Emergence of *Clostridium difficile* ribotype 106 in Western Australia

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Background & Objectives

Clostridium difficile is a common cause of antibiotic-associated diarrhoea, which is mediated by one or more of three toxins: toxin A (TcdA), toxin B (TcdB) and binary toxin (CDT).[1,2] *C. difficile* can be classified into different ribotypes (RTs). Recently, there has been a change in the epidemiology of *C. difficile* infection (CDI) in the USA, as *C. difficile* RT 106 has become the most common cause of both community-acquired and hospital-associated CDI supplanting RT 027.[3] In WA, there were no reports of RT 106 before September 2015, when the first strain was isolated. Since then, there has been a steady increase in the prevalence of RT 106 in WA (Figure 1).

The objectives of this study were to investigate the increased prevalence of *C. difficile* RT 106 in WA and to evaluate the screening tool used in the outbreak investigation of CDI.

Methods

- All *C. difficile* strains that had been reported as RT106 were sent to the reference laboratory where PCR ribotyping and toxin gene detection were performed as previously described.[4-7]
- Eleven *C. difficile* strains from 5 different hospitals in WA (Figure 2) were selected for further whole genome sequence analysis.
- *In silico* multi-locus sequence typing (MLST) was performed on 11 *C. difficile* strains and core genome single nucleotide polymorphisms (CGSNPs) analysis was performed on 11 *C. difficile* strains. For comparative analysis, we included genomes of RT 106 circulating in the United Kingdom (UK)[8] and the USA.[9]

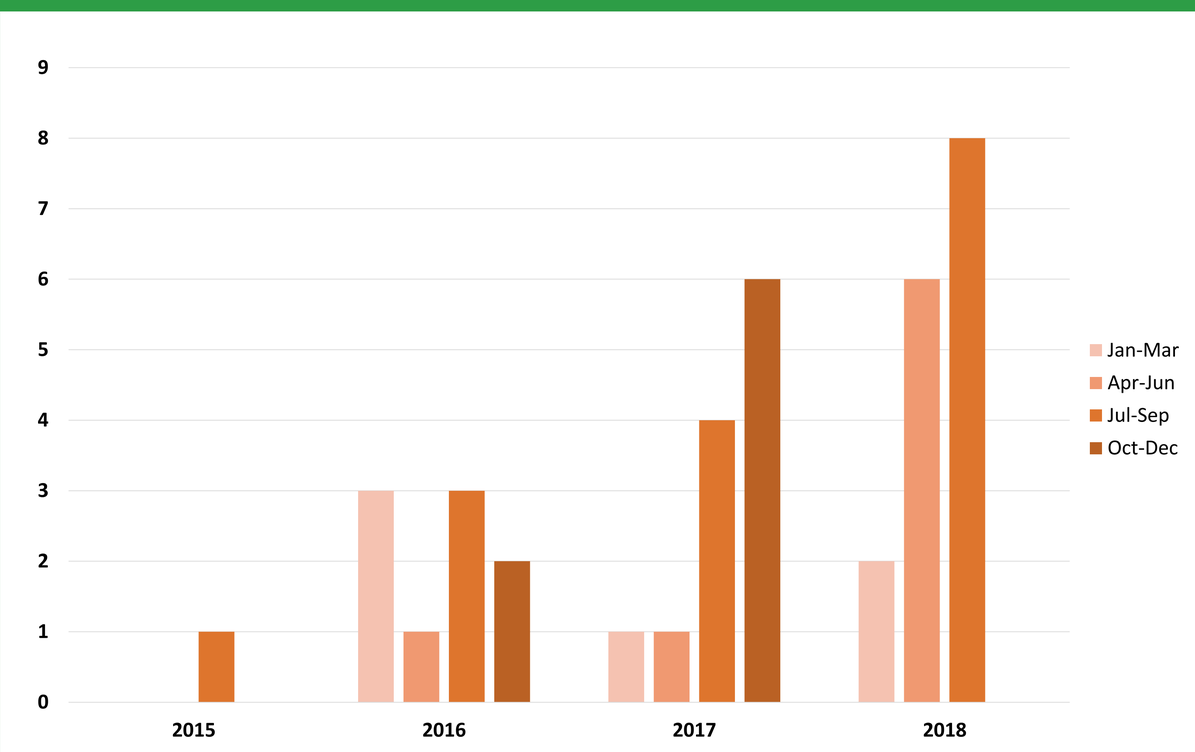


Fig 1 – Number of *C. difficile* RT 106 isolated in WA. The first and only strain in 2015 was isolated in September. Nine, twelve and sixteen strains were isolated in 2016, 2017 and 2018, respectively.

ST	*adk*	*atpA*	*dxr*	*glyA*	*recA*	*sodA*	*tpi*	clade
28	1	1	2	1	1	3	1	1
42	1	1	2	1	1	7	1	1

