

CASE REPORT

Clostridium spiroforme-induced diarrhea in a patient with acute myeloid leukemia: A case report

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Abstract

Clostridium spiroforme is a Gram-positive, obligate anaerobic bacillus. Previous studies have suggested that *C. spiroforme* mainly causes typhlocolitis and enterotoxemia in rabbits, with few documented cases of human infections. We present a rare case of acute diarrhea caused by *C. spiroforme* in a 78-year-old female patient with acute leukemia. In the early stages of infection, the patient experienced up to 10 episodes of diarrhea per day. She was subsequently treated with live *Clostridium butyricum* capsules to modulate the intestinal microflora, and her symptoms resolved after 7 days of treatment. This case report highlights an uncommon intestinal infection with *C. spiroforme* in a patient with a hematologic malignancy, contributing valuable clinical insights into this rare infection.

Keywords: *Clostridium spiroforme*; Diarrhea; Acute myeloid leukemia

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1. Background

Clostridium species are significant contributors to morbidity and mortality in both humans and animals, with intestinal infections being the most common type of infection. In humans, the main infections include food poisoning caused by *Clostridium perfringens* and antibiotic-associated diarrhea and colitis caused by *Clostridium difficile*. While *Clostridium spiroforme* can colonize the human intestines, infections caused by this bacterium in humans are extremely rare.¹ The first reported case of *C. spiroforme* infection in humans occurred in 1986.² Here, we present a case of intestinal infection caused by *C. spiroforme* in a patient with hematologic malignancy in mainland China.

2. Case presentation

A 78-year-old female patient with acute myeloid leukemia (AML) was hospitalized due to a 1-day history of fever. Laboratory examination on admission revealed a white blood cell count of $21.80 \times 10^9/L$, a neutrophil count of $9.09 \times 10^9/L$, a hemoglobin level of 75 g/L, a platelet count of $5 \times 10^9/L$, and a procalcitonin concentration of 3.27 ng/mL. Upon hospitalization, the patient was started on meropenem, micafungin,

voriconazole, and ubenimex for anti-infective and immune regulation purposes. On the 6th day of hospitalization, interferon combined with thalidomide, interleukin-2, and cytarabine was administered, followed by a 7-day course of harringtonine chemotherapy (harringtonine 2 mg/day and cytarabine 100 mg/day). The patient experienced severe diarrhea (approximately 10 episodes per day) without accompanying symptoms such as abdominal pain or bloating. A routine stool examination showed normal findings, with green, thin-paste stool, and negative results for occult blood, white blood cells, and red blood cells. Aerobic fecal cultures indicated no growth of pathogenic bacteria. Microscopic examination of stool smear samples revealed the presence of Gram-positive bacilli, whereas Gram-negative bacilli and Gram-positive cocci were absent.

The patient was prescribed live *Clostridium butyricum* capsules (Qingdao Donghai Pharmaceutical Co. Ltd., China) to modulate the intestinal microflora, with no changes to her other treatments. By the 3rd day of probiotic treatment, the patient's diarrhea symptoms gradually improved, with the frequency of diarrhea episodes reduced to 3 – 4 times/day. After 7 days of treatment, both the frequency and characteristics of the stool returned to normal.

At a follow-up microscopic examination (10 days after the onset of intestinal symptoms), *C. spiroforme* was no longer observed in the stool smear specimens. However, a large number of fungal spores and a small number of hyphae were observed. The patient continued treatment to modulate the intestinal microflora, and her intestinal symptoms improved without the need for additional specific treatments.

3. Discussion

C. spiroforme is a strictly anaerobic, Gram-positive, spiral-shaped bacillus. In this case, microscopic examination of Gram-stained stool smear samples revealed a large number of curved, semicircular, eight-shaped, and spiral-shaped Gram-positive bacilli, with a scant number of Gram-positive cocci (Figure 1A). After 24 h of anaerobic incubation, small colonies were visible on the blood agar plate (Figure 1B). After 48 h of anaerobic incubation, only the blood agar plate showed growth of gray-white, opaque colonies, 1 – 2 mm in size, with irregular edges (Figure 1C). The colonies were dry and waxy, without hemolysis.

