Case Report

Two cases of *Clostridium difficile* infection in unrelated oncology patients attributable to a single clone of *C. difficile* PCR ribotype 126

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Introduction: *Clostridium difficile* is a significant gastrointestinal pathogen and a leading cause of life-threatening diarrhoea in the developed world. Antibiotic therapy and immunodeficiency are key risk factors for *C. difficile* infection (CDI); consequently, oncology patients are at high risk.

Case presentation: We present two cases of CDI in unrelated oncology patients from different Western Australian hospitals in 2012. The first, a 59-year-old male, presented with diarrhoea 3 weeks after admission to hospital for treatment of a grade IV glioblastoma. Symptoms commenced after receiving prophylactic perioperative cephazolin. The second case was a 2-year-old female who presented with several episodes of diarrhoea after extended hospitalization following treatment for a stage 4 neuroblastoma. The patient had been exposed to regimens of piperacillin/tazobactam and ciprofloxacin for febrile neutropaenia and intra-abdominal sepsis. In both cases, the diarrhoea resolved after commencement of oral metronidazole. Both patients had an uncommon strain of *C. difficile* (PCR ribotype 126) detected in stool specimens, and both strains belonged to an unusual multilocus sequence type (ST), ST258. Comparison of the genomes of both strains by whole-genome sequencing showed them to be indistinguishable (no single-nucleotide variants). No epidemiological link between the patients was identified.

Conclusion: These data suggest that both cases resulted from exposure to a common source, most likely food contaminated with livestock faeces. Moreover, this is the first report of ribotype 126 isolates belonging to a ST (ST258) other than ST11. Our cases highlight the need for continued molecular surveillance of *C. difficile*, and the genetic analysis of emerging ribotypes.

Keywords: antibiotics; *Clostridium difficile* infection (CDI); diarrhoea; oncology; PCR ribotyping; whole-genome sequencing; zoonosis.

Introduction

Clostridium difficile is a significant gastrointestinal pathogen, the leading cause of life-threatening infectious diarrhoea in the developed world (Khanna & Pardi, 2014).

C. difficile infection (CDI) is a toxin-mediated disease that manifests as a variety of disorders ranging in severity from diarrhoea, cramps and nausea to more serious sequelae including pseudomembranous colitis, toxic megacolon and sepsis (Heinlen & Ballard, 2010). Risk factors for the development of CDI are well defined and include immunodeficiency, hospitalization and perturbation of the colonic microbiota by antibiotic exposure (especially with cephalosporins and clindamycin) (Heinlen & Ballard, 2010). Consequently, oncology patients undergoing chemotherapy and antibiotic treatment are at great risk of developing CDI (Palmore *et al.*, 2005). In particular, paediatric cases of CDI are significantly more common in children with malignancy (Kim *et al.*, 2012).

Surveillance of current and emerging *C. difficile* strains is a key component in the strategy for understanding and ultimately reducing the burden of CDI on global healthcare systems. In the past 20 years, CDI has come to prominence in the developed world due to large epidemics in North America and Europe attributable to PCR ribotypes (RTs) RT027 and RT078, which are associated with increased mortality and morbidity (Goorhuis *et al.*, 2008; He *et al.*, 2013; Walker *et al.*, 2013). In Australia, RT027 and RT078 have not become established, yet CDI rates have increased in all states since mid-2011 (Slimings *et al.*, 2014). In Western Australia (WA), epidemiological typing of all *C. difficile* isolates has been performed since 2011. During this time, we have observed an increase in the frequency of cases of CDI in haematology/oncology patients (Foster *et al.*, 2014) and also the emergence of previously uncommon strain types belonging to the same hypervirulent clonal lineages as RT027 (Eyre *et al.*, 2015; Lim *et al.*, 2014). This case report details the isolation and genetic characterization of a single clone of an uncommon *C. difficile* RT from two unrelated oncology patients with CDI in WA. These CDI cases were geographically and epidemiologically distinct, and there was no opportunity for direct transmission between the patients.

