Characterisation of a ribotype 251 strain of *Clostridium difficile* causing severe disease in the community

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Background

Clostridium difficile infection (CDI) has reached an epidemic state in many developed countries with high incidence and severe disease in not only traditional healthcare settings but also in the community[1]. Three patients with CDI caused by an unusual strain of *C. difficile* PCR ribotype (RT) UK 251 were identified in New South Wales. All cases presented with severe diarrhoea in the community. While one patient was elderly (ES 1207; 79 years) and suffered multiple comorbidities, two were young and previously healthy (ES 1205, ES 1209; 32 years and ES 1213; 22 years). One of the young cases died.

Methods

RT 251 strains (*n*=4) were isolated by toxigenic culture. Genetic characterisation was performed using a variety of techniques including; toxin gene profiling[2], whole genome sequencing (WGS)[3], *in silico* multilocus sequence typing (MLST)[4] and microevolutionary analysis by comparison of single-nucleotide variants (SNVs)[1] in the core genome of these isolates. Phenotypic characterisation of antimicrobial resistance was carried out using an agar incorporation method as described by the CLSI[5]. *In vitro* toxin production was confirmed by Vero cell cytotoxicity assay[6] and pathogenicity was confirmed in a murine model[7] .

Results

TABLE 1. Pairwise SNV analysis with associated genotypic results for RT 251 strains isolated from three independent cases in Australia.

ES1205	ES1207	ES1209	ES1213	R20291	ID	RT	TP$^{\infty}$	ST$^{\#}$	Clade*
	4	0	1	4511	ES1205	251	$A^+ B^+ CDT^+$	231	2
		4	5	4515	ES1207	251	$A^+ B^+ CDT^+$	231	2
			1	4511	ES1209	251	$A^+ B^+ CDT^+$	231	2
				4512	ES1213	251	$A^+ B^+ CDT^+$	231	2
					R20291	027	$A^+ B^+ CDT^+$	1	2

∞Toxin profiling by PCR for genes *tcdA* (A), *tcdB* (B) , *cdtA* and *cdtB* (CDT). #Sequence typing based on allelic polymorphisms of the 7 house keeping genes[3]. * Clustering of the ST into phylogenetic clades. R20291 (RT 027) - *C. difficile* reference strain used for SNV mapping (GenBank accession FN545816).

- All isolates were susceptible to metronidazole and vancomycin.
- One strain (ES 1209) was resistant to erythromycin and clindamycin.
- Cytopathic effect (CPE) in Vero cells appeared as cell rounding and lost of adhesion (data not shown).
- 90% CPE in Vero cells was recorded at an approximately 10^{-5} toxin titre across all RT 251 strains. The control RT 027 strain (R20219) caused 90% CPE at 10^{-8} dilution.
- Disease severity of RT 251 strains was similar to that observed with epidemic RT 027. Mice infected with RT 251 strains lost weight and showed severe disease signs in the colon and caecum within 48 h post-infection, with no mice surviving beyond this time point (*n*=5) (Figure 1).

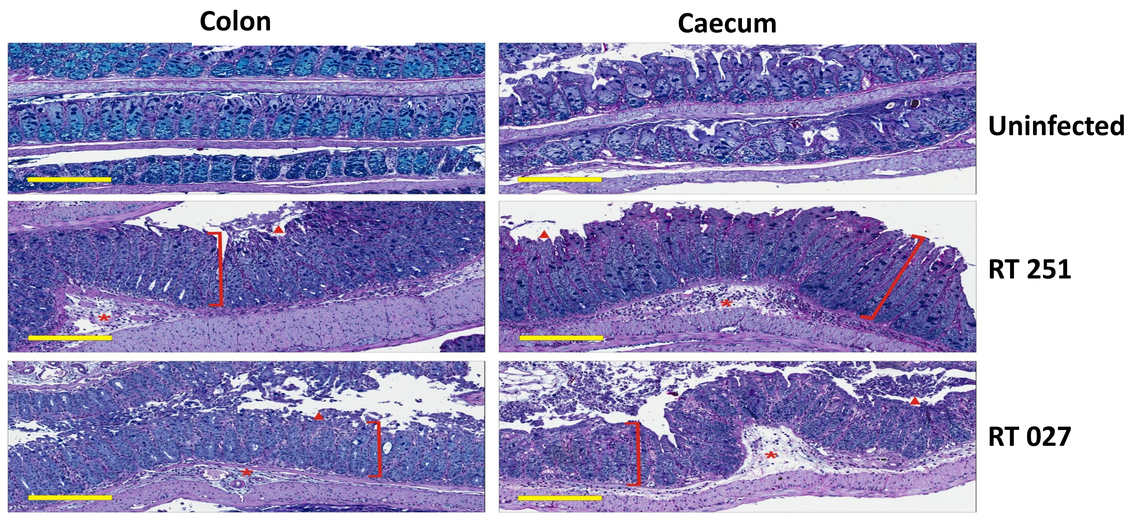

FIGURE 1. Infection with the RT 251 strain ES 1209 causes extensive damage to the caecum and colon of infected mice. (PAS-Alcian blue stained)

Conclusion

- Similarities between RT 251 and RT 027 indicate the potential for RT 251 to cause severe disease.
- The finding of indistinguishable strains of the same RT recovered from independent CDI cases is significant and suggests all patients were exposed to a common source, most likely in the community.
- Emergent clindamycin and erythromycin resistance showed the increased adaptability of *C. difficile* strains.
- Although toxin production of RT 251 was relatively low compared to epidemic RT 027 (R20291) *in vitro*, the death of mice 2 days post-infection suggests severe disease and significant pathogenic potential.
- Further surveillance and comparative genomic analyses are required to determine the significance of RT 251 in Australia and elsewhere.

References

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