Restriction fragment patterns and emm types of group G streptococci

Group G streptococci (GGS) are aetiological agents of exudative pharyngitis, pyoderma, invasive infections and post-streptococcal sequelae (Zaoutis et al., 2004; Steer et al., 2009). Like group A streptococci (GAS), GGS also express M protein and other virulence factors such as fibronectin binding proteins, IgG binding protein, streptokinase, streptolysin O, streptolysin S, streptococcal pyrogenic exotoxins, C5a peptidase and NADase (Chhatwal & Talay, 2000), and have emm genes that encode M protein similar to that of GAS. More than 60 sequence types have been described for group C streptococci and GGS (Streptococcus pyogenes emm sequence database: BLAST emm – Centers for Disease Control and Prevention, 2008 – http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm). Restriction profiles of emm amplicons of GAS have been used in the past to detect groups of isolates with identical emm types (Beall et al., 1998; Mylvaganam et al., 2009). We wished to study the correlation between the RFLP profiles of emm amplicons of GGS strains obtained using single (DdeI) and double digests (HincII and HaeIII) with the emm sequence types, and the possibility of using RFLP to type GGS strains, in a similar manner to the identification of emm types of GAS.

A total of 18 strains of GGS isolated from throat swabs (12), skin swabs (3), blood (2) and fluid (1) were included in the study. The study was approved by the institutional ethical committee. DNA extraction and amplification of the emm gene was carried out using standard techniques, and the emm types of the isolates was determined (Streptococcus pyogenes emm sequence database: protocol for emm typing – Centers for Disease Control and Prevention, 2008 – http://www.cdc.gov/ncidod/biotech/strep/protocol_emm-type.htm). emm amplicons were digested with restriction enzyme DdeI, and double digested with HincII and HaeIII. Restriction digestion with DdeI was carried out using 5 µl emm amplicon DNA, 0.5 µl DdeI and 0.5 µl 1× NE buffer 3 (New England BioLabs), which was incubated at 37 °C in a water bath for 40 min. For double digestion, 4 µl emm amplicon DNA was digested with 0.4 µl each of HincII and HaeIII in 0.5 µl 1× NE buffer 2 and 0.1 µl 100× BSA (New England BioLabs), at 37 °C for 40 min. The fragments were resolved using 2.5% agarose (Sigma Aldrich) with a 50 bp molecular mass marker.

Dendrogram analysis of RFLP fragments was carried out using DNA Fingerprinting II Informatix software version 3.0 (BioRad), using the UPGMA algorithm and Dice similarity coefficients. Simpson’s diversity index was applied to compare the discriminatory ability of the RFLP profiles obtained by single and double digestion of emm amplicons (Simpson, 1949).

A total of 8 different emm types were detected among 18 GGS strains [stG6792.3 (8), stG866.0 (3), stG1750.0 (2), stG652.0 (1), stG643.1 (1), stG653.0 (1), stG2574.0 (1), stC2sk.0 (1)] resulting in a strain diversity of 44.4%. Dendrogram analysis of the 18 GGS strains using the single digest profiles showed 18 different clusters, giving a 100% strain diversity (Fig. 1). Simpson’s diversity index for single digest profiles was zero showing their poor discriminatory ability.

Fig. 1. Dendrogram of RFLP patterns of DdeI digests. The dendrogram was constructed with Fingerprinting II Informatix software version 3.0 with 0% optimization and 0.87% positional tolerance, by using the UPGMA algorithm and Dice similarity coefficients. Strain numbers and emm sequence types are indicated on the right.
Dendrogram analysis of strains using double digest profiles showed 10 restriction profiles at a 100% similarity coefficient, with a strain diversity of 55.6% (Fig. 2). Seven among the eight strains with emm type stG6792.3 belonged to a single cluster and exhibited identical profiles. Similarly, three strains belonging to the type emm866.0 showed 100% similarity in their restriction profiles. However, two strains with emm type stG1750.0 showed dissimilar restriction profiles. Simpson’s index of diversity for double digestion was 0.7.

Single digest profiles were less discriminatory, since none of the strains with a similar emm type (stG6792.3, stG866.0 and stG1750.0) showed similar RFLP profiles. These findings appeared to be similar to those found for GAS (Streptococcus pyogenes emm sequence database: protocol for emm typing – Centers for Disease Control and Prevention, 2008 – http://www.cdc.gov/ncidod/biotech/strep/protocol_emm-type.htm). Double digest profiles were more discriminatory, since strains belonging to the same emm type [stG6792.3 (7/8), stG866.0 (3/3)] belonged to the same cluster with 100% similarity in their restriction profiles. Though this study included only a small number of strains, preliminary results do show that RFLP profiles of double digests (HincII and HaeIII) of emm amplicons correlate with the emm sequence type, while single digests with Ddel do not discriminate between emm types. Hence, restriction profiling using HincII and HaeIII may be used to detect GGS with identical emm types and may serve as a less expensive method of molecular typing the strains.

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**Malathy Balaraman and Thangam Menon**

Department of Microbiology, Dr A. L. Mudaliar Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 113, India

**Correspondence**: Thangam Menon (thangam56@gmail.com)


