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Clostridium tertium isolated from gas gangrene wound; misidentified as *Lactobacillus* spp initially due to aerotolerant feature

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Abstract

Clostridium tertium has been increasingly reported as a human pathogen. This organism is an aerotolerant Gram-positive rod that is often mistaken for other organisms, such as *Lactobacillus* or *Bacillus* species. We describe a case of a patient with a history of intravenous drug use presenting to UCLA-Olive View Medical Center with gas gangrene of both upper extremities. The organism was initially misidentified as a *Lactobacillus* species on aerobic culture plates. However, terminal spore formation was detected in this isolate on a sub-cultured anaerobic culture plate and this isolate was confirmed as *C. tertium* biochemically and genetically by 16S rDNA sequencing. Additional DNA cloning libraries made from the formalin-fixed specimen revealed *Peptoniphilus* species and an *uncultured Clostridium* clone, but not *C. tertium*. *C. tertium* might be a causative organism of gas-producing myonecrosis but such an association has never been described. Clinicians should be aware of the phenomenon of aerotolerance of some anaerobes and need to clarify the identification of organisms if the clinical picture does not fit the isolated organism.

Keywords: Myonecrosis; Aerotolerance; *Clostridium tertium*; Gas gangrene; *Lactobacillus*

1. Case

A 51 year-old male with a history of intravenous drug use and cryptogenic hepatitis presented to the emergency department with bilateral upper extremity swelling and progressive pain associated with multiple skin abscesses. One month prior to admission he had skin abscesses on his left forearm and incision and drainage was performed; no cultures were obtained and the patient was treated empirically with oral antibiotics. One week prior to admission, the patient noticed one small "boil" on his right forearm; however, he continued to "skin pop" black tar heroin 5–6 times per day at alternate upper arm sites and began experiencing increased swelling and pain in both upper extremities. According to the patient, he obtained needles and sterile water from a needle exchange program and denied licking the needle, mixing the drugs with tap water or water from the toilet, or soil contamination of the wound. He stated that he had shared the same batch of heroin with another friend; this individual also developed a skin infection but improved following treatment with an unknown antibiotic. The patient denied fever or chills.

Initial physical examination demonstrated a "non-toxic appearing" male in no acute distress; vital signs showed a temperature of 37.6 °C, blood pressure of 152/69 mmHg, respiration rate 20/min, heart rate 100/min and oxygen saturation of 98% (room air). Oral examination showed very poor dentition with caries. Skin examination revealed multiple small skin abscesses with diffuse erythema and swelling in his both upper arms; there was marked erythematous swelling in his right forearm but no evidence of tissue gangrene. The remainder of the examination was within normal limits.

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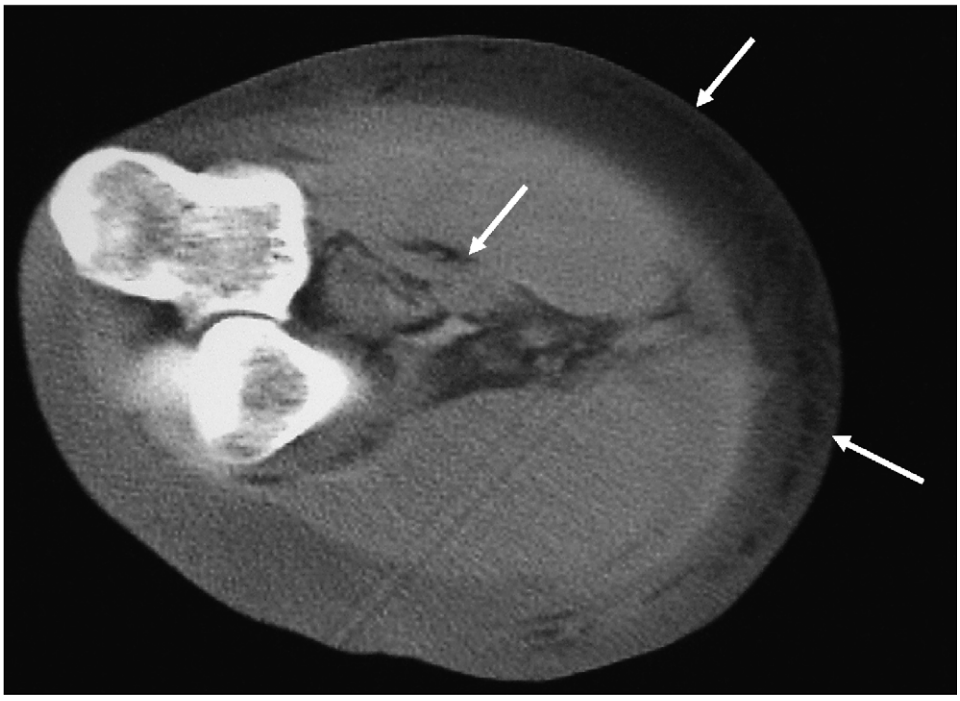

Fig. 1. CT scan of right upper extremity on admission. Arrows show gas collection.