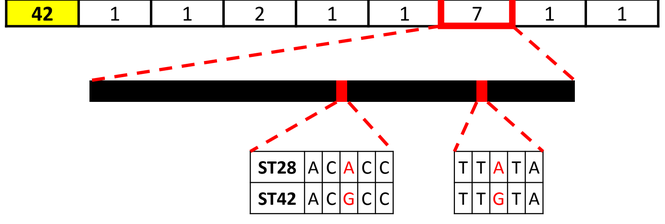
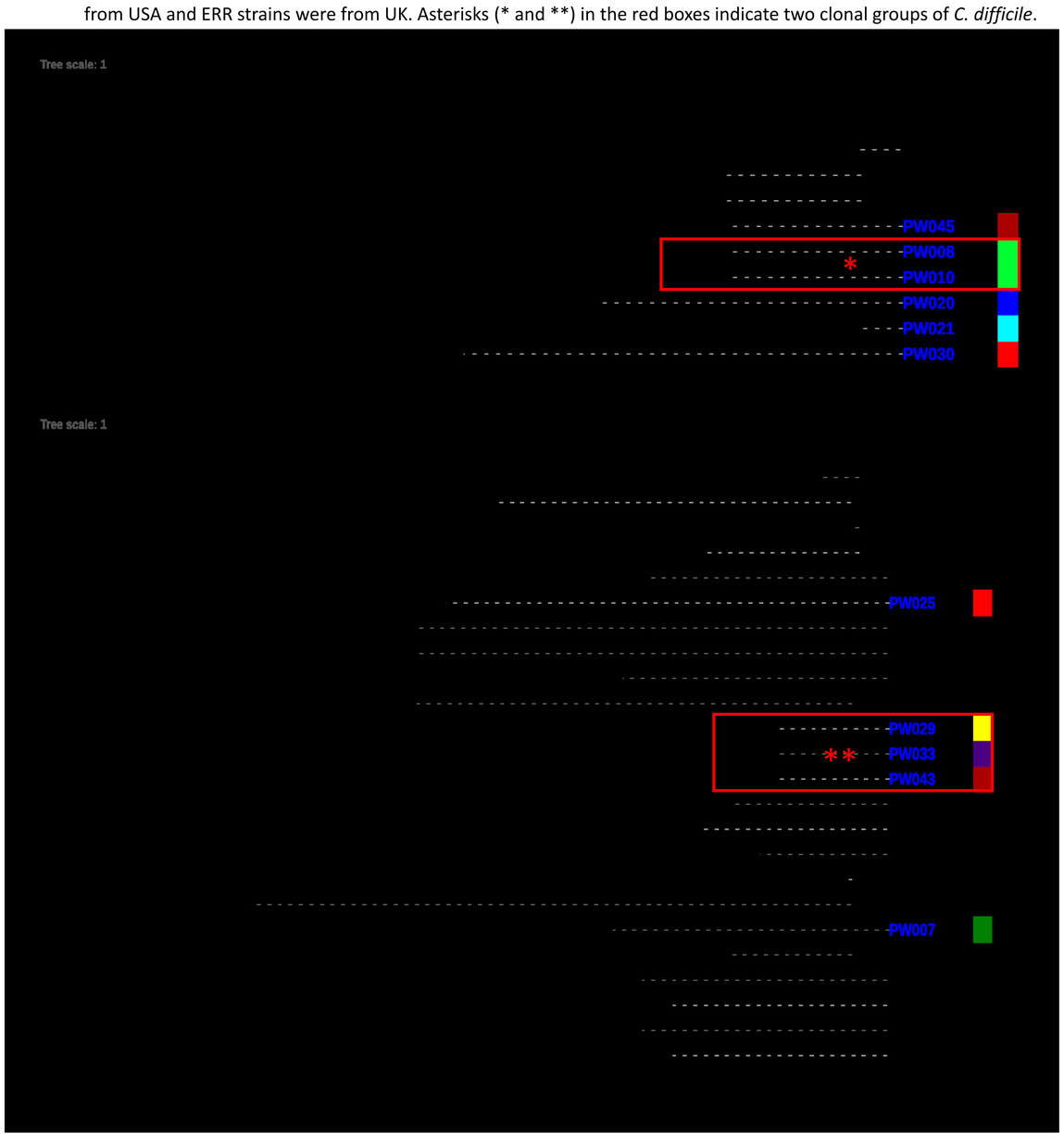


Fig 2 – Allele differences between STs 28 and 42. ST 28 differs from ST 42 by two different SNPs in a single allele.

Fig 3 – Phylogenetic tree of *C. difficile* ST 28 (A) and ST 42 (B) from CGSNPs analysis. Eleven WA isolates are displayed in blue lettering (PWs). The coloured squares correspond to individual wards in different hospitals. SRR and LK strains were from USA and ERR strains were from UK. Asterisks (* and **) in the red boxes indicate two clonal groups of *C. difficile*.



Symbol	Name	Details
*	Clonal group I	A group of 2 *C. difficile* isolates from 2 patients from a single ward in a hospital. The 2 strains were separated by 55 days.
**	Clonal group II	A group of 3 *C. difficile* isolates from 3 patients from 3 different hospitals. PW029 and PW033 were separated by 65 days. PW033 and PW043 were separated by 98 days.

Results

- Six RT 106 strains belonged to ST 28 and five belonged to ST 42. These two STs are single-locus variants of one another (Figure 2).
- The phylogenetic tree of ST 28 (Figure 3A) shows that ST 28 in WA formed a single cluster which was distinct from strains from both the UK and USA.
- Two ST 28 strains from two patients in the same ward (clonal group I in Figure 3A) were identical (CGSNP = 0).
- The phylogenetic tree for ST 42 (Figure 3B) showed that ST 42 in WA did not form a single cluster.
- Three ST 42 strains from three patients in different hospitals (clonal group II in Figure 3B) were identical (CGSNP = 0).

Discussion and Conclusions

- This study demonstrates two closely related lineages of *C. difficile* RT 106 (STs 28 and 42).
- Two clonal groups of *C. difficile* RT 106 were identified in this study. Clonal group I demonstrates a possible source of transmission within a hospital. Clonal group II demonstrates a possible source of transmission in the community.
- *C. difficile* RT 106 strains in WA were distinct from RT 106 strains found in other countries.
- PCR ribotyping can be used as a screening test for outbreak investigations, but confirmation testing (e.g. MLST or core genome typing) is also needed.

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