

Case Report

Human *Clostridium difficile* infection caused by a livestock-associated PCR ribotype 237 strain in Western Australia

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Introduction: *Clostridium difficile* infection (CDI) is a significant gastrointestinal disease in the developed world and increasingly recognised as a zoonotic infection. In North America and Europe, the PCR ribotype (RT) 078 strain of *C. difficile* is commonly found in production animals and as a cause of disease in humans although proof of transmission from animals is lacking. This strain is absent in Australian livestock. We report a case of human CDI caused by a strain of *C. difficile* belonging to known Australian livestock-associated RT 237.

Case presentation: A young male was admitted for multiple trauma following a motor vehicle accident and placed on piperacillin/tazobactam for pneumonia. After 4 days of treatment, he developed symptoms of CDI, which was confirmed in the laboratory. His symptoms resolved after 6 days of intravenous metronidazole. The strain of *C. difficile* isolated was identified as RT 237, an unusual RT previously found in with several Western Australia piggeries.

Conclusion: This case of CDI caused by an unusual livestock-associated *C. difficile* RT 237 supports the hypothesis of zoonotic transmission. The case highlights the potential of livestock to act as reservoir for *C. difficile* and the need for continued surveillance of CDI in both human and animal populations.

Keywords: *Clostridium difficile* infection (CDI); zoonosis; diarrhoea; PCR ribotyping; antibiotics; metronidazole.

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Introduction

Clostridium difficile is an anaerobic, spore-forming, Gram-positive bacillus that acts as an opportunistic pathogen causing diarrhoea and sometimes life-threatening conditions such as toxic megacolon and pseudomembranous colitis. Since the early 2000s, the number of cases of *C. difficile* infection (CDI) worldwide has increased dramatically, particularly in North America and Europe. In the USA alone, CDI has been identified as the major healthcare-associated infection with half a million patients infected and costing at least US\$1 billion annually (Lessa *et al.*, 2015). An

increasing number of infections are caused by strains belonging to PCR ribotypes (RTs) that are common to both humans and animals, the most prominent of which is RT 078 (Goorhuis *et al.*, 2008). Direct transmission of RT 078 between pigs and humans has been postulated in the Netherlands (Knetsch *et al.*, 2014) but never conclusively proven. In Australia, RTs associated with major outbreaks overseas such as RT 027 and RT 078 are rare; however, overall rates of CDI across Australia have increased recently (Slimings *et al.*, 2014). While RT 078 has not been detected in Australian livestock, a variety of other RTs has been isolated from production animals, including cattle, pigs and sheep (Knight & Riley, 2013; Knight *et al.*, 2015c; Knight *et al.*, 2013; Moono *et al.*, 2016; Squire *et al.*, 2013). In several piggeries in Western Australia (WA), an unusual RT of *C. difficile* (RT 237) is endemic (Squire *et al.*, 2013; Moono *et al.*,

Abbreviations: BRH, Bunbury Regional Hospital; CDI, *Clostridium difficile* infection; CLSI, Clinical and Laboratory Standards Institute; RT, PCR ribotype; WA, Western Australia.

2016). This report describes the isolation and characterisation of a *C. difficile* RT 237 strain in a case of human infection, initially identified in a cross-sectional study of CDI epidemiology in WA (Foster *et al.*, 2014).

Case report

A 19-year-old Caucasian male was admitted to Bunbury Regional Hospital (BRH) in WA during February 2012 for multiple trauma following a motor vehicle accident. The patient, a New Zealand national, was in Australia working as a dairy farmer. He had been hospitalised at BRH 3 months earlier for a crush injury of his hand. He had no known allergies and his only other medical history was of mild asthma requiring occasional inhaled salbutamol. He was transferred from BRH to the State Trauma Unit at Royal Perth Hospital via the Royal Flying Doctor Service.

