

# Atypical mycobacterial spondylitis in HIV-negative patients identified by genotyping

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**Non-tuberculous mycobacterial infections pose a significant diagnostic and therapeutic challenge. We report two cases of such infection of the spine in HIV-negative patients who presented with deformity and neurological deficit. The histopathological features in both specimens were diagnostic of tuberculosis. The isolates were identified as *Mycobacterium intracellulare* and *M. fortuitum* by genotyping (MicroSeq 16S rDNA Full Gene assay) and as *M. tuberculosis* and a mycobacterium other than tuberculosis, respectively, by culture. There is a growing need for molecular diagnostic tools that can differentiate accurately between *M. tuberculosis* and atypical mycobacteria, especially in regions of the developing world which are experiencing an increase in non-tuberculous mycobacterial infections.**

Non-tuberculous mycobacterial infections are closely associated with severe immunosuppression, usually because of HIV infection or poverty.<sup>1</sup> A recent rise in their reported incidence may be attributed to improvements in laboratory methods of diagnosis.<sup>2</sup> An accurate and timely identification of the infective organism is essential for a successful outcome. Currently, diagnostic assays for tuberculosis (TB) are often negative, non-specific or delayed.<sup>2,3</sup> The use of molecular diagnostics has improved the sensitivity and specificity of current technologies. We describe the use of genomic sequencing to diagnose spinal non-tuberculous mycobacterial infections in HIV-negative patients.

## Materials and Methods

The local Institutional Review Board approved the study and informed consent was obtained from each patient before sample collection.

A total of 35 biopsies of granulomatous tissue collected during spinal decompression (15 HIV-positive and 20 HIV-negative) was assessed by conventional diagnostic assays, including microscopy (Ziehl-Neelsen and auramine stains for acid-fast bacilli)<sup>4</sup> and culture (BACTEC MGIT; Becton Dickinson, San Jose, California). Specimens were further subjected to independent histopathological evaluation, and macrophage infiltration was quantified by CD68 monoclonal antibody immunolocalisation using a modified immunoperoxidase method.<sup>5</sup>

Bacterial DNA was extracted from all heat-inactivated (95°C, 10 minutes) positive cultures using the Qiagen DNA minikit (Qiagen, Hilden,

Germany). The 16S ribosomal (r)DNA region was polymerase chain reaction (PCR) amplified (Applied Biosystems, Foster City, California) and sequenced using the MicroSeq 16S rDNA Full Gene and 500 bp kits (Applied Biosystems). Appropriate controls were included in each run, and extension products were sequenced on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems) using the ABI Big Dye platform (Applied Biosystems). An independent laboratory performed confirmatory sequencing on five randomly selected sequence-positive isolates.

Satisfactory 16S rDNA sequences were codon aligned, and compared with published reference strains in the National Centre of Biotechnology Information, Ribosomal Differentiation of Medical Microorganisms and DNA Data Bank of Japan sequence repositories. An agreement between the query and reference sequences of more than 90% denoted a positive match. Confirmatory phylogenetic reconstruction was also performed using standard bioinformatics software such as the PAUP (Sinauer Associates Inc., Sunderland, Massachusetts), MODELTEST 3.0,<sup>6</sup> and TreeTool<sup>7</sup> bioinformatics software tools. Statistical analysis was performed using the SPSS 11.5 (SPSS Inc., Chicago Illinois) software package. A p-value of < 0.05 was considered to be significant.

## Results

All the specimens were microscopy-negative and 25 (71%) were culture negative. Eight (23%) of the culture-positive specimens were identified as *Mycobacterium tuberculosis* and

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Table I. Demographic, clinical and laboratory findings of the two patients infected with non-tuberculous mycobacteria

	<i>M. intracellulare</i>	<i>M. fortuitum</i>
Gender	Male	Male
Age (yrs)	47	71
HIV status	Negative	Negative
Culture identification (BACTEC MGIT)	<i>M. tuberculosis</i>	<i>Mycobacterium</i> other than tuberculosis
Level of pathology	T6/7	L3/4
Neurological deficit (Frankel grade <sup>9</sup> )	C	C
Previous tuberculosis contact	None	None
Microscopy (acid-fast bacilli)	Negative	Negative
Histopathology		
Granulation	Profuse	Sparse
Caseation	Profuse	Sparse
Lymphocytes	Sparse	Sparse
Epithelioid cells	None	Sparse
Plasma cells	None	None
Features diagnostic of tuberculosis	Yes	Yes
CD68 immunolocalisation (range)		
Mean area ( $\mu\text{m}^2/\text{field}$ )	416.1 (165.1 to 1140.8)	1805.0 (1124.0 to 2397.0)
Mean intensity* (mean intensity/field)	153.7 (2.0 to 241.0)	200.9 (106.0 to 255.0)

\* measured according to an intensity scale 0 to 255, where 0 = white and 255 = black. The greater the intensity, the greater the reactivity to CD38 antibody

the remaining two (6%) as a mycobacterium other than tuberculosis, using conventional techniques.

