The genomic epidemiology of novel virulent *Clostridium difficile* ribotype 251 strains in Australia

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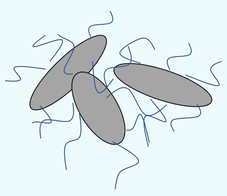
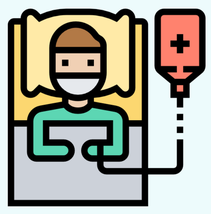
BACKGROUND

In the last 20 years, *Clostridium difficile* infection (CDI) has reached epidemic proportions across continents, with significant increases in incidence and severity of disease following the emergence and global dissemination of the "hyper-virulent" *C. difficile* ribotype (RT) 027 strain, initially in North America and then Europe[1]. This strain never established in Australia and there is a different repertoire of circulating *C. difficile* strains causing human infection[2]. One such strain that has increased in prevalence Australia-wide is RT251[3]. Herein, comparative genomics and high-resolution core genome phylogenetics were used to examine the genomic diversity, evolutionary history and virulence potential of *C. difficile* RT251 strains in Australia.



MATERIALS & METHODS

A total of 48 *C. difficile* RT251 strains were sourced from Australia (n=28), USA (n=11), Slovenia (n=4), New Zealand (n=3) and Canada (n=1).

Genetic characterisation was performed using a variety of techniques including; toxin gene profiling[2], whole genome sequencing (WGS), *in silico* multi-locus sequencing (MLST), core-genome single nucleotide polymorphism (cgSNP) analysis, and detection of antimicrobial resistance genes[4].

Using the *C. difficile* molecular clock (within-host mutation rate), a cut-off of 0 – 2 cgSNPs between strains represents a recent clonal transmission event[4].

RESULTS

Table 1. *C. difficile* RT251 sequence type (ST) sub-lineages and allelic profiles. *RT027 was included as a comparator strain.

RT	Toxin profile	Clade	ST (n)	Housekeeping genes/Allelic profiles						
				adk	*atpA*	*dxr*	*glyA*	*recA*	*sodA*	*tpi*
251	$A^+B^+CDT^+$	2	188 (5)	1	1	9	9	14	5 (C381G)	2
		2	230 (1)	1	1	9	9	14	3	1 (A403G)
		2	231 (40)	1	1	9	9	14	3	2
		2	365 (2)	1	1	9	23 (C19A)	14	3	2
027*	$A^+B^+CDT^+$	2	1	1	1	1	10	1	3	5

Key epidemiological questions answered using WGS:

- Where did Australian *C. difficile* RT251 strains come from?
- What is the genetic relatedness between RT251 strains isolated in CDI patients in Australia?

MAJOR FINDINGS

- *C. difficile* RT251 strains clustered with RT027 in clade 2, also known as the "hyper-virulent" clade[5].
- The *C. difficile* RT251 lineage was differentiated into four sub-lineages by MLST (STs 188, 230, 231 and 365) [Table 1].
- All Australian *C. difficile* RT251 strains belonged to ST 231, suggesting a highly clonal population.
- The methyltransferase gene *ermB* was found in 3/48 (6.3%) RT251 strains, all of which belonged to the ST231 sub-lineage. This genotype is associated with the MLS_B (macrolide-lincosamide-streptogramin B-resistant) phenotype[4].
- A cgSNP maximum-likelihood phylogeny of 40 RT251/ST231 strains revealed a highly clonal population, consisting of five clonal groups (CGs 1 – 5) [**Figure 1**].
- CGs 1 – 4 consisted of genetically indistinguishable strains (≤2cgSNPs difference) isolated across five States in Australia separated by thousands of kilometres without evidence of geographical or temporal clustering [**Figure 1**]. This finding is consistent with a point source of contamination likely associated with the national food chain.
- One strain (US48★) that originated from Virginia, USA, in 2009 shared ≤2 cgSNP difference with RT251 strains isolated in Australia (CG4), suggesting a cross-continental transmission event and shared evolutionary ancestry.