Initial computerised tomography and X-ray scans revealed a minor mandibular fracture, two severe distal phalangeal fractures and extensive pelvic fracturing with associated haematoma. Haemoperitoneum and lacerations to the skin, right kidney and bladder were also found. On admission, the patient was placed on 4.5 g intravenous (IV) piperacillin/tazobactam three times daily for 6 days to prophylax against infection complicating the pelvic and abdominal trauma. Pelvic injuries were initially managed conservatively, but on the second day of his admission, after worsening abdominal pain, he underwent an exploratory laparotomy. At the procedure, a bladder injury was noted and repaired. On the same day, he also underwent surgical debridement of the finger wounds. Mandibular fractures were managed conservatively.

On the fourth day of admission, the patient developed a fever with a temperature of 38.4 °C and associated tachycardia and tachypnoea. He began to exhibit abdominal distension and frequent bowel motions with loose stools. Blood cultures taken at this time were negative. At 7 days post-admission, IV piperacillin/tazobactam was ceased. The patient underwent an open reduction and internal fixation of the pubic symphysis and was commenced on IV cephazolin 1 g 6 hourly, as peri-operative prophylaxis for 48 h. His fevers continued while his bowel motions became more frequent and CDI was suspected. A stool sample was collected at 9 days post-admission for standard faecal microscopy and culture. At 10 days post-admission, he was noted to have pus discharging from the urethra and was recommenced on piperacillin/tazobactam 4.5 g IV three times daily for 5 days for treatment of presumed urinary tract infection. There was no significant pathogen identified from a urethral swab taken at the time.

PCR testing (BD GeneOhm™ Cdiff Assay, BD Diagnostics) of the stool specimen detected the presence of the *C. difficile* toxin B gene (*tcdB*). Vancomycin-resistant *Enterococcus* was also detected but *Aeromonas*, *Campylobacter*, *Norovirus*, *Salmonella*, *Shigella* and intestinal parasites were not. His haematological profile at this time showed thrombocytosis

($628 \times 10^9 \text{ l}^{-1}$) [normal range, 150 to $450 \times 10^9 \text{ l}^{-1}$], neutrophilia ($12.03 \times 10^9 \text{ l}^{-1}$) [normal range, 2 to $7.5 \times 10^9 \text{ l}^{-1}$], low haemoglobin (87 g l^{-1}) [normal range, 115 to 165 g l^{-1}] and hypo-albuminaemia (27 g l^{-1}) [normal range, 35 to 45 g l^{-1}]. Based on these laboratory results, the patient immediately began a 10 day course of metronidazole 400 mg IV three times daily and was placed under contact precautions. Ten days post-admission, the patient developed a deep infection complicating the pelvic plate. At this time, he was recommenced on piperacillin/tazobactam empirically. He underwent debridement of the wound on day 14 and day 19 of the admission, and he underwent an exchange of the pelvic plate on day 21 of his admission. No specimens for culture were obtained from the wound. The patient continued on piperacillin/tazobactam for 18 days, after which time he developed a presumed delayed hypersensitivity reaction to piperacillin/tazobactam (rash fever, eosinophilia and liver function derangement) and was subsequently changed to IV cephazolin 2 g 8 hourly. After 6 days of metronidazole treatment (16 days post-admission), his fever and CDI symptoms had subsided. A faecal specimen collected 23 days post-admission was PCR negative for the toxin B gene and the patient was discharged to the physical rehabilitation unit after a total of 46 days as an in-patient. The patient completed 6 weeks of IV antimicrobial therapy for the infection of the pelvic plate, after which time he continued on oral cephalixin, with the plan for this to continue until the pelvic plate could be definitively removed. No further episodes of CDI were noted.

