Case Reports

**Mycobacterium riyadhense infections**

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**ABSTRACT**

Mycobacterium riyadhense is a newly described slow-growing, non-tuberculous mycobacterium species. We describe 2 new cases of *Mycobacterium riyadhense* infections presenting with extra-pulmonary involvement, and reviewed all previously reported cases in the literature. We also describe the spectrum of the disease and explore treatment options based on the experience with the current and previously reported cases.


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Received 10th January 2015. Accepted 9th February 2015.

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Non-tuberculous *Mycobacteria* (NTM) are ubiquitous in the environment with a worldwide distribution. They are capable of causing infections in immunocompromised and immunocompetent individuals, and can also colonize and infect patients in healthcare settings.1 Although pulmonary involvement is the most common form of NTM infections, a range of extra-pulmonary presentations has been reported.2 Despite the recent advances in diagnostic tools, infections with NTM continue to represent diagnostic and therapeutic challenges. *Mycobacterium riyadhense* (*M. riyadhense*) is a newly described NTM species that was first isolated from a patient in Riyadh, Kingdom of Saudi Arabia (KSA) who presented with maxillary sinus infection in the year 2009.3 Ironically, a commercial line-probe assay misidentified this isolate as a member of the *Mycobacterium tuberculosis* (*M. tuberculosis*) complex. Further testing of the isolate as part of a second-line quality assurance program has lead to the recognition of its unique biochemical and molecular characteristics that are different from those of all previously recognized mycobacteria species. Consequently, a novel species was discovered, and was given the name *M. riyadhense*, after the city of Riyadh, KSA where it was first isolated.3 The report of this novel species was followed by 2 other reports describing 3 additional cases from France, Bahrain,4 and Korea,5 all presenting with pulmonary infections. Our current knowledge regarding this newly recognized NTM species, the diversity of its clinical presentations, and available treatment options are limited. In this report, we describe 2 additional cases of *M. riyadhense* infections with unusual extra-pulmonary presentations, and review all previously reported cases in the English literature to shed more light on this newly described NTM species.

**Case Report. Patient 1.** In July 2012, a previously healthy 18-year-old Saudi female patient was referred to the neurosurgery service at King Faisal Specialist Hospital and Research Center in Jeddah, KSA for evaluation of a right frontal cerebral lesion. She had a 3-year history of non-progressive headache, and a 3-week history of generalized tonic-clonic seizures but no other neurological complaints. The patient had no fever, excessive sweats, anorexia, or weight loss. She denied exposure to patients known to have tuberculosis (TB), and did not travel outside the kingdom. Her physical examination was unremarkable with no focal...
neurological deficit. Her baseline complete blood count, renal profile, hepatic profile, and chest x-ray were all within normal limits. Her inflammatory markers showed a C-reactive protein (CRP) of 180 mg/L, and an erythrocyte sedimentation rate (ESR) of 20 mm/hour. Her laboratory investigations also showed positive serology for hepatitis B surface antigen (HBsAg) and hepatitis B e-antigen (HBeAg). A brain magnetic resonance imaging (MRI) study showed extra-axial enhancing lobulated masses with destructive bony changes in the right frontal bone (Figure 1). The lesion measured 5.0 x 4.8 x 2.0 cm. On 9th July 2012, she underwent craniotomy and excision of the lesion. Intra-operatively, she was noted to have granulation tissue over the right frontal dura with invasion of the underlying cerebral surface. Her dural lesion pathology showed chronic necrotizing granulomatous inflammation suggestive of TB. Special stains for fungi and acid fast bacilli (AFB) on the obtained tissue were negative, and there were no pathological findings suggestive of a malignant condition. On 15th July 2012, the patient was started on standard anti-TB therapy of rifampin, isoniazid, pyrazinamide, and ethambutol, and discharged home a few days later. At the outpatient review 2 weeks later, she complained of severe nausea, vomiting, and epigastric pain. Her laboratory tests showed hepatic dysfunction with elevated aspartate aminotransferase (AST) (232 IU/L), and alanine aminotransferase (ALT) (166 IU/L). Other hepatic profile parameters remained within normal. Anti-tuberculous drugs were withheld temporarily, and then restarted sequentially after her liver enzymes improved. Two months later, her dural cultures yielded a growth of non-tuberculous Mycobacterium; so the regimen was modified to include moxifloxacin in addition to isoniazid, ethambutol, and rifampin. The patient was continued on this regimen until the final susceptibility testing was available in April 2013, when the regimen was modified to include only rifampin and ethambutol; both of which were continued for 6 more months. The patient received a total of 15 months of antimicrobial therapy with good clinical response. A follow-up brain MRI 3 months after completion of therapy showed no evidence of residual, or recurrent infection.

