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Clostridium tertium Bacteremia in a Patient with Glyphosate Ingestion

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Conflict of interest: None declared

Patient: Female, 44
Final Diagnosis: *Clostridium tertium* bacteremia
Symptoms: Fever
Medication: Ertapenem • Metronidazole
Clinical Procedure: —
Specialty: Infectious Disease

Objective: Unknown etiology

Background: *Clostridium tertium* is distributed in the soil and in animal and human gastrointestinal tracts. *C. tertium* has been isolated from patients with blood diseases, immune disorders, and abdominal surgeries. Glyphosate is toxic, causing cause eye and skin irritation, gastrointestinal pain, and vomiting. Ingestion of herbicides modifies the gastrointestinal environment, which stresses the living organisms. However, there has been little attention to cases of bacteremia in patients recovering from suicide attempt by ingesting herbicide.

Case Report: *Clostridium tertium* was identified in a 44-year-old female who attempted suicide by glyphosate (a herbicide) ingestion. The 16S rRNA sequences from all colonies were 99% identical with that of *C. tertium* (AB618789) found on a BLAST search of the NCBI database. The bacterium was cultured on TSA under aerobic and anaerobic conditions. Antimicrobial susceptibility tests performed under both aerobic and anaerobic conditions showed that the bacterium was susceptible to penicillin, a combination of β-lactamase inhibitor and piperacillin or amoxicillin, and first- and second- generation cephalosporins. However, it was resistant to third- and fourth-generation cephalosporins.

Conclusions: Glyphosate herbicide might be a predisposing factor responsible for the pathogenesis of *C. tertium*. The results highlight the need for careful diagnosis and selection of antibiotics in the treatment of this organism.

MeSH eywords: Bacteremia • *Clostridium tertium* • Herbicides

Full-text PDF: http://www.amjcaserep.com/abstract/index/idArt/891287

Background

Clostridium tertium is an aerotolerant gram-positive bacillus that is capable forming spores under anaerobic conditions for its growth. The bacterium is widely distributed in the soil [1] and in animal and human gastrointestinal tracts [2–5]. It is a non-toxin-producing bacterium and is regarded to be a low-virulence pathogen, in contrast to *C. perfringens*. In fact, infection with this pathogen has been rare in humans after being first described in 1917 [6]. However, there have been human cases of *C. tertium* infection reported in last 2 decades. *C. tertium* has been isolated from patients with blood diseases such as leukemia, hepatic failure, and immune disorders. There are some reports of *C. tertium* infection in patients experiencing abdominal surgeries such as gastrostomy.

There have been few reports of bacteremia in patients recovered from suicide attempt by ingesting herbicides. Glyphosate (N-(phosphonomethyl) glycine) is a highly effective herbicide because of its potent and specific inhibition of 5-enolpyruvyl shikimate 3-phosphate synthase and enzyme of the shikimate pathway, which governs the synthesis of aromatic amino compounds in higher plants, algae, bacteria, and fungi [7]. Glyphosate-containing products are acutely toxic to humans. Various microorganisms have different sensitivities to glyphosate [8–10]. Herbicides modify the environment, which stresses living organisms [11,12]. Herein, we report a case of bacteremia due to *C. tertium* from a patient who had recovered from a suicide attempt by glyphosate ingestion. In addition, *C. tertium* infection might be involved in acute bronchopneumonia.

Case Report

A 44-year-old woman attempted suicide by glyphosate (herbicide) ingestion on May 15, 2012 and was admitted to Chonbuk National University Hospital. The amount of glyphosate ingested was about 20 ml. Twelve days after the suicide attempt, the patient presented with a high fever and general myalgia. Due to her symptoms, she visited the emergency room. At that time, her blood pressure was 80/60 mmHg, pulse was 70/min, respiration rate was 18/min, and temperature was 38.0°C. Laboratory studies revealed a white blood cell (WBC) count of 2010/ml, hemoglobin level of 14.2 g/dl, platelet count of 80 000/ml, serum creatinine of 3.59 mg/dl, aspartate aminotransferase level of 2428 IU/l, alanine amino transferase level of 1213 IU/l, total bilirubin level of 0.30 mg/dl, hs-CRP level of 20.77 mg/l, and PCT level of 1.08 ng/ml. Urine analysis revealed pyuria (WBC count >30/HPF). In addition, high-resolution computed tomography (CT) of the chest revealed acute bronchopneumonia in the left lower lobe. The initial antibiotic therapy included cefepime and azithromycin for 8 days. However, fever persisted, hs-CRP level increased abruptly to 107.49 mg/l, and PCT level increased to 3.53 ng/ml during antibiotic treatment. *C. tertium* was isolated from initial blood samples from a central catheter. Antibiotics were changed to ertapenem and metronidazole. After 16 days of appropriate antibiotic therapy, her clinical symptoms and signs completely disappeared and she was discharged.

