Case Report

Pulmonary *Mycobacterium kyorinense* Disease: A Case Report and Review of Literature

Rajagopalan Saranathan, Chandrasekaran Padmapriyadarsini[1], Gomathi Narayanan Sivaramakrishnan[2], Bhavani K. Perumal[1], Silambuchelvi Kannayan[2], Bency Joseph [1], Narendran Gopalan[1], Luke Elizabeth Hanna

Department of HIV, ICMR-National Institute for Research in Tuberculosis, Departments of [1]Clinical Research and [2]Bacteriology, ICMR-National Institute for Research in Tuberculosis, Chennai, Tamil Nadu, India

Abstract

We report here the first case of pulmonary infection due to *Mycobacterium kyorinense* in a 55-year-old hypertensive woman treated for pulmonary tuberculosis earlier on two occasions. She presented with productive cough, intermittent episode of left-sided chest pain, loss of appetite, low-grade fever, and breathlessness. Sputum cultures revealed non-tuberculous mycobacteria (NTM). She remained persistently symptomatic with sputum cultures positive for acid-fast bacilli even after 6 months of treatment. Hence, a 16SrRNA gene amplification and sequencing were done that revealed *M. kyorinense*. Based on the guidelines of the American Thoracic Society, she was started on weight-based dosing of clarithromycin, levofloxacin, ethambutol, isoniazid and injection amikacin daily. The patient improved symptomatically and became culture-negative after 3 months of therapy with the above regimen and continued to be culture negative for 12 months of treatment. She continues to remain symptom-free without evidence of any clinical or bacteriological relapse.

Keywords: 16SrRNA, non-tuberculous mycobacteria, rifampicin resistance

Introduction

Mycobacterium kyorinense, a rare pathogenic *Mycobacterium*, was first isolated from a Japanese patient suffering from pneumonia in 2007.[1] Till date, 16 cases of *M. kyorinense* infection have been reported from regions including Japan, Brazil, Australia and Saudi Arabia. Majority of them were pulmonary infections while three were extra-pulmonary infections, such as lymphadenitis and arthritis.[2-7] Laboratory identification of this bacterial species seems to be critical for the successful management of this disease as it does not respond to the conventional non-tuberculous mycobacteria (NTM)/ anti-tuberculosis (TB) regimen.[3] *M. kyorinense* infection has never been reported from India.[3-7] This article reports the first case of *M. kyorinense* pulmonary infection in an immunocompetent woman from India and its successful treatment with clarithromycin and levofloxacin.

Case Report

A 55-year-old female was referred to the National Institute for Research in Tuberculosis (NIRT), Chennai, India, for recurrent episodes of productive cough of 2 months duration, loss of appetite for a month and low-grade fever with breathlessness for 10 days. She also experienced intermittent episodes of left-sided chest pain for 4 years. She had been treated for pulmonary TB twice before – once before 10 years and again before 1 year. During both episodes of treatment, she had been regular with her anti-TB treatment and was declared cured of the disease at the end of treatment. She was on medications for systemic hypertension and ischemic heart disease with dilated cardiomyopathy for the past 3 years. She worked as a housemaid and had the habit of using nasal snuff.

Physical examination revealed a tall, pale, and thin built female with a height of 156 cm and weight of 40.9 kg. On chest auscultation, coarse crackles were heard in both lung

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Address for correspondence: Dr. Chandrasekaran Padmapriyadarsini, Department of Clinic Research, ICMR-National Institute for Research in Tuberculosis, 1, Mayor Sathyamoorthy Road, Chetpet, Chennai - 600 031, Tamil Nadu, India.
E-mail: pcorchids@gmail.com

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fields. There were no abnormal laboratory findings except for low hemoglobin (11.5 g/dL). Her chest radiograph showed infiltration in both lungs, involving five zones of the lung [Figure 1a]. Computed tomography imaging showed patchy areas of consolidation with calcification in both lungs [Figure 1b] and a cavity in the left upper lobe. Sputum smear and culture for acid-fast bacilli (AFB) were positive in three consecutive expectorated sputum samples. Following growth of *Mycobacterium* after 6 weeks in solid culture, it was sent for species identification by the line probe assay (LPA). LPA was negative for *Mycobacterium tuberculosis* complex and LPA for NTM did not match with any pattern from both GenoType *Mycobacterium* CM and GenoType *Mycobacterium* AS (Hain life sciences, Nehren, Germany). Instead, varying bands in the region for *M. kansasii* were seen raising the doubt of a variant of *M. kansasii* or mixed infection or a rare mycobacterial infection.