Microbiological diagnosis. The microbiology laboratory received tissue samples that were collected from the dural lesion of the right frontal cerebral lobe. Auramine-rhodamine fluorochrome stain of the processed specimen for AFB was negative. The specimen was cultured for mycobacteria on solid (Lowenstein-Jensen [LJ] Bio-Rad, Marnes-la Coquette, France) and liquid (Bectec MGIT 960 [mycobacteria growth indicator tube], Becton Dickinson, Franklin Lakes, NJ, USA) media, and was incubated at 37°C. After 6 weeks of incubation, the MGIT tubes were positive. A Kinyoun stain of the MGIT broth was positive for AFB, but tested negative for Mycobacterium tuberculosis complex (MTBC) DNA using the BDPProbe Tec ET system for MTBC (ctb) culture identification (Becton Dickinson, Franklin Lakes, NJ, USA). The LJ subculture was identified as Mycobacterium species by the Line Probe assay using INNO-LiPA Mycobacteria (LiPA) (Innogenetics, Ghent, Belgium). A battery of biochemical tests were performed and failed to identify the exact mycobacterium species. The isolate was subcultured onto LJ slopes and sent to Mayo Medical Laboratories (Rochester, MN, USA) for further identification. Using nucleic acid sequencing of a 500-base region of the 16s ribosomal RNA gene, the isolate was identified as M. riyadhense based on MicroSeq, GenBank, and Mayo Clinic mycobacteria databases. Susceptibility testing was performed at the National Jewish Health Advanced Diagnostic Laboratories (Denver, CO, USA) using the microtiter broth dilution. The isolate showed the following susceptibility results: isoniazid - 0.2 mcg/ml resistant, isoniazid - 1.0 mcg/ml sensitive, rifampin - 1 mcg/ml sensitive, streptomycin - 2 mcg/ml sensitive, capreomycin - 10 mcg/ml sensitive, amikacin - 6 mcg/ml sensitive, cyclonosine - 60 mcg/ml sensitive, ciprofloxacin <1.0 mcg/ml sensitive, clarithromycin <4.0 mcg/ml sensitive, ethambutol - 7.5 mcg/ml sensitive, ethionamide - 10 mcg/ml sensitive, doxycycline - 8 mcg/ml intermediate, kanamycin - 6 mcg/ml resistant, para-aminosalicylate (PAS) - 8 mcg/ml resistant, and imipenem >16 mcg/ml resistant. Patient 2. A previously healthy 24-year-old Saudi female patient was admitted to the Prince Sultan Military Medical City, Riyadh, KSA on 25th December 2012 with progressive low back pain of 2-months duration. Two days before admission, the pain became intolerable and was associated with progressive weakness, numbness, and decreased sensations in both lower extremities, and urinary retention. The patient reported having on and off night sweats for

