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Anaerobe 13 (2007) 161–165

Anaerobe

www.elsevier.com/locate/anaerobe

Short communication — Anaerobiosis: Molecular Biology, Genetics and Other Aspects

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

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Received 19 January 2007; received in revised form 24 February 2007; accepted 5 March 2007
Available online 12 March 2007

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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doi:10.1016/j.anaerobe.2007.03.002

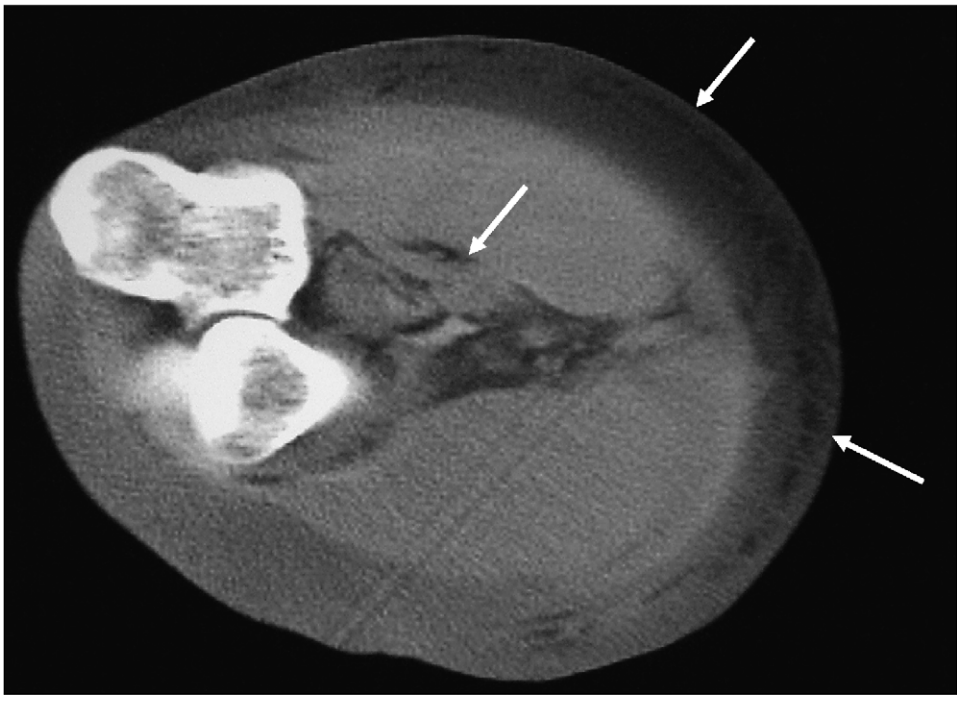

Fig. 1. CT scan of right upper extremity on admission. Arrows show gas collection.

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