Traditional phenotypic characterization and identification of *Clostridium* species involve the use of pre-reduced anaerobically sterilized (PRAS) medium combined with gas-liquid chromatography to analyze end-metabolic products and reveal fermentation profiles. However, few laboratories have access to PRAS medium

or the capacity to perform gas-liquid chromatography.^{3,4} Commercial kits based on enzyme detection of chromogenic or fluorescent substrates can be used for the rapid identification of anaerobic bacteria, but their overall performance is variable and often inadequate for precise identification.^{5–11} We attempted to identify *C. spiroforme* using the VETIK Compat2 System (Bio Merieux, USA) and the API anaerobic bacteria identification card (API System, France), but both methods failed.

Although the matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF) MS system (Bruker, Germany) has not been approved by the United States Food and Drug Administration for anaerobic bacteria identification, it has been used to analyze 68 strains of *Clostridia*, accurately identifying 67 of them using the manufacturer's recommended cut-off values.¹² In our study, the MALDI-TOF identification score for *C. spiroforme* was 1.503 (Figure 2A), which falls below the threshold for reliable identification. Further 16sRNA sequencing analysis showed 99.85% sequence similarity to NR_114393.1 in the GeneBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), with the phylogenetic tree of *C. spiroforme* shown in Figure 2B. Overall, sequencing technology is highly recommended for accurate identification of *Clostridium* species.

Metagenomic sequencing suggests that *C. spiroforme* is part of the normal human intestinal microflora.¹³ Previous studies have shown that *C. spiroforme* mainly causes diarrhea in weaning rabbits.¹⁴ It has also been isolated from human feces and, more recently, from human blood cultures.^{15,16} In one case, a patient presented with severe abdominal pain and a 3-day history of obstipation, nausea, and vomiting, with a medical history significant for IgG kappa monoclonal gammopathy.

In our report, the patient, who had AML, had undergone six rounds of chemotherapy, leading to a severely compromised immune system. While receiving immunomodulators, including ubenimex and Bozhi glycopeptide injection, her immune parameters showed significant suppression: T (CD3⁺) cells at 65.7% (normal range: 65 – 79%), Th (CD3⁺CD4⁺) cells at 27.8% (normal range: 34 – 52%), Ts (CD3⁺CD8⁺) cells at 37.4% (normal range: 21 – 39%), and a Th/Ts (CD4⁺/CD8⁺) ratio of 0.74 (normal range: 1 – 2).

The patient experienced severe diarrhea, and pure growth of *C. spiroforme* was detected in anaerobic fecal cultures. Next-generation sequencing of the pure *C. spiroforme* isolate revealed that the strain carried the *C. spiroforme* toxin (CST), which is considered the virulence determinant of this pathogen.¹⁷ CST is structurally similar to other clostridial binary toxins,

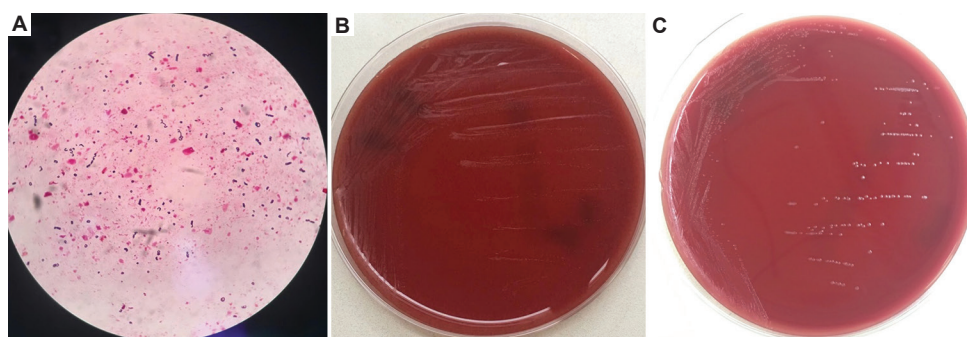


Figure 1. Gram staining and culture characteristics of *Clostridium spiroforme*. (A) Gram staining of *C. spiroforme*; (B) 24-h anaerobic culture on blood agar plate; (C) 48-h anaerobic culture on blood agar plate