In the initial blood culture, we observed slender Gram-positive rods under aerobic conditions, identified as Lactobacillus sp. by using the Vitek2 identification system (BioMérieux Inc., Hazelwood, USA). The blood culture was subcultured, resulting in a pure colony on tryptic soy agar (Sigma Aldrich, St. Louis, USA) under aerobic conditions. The randomly selected colonies were separately cultured in TSB (Sigma Aldrich, St. Louis, USA) for bacterial DNA extraction, followed by identification using 16S rRNA sequencing. The resultant colonies were submitted for spore staining according to the Schaeffer-Fulton method using malachite green (Life Technologies, Grand Island, USA). The 16S rRNA sequences from all colonies showed 99% identity with that of *C. tertium* (AB618789) on BLAST searching of the NCBI database. The bacterium was carefully cultured on TSA under aerobic and anaerobic conditions

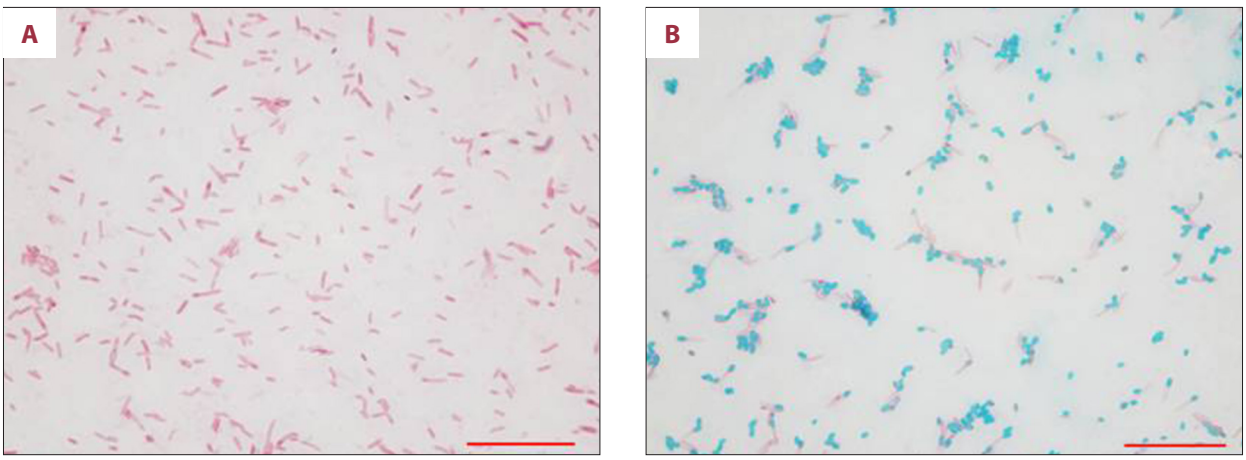

Figure 1. Spore stain of *Clostridium tertium* on two different cultivations, aerobic (**A**) and anaerobic (**B**) conditions.

Table 1. Antibiotic resistance of *Clostridium tertium* under anaerobic and aerobic cultivations.

Antibiotics	Potency	Anaerobic		Aerobic	
		Clear zone	Interpretation	Clear zone	Interpretation
Penicillin	10 IU	23.5 mm	S	22.5 mm	S
Piperacillin/tazobactam	110 ug	22.5 mm	S	28.5 mm	S
Amoxicillin/clavulanic acid	30 ug	40 mm	S	37 mm	S
Cephalothin	30 ug	22 mm	S	25 mm	S
Cefoxitin	30 ug	28 mm	S	32 mm	S
Imipenem	10 ug	35 mm	S	40 mm	S
Moxifloxacin	5 ug	24 mm	S	28 mm	S
Vancomycin	30 ug	27 mm	S	28 mm	S
Tetracycline	30 ug	39 mm	S	34 mm	S
Rifampicin	5 ug	26 mm	S	28 mm	S
Sulfamethoxazole/Trimethoprim	25 ug	36 mm	S	36 mm	S
Ceftiofur	30 ug	12.5 mm	R	10 mm	R
Cefotaxime	30 ug	0 mm	R	0 mm	R
Ceftazidime	30 ug	0 mm	R	0 mm	R
Cefepime	30 ug	0 mm	R	0 mm	R
Amikacin	30 ug	11 mm	R	15.5 mm	R
Gentamicin	5 ug	11 mm	R	16.5 mm	R
Clindamycin	2 ug	10 mm	R	10 mm	R
Metronidazole	5 ug	0 mm	R	0 mm	R

S – susceptible, R – resistant, mm; millimeter.

The 16S rRNA sequences from all colonies showed 99% identity with that of *C. tertium* (AB618789) on BLAST searching of NCBI database. The bacterium was separately cultured on TSA under aerobic and anaerobic conditions.

