>emm93.0</i> (T8)	1
<i>emm95.0</i> (T3/13/B3264)	1
<i>emm103.0</i> (T2/4)	1
<i>emm104.0</i> (T-nontypeable)	1
<i>emm106.0</i> (T5/27/44)	1
GCS	
<i>stC1400.2</i>	3
<i>stC1400.0</i>	1
<i>stG245.0</i>	4
<i>stCK401.2</i>	1
<i>stC922.0</i>	1
<i>stG653.0</i>	2
GGs	
<i>stG485.0</i>	12
<i>stC1400.0</i>	3
<i>stGLP1.0</i>	6
<i>stC5345.0</i>	4
<i>stC5345.2</i>	1
<i>stG6792.0</i>	4
<i>stG6792.3</i>	1
<i>stC36.0</i>	3
<i>stG2078.0</i>	2
<i>stG2078.2</i>	1
<i>stC74a.0</i>	2
<i>stG4831.0</i>	2
<i>stG643.0</i>	1
<i>stG643.1</i>	1
<i>stKNB7.0</i>	2
<i>stG866.0</i>	2
<i>stG2574.0</i>	1
<i>stG10.0</i>	1

with invasive disease. As with other limited studies of non-invasive isolates recovered in tropical or subtropical climates (Abdissa *et al.*, 2006; Sakota *et al.*, 2006), we found a high degree of GAS *emm* type diversity, indicating a potential challenge for a multivalent M type specific vaccine strategy and further need for surveillance of GAS isolates from disease cases in these regions.

Thangam Menon,¹ Charmaine Lloyd,¹ Balaraman Malathy,¹ Varja Sakota,² Delois Jackson² and Bernard Beall²

¹Department of Microbiology, Dr A. L. Mudaliar Post Graduate Institute of Basic Medical Sciences, Taramani, University of Madras, Chennai, India

²Respiratory Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Correspondence: Thangam Menon (thangam56@gmail.com)

Abdissa, A., Asrat, D., Kronvall, G., Shittu, B., Achiko, D., Zeidan, M., Yamuah, L. K. & Aseffa, A. (2006). High diversity of group A streptococcal *emm* types among healthy school children in Ethiopia. *Clin Infect Dis* **42**, 1362–1367.

Bisno, A. L., Collins, C. M. & Turner, J. C. (1996). M proteins of group C streptococci isolated from patients with acute pharyngitis. *J Clin Microbiol* **34**, 2511–2515.

Cohen-Poradosu, R., Jaffe, J., Lavi, D., Grisariu-Greenzaid, S., Nir-Paz, R., Valinsky, L., Dan-Goor, M., Block, C., Beall, B. & Moses, A. E. (2004). Group G streptococcal bacteremia in Jerusalem. *Emerg Infect Dis* **10**, 1455–1460.

Collins, C. M., Kimura, A. & Bisno, A. L. (1992). Group G streptococcal M protein exhibits structural features analogous to those of class I M protein of group A streptococci. *Infect Immun* **60**, 3689–3696.

Cunningham, M. W. (2000). Pathogenesis of group A streptococcal infections. *Clin Microbiol Rev* **13**, 470–511.

Davies, M. R., Tran, T. N., McMillan, D. J., Gardiner, D. L., Currie, B. J. & Sriprakash, K. S. (2005). Inter-species genetic movement may blur the epidemiology of streptococcal diseases in endemic regions. *Microbes Infect* **7**, 1128–1138.

Hashikawa, S., Iinuma, Y., Furushita, M., Ohkura, T., Nada, T., Tori, K., Hasegawa, T. & Ohta, M. (2004). Characterization of group C and G streptococcal strains that cause streptococcal toxic shock syndrome. *J Clin Microbiol* **42**, 186–192.

- Horii, T., Izumida, S., Takeuchi, K., Tada, T., Ishikawa, J. & Tsuboi, K. (2006). Acute peritonitis and salpingitis associated with streptococcal toxic shock syndrome caused by Lancefield group G α -haemolytic *Streptococcus dysgalactiae* subsp. *equisimilis*. *J Med Microbiol* 55, 953–956.
- Johnson, D. R., Kaplan, E. L., VanGheem, A., Facklam, R. R. & Beall, B. (2006). Characterization of group A streptococci (*Streptococcus pyogenes*): correlation of M-protein and *emm*-gene type with T-protein agglutination pattern and serum opacity factor. *J Med Microbiol* 55, 157–164.
- Kalia, A. & Bessen, D. E. (2003). Presence of streptococcal pyrogenic exotoxin A and C genes in human isolates of group G streptococci. *FEMS Microbiol Lett* 219, 291–295.
- Krucsó, B., Csizsár, K., Gacs, M. & Pászti, J. (2005). Molecular typing of Hungarian *Streptococcus pyogenes* isolates. In *Abstracts of the 15th European Congress of Clinical Microbiology and Infectious Diseases Copenhagen, Denmark*, abstract 1134–02–364. European Society of Clinical Microbiology and Infectious Diseases.
- Lopardo, H. A., Vidal, P., Sparo, M., Jeric, P., Centron, D., Facklam, R. R., Paganini, H., Pagniez, N. G., Lovgren, M. & Beall, B. (2005). Six-month multicenter study on invasive infections due to *Streptococcus pyogenes* and *Streptococcus dysgalactiae* subsp. *equisimilis* in Argentina. *J Clin Microbiol* 43, 802–807.
- McDonald, M., Towers, R. J., Andrews, R. M., Carapetis, J. R. & Currie, B. J. (2007). Epidemiology of *Streptococcus dysgalactiae* subsp. *equisimilis* in tropical communities, Northern Australia. *Emerg Infect Dis* 13, 1694–1700.
- Moses, A. E., Hidalgo-Grass, C., Dan-Goor, M., Jaffe, J., Sheitzigovsky, I., Ravins, M., Korenman, Z., Cohen-Poradosu, R. & Nir-Paz, R. (2003). *emm* typing of M nontypable invasive group A streptococcal isolates in Israel. *J Clin Microbiol* 41, 4655–4659.
- Pinho, M. D., Melo-Cristino, J. & Ramirez, M. (2006). Clonal relationships between invasive and noninvasive Lancefield group C and G streptococci and *emm*-specific differences in invasiveness. *J Clin Microbiol* 44, 841–846.
- Sakota, V., Fry, A. M., Lietman, T. M., Facklam, R. R., Li, Z. & Beall, B. (2006). Genetically diverse group A streptococci from children in far-western Nepal share high genetic relatedness with isolates from other countries. *J Clin Microbiol* 44, 2160–2166.
- Shulman, S. T., Tanz, R. R., Kabat, W., Kabat, K., Cederlund, E., Patel, D., Li, Z., Sakota, V., Dale, J. B., Beall, B. & the US Streptococcal Pharyngitis Surveillance Group (2004). Group A streptococcal pharyngitis serotype surveillance in North America, 2000–2002. *Clin Infect Dis* 39, 325–332.