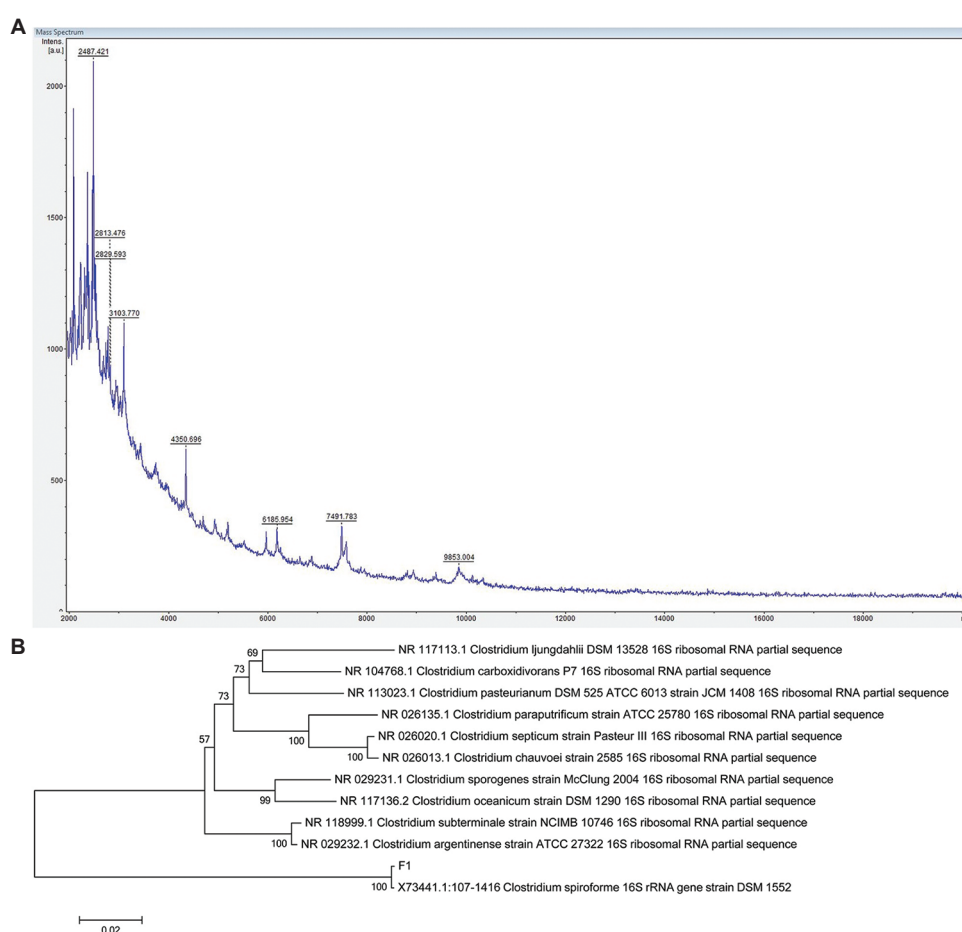


Figure 2. Identification and phylogenetic analysis of *Clostridium spiroforme* using matrix-assisted laser desorption ionization–time-of-flight MS and 16S rRNA sequencing. (A) Identification results using mass spectrometry; (B) Phylogenetic tree based on 16S rRNA sequencing of *C. spiroforme*

including *C. perfringens* type E iota-toxin and *C. difficile* transferase.^{18,19} An inquiry into the patient's lifestyle revealed no direct ownership of rabbits, but she had contact with rabbits at a neighbor's home. Given the patient's severely compromised immune system, this incident may have contributed to the *C. spiroforme* infection.

In addition, the patient's symptoms improved following probiotic treatment, and spirochetes were cultured. Based on these observations, we speculate that *C. spiroforme* was the cause of the patient's diarrhea. This case is the 1st time *C. spiroforme* has been isolated from human specimens in mainland China.

4. Conclusion

This case report shares our experience of the diagnostic and clinical treatment of diarrhea caused by *C. spiroforme*, providing guidance for laboratory testing and clinical management of this rare human infection.

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Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Chunyan Gao, Panfei Hou

Formal analysis: Jing Yu

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Writing-original draft: Yukai Jing

Writing-review & editing: All authors

Ethics approval and consent to participate

The experimental protocols were approved by the institutional review board of Shanxi Bethune Hospital.

Consent for publication

Informed consent of the patient was obtained for publishing her data in this paper.

Availability of data

The near-complete 16S rRNA sequence for *C. spiroforme*, available in GenBank, supports the conclusions of this report and can be obtained from the corresponding author upon reasonable request. All other data generated or analyzed during this study are included in this published article.

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