Disclosure. Authors have no conflict of interests, and the work was not supported or funded by any drug company.
approximately 6 months with weight loss of 10 kg. She denied having fever, chills, rigors, weakness, fatigue, anorexia, headache, seizures, or fecal incontinence. She denied exposures to patients known to have TB, and had not traveled outside of KSA. Physical examination was remarkable for decreased sensation, decreased power (3/5), and brisk reflexes in both lower extremities. The rest of the physical examination was unremarkable. Her baseline complete blood count, renal profile, hepatic profile, and chest x-ray were normal, except for low hemoglobin (8.6 g/dl). Her inflammatory markers showed CRP of 37 mg/L, and ESR of 46 mm/hour. An MRI study of her spine showed multiple sclerotic lesions involving the vertebrae of the cervical, thoracic, and lumbar spines (Figure 2) with enhancement in the post-contrast films. Furthermore, there were extradural mass lesions involving the anterior and posterior intra-spinal regions from T7-T10, which caused compression of the neural foramina. A bone scan showed abnormal uptake in T9, T12, L1, and L4; a pattern that was reported to be suspicious of a metastatic tumor. A computed axial tomography scan of the chest, abdomen, and pelvis showed enlarged pre-tracheal, sub-carinal, and mediastinal lymph nodes, subtle tree-in-bud appearance in the right upper lobe, and sclerotic bony lesions involving the sternum and the left iliac bone. It also re-demonstrated the spinal sclerotic lesions with the paravertebral soft tissue masses as initially seen in the spine MRI. Work-up for possible malignant etiology was negative. On 28th January 2013, a biopsy was obtained from the T12 bone and para-vertebral soft tissue mass. Histopathologic examination showed necrotizing granulomatous inflammation consistent with TB. Special stains for fungi and AFB on the obtained tissues were negative, and the tissue showed no pathological changes suggestive of a malignant condition. On 4th February 2013, the patient was started on a standard anti-TB therapy for presumed TB. The regimen included rifampin, isoniazid, pyrazinamide, and ethambutol, and the patient was later discharged home. She had regular follow up in the out-patient department and tolerated treatment well. Her back pain improved over time, she was gaining weight appropriately, and the neurological deficits gradually resolved. One month later, the T12 biopsy culture result was reported as NTM species. The isolate was sent to Bioscientia Reference Laboratory (Ingelheim, Germany) for further identification. The patient was continued on her initial 4-drug regimen since she was showing a remarkable clinical response and was tolerating treatment well. The isolate was reported to be \textit{M. riyadhense} in June 2013, but the final results of drug susceptibility testing were only available in August 2013. At that time, the patient was also found to be pregnant, and the regimen was changed to include rifampin and ethambutol only. The patient received 13 months of therapy and is back to normal with no residual symptoms or neurological deficits.

\textbf{Microbiological diagnosis.} The microbiology laboratory received a tissue biopsy from the T12 lesion on 28th January 2013. The sample was mixed with sterile saline, vortexed, and a slide stained with auramine-rhodamine fluorochrome stain was negative for AFB. The specimen was inoculated for mycobacteria culture onto 2 media; solid (LJ media, Saudi Prepared Media Laboratory, Riyadh, KSA) and liquid (Bectec MGIT 960, Becton Dickinson, Franklin Lakes, NJ, USA) media, and was incubated at 37°C. After 4 weeks, the MGIT flagged positive, and a slide stained with Ziehl-Neelsen stain showed AFB. The broth tested negative for \textit{M. tuberculosis} complex by real-time PCR.
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(Gene Xpert MTB/RIF, Cepheid, Sunnyvale, CA). The isolate was subcultured on a new LJ media and was sent to Bioscientia Reference Laboratory (Ingelheim, Germany) for further identification and susceptibility. The organism was identified as M. riyadhense at the National Reference Center for Mycobacteria (Borstel, Germany) by nucleic acid sequencing of a 500-base-region of the 16S ribosomal RNA gene as described by Richter et al., and comparing the resulting sequences to those of the reference strains of the most closely related mycobacterial species in the international nucleotide sequence databases (www.ncbi.nlm.nih.gov/blast). The isolate was tested with Bactec MGIT system (drug/concentration) for anti-mycobacterial drug susceptibility, and was found to be sensitive to rifampin (1.0 mcg/ml), streptomycin (1.0 mcg/ml), and ethambutol (5.0 mcg/ml), and resistant to isoniazid (0.1 mcg/ml and 0.4 mcg/ml) and pyrazinamide (100 mcg/ml). It was intermittently sensitive to Clarithromycin (minimum inhibitory concentration equal 16 mcg/ml) by E-test (bioMérieux, Marcy l’Etoile, France).

Discussion. A review of the available literature identified 4 more cases of M. riyadhense (Table 1). A case reported by Tortoli et al., which was included as a case of M. riyadhense by Choi et al. is actually a case of a novel species of NTM for which Tortoli et al. proposed the name Mycobacterium simulans; so it was not included in this review. In addition to the current 2 cases, one previous M. riyadhense infection was reported from each country of KSA, France, Bahrain, and South Korea. Infections occurred in younger age groups (median 31, range 18-43 years), and in females more than males (ratio 3:1). All patients with M. riyadhense infections that were reported from Saudi Arabia had extra-pulmonary infections, while those from other countries presented with pulmonary involvement. All patients had clinical presentations indistinguishable from that of TB, and, except for case 3, were initially treated with anti-TB regimens for presumed, or suspected TB. One patient (case 3) who received an initial regimen that targets NTM (ciprofloxacin plus clarithromycin) relapsed after 12 months of treatment. Nonetheless, he responded to a regimen that included the standard first-line anti-TB agents, in addition to ciprofloxacin and clarithromycin. All 5 patients who were empirically treated with the standard first-line anti-TB therapy had good initial responses, and were all eventually cured. Therapy was given for a median duration of 13.5 months (range 9-20 months).