Of the positive sequences, two were characterised as non-tuberculous mycobacteria by genotyping. BLAST analysis identified them as *M. fortuitum* and *M. intracellulare*. (The basic local Alignment Search Tool (BLAST) is a search tool which enables the comparison of the nucleotide or amino acid query sequences of an organism with a library or database of known sequences, thus identifying the unknown organism).<sup>8</sup> These findings were confirmed by phylogenetic reconstruction. There was concordance between genotyping and culture identification in one isolate, which was genotyped *M. fortuitum* but identified as a mycobacterium other than tuberculosis by culture assays (BACTEC MGIT). In direct contrast with the *M. fortuitum*-infected specimen, granulomas and caseation were profuse in the *M. intracellulare*-infected tissue (Table I). However, macrophage infiltration quantified by CD68 immunolocalisation was more abundant in the *M. fortuitum*-infected tissue (Table I).

Both patients presented with a neurological deficit (Frankel grade C<sup>9</sup>), were HIV-negative and immunocompetent (CD4+/CD8+ T-cell counts > 500 cells/ $\mu\text{l}$ ), and made a full recovery after surgery and adjunctive treatment.

## Discussion

Atypical mycobacterial infections pose a significant challenge to the immunocompromised patient, as their capacity for recruitment, activation and function of key effector cells is severely impaired.<sup>10</sup> The additional impact of poverty on incidence rates is particularly significant in developing nations, where the likelihood of exposure to atypical opportunistic organisms is high.<sup>1</sup>

The inability to establish an accurate early diagnosis remains the single most limiting factor in the patient's treatment. Genotyping identifies pathogens more accurately

than conventional diagnostic methods.<sup>11,12</sup> In our study, the discordance between culture identification and genotyping may reflect the limited specificity of conventional assays (BACTEC MGIT) and emphasise the need for a wider-ranging diagnostic tool. Culture-based diagnostic assays are positive in only a third of cases of paucibacillary disease, thereby delaying diagnosis by several weeks.<sup>13</sup> This is either because of earlier antibiotic treatment, which renders specimens negative by conventional culture assays, or because of infection by fastidious organisms which are difficult to culture.<sup>3</sup> In our patients, anti-TB medication was started at the referral hospital before surgery, yet did not prevent the disease process that ultimately necessitated surgery. Previous studies describe similar findings.<sup>10,14</sup> On the other hand, molecular diagnostic assays, despite being highly sensitive and specific, are often designed for a single organism or genus; consequently, multiple assays are required.<sup>3</sup> Harris and Hartley<sup>3</sup> used the broad-spectrum 16S rDNA genotyping assay to identify pathogens from clinical samples and were able to characterise approximately 25% of culture-negative organisms by genotyping alone, 82% of which were clinically significant. Direct genotyping is also quick and can give a result within 48 to 72 hours, compared with several weeks for a culture diagnosis.<sup>3,13</sup>

*M. intracellulare* has been increasingly implicated in pulmonary and extrapulmonary infections.<sup>15,16</sup> As part of the *M. avium* complex, *M. intracellulare* and *M. avium*, cause the highest number of associated infections, including those of bone and joints.<sup>14</sup> Although infections have been reported in HIV-positive and HIV-negative patients, *M. intracellulare* displays an increasing predilection for the latter.<sup>14</sup>

*M. fortuitum* is characteristically resistant to anti-TB medication, and poses a significant therapeutic challenge which often requires surgical intervention as well as the

appropriate antibacterial agents.<sup>17,18</sup> A recent account described a *M. fortuitum* infection of the spine in an HIV-negative woman with paraplegia.<sup>18</sup>

Our study highlights the need for accurate early identification of the infective pathogen in order to facilitate appropriate treatment. It is proposed that genotyping be used as an adjunct to conventional culture assays in regions with a high risk of exposure to atypical mycobacteria.

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