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

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Background

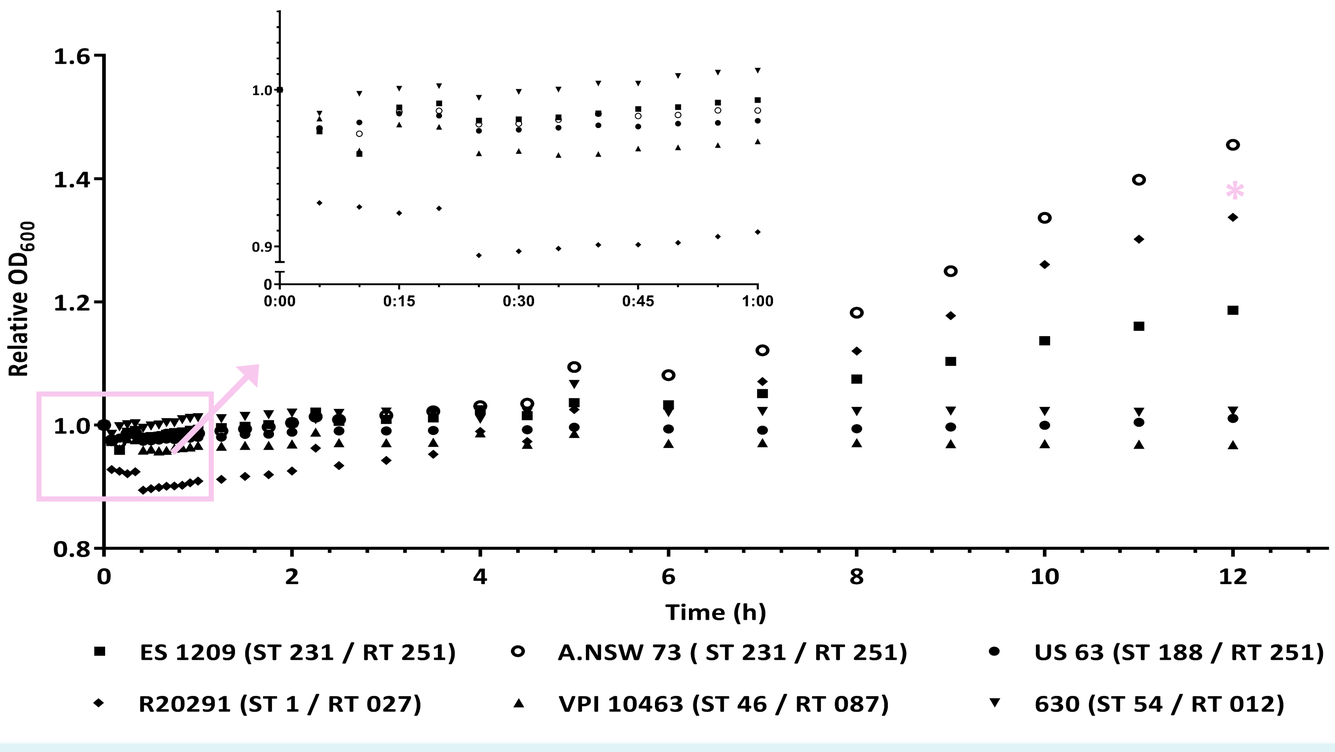
The binary toxin positive strain of *C. difficile* ribotype (RT) 027 caused major outbreaks in North America and remains highly prevalent in the USA. Although RT 027 has never established in Australia, a genetically related strain, RT 251, has increased in prevalence Australia-wide since 2010. Herein, we phenotypically characterised a selection of RT 251 strains, categorised under three MLST profiles (STs 188, 231 and 356) to ascertain virulence and significance in human infection.

