Infection due to a novel mycobacterium, mimicking multidrug-resistant
*Mycobacterium tuberculosis*

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**Abstract**

The treatment of multidrug-resistant tuberculosis (TB) requires the use, for long periods, of drugs liable to cause significant side effects. In the case of misdiagnosis of multidrug-resistant TB, the patient is exposed to toxic substances without any benefit. In low-income countries, where the microbiological diagnosis of TB relies on microscopy only, the misdiagnosis of multidrug-resistant TB is very frequent in patients persistently smear-positive despite anti-TB treatment, with the possibility of an infection due to non-tuberculous mycobacteria (NTM) being neglected. The isolation of a mycobacterium from the sputum of a Somali patient apparently confirmed the previous diagnosis of cavitary pulmonary disease. Preliminary investigations led, at first, to the strain being identified as multidrug-resistant *Mycobacterium tuberculosis*, with findings fully in agreement with the patient’s history, which was characterized by repeated interruptions of anti-TB treatment. Thorough phenotypic and genotypic analyses led subsequently to the recognition that the strain was a previously unreported non-tuberculous mycobacterium. The patient, who was unresponsive to the anti-TB treatment, dramatically improved once a drug combination active against NTM was used. A major objective of this article is to alert the medical community to the risk, present also in settings in which sophisticated diagnostic techniques are used, that a cavitary infection due to NTM, and consequently not responding to the anti-TB standard regimen, will be mistaken for multidrug-resistant TB.

**Keywords:** Case report, line probe assay, multidrug-resistant tuberculosis, *Mycobacterium simulans*, non-tuberculous mycobacteria

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**Introduction**

A belief that is still held by infectious diseases physicians and microbiologists is that the incidence of non-tuberculous mycobacteria (NTM) is increasing in countries with low tuberculosis (TB) endemicity, whereas it is close to zero in developing countries characterized by a high TB burden. It is much more likely that the increased isolation of NTM in the developed world is a consequence of the advanced diagnostic technologies that can be afforded, and the apparent absence of NTM in developing countries is imputable to the very limited use of culture for diagnosis in the large majority of such settings [1]. The legitimacy of the latter hypothesis is confirmed by a number of studies reporting the isolation of NTM in Africa once sensitive cultural methods had been adopted [2–5].

We report here the case of a African patient who was repeatedly, and unsuccessfully, treated for pulmonary TB, and who rapidly improved once the non-tuberculous aetiology was acknowledged and suitable therapy was introduced.

**Case report**

A 62-year-old Somali man was admitted to the Infectious Diseases Unit because of a long-standing history of low-grade fever, weakness, weight loss, dyspnoea and productive cough. He had joined his son and his daughter in Italy 2 months previously. His medical history included malaria, approximately 40 years before, and pulmonary TB. The diagnosis of TB had been made in Somalia, when the patient was 58 years of age; he carried no medical records, but reported that he had...
been given chemotherapy. Although he was unable to provide details about the drugs he had been taking, he reported at least five interruptions to the therapy because of the war in progress in his country.

Computed tomography showed extensive bilateral lesions, consistent with the diagnosis of pulmonary TB. This diagnosis, apparently supported by sputum smears strongly positive for acid-fast bacilli and by the first culture data, led to standard treatment with isoniazid, rifampicin, pyrazinamide and ethambutol. After 4 weeks of such treatment, which was well tolerated, the results of the susceptibility testing (Table 1) led to discontinuation of rifampicin, isoniazid and pyrazinamide (the susceptibility test result for the last of these was not yet available) and the addition of amikacin, ethionamide, moxifloxacin and linezolid to ethambutol [6].

The general condition of the patient rapidly improved and, again, the treatment was well tolerated. Three weeks later, the sputum smears became negative and the patient was discharged. Following the final recognition of the strain as a non-tuberculous mycobacterium, clarithromycin (shown, in the meantime, to be effective in vitro) was substituted for linezolid because of the high risk of toxicity associated with prolonged treatment with the latter drug. Two months later, amikacin was discontinued. The patient is now receiving clinical follow-up, and his biochemical and microbiological parameters are being checked regularly; continuation of the treatment is planned for at least 12 months.