1.1. Initial work-ups

Initial laboratory results included: haemoglobin 15.7 g/dL; white blood cell count 15,200/mm^3 (differential was neutrophils 83%, lymphocytes 8%, monocytes 7%); platelet count 461,000/uL; normal coagulation function; sodium 125 mmol/L, potassium 4.8 mmol/L, chloride 97 mmol/L, bicarbonate 25 mmol/L, creatinine 0.7 mg/dL, glucose 86 mg/dL, normal liver function tests and a CK 96 units/dL (normal). On admission, radiographs of both upper extremities showed marked swelling of soft tissue without gas production; however, a subsequent computerized tomographic scan taken within a few hours of radiographs showed marked edema of both proximal upper extremities, more prominent on the right side, with gas collections in both upper extremities (Fig. 1).

1.2. Hospital course

Blood cultures were obtained and the patient was started on piperacillin/tazobactam (3.375 g iv. every 6 h), clindamycin (900 mg iv. every 8 h) and vancomycin (1 g iv. every 12 h). Since the CT scan of both upper extremities suggested the possibility of gas gangrene, the patient underwent an emergent incision and drainage of the both upper extremities on the day of admission; this revealed extensive myonecrosis of both upper extremities with purulent drainage. The initial Gram stain of the drainage showed polymorphonuclear leukocytes with an occasional slender Gram-positive rod without evidence of spore formation; the pathology report demonstrated myonecrosis.

Material from the wound was submitted for only aerobic bacterial cultures, including blood agar media, chocolate agar, MacConkey agar, and colistin-nalidixic acid agar, with growth subsequently noted on aerobic plates; a Gram stain again demonstrated slender Gram-positive rods without spore formation. From the morphology of the organisms and the initial growth on aerobic plates, the organism was initially identified as a *Lactobacillus* species. Because of continued soft tissue pain/swelling and leukocytosis, the patient underwent repeat surgical exploration on hospital day 3 when he was found to have additional soft-tissue necrosis that required further debridement (Fig. 2). Following the second surgery, the infectious disease service was consulted and we changed the antibiotics to penicillin G plus clindamycin and reviewed the microbiology with laboratory personnel. The initial Gram stain of the aerobic culture showed no spores present (Fig. 3). Review of the Gram stain of the anaerobic subculture of the initial "aerobic" isolate showed a slender Gram-positive rod with terminal spores (Fig. 4), a finding not consistent with *Lactobacillus* species and suggesting a *Clostridium* species. The organism grew well on anaerobic media and biochemical studies demonstrated glucose fermentation with negative catalase, indole and gelatin tests; the biochemical test (VITEK ANI Card (Anaerobes) V1309®, Biomerieux Inc., Hazelwood, MO) suggested a 98% probability of *Clostridium tertium* (Bio number 4100200400). Preliminary antibiotic susceptibility tests (Kirby–Bauer disk diffusion) were now available and demonstrated susceptibility to vancomycin (30 mcg), ampicillin (10 mcg), cefazolin (30 mcg), levofloxacin (5 mcg), tetracycline (30 mcg) and trimethoprim/sulfamethoxazole