As the patient's respiratory symptoms were worsening, it was decided to give her the benefit of doubt and as *M. kansasii* is the most common pulmonary NTM in this part of the country; she was initiated on treatment for *M. kansasii* with a daily regimen of isoniazid (300 mg), rifampicin (450 mg) and ethambutol (800 mg). However, as even after 6 months of treatment, there was no clinical improvement and she remained symptomatic with sputum smears and cultures positive for AFB, two cultures were sent for 16SrRNA gene amplification and sequencing. The genomic DNA extracted from the cultures was used as a template for 16SrRNA gene amplification with 16S forward (5' GAGAGTTTGATCCTGGCTCAG 3') and 16S reverse (5' ACGGCTACCTTGTTACGACTT 3') primers. The amplicons were purified, sequenced in AB 3100 capillary sequencer (Applied Biosystems, USA) and aligned by NCBI-Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). We obtained nearly full-length sequence (1433 bp) which showed 99% identity with *M. kyorinense*. The sequence was submitted to Genbank (Accession number: MH005065.1). Phylogenetic analysis with the closely related *Mycobacterium* sp. 16SrRNA gene sequences was performed using MEGA v 5.05 tool. *M. kyorinense* strain NTM_1 showed proximity towards *M. kyorinense* reference strain (Accession n. JQ717033) [Figure 1c]. Drug susceptibility test by MGIT960 using single concentrations of drugs showed susceptibility to levofloxacin (1.0 µg/ml), moxifloxacin (2.0 µg/ml), ethambutol (5.0 µg/ml) and resistant to isoniazid (0.1 µg/ml), rifampicin (1.0 µg/ml), amikacin (1.0 µg/ml) and para-aminosalicylic acid (2.4 µg/ml). Based on the recommended regimen in literature and the drug susceptibility profile, she was started on clarithromycin (500 mg twice daily), levofloxacin (500 mg once daily), ethambutol (800 mg once daily) and amikacin (500 mg once daily).[3-7]

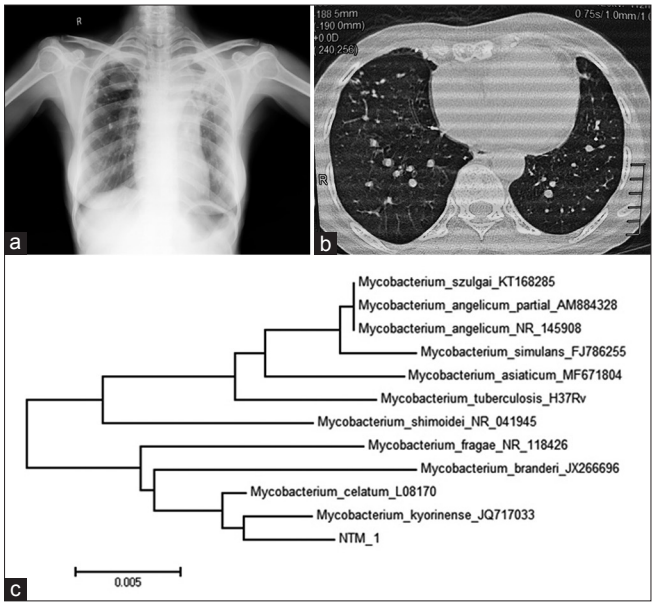

Figure 1: (a) Chest x-ray showing calcification in both lung fields, cavity in left upper lobe with dilated vessels and cardiac enlargement. (b) Computed tomography scan showing plethora of mosaic pattern in both the lungs with medium thick wall and calcification in left upper lobe. (c) Phylogenetic tree constructed using MEGA V 5.05 by neighbourhood joining method with the 16SrRNA gene sequences of representative mycobacterium species closely related to *Mycobacterium kyorinense* and NTM_1 strain obtained in this study

Following the initiation of combination therapy with clarithromycin and levofloxacin along with other drugs, the patient showed good clinical response with subsidence of clinical symptoms. There was an improvement in radiological findings and conversion of sputum smear and cultures, 3 months after treatment initiation. Injection amikacin was stopped after 9 months of treatment, and oral medicines alone were continued for 12 months post-sputum culture negativity; pulmonary *M. kyorinense* disease has not relapsed.

