Brucellosis is a zoonotic disease of worldwide distribution affecting mainly those working with domestic animals and/or their products. Although it is controlled in many developed countries, the disease remains endemic in many parts of the world including Saudi Arabia. The clinical presentation can vary from asymptomatic infection with seroconversion to systemic disease with fever, night sweats, and musculoskeletal manifestations. Rarely, the liver, heart, and eyes or the central nervous system may be involved.

Brucellosis remains endemic in many countries including Saudi Arabia. The disinfection of objects and surfaces contaminated with Brucella spp is not difficult, but we encountered a situation in which the organism survived the decontamination and liquefaction procedure adopted for AFB culture. A sputum specimen from a patient was sent for TB culture and the BACTEC MGIT 960 system isolated an organism identified as Brucella spp. The blood cultures and the serological testing had confirmed this case to be brucellosis. Isolation of Brucella spp from sputum samples is rare; this case appears to be the first of its kind.

As the clinical presentation of TB may mimic brucellosis and vice versa, we recommend that handling specimens from all cases of undiagnosed PUO should be done with care because of the possibility that it may contain either of these organisms.

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Although Brucella spp can survive in dust and dry environments, they are considered fastidious organisms because they are susceptible to routine disinfection procedures such as household bleach and 70% ethanol. The decontamination procedure was adapted for setting up mycobacterial cultures in the BACTEC MGIT 960 system that is designed to kill all bacteria except Mycobacterium spp. We describe a case in which a sputum specimen submitted for mycobacterial culture and decontaminated by the usual procedure grew Brucella spp hence showing the resistance of Brucella spp to both the recommended decontamination procedure and the antibiotics, PANTA (polymyxin B, amphotericin B, nalidixic acid,trimethoprim, azlocillin) in the MGIT tube.
negative, alkaline phosphatase was 193 U/L (normal range 50-180 U/L), alanine aminotransferase 67 U/L (normal range 20-65 U/L), aspartate aminotransferase 76 U/L (normal range 12-37 U/L). Brucella serology was B abortus titer was 1:2560 while B melitensis titer was 1:320. The urinalysis was normal and there was no growth from urine culture. Three sets of specimens for blood culture (BacT/Alert System) were submitted to the laboratory.

Although the patient had no specific respiratory symptoms or signs, two days later a sputum sample was collected for acid fast bacilli (AFB) smear and mycobacterial culture to rule out pulmonary tuberculosis considering the endemicity of the disease in the area. The sputum specimen was processed in accordance with the laboratory protocol for mycobacteriology culture. Decontamination and liquefaction was performed using 2% NALC-NaOH solution. On the same day a total of 12 specimens were processed using the same procedure. The specimen was then centrifuged and the sediment was inoculated into one tube of liquid MGIT medium containing PANTA supplement and one slope each of Lowenstein-Jensen LJ- glycerol and LJ-pyruvate medium. The MGIT tube was incubated in BACTEC MGIT 960 (Becton, Dickinson and Company Diagnostic Systems, Maryland USA) system and LJ slopes were incubated separately at 37°C. The smear was stained with auramine O fluorescent technique and examined under fluorescent microscope. No AFB were detected in the smear.

On February 27, all three sets of blood cultures gave a positive signal. Gram-stain smears from all bottles showed gram-negative cocccobacilli morphologically resembling Brucella spp. Subcultures were done onto a solid blood agar plate (BAP), a chocolate agar and MAC plates (CAP), and MacConkey agar media. The BAP and CAP yielded growth of organisms subsequently identified as Brucella spp based on positive oxidase and urease tests, along with positive serological typing as Brucella melitensis.

After 9 days of incubation in the BACTEC MGIT 960 system, the MGIT tube containing the sputum specimen gave a signal of positivity. The tube was retrieved and processed for smear and subculture in accordance with the manufacturer’s instructions. Smears were stained with Ziehl-Neelsen (ZN) stain for AFB and with Gram stain. Subcultures were done on LJ slopes and on BAP and MacConkey agar plate to rule out contaminants and fast growing mycobacterial species. The ZN stained smear did not show AFB, but the Gram-stained smear showed gram-negative cocccobacilli morphologically resembling Brucella spp. The subculture on BAP grew typical colonies that were identified as Brucella melitensis using the same criteria as described above. The batch set up for mycobacterial cultures done on the same day was reviewed and technical errors were excluded as all other specimens remained negative except three specimens from other patients that grew AFB subsequently identified as Mycobacterium tuberculosis.

To validate the resistance of the isolate to the decontamination and PANTA, a suspension (1.0 Macfarland) was prepared and subjected to the same decontamination procedure once again. The second exposure to a decontamination procedure and antibiotics contained in the PANTA supplement failed to kill the organism. This further reinforced our initial observation.

The patient was started on streptomycin injections for two weeks and oral doxycycline for four weeks. He was discharged from the hospital after three days, being afebrile, and advised to complete antimicrobial therapy at home. The susceptibility performed on this isolate showed that it was resistant to trimethoprim, but fully susceptible to streptomycin, sulfamethoxazole, tetracycline and rifampicin.

**DISCUSSION**

The major routes of Brucella infection to humans are ingestion of dairy products, inhalation of aerosols, and inoculation through the skin or mucous membranes or contact with infected animals and/or their products. Although the inhalation route of entry of Brucella into the body is not an uncommon route of transmission, the disease seldom involves the respiratory system and the isolation from sputum is only rarely reported. N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) mixture is used for liquefaction and decontamination of the sputum samples before setting up mycobacterial cultures. The liquid broth medium for mycobacterial culture is known to yield better recovery and faster growth of mycobacteria. The MGIT contains modified Middlebrook 7H9 broth base, and enrichment. The presence of antibiotics in the MGIT tube [polymyxin B (2,000U/ml), amphotericin B (200µg/ml), nalidixic acid (800µg/ml), trimethoprim (200µg/ml), azlocillin (200µg/ml)] is necessary to suppress contamination of culture with other bacteria. It was unexpected that Brucella survived the liquefaction and decontamination steps of setting up mycobacterial cultures and further survived exposure to the PANTA mixture in the MGIT tubes. It was not a case of decontamination failure because the batch of samples processed on the same day remained nega-
tive except in this case and the three other specimens that grew mycobacterium subsequently identified as *M tuberculosis*. In our laboratory, the decontamination process is checked regularly by quality control for each batch and our contamination rate with other respiratory flora matches the international standard.

It is of interest to mention that of the five antibiotics used in PANTA, trimethoprim is a component of cotrimoxazole, a recognized therapy for brucellosis. The concentration of trimethoprim in cotrimoxazole tablets is 160 mg while in a study the MIC90 of *Brucella* spp was found to be 0.125µg/mL. 11 and the amount present in PANTA is expected to inhibit *Brucella* spp.

In countries where brucellosis and TB remain endemic, brucellosis can mimic clinically TB and sputum specimens processed for *M tuberculosis* may contain *Brucella* spp. Brucellosis is still one of the top differential diagnoses of pyrexia of unknown origin in Saudi Arabia and treating physicians should have a high index of suspicion and discuss the case with the clinical microbiology laboratory to collect the best specimen according to the clinical syndrome to avoid a delay in the diagnosis and increased morbidity for the patient.

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