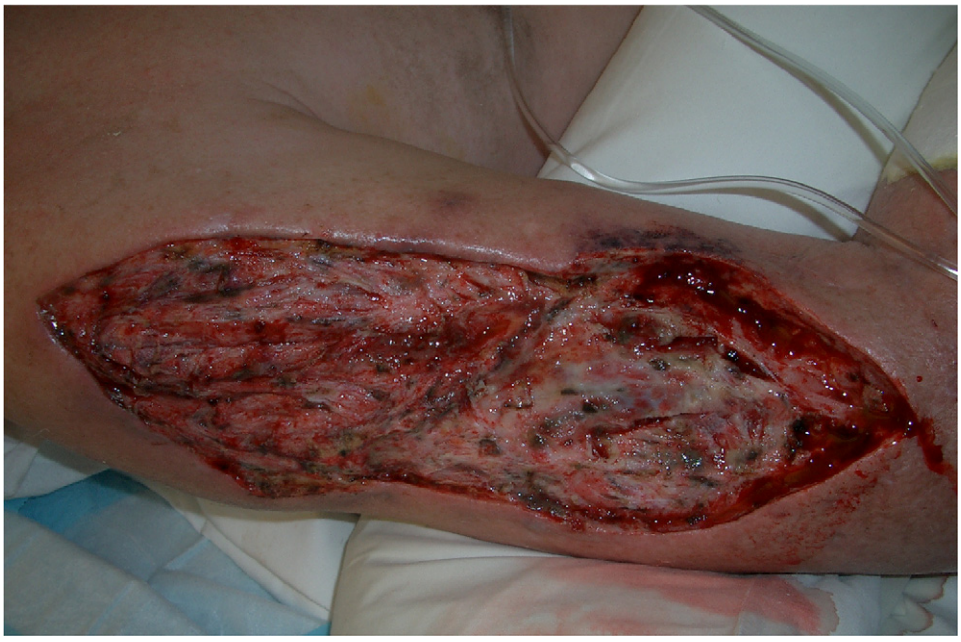

Fig. 2. Right upper arm after second debridement. Right upper arm after 2nd debridement which showed extensive myonecrosis.

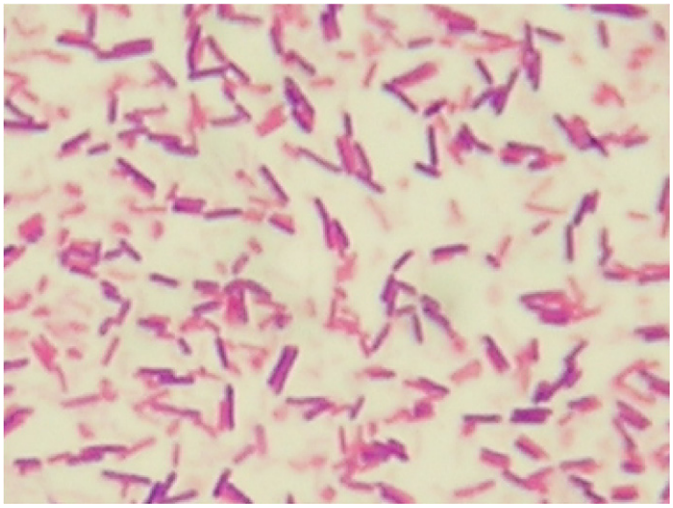

Fig. 3. Gram stain of aerobic culture. Gram positive rods without spore formation.

(23.75/1.25 mcg) and resistance to gentamicin (10 mcg), penicillin (10 mcg) and clindamycin (2 mcg).

Following these results, the antibiotics were changed from penicillin G and clindamycin to intravenous ampicillin/sulbactam (3 gm IV q 6 h). Although the patient remained afebrile, he continued to complain of arm pain and progressive swelling. Because of the slow clinical improvement, metronidazole (500 mg PO q 8 h) was added to the antibiotic regimen on hospital day 6. By hospital day 10, the swelling had improved dramatically and the patient was discharged home on oral amoxicillin/clavulanate and metronidazole. Blood cultures obtained on admission were reported as negative.

Because of the uncertainty surrounding the initial identification, the organism was sent to the Wadworth Anaerobic Bacteriology Laboratory (Los Angeles, CA) for additional studies. The organism subsequently underwent 16S rRNA gene sequencing on an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA) and was identified as *C. tertium* (16S rDNA sequence accession number was Y18174). Agar dilution susceptibility tests demonstrated susceptibility to vancomycin (MIC 2 mcg/mL) and amoxicillin/clavulanate (MIC 0.5 mcg/mL) but only intermediate susceptibility to ampicillin (MIC 1 mcg/mL). E-test demonstrated susceptibility to ampicillin/sulbactam (MIC 0.5 mcg/mL), metronidazole (MIC $\leqslant$ 2 mcg/mL), piperacillin/tazobactam (MIC 4 mcg/mL) and cefoxitin (MIC 0.75 mcg/mL) with resistance to clindamycin (MIC $>$ 256 mcg/mL). The repeat beta-lactamase test was negative. As surgical material collected from the initial and second debridements were only cultured aerobically, we carried out DNA extraction (QIAamp DNA Extraction Kit, Qiagen, Valencia, CA) from the formalin-fixed surgical material collected from the second debridement. To obtain sequence information from individual members of the bacterial community of wound infection, 16S rDNA clone libraries were constructed by cloning 5 μl of PCR product amplified with primers 8UA and 1392B. PCR products were purified and concentrated with a QIAquick spin PCR purification kit (Qiagen Inc., Chatsworth, CA). The purified PCR products were ligated into PCR®2.1-TOPO® (Invitrogen, Carlsbad, CA), as specified by the manufacturer. DNA preparations for sequencing were made with a QIAprep Spin Plasmid Kit (Qiagen Inc., Chatsworth, CA). Plasmids were eluted with 50 μl of water, and the plasmid inserts were sequenced with an

