First case of *Mycobacterium heckeshornense* lymphadenitis


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ABSTRACT

Mycobacterium heckeshornense is a slow-growing nontuberculous mycobacterium first characterised in 2000. It is reported to cause lung disease and tenosynovitis. We report a case of isolated massive axillary lymphadenopathy in an elderly woman, where histology showed necrotising granulomata and *M. heckeshornense* was isolated as the causative organism.
CASE REPORT

An 84 year old woman presented to her general practitioner in November 2006 with fatigue and subjective weight loss over several months and a mass in her left axilla. The mass had been progressively enlarging since she first noticed it several weeks earlier. Her doctor attempted to aspirate the mass and the procedure was complicated by significant blood loss after the patient returned home. She developed palpitations and light-headedness which led to her admission to Middlemore Hospital.

The patient had a past history of treated hypertension and a squamous cell carcinoma of the left side of the neck which had been resected in 2005. Physical examination at the time of presentation to the emergency department revealed pallor and postural hypotension, as well as a large palpable mass in the left axilla. Bleeding from the attempted aspiration site had resolved spontaneously. Investigations showed her electrolytes, creatinine and liver function tests were normal. A full blood count revealed a normochromic, normocytic anaemia with a haemoglobin of 82 g/L and thrombocytopenia with a platelet count of 65x10^9/L. Iron studies were consistent with anemia of chronic disease. Computed tomography of the neck, chest, abdomen and pelvis revealed enlarged left axillary lymph nodes up to 6cm in diameter but no other lymphadenopathy and no lung pathology. A fine needle aspirate of the left axillary lymph nodes was performed. Cytology showed only atypical lymphoid cells and flow cytometry suggested a reactive lymphoid population within the sample.

The patient proceeded to formal left axillary node dissection in December, 2006. The operative findings were of two large nodes which appeared infected and were described as “possible cold abscesses”. One of the nodes ruptured intraoperatively,
discharging frank pus. A swab of this pus and tissue samples of the excised nodes were sent for routine and mycobacterial culture, *Mycobacterium tuberculosis* PCR testing and histology. Mycobacterial culture included inoculation of a BacT/Alert MP broth (BioMérieux, Marcy L’Etoile, France) as well as Lowenstein-Jensen slopes (Fort Richard Laboratories, Auckland, New Zealand) incubated at 30°C and 35°C and a chocolate agar slope (Fort Richard Laboratories) incubated at 30°C.

Histological examination of the resected lymph nodes showed necrotising granulomatous inflammation with cystic abscess formation. No organisms were seen on Gram, PAS or Ziehl-Neelsen stain. No evidence of malignancy was seen. Routine bacteriological cultures of the pus swab taken from the ruptured node and the direct culture of the tissue sample were sterile however an enrichment broth culture of the tissue specimen grew *Staphylococcus epidermidis* after 4 days. This was considered to be a contaminant. PCR for *M. tuberculosis* DNA was performed on an operative tissue sample, using an in-house real-time PCR assay with primers targeted at the IS6110 insertion sequence. The following primers and probes (Applied Biosystems, CA) were selected: IS6110TF 5’ GGAAGCTCCTATGACAATGCACTA 3’, IS6110TR 5’ GGCTTGCCGGGTGTGAT 3’ and IS6110TP MGB-probe 6-FAM-AACCGGCTATACAGAC-MGBNFQ. Real-time PCR conditions (50-µl format) for TaqMan were as follows: 2× TaqMan Universal Mastermix (Applied Biosystems, CA), 1× Taqman Exogenous Internal Positive Control (Applied biosystems, CA), 45 pmol each primer, 12.5 pmol of probe and 2 or 5 µl of DNA; 2 min at 50°C, 10 min at 95 °C, and 45 cycles of 15 sec at 95 °C and 1 min at 60°C. Samples were also amplified with 1× Taqman RNaseP Control (Applied Biosystems, CA) and 2× TaqMan Universal Mastermix under same PCR conditions as above to verify...
extraction process. The PCR for *M. tuberculosis* DNA was negative by this method. PCR for other mycobacteria was not performed on the operative tissue samples. Auramine-rhodamine stains of the lymph node tissue and swabbed pus were negative for acid-fast bacilli. Mycobacterial culture of the excised nodal tissue grew an acid-fast bacillus from the BacT/Alert MP broth on day 39. The positive broth culture was referred to the local reference laboratory for speciation of the isolate which involved HSP 65 kDa and 16S rRNA sequence analysis. Lowenstein-Jensen and chocolate agar slopes were negative after 6 weeks incubation.

