Clostridium glycolicum Bacteremia in a Bone Marrow Transplant Patient

Running title: Clostridium glycolicum bacteremia

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Abstract

We describe a case of *Clostridium glycolicum* bacteremia and septic shock in an adult woman with a recent bone marrow transplant for relapsed Hodgkin’s disease. The bacterium was identified by 16S rRNA gene sequencing. This is the first published report of the recovery of this organism from human clinical material.
The patient was a 43-year-old woman with biopsy-proven nodular sclerosing Hodgkin’s disease diagnosed in early 2003 after she presented with progressive left anterolateral chest discomfort, chest wall swelling, and eventually dyspnea. She was found to have a 10 cm mediastinal mass associated with left axillary lymphadenopathy, a left pleural effusion, and involvement of the left anterior chest wall. Despite completing 8 cycles of chemotherapy with doxorubicin/bleomycin/vinblastine/dacarbazine (ABVD), she failed to achieve a remission. She eventually underwent an unsuccessful autologous stem cell transplant in February 2004, after which she required radiotherapy and a 6-month course of chemotherapy to help control her disease but her symptoms persisted. She received an allogeneic bone marrow transplant in October 2005 after marrow ablation with fludarabine, busulfan, and anti-thymocyte globulin (ATG) but developed septic shock on day 1 post-transplant, necessitating transfer to the intensive care unit (ICU). In addition to supportive measures, empiric therapy with intravenous vancomycin, gentamicin, and ciprofloxacin was initiated after three sets of BacT/Alert (bioMerieux Inc., Durham, NC) FA (aerobic) and FN (anaerobic) blood cultures were collected. Within 24 hours, all 3 anaerobic blood culture bottles were positive for large, gram-positive, rod-shaped bacteria resembling *Clostridium* spp., while two blood culture sets also demonstrated the presence of gram-positive cocci resembling streptococci. Forty-eight hours after subculture to brucella blood agar plates (PML Microbiologicals, Wilsonville, OR) incubated anaerobically at 35°C, tiny colonial growth (colonies approximately 2 mm in diameter) of a grey-white, non-hemolytic, motile, obligately-anaerobic gram-positive
bacillus with terminal endospores was observed. These observations along with the results of other biochemical tests confirmed the identity of the organism as a member of the genus *Clostridium* although definitive identification to the species level relied on the results of partial sequencing of the 16S ribosomal RNA gene using MicroSeq 500 kits and an ABI Prism 3100 sequencer (Applied Biosystems, Foster City, CA). A GenBank BLAST search and detailed phylogenetic analysis supported an identification of *C. glycolicum*, based on 99.0% to 99.7% sequence identity of our 387 base pair sequence (GenBank accession no. DQ986354) to those of four other strains of *C. glycolicum* in the GenBank database. The isolate was susceptible to penicillin G (minimum inhibitory concentration (MIC) = 0.125 µg/ml), clindamycin (MIC = 0.064 µg/ml) and metronidazole (MIC = 0.19 µg/ml) by Etest methodology. Additionally, a gram-positive coccus identified as *Enterococcus* spp. (ampicillin-susceptible) was recovered aerobically and anaerobically from 2 blood culture sets. After the blood culture Gram stain results were reported, the patient’s antibiotics were changed to intravenous ampicillin, aztreonam, and metronidazole; after susceptibility test results were available, the antibiotics were switched to ampicillin, gentamicin, and metronidazole to complete a 2-week course of therapy. After 36 hours in the ICU, she was transferred to the general medical ward. Her clinical condition gradually improved over the course of her stay in hospital, and she eventually demonstrated evidence of bone marrow engraftment but required ongoing standard post-bone marrow transplant medical care.
Discussion

The genus *Clostridium* is a group of anaerobic, endospore-forming Gram-positive rod-shaped bacteria with variable phenotypic characteristics (1). Over 150 species of *Clostridium* have been described to date (13) and while most have typically been considered as harmless soil saprophytes or inhabitants of human or animal gut, an increasing number are being reported as causes of human disease (1, 8-11, 16). Most human clostridial infections are endogenous, usually occurring secondary to local or widespread dissemination of gut-colonizing strains as a result of perturbed host defenses caused by trauma, hypoxia, diabetes mellitus, alcoholism, chemotherapy, radiotherapy, and/or malignancy (1, 22).

