

b Brucellosis

Case of Brucellosis: Laboratory diagnosis and safety

An isolate from the blood culture of a 21-year-old female patient who presented with fatigue, fever, headache and confusion was submitted to NICD from the NHLS Helen Joseph Microbiology Laboratory on 23 July 2015 for MALDI-TOF* identification. Unfortunately, the agar plates (chocolate and blood) had become contaminated with other organisms (possibly during transportation) and the following day, colonies from the mixed culture plate were carefully selected on the open bench, and prepared for testing on MALDI-TOF. Although the isolate yielded a spectrum of mass peaks on the MALDI-TOF, there was no reliable identification. This usually indicates that the genus and/or species are not represented in the database of the instrument.

The lack of a conclusive MALDI-TOF identification prompted the need for further investigation. Staff of the NICD commenced with bench-top tests. The Gram stain showed a tiny Gram-negative coccobacillus. The isolate was oxidase and catalase positive, urease positive (within a few hours), non-oxidative, rapid indole negative and failed to grow on MacConkey agar. The laboratory report on the patient indicated that the incubation time of the blood culture was 87 hours. As these results were compatible with *Brucella* species, all laboratory workers were informed, and appropriate laboratory safety measures implemented. Up to this point, testing and plating out had been performed on an open bench using only basic personal protective equipment i.e. lab coat and gloves. The isolate was submitted for 16sRNA sequencing, which identified it as a *Brucella* species. Subsequently, Onderstepoort Veterinary Institute confirmed the organisms to be *Brucella melitensis* biotype 3

Brucella spp. primarily infect animals, most commonly sheep, cattle, goats, pigs and dogs. Infection is transmitted to humans who come into contact with infected animals or animal products. Initial symptoms are generalized muscle and joints pain, headache and fever. A full description of clinical presentation of brucellosis may be found in the January 2011 Communiqué on the NICD website (www.nicd.ac.za). *Brucella* species pose a significant risk to laboratory workers (see reference below). The organism is easily aerosolised through

bench-top manipulation of cultures especially through the catalase test (detection of oxygen production through the addition of a dilute solution of hydrogen peroxide to colonies on agar) and through flaming of a nichrome wire loop. For this reason, it is conventional to conduct laboratory manipulation of *Brucella* species in a biosafety level 3 environment. This implies the use of personal protective equipment, a class II biological safety cabinet (BSC), and a facility with positive air-pressure ventilation. In addition, access to the laboratory should be restricted. Procedures that generate splashes or aerosols minimized should be performed in a BSC. The sniffing of opened cultures plates to assist in the identification of the isolate should not be done.

Upon suspicion of *Brucella* spp., all the laboratory staff that had been working on the isolate was questioned to ascertain risk. Four staff members had worked directly with the isolate or had come within 2.5m of the organism on an open bench and were thus identified as being at high risk of acquisition of *Brucella* infection. Fortunately only disposable inoculating loops had been used to pick off and plate out the isolate, minimising the risk of aerosolisation from flaming a nichrome wire loop. These staff members were prescribed doxycycline 100 mg orally, twice daily for 3 weeks, and are presently conducting daily symptom checks for fever, under the supervision of the NICD Occupational Health nurse. Blood has also been taken for *Brucella* serology at baseline, and will be repeated at 6, 12, 18 and 24 weeks after exposure. *Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF)

Reference: Yagupsky, P., Baron, EJ. Laboratory exposures to *Brucellae* and implications for bioterrorism. EID 2005; (11)8: 1180-1185.

Source: Centre for Opportunistic, Tropical and Hospital Infections, NICD-NHLS; Helen Joseph Hospital laboratory and Infectious Diseases Department.