Investigations

C. difficile was cultured from the PCR-positive stool specimen as previously described (Boseiwaqa *et al.*, 2013); the strain isolated was designated WA 1016. PCR ribotyping and PCR detection of the genes encoding toxin A (*tcdA*), toxin B (*tcdB*) and the binary toxin (CDT) component genes (*cdtA*, *cdtB*) were performed as described previously (Knight *et al.*, 2013). WA 1016 gave a PCR ribotyping pattern consistent with RT 237 (Fig. 1) and was positive for *tcdB* and both CDT genes but negative for *tcdA* ($A^-B^+ \text{ CDT}^+$), a toxin profile also consistent with *C. difficile* RT 237. Antimicrobial susceptibility testing was performed using the agar incorporation method as described by Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2011; CLSI, 2013). All clinical breakpoints were provided by CLSI (CLSI, 2013) with the exception of those for vancomycin and rifaximin that were recommended by the European Committee on Antimicrobial Susceptibility Testing [EUCAST (2016) and O'Connor *et al.* (2008), respectively]. WA 1016 was resistant to trimethoprim but susceptible to vancomycin, metronidazole, rifaximin, clindamycin, erythromycin, moxifloxacin and piperacillin/tazobactam. The fidaxomicin minimum inhibitory concentration of WA 1016 was 0.008 mg l^{-1} (no clinical breakpoints for fidaxomicin are currently available). The antimicrobial susceptibility profile of WA 1016 matched those typical of animal RT 237 isolates (Knight & Riley, 2016).

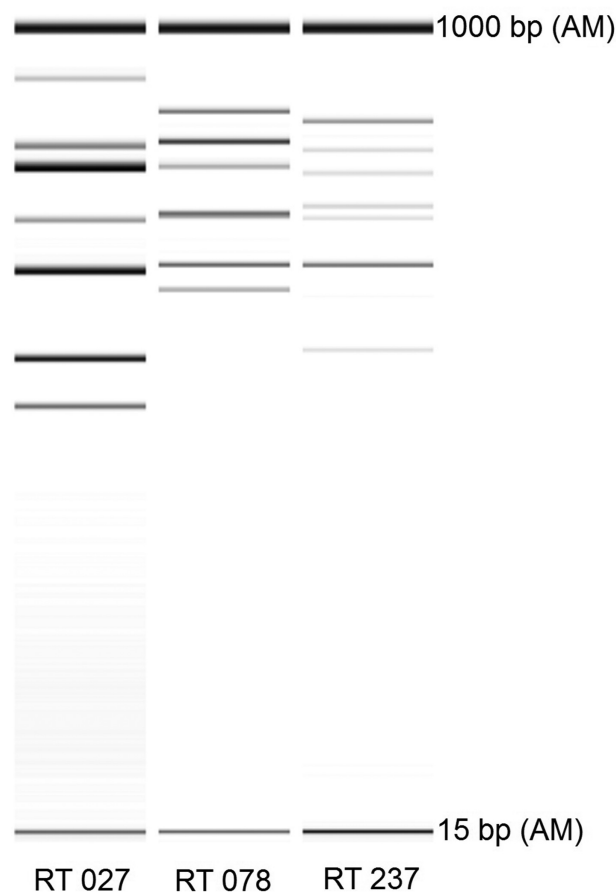


Fig. 1. PCR ribotyping (RT) pattern of *C. difficile* RTs 027, 078 and 237 when visualised by capillary electrophoresis. AM, alignment marker.

Discussion

The epidemiology of CDI is complex and ever-changing, with recent evidence pointing to genetically diverse sources of infection (Eyre *et al.*, 2013) and possible zoonotic transmission in Europe (Knetsch *et al.*, 2014). The potential for zoonotic transmission of *C. difficile* in Australia has also been suggested (Knight *et al.*, 2015b). In Australian livestock, the molecular epidemiology of *C. difficile* is different from that seen elsewhere in the world. The strain most commonly associated with production (food) animals in the global setting is RT 078 and this strain is not found in Australian livestock, although many related strains also belonging to phylogenetic clade 5 are present (Knight *et al.*, 2013). In Australian piglets, RTs 014 and 033 were the most common RTs recovered, followed by RTs 281 and 237 (Knight *et al.*, 2015c). In Australian veal calves slaughtered at 5–7 days old, the most frequently encountered RTs were RTs 127 and 033 followed by RTs 056 and 126 (Knight *et al.*, 2013). *C. difficile* RT 237 was absent in veal calves (Knight *et al.*, 2013). Crossover between animal and human RTs of *C. difficile* in Australia appears to be occurring with

RTs 014 and 056 being common RTs found in both humans and animals (Foster *et al.*, 2014); however, this needs to be confirmed with whole genome sequencing. The high prevalence of RT 033 in livestock does not seem to be mirrored in humans; however, the absence of toxin A and B production in this strain may preclude its detection in many diagnostic laboratories (Androga *et al.*, 2015).

