

Emerging *Clostridium difficile* PCR ribotype 251 infection in Western Australia

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INTRODUCTION

Recently, there have been reports from some regions of the world of a decline in *Clostridium difficile* infection (CDI) caused by PCR ribotype (RT) 027 and an increase in frequency of CDI due to related strain types belonging to clade 2 (RTs 176, 198 and 244)¹. All states in Australia have reported a significant increase in rates of CDI since mid-2011². In Western Australia (WA), all isolates of *C. difficile* are ribotyped, allowing us to document the emergence of new strains. One such strain that has increased in frequency Australia-wide is RT251 – a strain seen rarely outside North America, and rarely recognised within North America. The reasons for the emergence of RT251 in Australia remain unclear. Herein, we report the results of some preliminary studies looking at RT251 prevalence, genetic background and clinical significance in WA.

METHODS

RT251 strains (*n*=15) were isolated from 12 patients in WA between 2011 and 2014. Genetic characterisation of two isolates of RT251 from two different hospitals in WA in 2012 (one in Perth and one a regional hospital 450 km from Perth) was carried using a variety of techniques including; toxin profiling³, whole genome sequencing (WGS)⁴, *in silico* multilocus sequence typing (MLST)⁵, phylogenetic analysis and comparison of single-nucleotide variants (SNVs)⁶ between isolates. For comparative purposes, a RT251 strain isolated from a patient in Brisbane, Australia in 2012 (some 4,400 km from Perth), was also studied.

RESULTS

There was anecdotal evidence of RT251 strains being isolated in all states of Australia as well as New Zealand.

Clinical

- Five of the 12 cases of RT251 infection were community-onset.
- All cases were not associated with severe CDI (indicated by colectomy, admission to ICU or death).
- One patient presented three times with recurrent hospital-acquired but community onset CDI of the same RT251.
- Only one case presented with high white cell count (>15 x 10⁹ g/L).

Toxin Profile

- All isolates carried the virulence genes *tcdA/tcdB* and *cdtA/B* and were negative for *tcdC* deletion (Table 1).

MLST

- MLST showed RT251 to belong to ST231 (MLST clade 2, Figure 1).
- ST phylogeny revealed ST231 clusters with ST41 (RT244 – single loci variant) and is different at four loci from ST1 (RT027) (Table 1 & Figure 1).

Whole-genome SNV-analysis

- Comparison of the genomes of three geographically and temporarily distinct isolates of RT251 showed them to be indistinguishable – zero SNVs in the core genome (data not shown).

CONCLUSIONS

- Similarities between RT251 and other hypervirulent clade 2 RTs indicate its potential to cause severe disease.**
- The finding of indistinguishable strains recovered from independent CDI cases, hundreds and thousands of km apart, is significant and suggests all patients were exposed to a common source, most likely in the community.**
- Further surveillance and comparative genomic analyses are required to determine the significance of RT251 in Australia and elsewhere.**



Table 1. Genetic features of STs: 231 (RT251), 1 (RT027) and 41 (RT244)

ID	Ref	RT	Toxin profile	MLST clade	ST	ST							S-layer cassette type	ΔQRDR	<i>tcdC</i> allele
						<i>adk</i>	<i>atpA</i>	<i>dxr</i>	<i>glyA</i>	<i>recA</i>	<i>sodA</i>	<i>tpi</i>			
ES666/WA803/WA1086	7	251	A*B*CDT+	2	231	1	1	9	9	14	3	1	8	WT	WT
ES565	6	244	A*B*CDT+	2	41	1	1	9	9	1	3	2	4	WT	Δ18bp
2007855	8	027	A*B*CDT+	2	1	1	1	1	10	1	3	5	10	FQR	Δ18bp

QRDR, quinolone resistance determinant region; WT, wild type; FQR, fluoroquinolone resistant.