All isolates of M. riyadhense were tested for antimicrobial susceptibility, but using different methods. Table 2 summarizes reported susceptibility testing results for the 6 cases of M. riyadhense. Among the first line anti-TB drugs, rifampin and ethambutol are highly active against tested strains of M. riyadhense. On the other hand, resistance to isoniazid seems to be common, and pyrazinamide was only tested in case 6. Among other anti-TB drugs, rifabutin, ethionamide, cycloserine, and moxifloxacin all appear most active, followed by aminoglycosides, clarithromycin, and ciprofloxacin. Para-aminosalicylate does not seem to be effective in-vitro against M. riyadhense. Other drugs that were reported were only occasionally tested, and therefore, we cannot evaluate their activity.

Table 1 - Clinical characteristics of all Mycobacterium riyadhense cases reported in the literature.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Reference</th>
<th>Country</th>
<th>Gender, age</th>
<th>Site of infection</th>
<th>Initial regimen</th>
<th>Modified regimen</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>van Ingen et al</td>
<td>Saudi Arabia</td>
<td>Male, 19</td>
<td>Maxillary sinus with bone involvement</td>
<td>INH, RIF, EMB (2 months)</td>
<td>INH, RIF (7 months)</td>
<td>Cure</td>
</tr>
<tr>
<td>2</td>
<td>Godreuil et al</td>
<td>France</td>
<td>Female, 39</td>
<td>Pulmonary infection</td>
<td>INH, RIF, EMB, PZA (2 months)</td>
<td>INH, RIF (10 months)</td>
<td>Cure</td>
</tr>
<tr>
<td>3</td>
<td>Godreuil et al</td>
<td>Bahrain</td>
<td>Male, 43</td>
<td>Pulmonary infection</td>
<td>CLR, CIP (12 months)</td>
<td>INH, RIF, PZA, EMB, CLR, CIP* (8 months)</td>
<td>Relapse then cure*</td>
</tr>
<tr>
<td>4</td>
<td>Choi et al</td>
<td>South Korea</td>
<td>Female, 38</td>
<td>Pulmonary infection</td>
<td>INH, RIF, EMB, PZA (8 months)</td>
<td>RIF, EMB, PZA (6 months)</td>
<td>Cure</td>
</tr>
<tr>
<td>5</td>
<td>Current</td>
<td>Saudi Arabia</td>
<td>Female, 18</td>
<td>Brain with bone involvement</td>
<td>INH, RIF, EMB, PZA-MOX (9 months)</td>
<td>RIF, EMB (6 months)</td>
<td>Cure</td>
</tr>
<tr>
<td>6</td>
<td>Current</td>
<td>Saudi Arabia</td>
<td>Female, 24</td>
<td>Spine infection</td>
<td>INH, RIF, EMB, PZA (6 months)</td>
<td>RIF, EMB (7 months)</td>
<td>Cure</td>
</tr>
</tbody>
</table>

CIP - ciprofloxacin, CLR - clarithromycin, EMB - ethambutol, INH - isoniazid, MOX - moxifloxacin, PZA - pyrazinamide, RIF - rifampin. *PZA and EMB were stopped in the last 2 months of treatment, †patient relapsed after the initial 12 months of therapy with CLR and CIP, but he was cured after the second regimen for 8 months, ‡PZA was given in the first 2 months but then was replaced by MOX.
Mycobacterium riyadhense seems to be capable of causing a spectrum of clinical presentations that are clinically indistinguishable from TB. This is obvious from the 2 new cases reported herewith; both of which presented with unusual extra-pulmonary involvements. These cases represent the first reports of brain and spine infections due to M. riyadhense. Although the second case had subtle pulmonary changes that may suggest lung involvement, the main presentation was of an extra-pulmonary disease. Both patients had serious infections that resembled TB, tissue biopsies from both patients showed necrotizing granulomatous inflammation suggestive of TB, and both were initially treated with standard 4-drug regimens for presumed TB. Only culture could differentiate M. riyadhense from M. tuberculosis. The same diagnostic challenges were faced with other cases reported in the literature (namely; cases 1, 2, and 4). Furthermore, the latter cases were initially misidentified by commercially available DNA probes as members of the MTBC. This finding is important since the diagnosis of TB is not always confirmed with cultures, and treatment is frequently started on clinical grounds alone or based on histopathologic changes suggestive of TB without obtaining proper samples for TB cultures. If we combine this to reports indicating misidentification of NTM as M. tuberculosis, then it becomes clear that at least some of the patients that are being treated and reported as TB, may actually be NTM infections.