DNA for sequencing was extracted as previously described (5) and the 16S rRNA and HSP 65 genes were amplified with primers 27F and 519R (9) and Tb11 and Tb12 (2). Negative control was provided by use of negative blanks and PCR blanks. Amplification was performed in a 0.2 ml PCR tube with a total reaction volume of 50 µl by using a GeneAmp PCR system 9700 thermocycler (PE Applied Biosystems, CA). Each reaction tube contained; 250µM (each) dATP, dCTP, dGTP, and dTTP; 20 mM Tris-HCl; 50 mM KCl; 1.5 mM MgCl₂; primers and sample DNA. The gene amplification PCR round was conducted with initial 5 min denaturation step at 94°C coupled to repeating cycle of 45 s at 94°C, 45 s at 55°C (60°C for HSP65), and 45 s at 72°C for 35 cycles, followed by a 2 min final extension step at 72°C. The quality of DNA extraction was monitored using extraction blank, PCR blank and positive controls, and inhibitors were monitored for using differing concentrations of DNA.

The oligonucleotide primer 27F and Tb 11 (5µM each) were used for sequencing. PCR products used as templates for sequencing were purified using High Pure PCR Purification Kit (Roche Diagnostics GmbH, Mannheim, Germany). Sequencing
reactions were performed by using an ABI Prism BigDye terminatory cycle sequencing kit (PE Applied Biosystems, CA) in a GeneAmp PCR System 9700 thermal cycler and were electrophoresed by using an ABI Prism 310 Genetic Analyser (PE Applied Biosystems). The sequence data was edited by using DNASTAR software (DNASTAR, Madison, WI). A blast search was performed using the National Center of Biotechnology Information (NCBI) database. On 16S sequencing the isolate showed 100% identity with Genbank sequences AF547934.1 (Mycobacterium heckeshornense), AF174290.1 (M. heckeshornense), AF101243.2 (Mycobacterium sydneyiensis) and AJ243481.1 (Mycobacterium cf. xenopi). On HSP65 sequencing, the isolate showed 100% identity with only AF547843.2 (M. heckeshornense). We therefore concluded that the isolate was M. heckeshornense.

A swab of the purulent discharge from the node which ruptured during the operation also grew an acid-fast bacillus in the BacT/Alert MP broth after 39 days incubation; however this isolate was not referred or stored for duplicate testing.

The patient was followed up as an outpatient by the Infectious Diseases service at two and four months post-operatively. Her axillary wound healed well, and there was no residual clinical evidence of infection. She had no palpable lymphadenopathy and remained systemically well. Her anaemia resolved, however her platelet count remained consistently between 51 and 71. She declined investigation of her thrombocytopenia. Given her clinical status and the uncertainty regarding whether treatment would be of any benefit in this patient, she has not been treated with antimycobacterial agents.
Mycobacterium heckeshornense was first described in 2000 by Roth et al (10). It is a slow-growing, scotochromogenic mycobacterium initially isolated from an immunocompetent patient with severe bilateral cavitary lung disease (10). It is phenotypically very similar to Mycobacterium xenopi, therefore the burden of human disease caused by M. heckeshornense may have been underestimated in the past due to misidentification of M. heckeshornense as M. xenopi (3). It has since been described as a cause of lung disease in other patients (6, 7, 12) and as a cause of tenosynovitis (3). After a search of the published literature we find this case is the first report of M. heckeshornense as a cause of lymphadenitis.

M. heckeshornense can be identified by a number of methods, including sequencing of ribosomal 16S (10), heat shock protein 65 (4) and rpoB genes (8), and gas-chromatographic lipid profiling (11). It is important that more than one method is used as different methods may reveal conflicting results (11). In this case, the isolate from surgically resected tissue had 16S and HSP65 sequencing performed, which were 100% consistent with M. heckeshornense, confirming the identification. Gas-chromatographic lipid profiling and rpoB gene sequencing are not used routinely in our reference laboratory for the identification of mycobacteria, and as 16S and HSP65 gene sequencing results were concordant it was felt that further testing was not required. Culture of the swab taken during the operation also grew a mycobacterium, but this organism was not stocked and not further identified. This case fulfils the criteria of the American Thoracic Society for diagnosis of non-tuberculous mycobacterial lymphadenitis (1).
The limited reports regarding *M. heckeshornense* in the current medical literature make it impossible to provide definitive information on treatment of infections with this organism. Given the phenotypic similarities to *M. xenopi* similar treatment may be reasonable. However, in localised non-tuberculous mycobacterial lymphadenitis, once the infected nodes have been resected, antimycobacterial therapy is usually not indicated (1).

*M. heckeshornense* is rarely isolated as a pathogen and previous cases involved pulmonary infection and tenosynovitis. We have described the first recognized case of this organism causing lymphadenitis. Molecular identification methods may lead to more widespread identification of this organism from clinical specimens.
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References:


