

# *Clostridium glycolicum* Wound Infections: Case Reports and Review of the Literature<sup>∇</sup>

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**We describe two cases of *Clostridium glycolicum* wound infections in immunocompetent adults. The bacterium was identified by 16S rRNA gene sequencing. This is the third published report of the recovery of this organism from human clinical material and highlights the importance of the organism as a potential human pathogen. Our report extends the spectrum of the diseases caused by *C. glycolicum*.**

## CASE REPORTS

**Case 1.** The patient was a 24-year-old man who presented to the clinic with a painful wound in his scalp. He stated that he was pushed into some shrubs and, as a result, sustained a deep laceration. The patient initially did well, but after about 2 weeks, he noted significant drainage from the wound as well as increasing pain, which prompted him to present to the clinic.

On examination, the patient appeared alert and oriented. Vital signs were normal, and the patient was afebrile. The physical examination was normal except for the scalp. On the left temporal scalp, there was a 1.5-cm laceration draining purulent material. A piece of foreign material (which appeared to be a piece of wood) was impacted in the wound, and it penetrated through the skin, soft tissue, and fascia, deep into the belly of the temporalis muscle. The patient underwent surgery, during which he had debridement of the skin, subcutaneous tissue, muscle, and a portion of the fascia. The piece of wood was removed.

Swabs from the wound tissue were transported to the microbiology laboratory in anaerobe transport medium for bacterial culture. The aerobic culture was positive after 1 day for *Bacillus laterosporus* and a second *Bacillus* species. The anaerobic cultures were positive after 6 days for *Clostridium glycolicum* and a second *Clostridium* species. The patient was treated with 875 mg amoxicillin-clavulanic acid orally twice daily for 14 days. He responded well to debridement and had good wound healing on follow-up visits.

**Case 2.** The patient was a 20-year-old woman who reportedly sustained multiple injuries, including an open left radius and ulnar shaft compound fracture, following a motor vehicle accident. She was treated with open reduction and internal fixation of her left forearm at an outside hospital. She had delayed the union of both of the left forearm fractures. She was scheduled for a bone graft 2 months later; however, during the appointment, they noted on the plain radiograph that the hardware was loose, which delayed the procedure.

She presented to the emergency department of the Johns Hopkins Hospital (JHH) on 19 January 2008 with worsening pain in her forearm and pus draining through a wound at the surgical site for a week. She was started on cephalixin and was admitted to JHH for nonunion of the left radius and ulnar fractures and possible osteomyelitis. Other than the forearm, there were no significant findings in a systemic review. She underwent irrigation and debridement, hardware removal, antibiotic spacer placement, and provisional fixation on 22 January 2008. Intraoperative findings included nonunion of the left radius and ulnar fractures and chronic osteomyelitis, with a large amount of purulence encountered within the subcutaneous tissue. Cultures were obtained from the wound. The anaerobic cultures were positive for *Clostridium glycolicum* and very light *Veillonella* spp. after 7 days. Two blood cultures were positive for oxacillin-resistant coagulase-negative *Staphylococcus* spp. susceptible to vancomycin. She was treated with parenteral vancomycin for 6 weeks.

Three months later, the patient returned to JHH for irrigation and debridement of the left radius and ulna with removal of the nonbiodegradable antibiotic implants and iliac crest bone grafts to both bones. Cultures were obtained from both the left radius and ulna, and they were negative after 5 days of incubation.

Both isolates of *C. glycolicum* had identical morphologies and biochemical characteristics. After 3 to 4 days of incubation at 35°C on anaerobic T-soy agar plates with 5% sheep blood (Becton-Dickinson, Sparks, MD), colonies appeared as slightly white to gray in color, 1 to 3 mm in size, transparent, flat to slightly raised, nonhemolytic, and with a scalloped edge. Gram staining of the colonies revealed a large gram-positive rod with central-to-subterminal spore formation. The isolates were negative for catalase, nitrate reduction, growth in 5% CO<sub>2</sub>, lecithinase, lipase, gelatin liquefaction, and indole production. Further characterization was performed using the RapID ANA II system (Remel, Lenexa, KS). This system produced a reaction code of 000170, which is a match for *Clostridium difficile*. *C. glycolicum* and *C. difficile* differ in RapID ANA II reactions by only one test, the phenylalanine assay. The product package insert information lists *C. glycolicum* as being negative for this reaction. Both of our isolates and a subsequently recovered strain not included in this report were phe-