The incidence and epidemiology of M. riyadhense infections remain unknown. It seems the infection is not limited to certain geographical locations since cases have already been reported from France, Bahrain, and South Korea in addition to Saudi Arabia. We expect more cases to be diagnosed and reported due to the increased awareness of this new species. A recent study has addressed the occurrence of NTM at a national level in Saudi Arabia, and prospectively collected cultures from patients all over the country over a period of one year (from July 2009 to June 2010). The authors identified 95 cases of NTM infections, yet no cases of M. riyadhense were identified in the study. All reported cases of M. riyadhense occurred in a younger age groups with a median age of 31 years. In addition, more female patients were infected with M. riyadhense than males. Yet the limited number of reported cases does not allow us to establish general statements regarding age and gender predisposition. Furthermore, there is no clear explanation for this observation. All of the patients with M. riyadhense infections who received empirical

### Table 2 - Reported antimicrobial susceptibility testing for 6 Mycobacterium riyadhense isolates.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
<th>Case 6</th>
<th>Percent susceptible*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>33.3</td>
</tr>
<tr>
<td>Rifampin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>100</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>100</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>R</td>
<td>Not provided</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>NR</td>
<td>NR</td>
<td>100</td>
</tr>
<tr>
<td>Amikacin</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>NR</td>
<td>80</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>S</td>
<td>S</td>
<td>NR</td>
<td>Not provided</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>S</td>
<td>R</td>
<td>NR</td>
<td>Not provided</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>83.3</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>NR</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>NR</td>
<td>100</td>
</tr>
<tr>
<td>Prothionamide</td>
<td>S</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Not provided</td>
</tr>
<tr>
<td>Cycloserine</td>
<td>S</td>
<td>NR</td>
<td>NR</td>
<td>S</td>
<td>S</td>
<td>NR</td>
<td>100</td>
</tr>
<tr>
<td>Clofazimine</td>
<td>S</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Not provided</td>
</tr>
<tr>
<td>PAS</td>
<td>R</td>
<td>NR</td>
<td>NR</td>
<td>R</td>
<td>R</td>
<td>NR</td>
<td>0.0</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>I</td>
<td>83.3</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>NR</td>
<td>80</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>NR</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>ND</td>
<td>NR</td>
<td>100</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>NR</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>NR</td>
<td>25</td>
</tr>
<tr>
<td>Imipenem</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>R</td>
<td>NR</td>
<td>Not provided</td>
</tr>
<tr>
<td>Linezolid</td>
<td>NR</td>
<td>S</td>
<td>S</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Not provided</td>
</tr>
</tbody>
</table>

S - susceptible, R - resistant, I - intermediate susceptible, NR - not reported, PAS - para-aminosalicylate.

*Only provided for antimicrobials that had been tested in 3 or more cases

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treatment with standard first-line anti-TB regimens have responded well to treatment. On the other hand, the only patient who was initially treated with NTM regimen (clarithromycin and ciprofloxacin) relapsed after 12 months of treatment, and was only cured after adding first-line anti-TB drugs to his failing regimen. This happened despite the fact that his isolate tested susceptible to both drugs. This raises concerns regarding the interpretation of the results of susceptibility testing for *M. riyadhense*, which is usually based on the Clinical and Laboratory Standards Institute (CLSI) breakpoints for other slowly growing NTM.

In conclusion, *M. riyadhense* is a newly recognized, slowly growing NTM that is capable of causing a range of infections in humans clinically indistinguishable from TB. Although resistance to isoniazid is common, the infection responds well to the standard first-line anti-TB drug regimens.

**References**


**Case Reports**

Case reports will only be considered for unusual topics that add something new to the literature. All Case Reports should include at least one figure. Written informed consent for publication must accompany any photograph in which the subject can be identified. Figures should be submitted with a 300 dpi resolution when submitting electronically or printed on high-contrast glossy paper when submitting print copies. The abstract should be unstructured, and the introductory section should always include the objective and reason why the author is presenting this particular case. References should be up to date, preferably not exceeding 15.