Methods

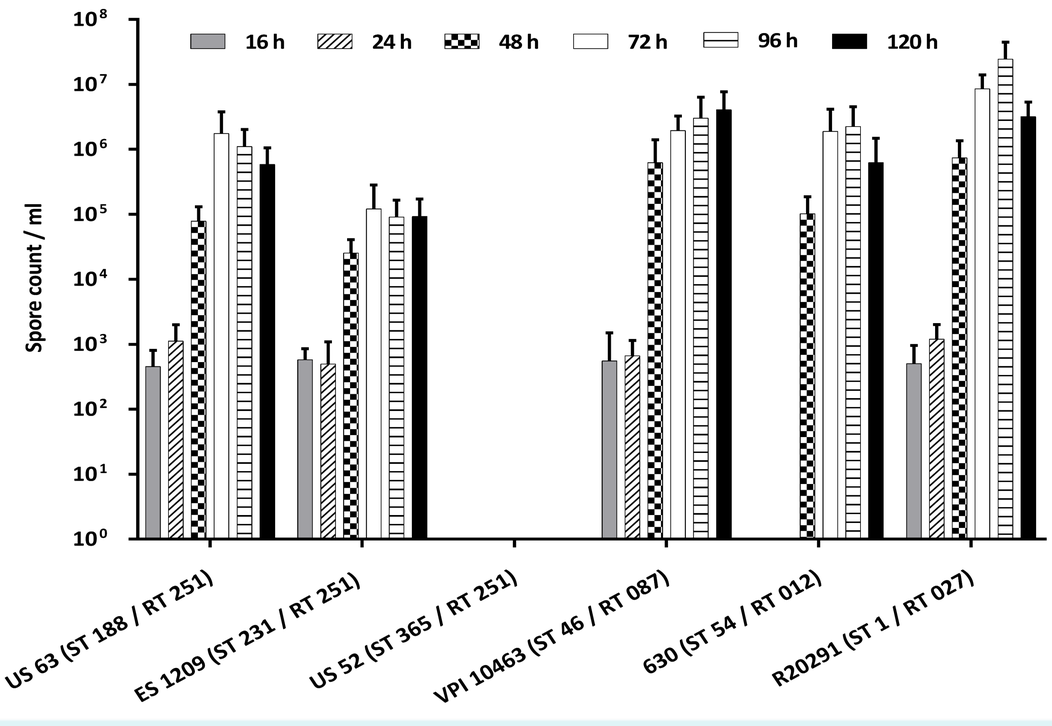
C. difficile spore germination and outgrowth were monitored at OD_{600}. The rate of sporulation and total spore count was determined using differential spore-plating CFU/ml measurements. Toxin A and toxin B production were quantified using tgcBIOMICS ELISA kit. *In vitro* toxin production was confirmed 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).

Results

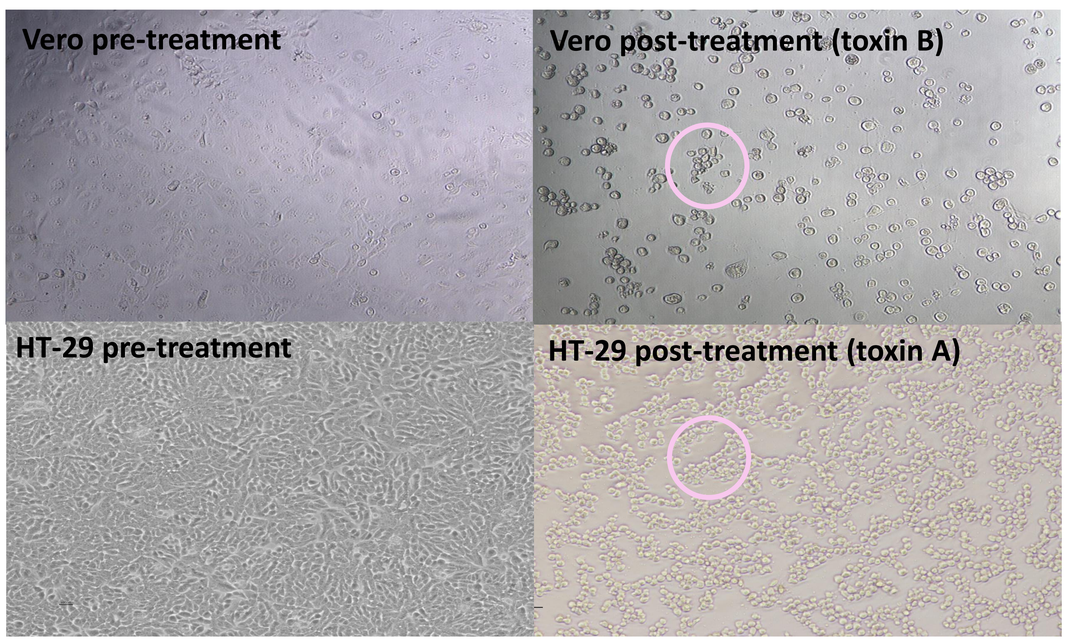
- RT 251 strains demonstrated robust germination and spore outgrowth ($p<0.05$; 12 h*).