### Radiology

The chest computed tomography scan showed marked retraction of the right lung, with deviation of the mediastinum and consolidation of the right pulmonary lobes. These showed lesions characteristic of tuberculous infection, with multiple cavitations in a breadcrumb-like pattern, and a large cavity, communicating with the bronchial tree, clearly visible in the upper lobe (Fig. 1). In the upper left lobe, parenchymal infiltrates with aerial bronchogram were present, and opacities in a ‘tree in bud’ pattern [7] affected the lingula (Fig. 2).

#### TABLE 1. Susceptibility testing pattern of FI-09026 obtained using the proportion method in liquid MGIT medium and the broth microdilution for the determination of the MICs

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Proportion method</th>
<th>MIC (mg/L) (interpretation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>S</td>
<td>8 (S)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>2 (I)</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>S</td>
<td>0.3 (S)</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>S</td>
<td>4 (I)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>S</td>
<td>0.5 (S)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>S</td>
<td>0.5 (S)</td>
</tr>
<tr>
<td>Minocycline</td>
<td></td>
<td>4 (I)</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>S</td>
<td>0.3 (S)</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>R</td>
<td>0.25 (S)</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>R</td>
<td>4 (I)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>R</td>
<td>&gt;64 (R)</td>
</tr>
<tr>
<td>Trimethoprim–sulphathiazole</td>
<td></td>
<td>4–76 (R)</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Ethionamide</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Isoniazid</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Kanamycin</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>S</td>
<td></td>
</tr>
</tbody>
</table>

#### FIG. 1. Computed tomography scan showing, in the right lung, a large cavity communicating with the bronchial tree and tuberculous inflammation of the lobes with multiple cavitation.

#### FIG. 2. Computed tomography scan showing, in the lingula (right lung), opacities in a ‘tree in bud’ pattern.
Microbiological investigations were initially characterized by contradictory results. Acid-fast microscopy (auramine–rhodamine stain) revealed a heavy load of elongated and curved bacilli, in morphology fully compatible with *Mycobacterium tuberculosis*. Real-time PCR (COBAS TaqMan; Roche Diagnostics, Basel, Switzerland) for *M. tuberculosis* complex (MTBC), performed directly on the sputum, gave a negative result. Various cultures in liquid medium (MGIT; Becton Dickinson, Towson, MD, USA) [8] grew acid-fast bacilli in 5–9 days, and showed the cored arrangement typical of *M. tuberculosis*. On Lowenstein–Jensen medium, non-pigmented rough colonies, indistinguishable from *M. tuberculosis*, grew in approximately 20 days.

The identification of the strain (FI-09026) as belonging to the MTBC was apparently confirmed by the reverse hybridization line probe assay GenoType Mycobacterium CM (Hain Lifescience, Nehren, Germany) [9] targeting the 23S rDNA. The use of a further reverse hybridization test, GenoType MTBC (Hain Lifescience) [10], suitable for differentiating the species included in the MTBC, however, produced further confusion. Again, a positive result was obtained with the MTBC-specific line probe, but none of the nine *gyrB* stretches harbouring polymorphisms characterizing single MTBC species could be amplified.

Genetic investigation of mutations responsible for rifampicin and isoniazid resistance, performed using GenoType MTBDRplus (Hain Lifescience) [11], to verify the suspicion of multidrug-resistant TB, was not able to detect any, either mutant or wild type, of the specific targets (*rpoB*, *inhA* and *katG*). The test confirmed, however, the presence of a sequence of 23S RNA specific for the MTBC.

Antimicrobial susceptibility testing was at first performed according to the proportion method (validated for *M. tuberculosis* strains only) in MGIT medium, using a wide panel of first-line and second-line drugs [12]. It revealed a pattern (Table 1) suggestive of multidrug-resistant TB.

When the MICs were determined using the microdilution method recommended by the CLSI for NTM [13], most of the second-line drugs, among them linezolid and amikacin, were shown to be effective (Table 1).

HPLC of cell wall mycolic acids, undertaken to try to resolve these identification uncertainties, revealed a pattern characterized by a single, late cluster of peaks, which differed from those obtained with *M. tuberculosis* [14] in the relative heights of several peaks (Fig. 3).

Despite the multiple indications that the strain belonged to the MTBC, several findings did not seem to support such a hypothesis. A genetic sequencing approach was therefore attempted to try to resolve the problem.

![FIG. 3. Representative HPLC pattern of FI-09026 in comparison with that of Mycobacterium tuberculosis (IS, internal standard).](image-url)
similarities with *M. tuberculosis* were clearly lower: 98% in the 16S rDNA, 86% in *hsp65*, 88% in the ITS, and 91% in *rpoB*.

Surprisingly, in the first part of the 23S rDNA (accession number FJ786255), which is known to include the target on which the GenoType identification probes rely, the similarity of FI-09066 to *M. tuberculosis* was also low (<97%).

The phylogenetic analysis conducted using the neighbour-joining algorithm (MEGA 4 software) [21] on the 1730-bp sequence, including the 16S rDNA and ITS, demonstrated the relatedness of FI-09026 and *M. riyadhense* (with the two strains belonging, however, to clearly distinct branches) and the sharp divergence from *M. tuberculosis* (Fig. 4).

**Discussion**

This is the second report of a mycobacterial strain, phenotypically mimicking *M. tuberculosis* but nevertheless clearly distinct from it, being responsible for severe disease that is indistinguishable from TB. The first case concerned a 19-year-old male with painful swelling of the left side of his face due to a tumour in the maxillary sinus extending into the nasal septum and the left orbit. Because of the isolation of a mycobacterium from lavage of maxillary sinuses, the patient was treated with a 9-month anti-TB regimen, which produced a clear improvement due to *M. tuberculosis*. The antimicrobial susceptibility is clearly different from that of *M. tuberculosis* but, paradoxically, the resistance of FI-09026 to the first-line anti-TB drugs, instead of suggesting a possible non-tuberculous mycobacterium, brought to mind a multidrug-resistant *M. tuberculosis* strain. The hybridization pattern obtained using the popular molecular identification system GenoType CM does not distinguish FI-09026 from the MTBC.

![Phylogenetic tree of selected Mycobacterium species](image)

**FIG. 4.** Phylogenetic tree of selected *Mycobacterium* species based on sequences (1730 bp) of the ribosomal operon. The scale bar represents a 0.2% difference in nucleotide sequences. Bootstrap values are indicated at the nodes. FJ786255 is the accession number of the current strain (FI-09024).

As a consequence of the above-mentioned features, the probability of misdiagnosis of multidrug-resistant TB is very high. This is true for low-income countries, in which the diagnosis of TB relies on smear microscopy only [23], but is equally true for a high proportion of laboratories in the developed world, including practically all those that base the identification of mycobacteria on methods other than genetic sequencing.

At present, we can only speculate about the real distribution of strains with the features of FI-09026. They may, indeed, not be very rare, and the major objective of this article is to alert the medical community to the possibility that some isolates considered to be multidrug-resistant *M. tuberculosis* may not actually be so.

Although we are unwilling to describe a new species until additional strains are detected, we propose, for the strain characterized here, the name ‘*Mycobacterium simulans*’ (*simulans* meaning ‘imitative’). The strain has been deposed in the German Collection of Microorganisms and Cell Cultures with the accession number DSM45395.

**Transparency Declaration**

No funding was used for this study, which was generated as part of routine activities. No commercial relationship or conflict of interest is expected for the present study.
References