Case report

Case 1

In late 2011, a 59-year-male from Kalgoorlie, WA, was admitted to Sir Charles Gairdner Hospital (SCGH) in Perth, WA, with acute-onset right-sided hemiplegia. A computed tomography scan demonstrated a left subcortical parietal mass. A biopsy of the mass by stealth-guided craniotomy revealed a grade IV glioblastoma/astrocytoma. The patient received 2 g cephazolin intravenously perioperatively for surgical prophylaxis but no other antibiotics during this admission. He was treated with dexamethasone and did not receive chemotherapy. Approximately 3 weeks after the initial admission, he developed non-severe CDI: he was afebrile, and had diarrhoea and abdominal pain,

and laboratory investigations revealed a white cell count of $12.30 \times 10^9 \ l^{-1}$ [normal range (NR) 4×10^9–$11 \times 10^9 \ l^{-1}$], neutrophil count of $11.28 \times 10^9 \ l^{-1}$ (NR 2×10^9–$7.5 \times 10^9 \ l^{-1}$), normal serum creatinine of 8 μmol l^{-1} (NR 60–110 μmol l^{-1}) and normal serum albumin of 38 g l^{-1} (NR 35–50 g l^{-1}). There was no evidence of shock, ileus or megacolon. The patient was commenced on oral metronidazole 4 days after symptom onset, by which time the diarrhoea had already improved. The patient's stool tested positive for *C. difficile* glutamate dehydrogenase by enzyme immunoassay (*C. DIFF CHEK*™-60; Alere) and positive for *C. difficile* toxin genes by real-time PCR (GeneOhm Cdiff Assay; Becton Dickinson). The stool sample was cultured for *C. difficile* on cycloserine-cefoxitin-fructose agar, and putative colonies were subcultured on blood agar for confirmation. Bacterial identification and isolation was based on characteristic colony growth and morphology, odour and fluorescence under long-wave UV light as described previously (Boseiwaqa *et al.*, 2013). The *C. difficile* isolate was submitted to the *C. difficile* reference laboratory (PathWest Laboratory Medicine, Perth, WA) for epidemiological typing.

Case 2

In 2012, a 2-year-old female from rural WA was admitted to Princess Margaret Hospital for Children (PMH), also in Perth, WA, but located several kilometres away from SCGH, for treatment of stage 4 neuroblastoma. Over a period of approximately 18 months, she was treated as both an inpatient and outpatient at PMH with multiple courses of intensive chemotherapy, including megatherapy with autologous stem-cell rescue, tumour resection, radiotherapy and immunotherapy. Treatment was complicated by extended hospital admissions requiring multiple courses of broad-spectrum antibiotics. This included an admission for *Klebsiella pneumoniae* bacteraemia from intra-abdominal sepsis after tumour resection, when she was treated with intravenous piperacillin/tazobactam and a short course of metronidazole, followed by oral ciprofloxacin. During these admissions, the patient repeatedly grew *C. difficile* from stool samples, with cytotoxin not detected by a cell culture cytotoxicity neutralization assay. As she did not have diarrhoea during these episodes, she was not specifically treated for CDI. Prolonged excretion of norovirus was also detected by PCR in all her stool specimens from April 2012 to January 2013. In December 2012, she was admitted with non-severe CDI: she had fluid diarrhoea and fever, and laboratory tests revealed a white cell count of $17.7 \times 10^9 \ l^{-1}$ (NR 6×10^9–$17.5 \times 10^9 \ l^{-1}$), neutrophil count of $10.21 \times 10^9 \ l^{-1}$ (NR 1×10^9–$8.5 \times 10^9 \ l^{-1}$), hypoalbuminaemia of 25 g l^{-1} (NR 32–48 g l^{-1}) and normal serum creatinine of 17 μmol l^{-1} (NR <45 μmol l^{-1}), and *C. difficile* was again isolated. There was no evidence of shock, ileus or megacolon. She was treated with oral metronidazole and the diarrhoea resolved. The *C. difficile* isolate was sent for typing.

