

## **Distribution of virulence genes and the molecular epidemiology of *Streptococcus pyogenes* clinical isolates by emm and multilocus sequence typing methods**

### **ABSTRACT**

Background: *Streptococcus pyogenes* has a variety of virulence factors and the predominant invasive strains differ according to specific emm types and geographical orientation. Although emm typing is commonly used as the gold standard method for the molecular characterisation, multilocus sequence typing (MLST) has become an important tool for comparing the genetic profiles globally. This study aimed to screen selected virulence genes from invasive and non-invasive clinical samples and to characterise the molecular epidemiology by emm typing and MLST methods. Materials and methods: A total of 42 *S. pyogenes* isolates from invasive and non-invasive samples collected from two different tertiary hospitals were investigated for the distribution of virulence factors and their molecular epidemiology by emm and multilocus sequence typing methods. Detection of five virulence genes (*speA*, *speB*, *speJ*, *ssa* and *sdaB*) was performed using multiplex polymerase chain reaction (PCR) using the standard primers and established protocol. Phylogenetic tree branches were constructed from sequence analysis utilised by neighbour joining method generated from seven housekeeping genes using MEGA X software. Results: Multiplex PCR analysis revealed that *sdaB/speF* (78.6%) and *speB* (61.9%) were the predominant virulence genes. Regardless of the type of invasiveness, diverse distribution of emm types/subtypes was noted which comprised of 27 different emm types/subtypes. The predominant emm types/subtypes were emm63 and emm18 with each gene accounted for 11.8% whereas 12% for each gene was noted for emm28, emm97.4 and emm91. The MLST revealed that the main sequence type (ST) in invasive samples was ST402 (17.7%) while ST473 and ST318 (12% for each ST) were the major types in non-invasive samples. Out of 18 virulotypes, Virulotype A (five genes, 55.6%) and Virulotype B (two genes, 27.8%) were the major virulotypes found in this study. Phylogenetic analysis indicated the presence of seven different clusters of *S. pyogenes*. Interestingly, Cluster VI showed that selected emm/ST types such as emm71/ST318 (n=2), emm70.1/ST318 (n=1), emm44/ST31 (n=1) and emm18/ST442 (n=1) have clustered within a common group (Virulotype A) for both hospitals studied. Conclusion: The present study showed that group A streptococci (GAS) are genetically diverse and possess virulence genes regardless of their invasiveness. Majority of the GAS exhibited no restricted pattern of virulotypes except for a few distinct clusters. Therefore, it can be concluded that virulotyping is partially useful for characterising a heterogeneous population of GAS in hospitals.

**Keyword:** Emm type; Multilocus sequence typing; *Streptococcus pyogenes*; Virulence genes