C. difficile RT 237 belongs to toxinotype XXXI and is missing a large portion of the 5' end of the *tcdA* gene yet retains an intact *tcdB* gene (Rupnik & Janezic, 2016). Certain diagnostics tests reliant on detection of the missing fragment of *tcdA*, such as the Illumigene[®] *C. difficile* test (Meridian Bioscience), will also not detect RT 237 (Androga *et al.*, 2015). Diagnostic issues such as these are compounded by recent trends away from culture and greater reliance on molecular tests (Burnham & Carroll, 2013). *C. difficile* RT 237 is an unusual ribotype rarely seen as a cause of disease in humans in Australia. This is in marked contrast with RT 078, which is commonly detected in both humans and animals outside Australia (Goorhuis *et al.*, 2008). Interestingly, RT 078 infections in Australian patients still occur (Knight *et al.*, 2015a) in the absence of overseas travel suggesting another reservoir of infection, perhaps imported food. Since routine surveillance began in WA in 2011, only six other cases of human CDI involving RT 237 have been recorded (TV Riley *et al.*, unpublished data). However, *C. difficile* RT 237 has been isolated from WA piggeries for many years and remains highly prevalent (Moono *et al.*, 2016; Squire *et al.*, 2013). To our knowledge, RT 237 has not been reported elsewhere in the world and was not mentioned in a recent publication describing strains of *C. difficile* isolated from animal populations around the world (Janezic *et al.*, 2014). To what extent that the lack of *C. difficile* RT 237 infections described in other parts of the world is due to detection issues remains unclear.

The source of infection in our case is difficult to determine. As symptoms began after 48 h of admission to a healthcare facility, the case was classified as hospital-associated healthcare facility onset according to McDonald *et al.* (2007). No cases of RT 237 had occurred at BRH prior to the patient's admission. The apparently exclusive distribution of *C. difficile* RT 237 in production animals in WA (Knight *et al.*, 2015c; Moono *et al.*, 2016; Squire *et al.*, 2013) is suggestive of transmission within the state. The patient lived in shared accommodation in a rural area working as a dairy farmer. RT 237 was not isolated from dairy (veal) calves in a previous Australian surveillance study (Knight *et al.*, 2013); however, this study did not include WA calves. *C. difficile* RT 237 has been isolated from several piggeries in WA and there is pig rearing in the region of WA where the patient lived. Comprehensive surveillance of *C. difficile* in livestock is not in place nor is it likely to be in the near future, making it difficult to get a complete picture of the distribution of this ribotype. We recently isolated *C. difficile* RT 237 from vegetables sold in several grocery stores in WA (Lim, Foster & Riley, unpublished data). This suggests contamination of vegetables by piggery effluent. Possible food-borne transmission of another clade 5 strain, RT 126, common in Australian cattle, has recently been reported (Knight *et al.*, 2015b). The

potential of contaminated food as a route of transmission of *C. difficile* has been gaining more attention (Gould & Limbago, 2010; Rupnik, 2007; Weese, 2010). Additionally, the role of the broader environment as a reservoir for *C. difficile* requires further investigation. There are difficulties in studying transmission of *C. difficile* outside the hospital; however, data from the limited number of surveys performed so far suggest that *C. difficile* is present widely in soil and water (Al Saif & Brazier, 1996).

This report demonstrates that *C. difficile* RT 237, an animal strain of *C. difficile* reported only in WA, can be associated with human infection. This finding supports our previous observation that *C. difficile* RT 237 caused significantly greater weight loss in mice than *C. difficile* RT 078 due to the production of a variant toxin (Squire *et al.*, 2013) and highlights the potential risk that animals carrying this strain may pose to humans. Surveillance of animal populations is needed to clarify the relationship between livestock-associated *C. difficile* and human CDI. Ultimately, the promotion of a dialogue between physicians, veterinarians and scientists in the development of a One Health approach will be essential to control CDI.

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