Investigations

In both cases, characterization of the *C. difficile* isolates by PCR (Hart *et al.*, 2014) showed the presence of the main *C. difficile* virulence factors *tcdA* (toxin A), *tcdB* (toxin B) and *cdtA/B* (ADP-ribosyltransferase, binary toxin). PCR ribotyping was performed as described previously (Knight *et al.*, 2013), and both isolates yielded a 16S–23S rRNA gene spacer banding pattern congruent with RT126 (Fig. 1) (Knight *et al.*, 2013). RT126 is an uncommon RT in humans in Australia, and the finding of two isolates of this RT within a short time period in seemingly unrelated oncology patients prompted further investigation. We examined the genetic background of both isolates using a variety of genomic and bioinformatic methods including whole-genome sequencing (WGS) (Eyre *et al.*, 2013), *de novo* assembly (Zerbino & Birney, 2008), *in silico* multilocus sequence typing (Griffiths *et al.*, 2010) and *tcdC* characterization (Curry *et al.*, 2007). The phylogenetic relationship between these isolates and a collection of *C. difficile* reference sequence types (STs) including ST11 (RT078) and ST1
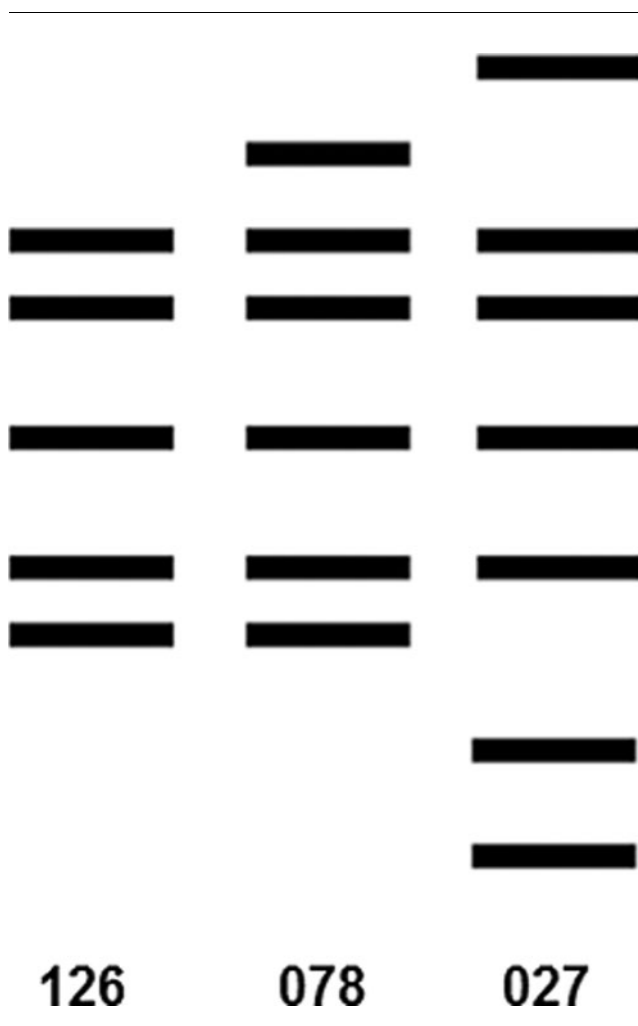
(RT027) was examined by ClonalFrame (Didelot & Falush, 2007). Furthermore, using a calculated *C. difficile* in-host mutation rate of 0.74 single-nucleotide variants per genome per year, the genomes of both isolates were compared for evidence of transmission (Didelot *et al.*, 2012; Eyre *et al.*, 2013).

Both isolates were determined to be ST258. Micro-evolutionary analysis of ST by ClonalFrame showed that ST258 belongs to clade 5, sharing a recent common ancestor with ST11, the clonal lineage containing RT078. Both STs appeared quite divergent from other STs in clade 5. Both isolates possessed a variant *tcdC* gene (C184T, Δ39 bp), which resulted in a significantly truncated TcdC protein – a negative regulator of toxin expression. Notably, WGS showed that the genomes of the two isolates were indistinguishable (no single-nucleotide variants identified), indicating that either transmission of a single *C. difficile* clone between patients had occurred, or that both cases were the result of exposure to a common source.

Outcome and follow-up

The first patient was transferred to a palliative care service and was not readmitted to hospital. The second patient had on-going *C. difficile* growth from stools throughout 2013 but did not require treatment.

Discussion

The global molecular epidemiology of CDI is complex and is continually evolving, with several new strain types emerging from existing virulent clonal lineages (Cairns *et al.*, 2012). Concomitantly, CDI in the community setting has emerged as a previously unrecognized but significant issue, which in the Northern Hemisphere has been reported to be frequently associated with *C. difficile* RT078 (Freeman *et al.*, 2010; Leffler & Lamont, 2012).

Our case report documents the isolation of a single clone of uncommon *C. difficile* RT126 from two apparently unrelated cases of non-severe, non-recurrent CDI in oncology patients in WA. Both isolates possessed genes encoding toxins A and B, key virulence factors in *C. difficile* (Heinlen & Ballard, 2010). In addition, the presence of binary toxin genes and a variant *tcdC* gene may have clinical significance, as they have been associated with severe and recurrent disease (Barbut *et al.*, 2005; Stewart *et al.*, 2014).

