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International Inter-laboratory Evaluation of Selected Variable-Number Tandem-Repeat Loci for Establishing a Standardised Multilocus Variable-Number Tandem-Repeat Analysis Protocol for Shigella Sonnei

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Abstract

Shigella sonnei is an important etiological agent of travel-associated diarrhea. It is monomorphic and occasionally some epidemiologically unrelated strains may be indistinguishable by some molecular subtyping tools such as PFGE. Multilocus variable-number tandem-repeat (VNTR) analysis (MLVA) can overcome this problem as it is a highly discriminatory sequence-based subtyping tool. MLVA is based on the inherent variability of short sequences organised as tandem repeats at multiple VNTR loci. The VNTR loci in S. sonnei have different degree of variability. We participated in an international inter-laboratory evaluation of 8 VNTR loci previously identified, as part of an effort to develop a standardised S. sonnei MLVA protocol for inter-laboratory comparison and communication of data. Thirty strains of S. sonnei obtained from Centre for Disease Control and Prevention, Taiwan, were analysed. Primers targeting 8 VNTR loci, SS1, SS3, SS6, SS9, SS10, SS11, SS12 and SS13 were used. The VNTR loci used exhibited different variability. Loci SS6 and SS3 are highly variable compared to the other loci used. Locus SS13 is the least variable. MLVA subtyping is rapid and easy compared to the laborious PFGE. Interpretation of result was less subjective, copy numbers were easily assigned and results were also readily comparable between laboratories. A standardised MLVA protocol can complement PFGE for S. sonnei surveillance and outbreak investigation and even may replace PFGE as a routine subtyping method for S. sonnei.

Introduction

Shigella sonnei is an important etiologic agent of travel-associated diarrhea (Ekdam and Andersson, 2005). This species is monomorphic and occasionally some epidemiologically unrelated strains may not be distinguishable by other molecular subtyping tools such as PFGE (Chou et al., 2006). MLVA can overcome this problem as it is a highly discriminatory sequence-based subtyping tool. This method is based on the inherent variability of short sequences that are organised as tandem repeats at multiple VNTR loci. The VNTR loci in S. sonnei were found to have different degree of variability. MLVA based on 4 to 8 highly variable VNTR loci exhibited a discriminatory power parallel to or higher than PFGE and combined loci with different variability values can be utilised to establish phylogenetic relationships among S. sonnei strains with different evolutionary timescales (Liang et al., 2007; Chou et al., 2009). To evaluate the usefulness of selected VNTR loci for molecular subtyping of S. sonnei, we participated in an international inter-laboratory evaluation of 8 VNTR loci previously identified. The evaluation was part of an international collaboration in the effort to