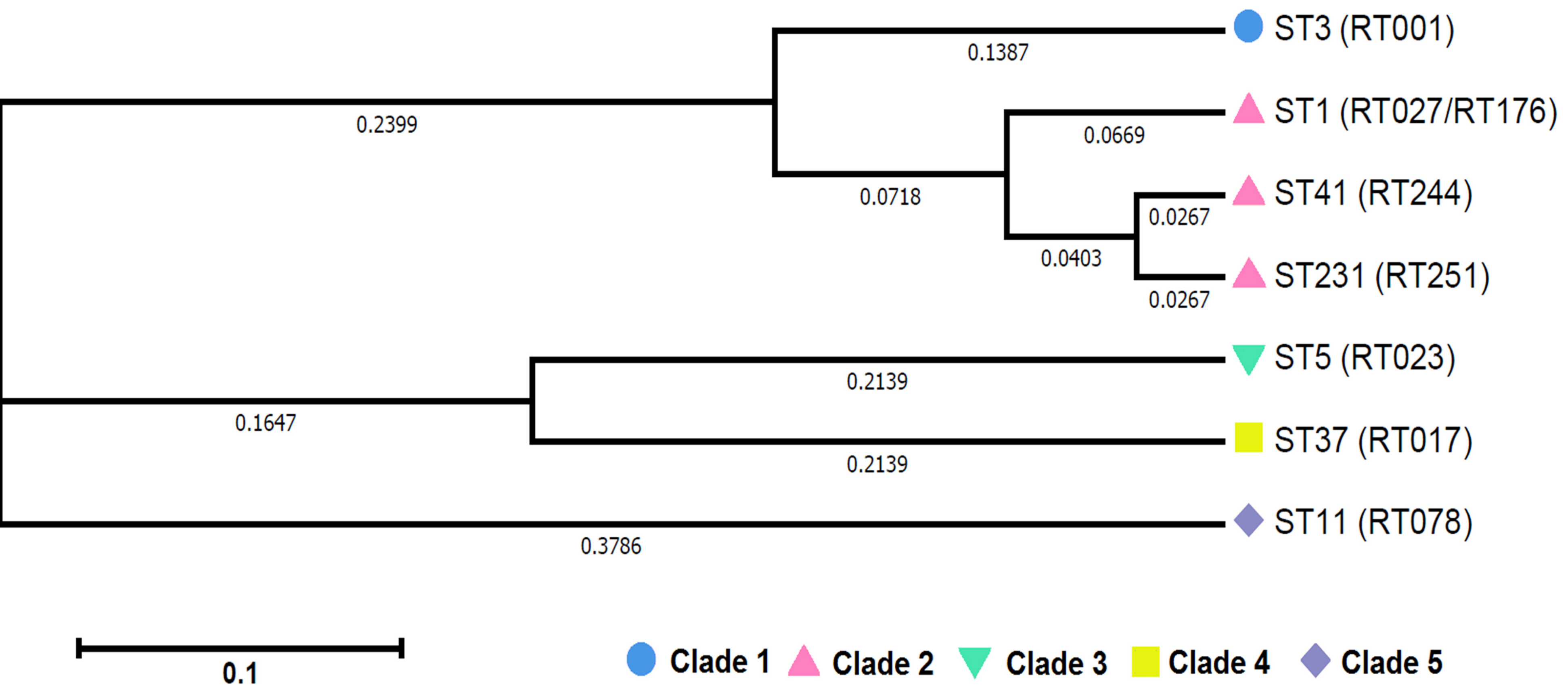


Figure 1. Microevolutionary analysis of clade 2 STs: 231 (RT251), 1 (RT027/RT176) and 41 (RT244) 50% consensus tree (*n* ≥1000) generated using ClonalFrame v1.2⁹ using default parameters and edited in FigTree 1.4.2. For phylogenetic context, STs representing clades 1 (ST3), 3 (ST5), 4 (ST37) and 5 (ST11) are also shown. Branch lengths are shown in coalescent units.

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