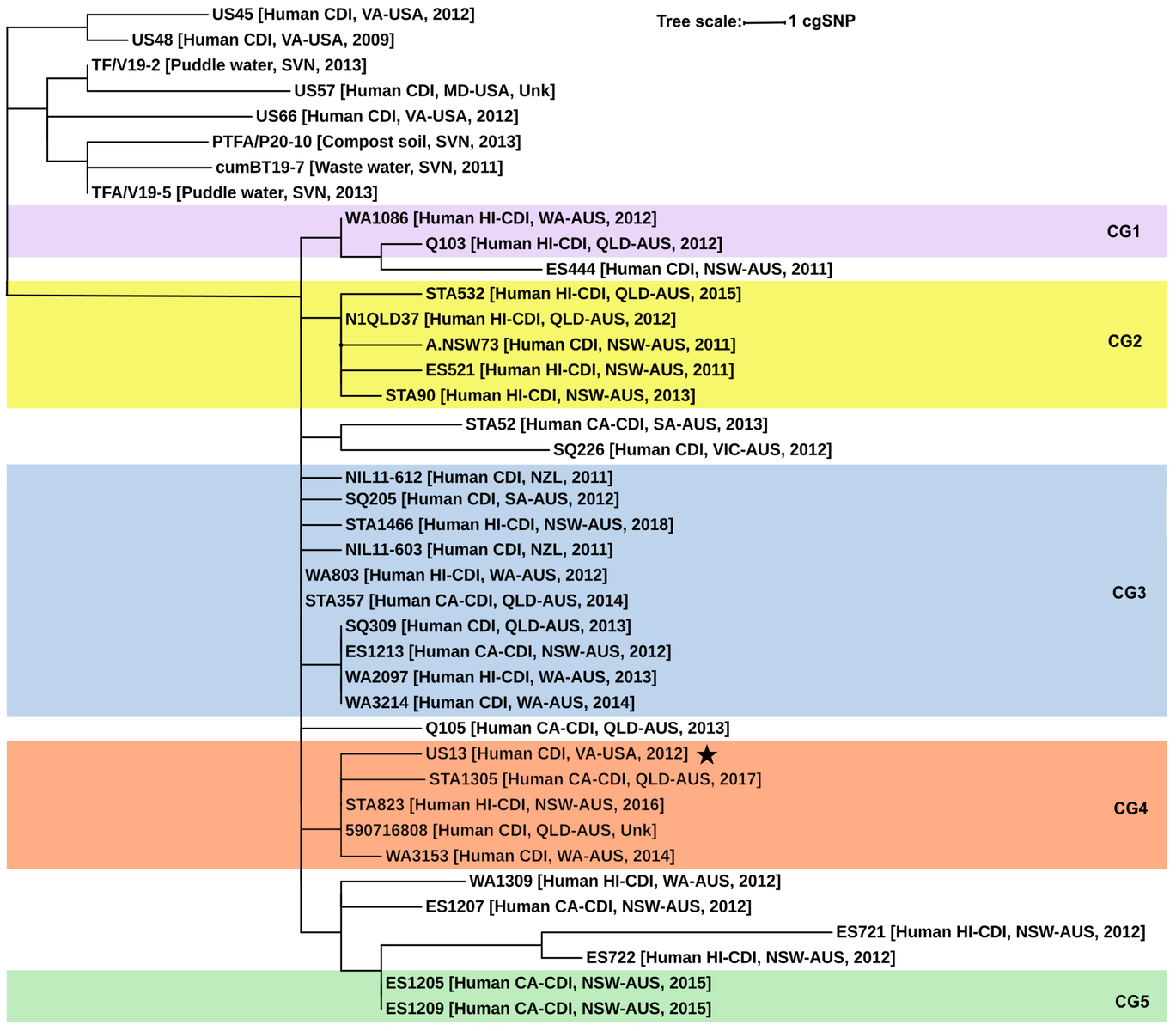


Figure 1. Population structure of 40 *C. difficile* RT251/ST231 strains based on cgSNPs.

Maximum-likelihood phylogeny based on non-repetitive, non-recombinant SNPs (n=86) identified after mapping all sequence reads against the R20291 (RT027) reference genome (accession number FN545816; 4,191,339 bp). RAxML tree is mid-point rooted and is supported by 100 nonparametric bootstrap replicates. Colour range based on CGs where all isolates differ by no more than four SNPs in their core genome.

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