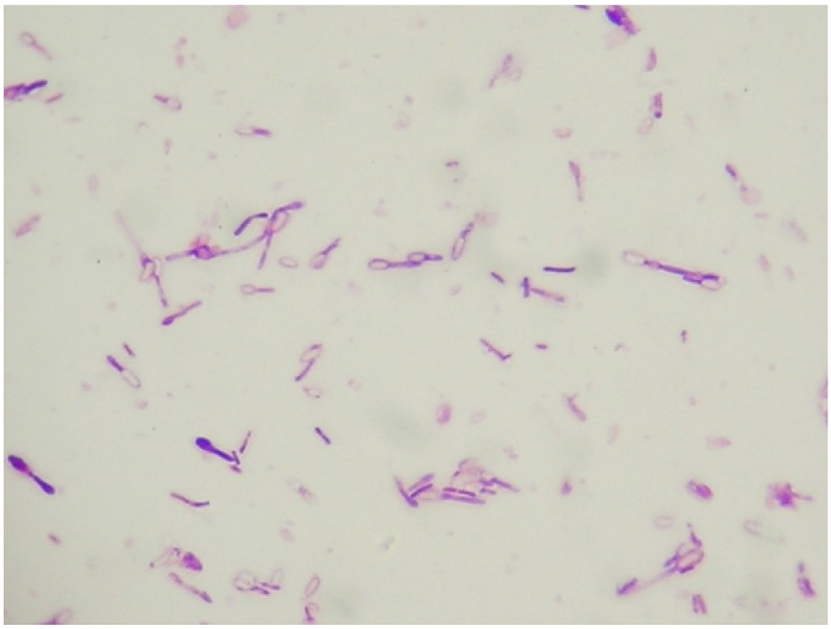

Fig. 4. Gram stain of anaerobic culture. Gram positive rods with oval terminal spores.

automated ABI Prism 377 DNA sequencer (Applied Biosystems, Perkin-Elmer Corporation). Newly determined sequences were compared to those available in public databases (Ribosomal Database Project [RDP] and Genbank). These 16S rDNA clone libraries detected *Peptoniphilus* sp (99.9% match) and uncultured *Clostridium* bacterium clone (99.9%), but not *C. tertium* or other *Clostridium* spp.

2. Discussion

2.1. General comments

Bacteria of the genus *Clostridium* are a diverse group of Gram-positive, spore-forming, anaerobic bacilli found in the soil and the gut of many animal species, including humans [1]. *C. tertium* was initially isolated from a war wound by Henry in 1917 [2] and is increasingly reported as a cause of bacteremia [3] and necrotizing soft tissue infection in humans. In the cases with necrotizing fasciitis, the patients had gangrene that required extensive debridement of skin, subcutaneous tissue and fascia, although the presence of soft tissue gas was not reported [4]. *C. perfringens* is the major cause of gas gangrene; however, other clostridial species may be associated with gas gangrene including *C. septicum, C. novyi, C. sordellii, C. histolyticum, C. fallax,* and *C. bifermentans* [5]. Our case is the first report which suggested that *C. tertium* might possibly be a causative organism of ''gas gangrene''; however, the data do not establish this association. As with other cases of ''gas gangrene'', the patient did not improve until repeated surgical debridement and treatment with antibiotics active against this organism.

