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Surgical-site infection with toxin A-nonproducing and toxin B-producing Clostridium difficile

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Abstract To date, few cases of extraintestinal infection with Clostridium difficile have been reported. We describe a case of surgical-site infection with C. difficile following a colonic operation. Administration of metronidazole was considered to be effective for treatment of the infection. The isolate was a toxin A-nonproducing and toxin B-producing strain.

Key words Surgical-site infection · Clostridium difficile · Toxin B

Introduction

Clostridium difficile is an anaerobic Gram-positive bacillus and is recognized as the leading cause of antibiotic-associated colitis. Clinical manifestations of C. difficile-associated colitis (CDAC) range from abdominal pain, diarrhea, and fever to severe complications, including toxic megacolon and colonic perforation. Two major toxins, toxin A and toxin B, are the main virulence factors associated with CDAC. Until recently, all pathogenic C. difficile strains were thought to produce both toxin A and toxin B. However, toxin A-nonproducing and toxin B-producing strains (A−/B+ strains) have been also reported to cause CDAC. Our previous surveillance study in the Tsukuba-Tsuchiura district in Japan showed that 6.3% of C. difficile strains isolated from specimens submitted for toxin A assay were A−/B+ strains.

In contrast to CDAC, extraintestinal infection due to C. difficile has been rarely reported. In the present report, we describe a case of surgical-site infection caused by C. difficile following a colonic operation. Toxigenic analyses revealed that the isolated C. difficile was an A−/B+ strain.

Case report

A 56-year-old Japanese woman without any remarkable medical history was hospitalized as an emergency because of sudden right hemiparesis. Magnetic resonance imaging revealed the presence of a hematoma in her cervical epidural space. During and after cervical surgery, performed on days 2 and 4 of hospitalization, cefazolin and cefditoren-pivoxil were administered. The patient suffered from diarrhea on day 10 and abdominal distension on day 15. Because computed tomography demonstrated distinct distension of the entire colon with air, she received an operation on her colon for decompression and construction of an enterostoma, on day 16. Inspection inside the colon during the operation and histopathological examination of the excised sigmoid colon revealed no apparent pseudomembrane formation. On day 31, her body temperature suddenly rose to 40°C. Purulent substances were discharged from the abdominal surgical wound; therefore multiple antibiotics, including ceftazidime, tobramycin, and ampicillin plus sulbactam, were administered, and the surgical wound was irrigated frequently. Administration of metronidazole, through a nasogastric tube (0.5 g every 6 h), was started on day 42, and she became afibrile immediately. The drug was given for 11 days and the patient was transferred to another hospital for rehabilitation on day 180.

Gram staining of purulent substances obtained from the abdominal surgical wound on day 32 demonstrated Gram-positive bacilli with terminal spores (Fig. 1). The specimen was inoculated on chocolate agar (Eiken Kagaku, Tokyo, Japan) and on Brucella HK agar (Kyokuto Kagaku, Tokyo, Japan) and incubated aerobically at 35°C for 18h and
anaerobically at 35°C for 48 h, respectively. No bacteria grew in the aerobic culture. In contrast, Gram-positive bacilli, Gram-negative bacilli, and Gram-positive cocci were isolated in the anaerobic culture. The two former organisms were identified as *Clostridium difficile* and *Bacteroides thetaiotaomicron*, respectively, with Api20A (BioMérieux, Marcy l’Etoile, France).

To examine toxigenicity, the *C. difficile* strain was inoculated in brain heart infusion broth (Becton Dickinson, Sparks, MD, USA) and incubated anaerobically at 35°C for 48 h. The broth was filtered through a 220-nm filter and inoculated on Vero cells. Deformity of the cells was observed after incubation at 37°C for 24 h, and the deformity was inhibited by the addition of anti-*C. difficile* toxin B antibodies (TOX-B TEST; TechLab, Blacksburg, VA, USA). Toxin A was not detected in the broth with an immunochromatography test (UniQuick; Kanto Kagaku, Tokyo, Japan). Analysis of the pathogenicity locus of the strain with a polymerase chain reaction, according to previous descriptions, demonstrated a defect in the repetitive region of the *tcdA* gene.

Fecal examination on days 10 and 36 showed negative results for *C. difficile* toxin A with the immunochromatography test. Although *C. difficile* was isolated from the fecal specimen obtained on day 36, the isolate could not be subcultured and analyzed in detail.

**Discussion**

*C. difficile* has rarely been isolated from extraintestinal sources, but such isolation may only represent potential fecal spillage and should be interpreted carefully. In the present patient, *C. difficile* was isolated from purulent sub-

stances in the surgical wound associated with the colonic operation. In addition, other anaerobes were simultaneously cultured from the purulent substances. However, Gram staining of the substances demonstrated only spore-forming Gram-positive bacilli with numerous leukocytes. This finding indicates that *C. difficile* was the main causative agent of the abdominal surgical-site infection.

For the treatment of CDAC, metronidazole is the first-line drug. In contrast, appropriate treatment for soft-tissue infections by *C. difficile*, including surgical-site infections, has not been determined. Bhargava and colleagues reported a case of necrotizing fasciitis and gas gangrene caused by *C. difficile* following multiple injuries due to a motor vehicle accident. The patient’s condition improved with extensive wound debridement and the administration of multiple antibiotics, including intravenous metronidazole. In the present patient, the administration of metronidazole after the identification of a causative agent was more effective than the irrigation of the surgical wound and the administration of other antibiotics. We consider that metronidazole may be also a key drug for the treatment of soft-tissue infections caused by *C. difficile*. In the present patient, we administered the drug through a nasogastric tube because only a tablet form of metronidazole has been commercially available in Japan.

The toxigenic analyses revealed that the *C. difficile* strain isolated from the abdominal surgical wound in our patient was an A+/B+ strain. To date, the epidemiology and pathogenicity of *C. difficile* A-/B+ strains have not been extensively examined in Japan because, until recently, only a rapid assay kit for detecting toxin A has been available commercially. However, several reports have described the detection of A−/B+ strains in fecal specimens submitted in Japanese hospitals. Therefore, in the present patient, we speculate that the A−/B+ strain isolated from the abdominal surgical wound originated from inside the patient’s colon and contaminated the surgical site during or after the abdominal operation. The fact that *C. difficile* was detected in a toxin A-negative fecal specimen submitted on day 36 may support our speculation. In contrast, although we suspect that the diarrhea after the cervical surgery was attributable to CDAC caused by the A+/B+ strain, it remains unknown whether the A−/B+ strain caused the colonic distension, because there was no evidence of pseudomembrane formation in the colon and, as far as we know, no report has described a case of toxic megacolon associated with *C. difficile* A−/B+ strains. Further epidemiological studies are necessary to clarify the clinical features of CDAC associated with A−/B+ strains.

Recently, increases in the numbers of patients with CDAC and in the prevalence of more severe CDAC due to a toxin gene-variant *C. difficile* strain have been reported from North America and Europe. As far as we know, these variations in the pathogenicity of *C. difficile* have been reported only in cases of enterocolitis. However, in the future, pathogenic factors associated with morbidity in other organs may appear in *C. difficile*. Although it is unclear whether *C. difficile* will become a major pathogen in surgical-site infections, we should pay attention to