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nylalanine positive. Further differentiation of *C. glycolicum* from *C. difficile* can be achieved by gelatin hydrolysis (*C. difficile* is positive), volatile fatty acid production (*C. difficile* produces a large butyric peak), and the characteristic horse barn odor that is produced by *C. difficile*. Furthermore, *C. difficile* produces a yellow-green fluorescence on cycloserine-cefoxitin fructose agar (PML Microbiologicals, Wilson, OR), while *C. glycolicum* produces a red fluorescence under long-wave UV light on anaerobe identification media (3). A cellular fatty acid analysis of our *C. glycolicum* isolates using gas-liquid chromatography and the MIDI Sherlock version 6.1 software with the Moore 3.90 database (MIDI, Inc., Newark, DE) revealed the major fatty acids to be 18:1 CIS 9 (cis-9-octadecenoic acid) (24 to 61%), summed feature 10 (an unconfirmed fatty acid, most likely a monosaturated 18-carbon fatty acid), 18:1 isomer c11, t9, or t6 (5 to 7%), and 16:0 (3 to 17%). The MIDI Sherlock software also creates a similarity index (SI) value from 0.001 to 1.000, in which 1.000 is a perfect match with profiles stored in the library. *C. glycolicum* was the top choice for both isolates, with a mean SI value of 0.431. Volatile fatty acid production using peptone-yeast extract-glucose broth with Tween 80 (Anaerobe Systems, Morgan Hill, CA) demonstrated a large acetic peak, a trace level of propionic acids, and large isobutyric acid and isovaleric peaks. 16S rRNA gene sequencing was performed on the first 500 bp using guidelines outlined by Applied BioSystems, Inc. (ABI, Foster City, CA). The consensus sequences generated were submitted to the Ribosomal Database Project (RDP, Michigan State University) and GenBank (National Center for Biotechnology Information [NCBI]) for identification. Both libraries showed *C. glycolicum* to be the best match with RDP, giving a mean score of 0.930, and GenBank, demonstrating a mean of 99% agreement. All of the above procedures were performed according to the manufacturer's instructions. Susceptibility testing was performed using the Etest (AB Biodisk-V, Solna, Sweden) and was interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (7). Susceptibility testing determined that all of the isolates were susceptible to metronidazole, clindamycin, chloramphenicol, and amoxicillin-clavulanic acid.

**Discussion.** The genus *Clostridium* contains obligate anaerobic, endospore-forming, usually gram-positive, rod-shaped bacteria (1). More than 150 species of *Clostridium* have been identified to date, and most of them are environmental organisms, found as soil saprophytes, or are part of normal human or animal intestinal flora (1). They are also some of the most commonly encountered anaerobic organisms isolated from human clinical specimens, and the members of this genus contribute significantly to human infections, especially wound infections. In a review of 368 specimens obtained from 340 trauma patients from 1973 to 1988, anaerobic bacteria only were isolated in 119 (32%) specimens, aerobic bacteria only in 58 (16%), and mixed aerobic-anaerobic in 191 (52%). *Clostridium* spp., most commonly *Clostridium perfringens*, *Clostridium septicum*, *Clostridium innocuum*, and *Clostridium ramosum*, were the third most frequently isolated anaerobes in this study (5).

*Clostridium glycolicum* was first described in 1963 by Gaston and Stadtman (16). They reported the isolation of an anaerobic organism from mud, which utilized ethylene glycol as a carbon and energy source, hence, the name of the species. It was a gram-positive, spore-forming rod with peritrichous flagella. It grew well from 22 to 37°C at pH 7.4 to 7.6 and fermented glucose, fructose, sorbitol, dulcitol, and cellulose. It did not reduce nitrates, form indole, or cause hemolysis or proteolysis (16). Since then, strains of *C. glycolicum* have been recovered from environmental sources such as soil, mud, sea grass roots, and olive mill wastewater (4, 6, 8, 18, 19). In animals, it is usually a part of the normal flora, having been isolated from bovine feces and snake venom (10). However, it can occasionally cause animal infections. It was isolated from a hepatic abscess in a yellow-footed tortoise (*Geochelone denticulata*) (20). Gulland and Parsons reported *C. glycolicum* causing severe acute myonecrosis in a young addax in 1987 (17), and in 2006, Bertelsen and Weese reported enterotoxemia caused by *C. glycolicum*, which resulted in circulatory collapse and death in a 3-year-old, female, captive-bred ornate Nile monitor (2).

*C. glycolicum* has been isolated from human feces and clinical specimens such as wounds, abscesses, and peritoneal fluid (9, 10, 15). There have only been two published case reports of *C. glycolicum* causing human infection. In 2007, Elsayed and Zhang reported a case of *C. glycolicum* bacteremia and septic shock in an adult woman with recent bone marrow transplantation for relapsed Hodgkin's disease (14). In their report, three consecutive sets of blood cultures grew *C. glycolicum*, and the patient was treated successfully with antibiotic coverage according to the susceptibility testing. The authors proposed that the source of their patient's *C. glycolicum* bacteremia was the gastrointestinal tract, although this remained to be proven. In 2009, Van Leer et al. reported isolation of *C. glycolicum* from a brain abscess in a previously healthy 62-year-old man (21). The patient had a brain abscess with gas formation following otitis media, and he treated himself by placing clay in his ear. Several bacterial species were cultured from the patient's material and *C. glycolicum* was among them.

In this study, we reported the isolation of *C. glycolicum* from two patients, one with a scalp wound infection and the other with chronic osteomyelitis. In both cases, there was recovery of additional organisms, such as *Bacillus laterosporus* in the first case and *Veillonella* spp. in the second case. This might obscure the clinical importance of the isolated *C. glycolicum*. However, both patients had evidence of clinical infection and specimens appeared to be collected appropriately. Both of our cases and the recent case reports should heighten the awareness of this species as a clinically important organism, not only in immunocompromised patients but also in immunocompetent hosts as a potential cause of wound infections. Furthermore, although many of the *Clostridium* species have been considered environmental organisms or harmless human flora, an increasing number of less commonly recovered species have been reported to cause serious infections, such as *C. intestinale* (13), *C. symbiosum* (11), and *C. hathewayi* (12). Our report extends the spectrum of the infections caused by *C. glycolicum* and highlights the fact that 16S rRNA gene sequencing may be required to definitively identify this organism.

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