

Abstract

In the present study, we developed a rapid, cost-effective pulsed-field gel electrophoresis (PFGE) method for molecular subtyping of *Streptococcus pyogenes* and used the new PFGE, Vir typing, and emm typing techniques to analyze the genotypes of 182 streptococcal isolates recovered from scarlet fever patients in central Taiwan from 1996 to 1999. With aid of a plug washing machine assembled in our lab, a technician a day could finish DNA preparation with good DNA quality for 30-60 bacterial isolates and obtained PFGE result in the second day. In addition, the usage of SmaI for a routine restriction digestion had been reduced from 40 units to 5 units when high quality of DNA was used. The improvement had helped to cut down the cost of PFGE. From the molecular subtyping of the 182 *S. pyogenes* isolates with the rapid PFGE method, Vir typing, and emm typing, we obtained 27 PFGE-SmaI types for 173 isolates and 6 PFGE-SfiI types for 9 isolates, whose DNA was resistant to SmaI digestion. We also obtained 20 VT types for the 182 isolates from which 10 emm genes were detected for 181 isolates and no emm gene for 1 isolates. To study the phylogenetic relationship for the isolates, a dendrogram was constructed with the PFGE-SmaI patterns. Six clusters were designated based on a cut off value of 60% identity. The clustering was related to the emm genes. Isolates with emm22, emm4, emm12, emm12, emm6, and emm1 were distributed in cluster A, B, C, D, E, and F, respectively. The fact that emm12 isolates located in two clusters suggested that they could be derived from two different origins. The dendrogram also showed that isolates with different emm genes were genetically distant from each other. The data also indicated that the isolates with different emm genes were different in PFGE patterns and isolates with the same emm gene could be different in PFGE patterns and different in VT types. Some isolates with the same VT types could further be subtyped into several PFGE types, in contrast, some isolates with the same PFGE pattern could be different in VT types. Although PFGE was the most discriminatory method for molecular subtyping of the *S. pyogenes* isolates tested, emm typing could show the most distinct differences between the phylogenetic groups and Vir typing could help to discriminate more genotypes of isolates. The new rapid PFGE method and the database of the genotypes created in this study can be applied for molecular epidemiology study and for setting up a molecular subtyping system for surveillance of *S. pyogenes*. In the next, we will applied the results to investigate the abruptly increase in cases of scarlet fever and in severe cases of invasive group A streptococci infections in the past year.

Keywords : pulsed-field gel electrophoresis ; Vir typing ; emm typing ; *Streptococcus pyogenes*