Under aerobic condition, morphology and staining of the bacterium were similar with that from initial blood culture (Figure 1A). Under anaerobic conditions, the bacterium showed a tennis racquet-like shape with terminally located ova with blue color, indicating spore formation (Figure 1B). Antibiotic susceptibility tests for the bacterium were also performed under aerobic and anaerobic conditions. Although there were differences in susceptibilities to most antibiotics between both conditions, there is no difference in susceptibilities of *C. tertium* strain to antibiotics on interpretation based on clear zone diameter. Under both aerobic and anaerobic conditions, *C. tertium* was sensitive to penicillin, piperacillin/tazobactam, Amoxicillin/clavulanic acid, Cephalothin, Cefoxitin, Imipenem, Moxifloxacin, Vancomycin, Tetracycline, Rifampicin, and Sulfamethoxazole/Trimethoprim but resistance to Ceftiofur, Cefotaxime, Ceftazidime, Cefepime, Amikacin, Gentamicin, Clindamycin, and Metronidazole (Table 1).

Discussion

We presented a case report of bacteremia and acute bronchopneumonia due to *C. tertium* in a patient recovering from deliberate ingestion of glyphosate herbicide. *C. tertium* was considered as the pathogen [2,13,14]. *C. tertium* isolates are usually found with other bacteria [3,13,15,16] and sometimes it is the only isolate [15,17]. Patients who died and who had *C. tertium* in their blood cultures had severe underlying diseases [13,18] that were potentially fatal in the short term. As a result, the bacterium's virulence has not yet been clearly determined [3]. The present *C. tertium* was misidentified as Lactobacillus sp. by using Vitek2 identification on initial blood culture under aerobic conditions. There are many reports of misidentification of *C. tertium* under aerobic conditions. *C. tertium* could be mistaken for a Gram-negative enteric organism because of its various degree of Gram straining. In addition, the bacterium is an aero-tolerant species [19]. It could share similar biochemical characteristics with *Bacillus sp*., *Lactobacillus sp*., and *Corynebacterium sp*. under aerobic growth. When identified as *Lactobacillus* species by phenotypic methods, clinicians should be aware of the possibility of aero-tolerant *Clostridium sp*. and

perform additional testing to rule out these organisms. *C. tertium* can be differentiated from other bacteria by many methods; for example, catalase and oxidase testing, anaerobically spore-formation, and gas chromatography profiling. In our case, we correctively identified *C. tertium* by the presence of spores under anaerobic growth and 16S rRNA sequencing from misidentification of the present strain using the Vitek2 system.

Two or 3 antibiotics have generally been used for controlling *C. tertium* in humans. However, there is no guideline for treatment to *C. tertium* infection. Based on antimicrobial susceptibility tests for *C. tertium* strains from the literature, there are differences among clinical *C. tertium* strains. Some previous studies showed resistance of *C. tertium* to β-lactams, clindamycin, and metronidazole. There is limited information about resistance of *C. tertium* in patients with bacteremia and pneumonia after glyphosate ingestion. There was a case of bacteremia reported due to *Bacillus licheniformis* from a convalescent patient after a suicide attempt [20].

Although there are many cases of *C. tertium* infection in humans, to the best of our knowledge, this report is the first from Korea. Most patients had various abdominal disorders associated with intestinal mucosa damage, a prerequisite providing a portal for entry for *C. tertium* from the gut. Intestinal pathology can therefore be considered the major risk factor for development of *C. tertium* bacteremia. In the previous cases and/or retrospective studies, intestinal mucosa damage has been suggested to be one of the major risk factors for bacteremia due to *C. tertium* [13].

In agreement with the previous studies, the bacteremia might be due to translocation of *C. tertium* from the gastrointestinal tract by intestinal mucosa damage from herbicide toxicity combined with excessive stress. The patient recovered from bacteremia and pneumonia and was discharged after 16 days of appropriate antibiotic therapy using ertapenem and metronidazole.

One study reported 2 cases, of which 1 patient was being treated for a first relapse of acute myeloblastic leukemia, and the second was receiving high-dose chemotherapy with hematopoietic stem cell support for non-Hodgkin lymphoma. The first patient was completely asymptomatic, whereas the other case improved clinically and bacteriologically despite *in vitro* evidence of inadequate antibiotic therapy [21]. However, they were reported as true pathogens in both cases because the patients were at risk (hematologic malignancies, leukopenia, and chemotherapy) for *C. tertium* bacteremia. Leukopenia is a known as risk factor for *C. tertium* bacteremia, as shown in the present case.

Conclusions

This report was limited because we did not perform blood or urine tests to detect glyphosate. It is not clear if the detected *C. tertium* was a contaminant or a true pathogen. However, the patient had a definite risk factor for *C. tertium* bacteremia as a complication of glyphosate ingestion. Ingestion of glyphosate might be a predisposing factor for the pathogenesis of *C. tertium* bacteremia.

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