Phenotypic characterisation of an emerging MLST clade 2 lineage of *Clostridium difficile*, ribotype 251

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**Background**

The binary toxin positive strain of *C. difficile*, ribotype (RT) 027, caused major outbreaks in North America and remains highly prevalent in the USA. Although RT 027 has never established in Australia, a genetically related strain, RT 251, has increased in prevalence Australia-wide since 2010. Herein, we phenotypically characterised a selection of RT 251 strains from Australia and USA, categorised under three MLST profiles (STs 188, 231 and 365) to ascertain virulence and significance in human infection.

**Results**

- RT 251 strains demonstrated robust germination and spore outgrowth (*p<0.05; 12 h*).

![Graph showing relative OD values over time for different strains.](image)

- RT 251 strains induced cytopathic effect (CPE) in cell culture.
- RT 251 strains have lower 90% CPE toxin titres compared to RT 027, VPI 10463 and 630 strains.

![Image showing Vero pre- and post-treatment with toxins A and B](image)

- Toxin production (toxin A – solid line; toxin B – dashed line) was highest at 96 h.
- RT 251 strains showed low levels of toxin A and toxin B in vitro.

![Graph showing toxin concentration (µg/ml) over time](image)

**Methods**

*C. difficile* spore germination and outgrowth were monitored at OD 600. The rate of sporulation and total spore count was determined using differential spore-plating CFU/ml measurements. Toxin A and toxin B production were quantified using IgGBIOMICS ELISA kit. *In vitro* toxin production was confirmed by Vero cell and HT-29 cytotoxicity assays. Motility assays (0.175% BHIA) were performed and antimicrobial susceptibility was determined using agar incorporation methods (data not shown).

**Discussion & Conclusion**

*C. difficile* RT 251 strains produced spores that germinated faster than those of an epidemic RT 027 strain. Despite lower toxin production, RT 251 strains produce cytotoxin that induced significant CPE in cell culture. One RT 251 strain (ST 365) did not form spores and demonstrated robust toxin production at 120 h. All but one RT 251 strain showed erythromycin and clindamycin resistance. Phenotypic assays did not reveal greater virulence potential of RT 251 strains, however, further epidemiological and in vivo studies are required to elucidate the true significance of RT 251 strains in human infection.