*Clostridium glycolicum* is a species that was first described in 1963 by Gaston and Stadtman (15). These investigators reported the isolation of a unique anaerobic gram-positive bacterium from a specimen of mud obtained from a pond in Maryland, USA (15). Their isolate was a long, slender, motile, gram-positive endospore-forming rod-shaped bacterium capable of utilizing ethylene glycol as a source of energy and carbon, hence the species name (15). *Clostridium glycolicum* has since been recovered from a variety of soils and environmental niches in different geographic regions of the world (4, 6, 20, 23). The organism has also been isolated from human and bovine feces (7). In 1987, the first and only published report of animal infection due to *C. glycolicum* was described in a young addax with myonecrosis of the buttock and hindlegs (17). As far as we know, there are no published reports of *C. glycolicum* human infection in the world.
literature although recovery of this organism from clinical sources (wounds, peritoneal fluid) has previously been documented in published texts (1, 12).

Microscopically, cells of *C. glycolicum* are Gram-positive straight or slightly curved motile, rod-shaped bacteria (0.3-1.3 µm wide x 2-15 µm long) that occur singly or in pairs and commonly display sub-terminal or terminal endospores (4, 5, 12, 15, 18, 20, 24). Colonies are approximately 2 mm in diameter and are typically flat and round with smooth symmetrical borders (15). Virtually all strains demonstrate strict anaerobic growth properties (4, 5, 18) although an aerotolerant strain of *C. glycolicum* recovered from sea grass roots has been reported (20). Phenotypically, *C. glycolicum* is a non-hemolytic, weakly saccharolytic organism which demonstrates negative tests for lecinthinase and lipase production, gelatin liquefaction, indole production, nitrate reduction, catalase production, and esculin hydrolysis, characteristics that differentiate it from most other clinically important *Clostridium* spp. except for *C. symbiosum* from which it may be distinguished using gas-liquid chromatography (4, 5, 15, 19, 24). An additional taxonomically recognized characteristic of *C. glycolicum* is its ability to metabolize cinnamic acids (4). However, reliance on an extensive battery of biochemical and other phenotypic tests for organism identification may be considered too time consuming and labor intensive in today’s clinical microbiology laboratory. Molecular techniques such as automated DNA sequencing are playing an increasingly important role in infectious diseases diagnostics worldwide. Many labs are resorting to 16S rRNA gene sequencing for definitive identification of bacteria that cannot easily be identified to the genus or species level using standard phenotypic tests.
Although *C. glycolicum* was recovered from 3 consecutive blood culture sets in our patient, the concomitant recovery of an *Enterococcus* sp. from 2 of these sets may obscure the potential clinical importance of *C. glycolicum* in humans. Like many *Clostridium* spp., *Enterococcus* spp. are also found as normal inhabitants of the human colon and may similarly cause bacteremic disease in patients with perturbed host defense mechanisms (22). Polymicrobial bacteremia is frequently observed in patients with *Clostridium* bloodstream infections, particularly in the setting of severe sepsis or septic shock (3, 21, 22, 25), whereby *Clostridium* spp. and infectious agents such as *Enterococcus* spp. may act as co-pathogens. Severe sepsis/septic shock occurs in approximately 40% of patients with *Clostridium* bacteremia and, in this setting, is associated with a mortality rate of over 50% (2). Patients with haematological malignancies, particularly those experiencing partial failure of chemotherapy, have been shown to be at increased risk for *Clostridium* bacteremia, with or without sepsis/septic shock (2, 3, 25). The rigorous chemotherapeutic regimens used for the treatment of haematological malignancies is typically associated with mucosal damage to the gastrointestinal tract, leading to potential translocation of anaerobic bacteria such as *Clostridium* spp. from the gut to the bloodstream (2, 25). Presumably, the source of our patient’s *C. glycolicum* was the gastrointestinal tract although this remains to be proven. Further study, presumably using an animal model, is required to determine the true pathogenic potential of *C. glycolicum* although the clinical importance of this organism in our patient appears to be supported by the patient’s clinical presentation and the recovery of the organism from multiple blood collections.
Limited information is available regarding the putative antimicrobial susceptibility profiles of *C. glycolicum* strains. However, Finegold and colleagues reported agar dilution susceptibility results for 9 fecal isolates of *C. glycolicum* wherein all strains demonstrated susceptibility to amoxicillin-clavulanate, clindamycin, metronidazole, and vancomycin (14). In a similar regard, our isolate was susceptible to penicillin G, clindamycin, and metronidazole, which is typical of most *Clostridium* spp. Phylogenetically, *C. glycolicum* does not display very close 16S rRNA gene relationships with other *Clostridium* species of known medical importance.

In summary, *C. glycolicum* is an anaerobic gram-positive rod-shaped bacterium that may be implicated as a cause of bacteremia and septic shock in immunocompromised patients. The clinical significance and pathogenic potential of *C. glycolicum*, however, awaits further study.

References


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