

## **HYPERVIRULENT EPIDEMIC STRAINS OF *Clostridium difficile* HAVE ALTERED HOST CELL ADHERENCE AND PROTEIN EXPRESSION**

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**Introduction:** Hypervirulent strains of *Clostridium difficile* (CD) are being identified from epidemics in the USA, Canada, and Europe. These isolates have been restriction endonuclease typed as group BI. BI strains produce 16-23 fold more toxin, can cause a fulminant colitis, and are associated with increased mortality. Because BI strains appear to rapidly cause epidemics when present in hospitals, we hypothesized that (a) BI strains may have increased ability to adhere to host intestinal epithelia and (b) non-toxin proteins important for persistence may also be differentially expressed.

**Methods and Results:** First, we developed an *in vitro* anaerobic adherence assay to assess the ability of the BI-6, BI-8 and BI-17 strains to attach to the Caco-2 human intestinal epithelial cells. The non-toxigenic CD strains M3 and T7, and the toxigenic but non-epidemic strains K14 and 630 were used as controls. M3 exhibited the highest adherence, followed by the BI strains. Both K14 and 630 had the lowest adherence. These results corroborate hamster studies where it was shown M3 conferred greater protection against BI strain challenge than T7. Hamster protection differences may thus be due to increased ability of the non-toxigenic strain to adhere.

We also identified proteins dysregulated in the BI strains. Exponentially growing BI-6, BI-17, M3, 630 and K14 strains were fractionated to obtain soluble and membrane proteins. SDS-PAGE revealed that expression of BI strain proteins was altered, and that several proteins were distinctly up- or down-regulated. Using MALDI mass spectrometry, we determined that one over-expressed protein in BI-6 and BI-17 was the SlpA surface layer protein.

**Conclusions:** Epidemic stains of CD have enhanced adherence to human intestinal epithelial cells, and this may be mediated by an increased expression of surface layer proteins. Our data may provide insights into how the BI strains predominate in their environment, and how they are able to cause more severe disease outcomes that are not solely toxin-related.