Two cases of fulminant colitis due to binary toxin-positive *Clostridium difficile* that are not PCR ribotype 027 or type 078

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Two cases of fulminant colitis due to *Clostridium difficile* occurred within ten weeks of each other on the same ward of a hospital in Japan. The patients died 2 and 4 days after the onset of colitis. *C. difficile* isolates obtained from both patients were toxin A-positive, toxin B-positive and binary toxin-positive. These isolates yielded identical results by both PCR ribotyping and *slpA* sequence typing. However, the banding patterns and *slpA* sequences of the isolates differed from those of PCR ribotype 027, as well as those of PCR ribotype 078. The *tcdC* sequences of the isolate differed from those of *C. difficile* 027, but a single base-pair deletion at position 117 and an 18 bp deletion, both of which were identical to the sequence of the reference strain of 027, were found. This type may be a new hypervirulent strain, but further studies of the epidemiology and pathogenicity of the strain are needed.

**Introduction**

*Clostridium difficile* infection (CDI) can develop into a serious disease (Morris et al., 2002; Redelings et al., 2007). It has been documented that patients infected with *C. difficile* that produce the binary toxin (CDT), including PCR ribotype 027 (NAP1/BI/027) and PCR ribotype 078, are more likely to develop a severe disease (Goorhuis et al., 2007, 2008; Miller et al., 2010). In contrast, other studies have noted the lack of an association between PCR ribotypes 027 and 078 with severe CDI (Goorhuis et al., 2007, 2008; Miller et al., 2010). In addition, genotypes negative for CDT, such as PCR ribotypes 056 and 018, have been associated with complicated diseases (Bauer et al., 2011). In Japan, it was reported that two of 86 CDI cases examined had severe complications, and *C. difficile* isolates from both cases were toxin A-negative, toxin B-positive and CDT-negative (Kato et al., 2010). In another report, comparative analysis of the clinical characteristics of patients harbouring three types was performed. The duration of CDI was longer in patients with PCR ribotype yok (UK PCR ribotype 002), which is toxin A-positive, toxin B-positive and CDT-negative (Iwashima et al., 2010). So far, any association between complicated CDI and CDT-positive *C. difficile* isolation has not been documented in Japan.

In a non-epidemic setting, fulminant colitis due to *C. difficile* occurred in two cases within a ten-week period on the same ward. Here, we describe two fatal cases due to CDT-positive *C. difficile*, which was neither PCR ribotype 027 nor type 078.

**Case presentation**

**Case 1**

A 60-year-old Japanese man suffered from subcortical haemorrhage and aspiration pneumonia. He was admitted to hospital in December 2010. When he was in a nursing home, he was treated for alcoholic liver cirrhosis and alcoholic psychosis. Upon admission to the hospital, ampicillin-sulbactam was started for aspiration pneumonia, as well as an anti-ulcer drug for the prophylaxis of stress ulcer. Six days after admission, a laxative was initiated for obstinate constipation. After a week of antimicrobial therapy, he showed symptoms of severe watery diarrhoea and hypovolemic shock and was admitted to the intensive care unit (ICU).

Subsequent to transfer to the ICU, laboratory test results revealed a rapid increase in white blood cells (WBCs) from 5500 to 40 300 mm$^{-3}$, a rapid decrease in albumin from 3.1 to 0.6 g dl$^{-1}$ and acute renal injury. The stool specimen was positive for toxins A and B of *C. difficile* by enzyme immunoassay (EIA), and vancomycin therapy was initiated. The patient was intubated and was administered a strong dose of saline, noradrenaline and dopamine without resolution of septic shock. He died 28 h after the onset of...
diarrhoea. An autopsy revealed pseudo-membrane formation, oedema and submucosal haemorrhage from the ascending colon to the sigmoid colon (Fig. 1a).

Case 2
A 60-year-old Japanese woman was hospitalized in the same ward and experienced subarachnoid haemorrhage six weeks after the onset of colitis of case 1. Case 2 had brain surgery on the day after hospitalization. In the perioperative period, cefazolin was administered for four days. An anti-ulcer drug was also administered for the prophylaxis of stress ulcer. After the surgery, she was intubated with a feeding tube. Two weeks after the surgery, she developed pyelonephritis due to *Escherichia coli*, for which piperacillin-tazobactam was administered. After an 18-day antimicrobial therapy, she developed severe watery diarrhoea and needed to be admitted to the ICU. On admission to the ICU, she had indications of leukocytosis (29 400 mm$^{-3}$), hypoalbuminaemia (1.5 g dl$^{-1}$) and acute kidney failure. A strong dose of saline, noradrenaline and dopamine was required as she developed septic shock. Her stool specimen tested positive for toxins A and B of *C. difficile* by EIA; treatment with vancomycin and metronidazole was initiated. On the day after admission to the ICU, contrasted computed tomography of the chest and abdomen was performed, which showed pancolitis, ascites around the colon and the accordion sign (Fig. 1b). Colectomy was not performed and she died 4 days after the onset of colitis.

