Case Quiz

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Introduction: Clostridium tertium is present both in soil and in oral and faecal human flora and has been reported as a cause of various infections, such as brain abscess, sepsis and necrotizing soft tissue infections.

Case presentation: A 12-year-old boy arrived at a rural hospital in the highlands of south-western Ethiopia with two cutaneous lesions on the left foot and elbow. The father said that the boy had fallen from a tree 6 days ago. A sample was taken from both lesions and cultured on blood agar. For both specimens, Gram-positive rods were observed following Gram staining and the following day growth on solid medium showed an aerotolerant sporulated Gram-positive bacillus. Treatment with intravenous penicillin was started and the boy recovered in 3 days. A concentrate of the culture inactivated with methanol was sent to a reference laboratory and identified by mass spectrometry as Clostridium tertium.

Conclusion: The combined use of basic clinical microbiology tools with new and simple diagnostic technologies is redefining our knowledge about the aetiology of some common infectious diseases.

Keywords: bacterial disease; cutaneous ulcers; intravenous penicillin.
A few bacterial colonies were suspended in 300 ml sterile water and vortexed. The bacterial suspension was inactivated with 900 ml absolute ethanol and sent to a reference laboratory in Spain. The colonies were identified with a high level of reliability (99.9%) using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) Bruker Biotyper system (Bruker Daltonik).

**Discussion**

Differential diagnosis in this case was guided by the Gram stain of the exudative material of the ulcers. The presence of Gram-positive bacilli in this type of lesion in an African rural area could indicate a diagnosis of anthrax, although the disease is usually associated with direct contact with infected animal meat, wool and skin. The clinical picture suggested that it was not anthrax as there was no black scar. A non-specific bacterial infection due to a soil micro-organism was suspected. The patient was treated initially with penicillin to cover the possibility of anthrax, or another Bacillus sp., and this treatment was continued after obtaining the results of the antibiogram.

The colonies were identified as Clostridium tertium, with a high level of reliability (99.9%), employing MALDI-TOF MS, following the ethanol/formic acid extraction procedure recommended by the manufacturer. C. tertium has long been considered to be apathogenic, as it only rarely occurs in mixed infections of traumatic wounds. In recent years, the organism has been identified as a cause of bacteremia and other infections such as brain abscess, sepsis and necrotizing soft tissue infections in immunocompromised and immunocompetent patients (Fujitani et al., 2007; Miller et al., 2001; Salvador et al., 2013).

C. tertium is an endospore-forming, Gram-positive bacillus, which may grow aerobically and is distributed in the soil as well as in animal and human gastrointestinal tracts (Steensma et al., 2011; Wells & Wilkins, 1996). Because of its micromorphology and growth pattern, there are many reports of misidentification of C. tertium under aerobic conditions with Bacillus spp. or Lactobacillus spp., both considered to be contaminants or apathogenic (Salvador et al., 2013; You et al., 2015). C. tertium can be differentiated from other bacteria by methods such as catalase and oxidase testing, anaerobic spore formation and gas chromatography profiling (You et al., 2015). However, in this case, the patient was attended to in a basic rural setting without a specialized infrastructure for bacteriology.

The susceptibility pattern of the isolate was different from Bacillus cereus infections, as described in many previous case reports (You et al., 2015; Miller et al., 2001). The broad picture of B. cereus is one of resistance to penicillin, ampicillin, cephalosporins and trimethoprim with susceptibility to clindamycin, erythromycin, chloramphenicol, vancomycin, the aminoglycosides and, usually, tetracycline (Kemmerly & Pankey, 1993). Both pathogens have different susceptibility patterns.

Bacillus anthracis also produces a cutaneous ulcer, but the clinical appearance of the lesion is different when compared with the lesion in this case. Cutaneous anthrax, which is relatively common and under-reported in the rural areas of

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**Fig. 1.** (a) Coin-sized ulcer on the left elbow. (b) Gram-positive bacillus showing a subterminal swollen area (circled), probably due to the presence of an endospore.

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**Question**

Which pathogen is involved in this infection?

**Answer options**

a) Bacillus cereus  

b) Escherichia coli  

c) Actinomyces israelii  

d) Bacillus anthracis  

e) Clostridium tertium
Africa, typically presents a painless ulcer with a black necrotic central eschar (Chatterjee et al., 2014).

*Clostridium tertium* could also be mistaken for a Gram-negative enteric organism such as *Escherichia coli* because of its varying degree of Gram staining. However, the distinct characteristics of *C. tertium* as Gram-positive bacillus with a large size (1.5 × 10 μm) and its unusual ‘square’ morphology on Gram stained smear allow differentiation from *Escherichia coli* or *Actinomyces* spp.

As this case illustrates, the combined use of basic clinical microbiology tools along with new and simple diagnostic technologies can improve our understanding of the aetiology of infectious diseases.

**References**