In Europe, the incidence of CDI attributable to RT126 appears to be relatively low, accounting for 3 % of infections in a 2008 European survey (Bauer *et al.*, 2011). Outside of Europe, there are very few reports of RT126. In a recent study from Taiwan, Hung *et al.* (2014) describe three cases of *C. difficile*-associated diarrhoea attributed to RT126, one of which developed pseudomembranous colitis and refractory disease suggesting virulence potential. Notably, one of these patients was receiving treatment for rectal cancer. A small number of cases have also been reported in

Fig. 1. 16S–23S rRNA gene spacer banding pattern of *C. difficile* PCR RT126, RT078 and RT027.

the Middle East (Jamal et al., 2010). RT126 belongs to clade 5, as does RT078 (ST11) (Stabler et al., 2012). In addition, RT126 has a very similar ribotyping banding pattern to RT078 (Fig. 1), and these two RTs are often reported together as RT078/126 (Lipovsek et al., 2013; Schneeberg et al., 2015). Interestingly, in this current study, both RT126 isolates belonged to an unusual ST (ST258) rather than the more common ST11. The finding of a second ST within RT126 so closely related to ST11 (two-locus variant) further demonstrates the extent of genetic diversity within C. difficile. Further characterization of this ST is under way to examine its evolutionary relationship to ST11.

RT078 is not a common cause of human CDI in Australia, and this RT is not found in Australian production animals (Knight et al., 2013, 2014). However, in recent years, several uncommon RTs of the same genetic lineage (clade 5) have been isolated from patients with CDI in Australia including RT033, RT237, RT127 and RT126 (Elliott et al., 2011; Thomas V. Riley, unpublished data). These RTs are increasing in frequency and appear to have a provenance in the community (Thomas V. Riley, unpublished data). Moreover, CDI caused by these clade 5 isolates of C. difficile may have a zoonotic origin. We have previously identified a potential reservoir of this lineage in the gastrointestinal tracts of Australian veal calves and piglets (Knight & Riley, 2014; Knight et al., 2013, 2014). These data corroborate findings of European studies (Schneeberg et al., 2013a, b; Zidaric et al., 2012) and support the hypothesis that CDI has a food-borne aetiology and is a potential zoonosis (Rupnik, 2007).

WGS permits ultrafine resolution of strain types by comparing genomes at the single-nucleotide level. WGS is becoming an accessible and essential tool for infection control and outbreak investigations (Eyre & Walker, 2013) that can assist hospitals in identifying sources of infection, tracking transmission and providing meaningful strain characterization (Didelot et al., 2012; Eyre et al., 2013). Comparative analysis of the isolates from both cases found no differences in the core genome (no single-nucleotide variants), supporting the hypothesis of direct transmission (Eyre et al., 2013). Examination of the clinical histories of each case found no evidence of direct contact between patients. The respective treating hospitals (PMH and SCGH) are located several kilometres apart and the single visit by patient 2 to SCGH in October 2012 was several months after the diarrhoea in patient 1 had resolved; thus, transmission via this route seems highly unlikely. Whilst we cannot rule out transmission between hospital staff or other patients, the molecular epidemiology of the strain strongly suggests that transmission did not occur in the hospital, rather that acquisition took place in the community setting.

Many studies have shown CDI to be highly prevalent among adults and children with cancer (Chopra et al., 2010). For some oncology patients, treatment takes place over long periods of time in both community and hospital environments. C. difficile is ubiquitous in the environment, and patients would be exposed to a wide range of strain types in the community. Prior and continuing exposure to broad-spectrum antibiotics would render the patients highly susceptible to C. difficile colonization and onset of disease. Early recognition of CDI and treatment with antimicrobial agents effective in CDI (e.g. oral metronidazole and vancomycin) is imperative to reduce recurrences and the severity of disease. In both patients, symptoms resolved after the commencement of oral metronidazole.

In conclusion, in this study we identified a single clone of C. difficile, of an uncommon but emerging RT, in the stool of two unrelated oncology patients with diarrhoea. This provides evidence that either transmission of a single C. difficile clone between patients had occurred, or that both cases were the result of exposure to a common source. The genetic background of the RT suggests that the source is likely to be in the community, and the patients were possibly exposed to contaminated food of production animal origin. These cases highlight the need for continued molecular surveillance of C. difficile and for the genetic analysis of emerging RTs.

Acknowledgements

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