Analysis of *C. difficile* isolates
*C. difficile* isolates recovered from stool specimens of both patients were identified as toxin A-positive, B-positive and CDT-positive. Both isolates were indistinguishable by PCR ribotyping (PCR ribotype ash1101), but the banding patterns of both isolates differed from those of PCR ribotype 027 and PCR ribotype 078 (Fig. 2). In addition, the *slpA* sequences (Kato et al., 2010) were identical in both isolates (*slpA* sequence type; ts592-02; GenBank accession no. AB675076) but analysis indicated that these sequences were different from those of *C. difficile* 027 and 078. Sequence analysis of *tcdC* (Spigaglia & Mastrantonio, 2002) showed that the *tcdC* sequences of the isolates differed from those of *C. difficile* 027. However, we found a single base-pair deletion at position 117 and an 18 bp deletion, identical to the sequence of the reference strain of 027 (GenBank accession no. AB771925).

Discussion
Two fatal cases of fulminant colitis due to *C. difficile* occurred within ten weeks of each other on the same hospital ward. Notably, the isolates from the cases were
CDT-positive, but differed from PCR ribotypes 027 (NAP1/BI/027) and 078. Severe CDI is more frequent among individuals infected with PCR ribotype 027 (Goorhuis et al., 2007; Miller et al., 2010). Another report found that CDI due to PCR ribotype 078 has similar severity to type 027 (Goorhuis et al., 2008). A recent publication showed an increased case fatality rate with CDT, regardless of the PCR ribotype (Bacci et al., 2011).

To date, there have been no reports that CDT-positive C. difficile strains including PCR ribotypes 027 and 078 have been endemic or epidemic at healthcare facilities in Japan. Sawabe et al. (2007) reported that five of 148 isolates (3%) collected at a university hospital in Japan were CDT-positive and one was PCR ribotype 027 (Sawabe et al., 2007). Another report from Japan showed that 6% of isolates examined were CDT-positive, but none of them was PCR ribotype 027 or 078 (Iwashima et al., 2010). It was reported that three of 87 isolates (3%) from 86 CDI patients examined were CDT-positive and only one suffered from CDI due to PCR ribotype 027 (Kato et al., 2007; Kato et al., 2010). In none of the aforementioned reports there was a correlation between complicated CDI and CDT-positive C. difficile.

At the hospital where the cases presented in this study were admitted, no other CDI cases with severe complications, including ICU admission, colectomy and death directly related to CDI, were found in the last five years. As C. difficile isolates from other CDI patients at this hospital were not available, it was unknown whether this strain spread among other patients. More than 300 C. difficile isolates collected at Japanese medical facilities other than the hospital where the patients were admitted were examined, and none was characterized as PCR ribotype ash1101/slpA sequence type ts592-02 (data not shown). This indicates that isolation of the strain is infrequent, at least in Japan. As this rare strain caused CDI-associated death in two patients, it should be noted that PCR ribotype ash1101/slpA sequence type ts592-02, which is CDT-positive and has mutations in tcdC, may be problematic and hypervirulent. In this study, PCR ribotype ash1101 could not be characterized as a UK PCR ribotype (Stubbs et al., 1999), because reference strains of CDT-positive C. difficile other than PCR ribotypes 019, 027 and 078 were not available. For further studies of the worldwide epidemiology of CDT-positive C. difficile, sequence-based typing techniques may be more useful than typing schemes that depend on banding-pattern analysis.

By contrast, no correlation has been shown between tcdC type and CDT status with disease severity of CDI (Goldenberg & French, 2011). A hospital-based survey performed in 34 European countries suggested that PCR ribotypes 018 and 056, which are CDT-negative, were significantly associated with complicated disease outcome (Bauer et al., 2011). Walk et al. (2012) documented that ribotype is not a significant predictor of severe CDI, while WBC count and albumin level are the most clinically relevant predictors. The patients in this study had leukocytosis and hypoalbuminaemia, which are predictive of severe diseases. Unfortunately, we could not cure either of the patients. Studies into the association between specific strain types, such as the type presented in this study, and disease severity may provide valuable information for examining CDI from an epidemiological standpoint. However, a severity score using clinical and laboratory parameters is needed for accurate clinical decision-making.

Fulminant colitis due to C. difficile positive for CDT, which was neither PCR ribotype 027 nor ribotype 078, occurred in two cases within ten weeks. The C. difficile isolates recovered from the patients may be a new hypervirulent strain. Further studies evaluating the virulence factors and epidemiology of this strain are required.

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**References**


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