Development and Evaluation of Next Generation Molecular Subtyping Methods (I): Development of Multilocus Variable Number Tandem Repeat Methods for Molecular Subtyping of Neisseria Meningitidis

Abstract

The multilocus variable-number tandem repeat (VNTR) analysis (MLVA) technique has been developed for fine typing of many bacterial species. The genomic sequences of *Neisseria meningitidis* strains Z2491, MC58 and FAM18 have been available for searching potential VNTR loci by computer software. In this study, we developed and evaluated a MLVA method for molecular subtyping and phylogenetic analysis of *N. meningitidis* strains.

A total of 12 VNTR loci were identified for subtyping and phylogenetic analysis of 100 *N. meningitidis* isolates, which had previously been characterized by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing. The number of alleles ranges from 3 to 40 for the 12 VNTR loci; theoretically, the numbers of alleles can generate more than 5×10^{11} MLVA types. In total, 93 MLVA types were identified in the 100 isolates, indicating that MLVA is powerful in discriminating *N. meningitidis* strains. The discriminatory index for MLVA is 0.998, which is higher than PFGE (0.95) and MLST (0.87). In phylogenetic analysis with the minimal spanning tree method, clonal relationships, established with MLVA types, agreed well with those built with ST types.

Our study indicates that the MLVA method has a higher degree of resolution than PFGE in discriminating *N. meningitidis* isolates. It is powerful tool for fine typing of *N. meningitidis* isolates for epidemiological and forensic investigation. MLVA may also be a useful tool for phylogenetic studies of strains evolving over different time scales.

Keywords: *Neisseria meningitidis*, multilocus VNTR analysis (MLVA), variable-number tandem repeat (VNTR), pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), DNA fingerprint database, molecular epidemiology