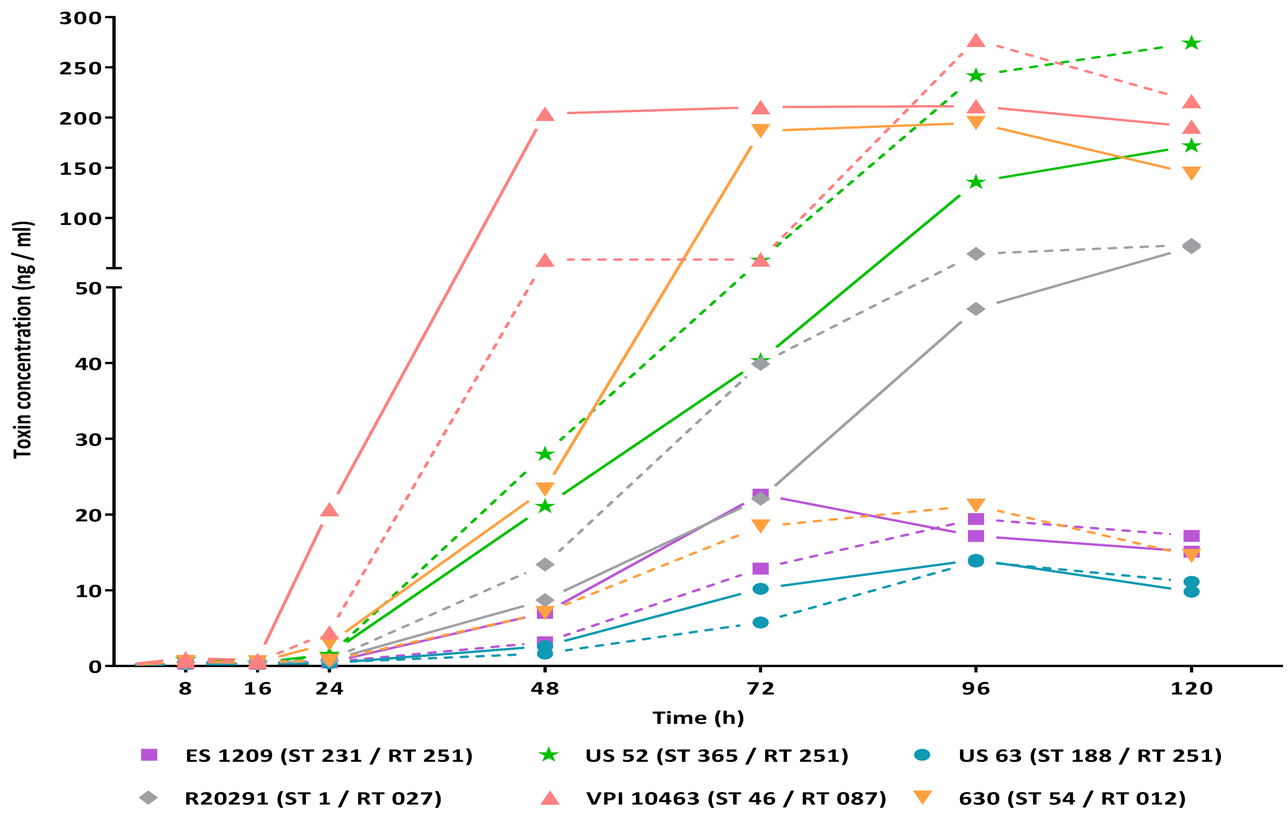
- Sporulation rate was highest at 48 h for all strains, except for US 52, where no spores were seen by 120 h.



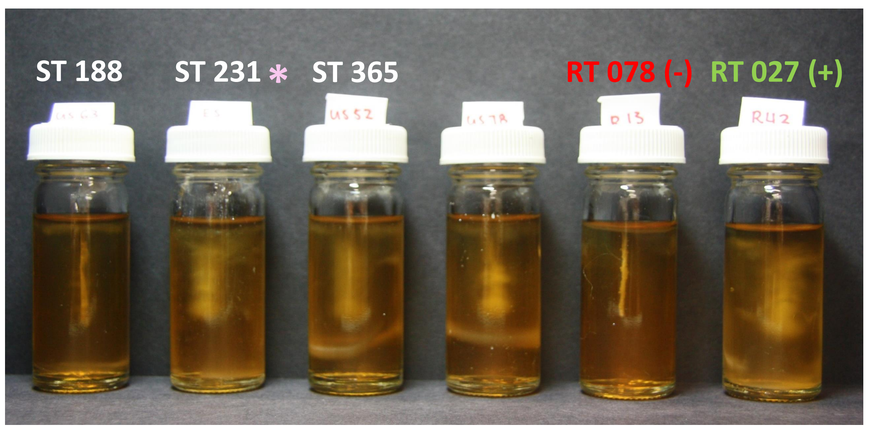
- RT 251 strains induced cytopathic effect (CPE) in cell culture.
- RT 251 strains have lower 90% CPE toxin titres compared to RT 027, VPI 10463 and 630 strains.



- 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.*



- All Australian RT 251 strains (ST 231) were highly motile.



Discussion & Conclusion

C. difficile RT 251 strains produced spores that germinated faster than those of an epidemic RT027 strain. Despite lower toxin production, RT251 strains produce cytotoxin that induced significant CPE in cell culture. One RT 251 strain (ST 356) did not form spores and demonstrate robust toxin production at 120 h. All but one RT 251 strain showed erythromycin and clindamycin resistance. Phenotypic assays did not reveal superior virulence for RT 251 strains, however, further epidemiological and *in vivo* studies are required to elucidate the importance of RT 251 strains in human infection.

Key references:

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