2.2. Identification of C. tertium

C. tertium is a difficult organism to identify because it is aerotolerant and can present with variable Gram stain results, thus causing incorrect early identification as a *Bacillus* spp, *Lactobacillus* spp or as a Gram-negative enteric organism [6–8]. Lack of catalase production, the gas chromatography profile and other biochemical tests (the organism is saccharolytic but not proteolytic), as well as spore production under anaerobic conditions, help to differentiate *C. tertium* from these other organisms. Other distinct characteristics are the large size ($1.5 \times 10\,\mu m$), and its unusual ''square'' morphology on Gram stained smear. Organisms such as *C. carnis, C. histolyticum*, and *C. tertium* are aerotolerant and able to grow on aerobic plates; however, they grow better anaerobically and can only sporulate in an anaerobic environment. In our case, the organism was initially misidentified as *Lactobacillus* because of its aerotolerance, lack of sporulation (on aerobic culture) and biochemical tests suggestive of *Lactobacillus* species (e.g., catalase negative; indole negative). When confronted with a tentative identification of ''*Lactobacillus* species'', clinicians should be aware of the possibility of confusion with aerotolerant *Clostridium* spp and the need to request additional testing to rule out these organisms. The other issue is that still many microbiology laboratories do not even set up anaerobic cultures or do not identify anaerobes accurately.

''Usually, a Gram stained smear of the wound exudates of gas gangrene due to *Clostridium* spp reveals many large, Gram-positive rod with blunt ends but few polymorphonuclear leukocytes. The absence of white cells locally is ascribed to the lecithinase, θ-toxin, or other toxins that lyse neutrophils [5]. A more recent work by Bryant et al.

indicates that the main reason we do not see white blood cells in the fluminant myonecrosis is changes in vascular flow and plugging of small vessels by platelets and neutrophils so that the neutrophils cannot be released [9]. *C. tertium* is a non-toxin-producing organism [4], which can be a reason for "non-toxic appearing".

2.3. Antimicrobial susceptibility of C tertium and antibiotic selection

In our case, the initial antimicrobial susceptibility testing may have provided a clue to the identity of the organism. In a previous study, antimicrobial susceptibility tests of six isolates of *C. tertium* demonstrated resistance to penicillin (100% of isolates), partial resistance to clindamycin (50% of isolates were of intermediate susceptibility; 50% of isolates were resistant) and 100% susceptibility to amoxicillin/clavulanate, carbapenem and metronidazole [10]. In the previously cited case report [4] of two patients with *C. tertium* necrotizing fasciitis/gangrene, the isolates were reported susceptible in vitro to penicillin, vancomycin and metronidazole and patients responded to these antibiotics. Our *C. tertium* was found to be susceptible to ampicillin/sulbactam and piperacillin/tazobactam, however, resistant to penicillin and intermediate to ampicillin despite negative beta-lactamase test. It is unclear why adding a beta-lactamase inhibitor changed the susceptibility results. The poor response to piperacillin/tazobactam and ampicillin/sulbactam could be due to inadequate debridement. Of note, the patient improved after adding metronidazole to ampicillin/sulbactam. The common resistance of this organism to penicillin is an important observation and stresses the need to obtain antimicrobial susceptibility testing of clostridial isolates, especially non-*perfringens* species where antimicrobial susceptibility may be difficult to predict. Usually, the aerotolerant *Clostridium* spp are sensitive to metronidazole, while *Bacillus* spp and *Lactobacillus* spp are not. This is a useful screening test for sorting out aerotolerant *Clostridium* spp from *Bacillus* spp and *Lactobacillus* spp.

2.4. Limitations

Anaerobic cultures were not undertaken from surgical specimens. Although we performed the DNA cloning libraries to identify possible co-infection, *Clostridium* spp, including *C. tertium*, were not detected from the formalin-fixed surgical specimen. Instead, *Peptoniphilus* sp and an uncultured *Clostridium* clone were detected in 16S rDNA clone libraries. However, there has been no report of gas gangrene caused by these organisms. It is possible that co-infecting anaerobes, including *C. tertium*, were not detected due to a sample error, because formalin fixation might have denatured the DNA of *C. tertium.*

3. Conclusion

Although *C. tertium* has been reported as a cause of necrotizing fasciitis, gas gangrene (myonecrosis) caused by *C. tertium* as the sole pathogen has not been reported. Our case suggested that *C. tertium* perhaps could cause gas gangrene but adequate evidence for this was not obtained. Clinicians must be aware of the possible confusion of this organism with other aerotolerant Gram-positive organisms (e.g., *Lactobacillus* species, *Bacillus* species) to avoid errors in choice of antibiotic therapy and should request additional cultures and biochemical tests to further confirm the identity of the suspected pathogen. Antimicrobial susceptibility testing is important since *C. tertium* is frequently resistant to penicillin G and may not respond to antibiotics (e.g., ampicillin, penicillin G, clindamycin) traditionally employed for clostridial infection.

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