Improved molecular typing of toxigenic *Clostridium difficile* strains affecting animal and human health

M Nowak-Roddy, J McKenna, DJ Fairley

School of Biological Sciences, Queen’s University Belfast
Department of Microbiology, Belfast Health & Social Care Trust, Kelvin Building, Grosvenor Road, BT12 6BA

**Background**

*Clostridium difficile* is a Gram-positive, spore forming bacterium, which remains a formidable pathogen as the etiological agent of *Clostridium difficile* infection (CDI). Substantial effort goes into diagnosis of CDI and characterisation of circulating toxigenic *C. difficile* strains for epidemiology and infection, prevention, and control. Currently, molecular typing of *C. difficile* requires 9 days following diagnosis through PCR ribotyping and multilocus variable number tandem repeat analysis (MLVA). There is a need for more rapid typing methods to investigate possible linkage between CDI cases in healthcare settings.

Through use of a one-step, closed tube qPCR-high resolution melt (HRM) assay targeting the intergenic spacer region (ISR) and variable number tandem repeats (VNTR) of toxigenic *C. difficile* isolates with results generated in less than 90 minutes, this study set out to:

1) Validate a new method for PCR ribotyping and/or MLVA of *C. difficile* directly from positive specimens.

2) Develop a ribotype profile library for direct ribotyping from specimens.

**Results**

Results were available in less than 90 minutes. Through comparison of HRM profiles targeting the ISR of isolates belonging to the 17 PCR ribotypes, 13 HRM genotypes were recognised with 11 PCR ribotypes resolved from each other. Isolates belonging to PCR ribotypes 014, 020, and 220, alongside 070, 078, and 193 could not be resolved from each other and were collectively placed into 2 HRM genotypes. Using correlation between melt temperature (Tm) and known VNTR repeat numbers at the B7, C6, and G8 loci (Figure 1), VNTR repeat numbers for isolates could be predicted within an average absolute difference of 1.8 at the B7 locus, 2.1 at the C6 locus, and 2.5 at the G8 locus. No correlation was found between the Tm and known repeat numbers of isolates at the A6 locus.

**Methods**

90 toxigenic clinical and animal *C. difficile* isolates were analysed within this study. All isolates were previously characterised into 17 PCR ribotypes. A subset of 19 *C. difficile* isolates were further characterised by MLVA with focus on the A6, B7, C6, and G8 loci. qPCR and HRM was performed on all isolates included within this study with targeting of the ISR in clinical isolates (n=79) and targeting of the aforementioned VNTR loci in both clinical and animal isolates (n=90).

**Conclusions**

The results of this study suggest that a qPCR-HRM assay with a multilocus panel targeting ISR and various VNTR loci generating results in less than 90 minutes could form part of an improved molecular typing scheme for toxigenic *C. difficile* strains affecting animal and human health that is more rapid than the standard molecular typing methods.