Discussion

Till date, only 16 cases had fulfilled the criteria for a clinically significant infection with *M. kyorinense*.[8] The reported 16 cases were from Japan, Brazil, Australia and Saudi Arabia and were isolated from immunocompetent patients with extensive clinical disease.[3-7] Among the 16 cases, 13 presented with respiratory failure/infection and two had lymphadenitis, while the other had arthritis. Five of the patients with respiratory infection died due to the infection and 9 improved in due course of treatment and recovered subsequently [Table 1]. The outcome of one patient from Australia and another patient from Japan is unknown.[1,4] However, the Australian patient remained culture positive even after 6 months of therapy with appropriate antibiotics which may be attributed to the uncertainties on adherence to the medication and her continued smoking during medication.[4]

Identification/diagnosis

The symptoms and clinical features of *M. kyorinense* infection are very similar to other NTM infections and

Table 1: Case details, antibiogram, treatment and outcome of *Mycobacterium kyorinense* infections reported till date

References	Age (years)/sex	Site of infection	Co-morbidities±	Susceptibility pattern[a]		Treatment (antibiotics prescribed)	Change in antibiotics after inappropriate therapy	Clinical prognosis
				S	R			
Okazaki *et al*., 2009[1]	62/male	Lung	-	STR, KAN, LVX, CLR, AMK	INH, RIF, EMB	-	-	Unknown
	70/male	Lung	-	STR, KAN, LVX, CLR, AMK	INH, RIF, EMB	RFB, EMB, CLR	-	Dead
	64/female	Cervical lymph node	Breast cancer	STR, KAN, LVX, CLR, AMK	INH, RIF, EMB	-	-	Dead
Wada *et al*., 2009[2]	89/male	Lung	COPD	STR, KAN, LVX, CLR, AMK	INH, RIF, EMB	BIP	-	Dead
Ohnishi *et al*., 2013[3]	81/male	Lung	-	STR, KAN	INH, RIF, EMB, LVX	INH, EMB, RIF	-	Dead
	50/male	Lymph node	MDS	CLR, LVX, AMK	INH, RIF, EMB	CLR, RIF, LVX, AMK	-	Recovery
	67/male	Lung	-	STR, KAN, INH, LVX, CLR, AMK	RIF, EMB	RIF, EMB, CLR, AMK, LVX	CLR, STR, MXF	Recovery
	72/male	Lung	-	STR, EMB, KAN, LVX	INH, RIF	CLR, RIF, EMB	-	Recovery
	48/female	Joint	RA, SLE	STR, KAN, LVX, AMK	EMB, INH, RIF	INH, RIF, EMB	LVX, EMB, CLR	Recovery
	66/male	Lung	-	STR, KAN, INH, LVX, CLR, AMK	EMB, RIF	INH, RIF, EMB	RIF, CLR, LVX	Recovery
	60/male	Lung	COPD	STR, KAN, LVX, CLR, AMK	EMB, INH, RIF	RFB, EMB	CLR, LVX	Recovery
Campos *et al*., 2012[9]	26/male	Lung	-	-	-	INH, RIF, EMB, PZA	-	Dead
Kobashi *et al*., 2012[5]	63/male	Lung	Past history of pulmonary adenocarcinoma and COPD	STR, KAN, LVX, CLR, AMK	EMB, INH, RIF, ETA	CLR, LVX	-	Recovery
Muruganandan *et al*., 2015[4]	46/female	Lung	Anxiety disorder	-	-	CLR, EMB, MXF	-	Not recovered
Ikeue *et al*., 2017[6]	48/male	Lung	Past history of FL and GVHD	STR, KAN, LVX, CLR, AMK, ETA	EMB, INH, RIF	INH, RIF, EMB, PZA	CLR, MXF	Recovery
Varghese *et al*., 2017[7]	14/male	Lymph node	HL	-	-	EMB, CLR	-	-
Saranathan *et al*., 2018	55/female	Lung	Ischaemic heart disease with dilated cardiomyopathy	LVX, MXF, EMB	INH, RIF, AMK, PAS	INH, RIF, EMB	CLR, LVX, EMB, AMK	Recovery

[a]COPD: Chronic obstructive pulmonary disease, MDS: Myelodysplastic syndrome, RA: Rheumatoid arthritis, SLE: Systemic lupus erythematosus, GVHD: Graft versus host disease, FL: Follicular lymphoma, HL: Hodgkin Lymphoma, [a]S: Susceptible, R: Resistant, EMB: Ethambutol, KAN: Kanamycin, INH: Isoniazid, RIF: Rifampin, LVX: Levofloxacin, CLR: Clarithromycin, AMK: Amikacin, RFB: Rifabutin, MXF: Moxifloxacin, BIP: Biapenem, AZM: Azithromycin, PZA: Pyrazinamide, ETA: Ethionamide, PAS: Para-aminosalicylic acid, STR: Streptomycin, AMK: Amikacin

therefore, most clinicians usually start the patients on NTM regimen with clarithromycin tablets. Uncertainties in species identification of *M. kyorinense* prevail in most clinical set up as it is not included in the GenoType *Mycobacterium* CM. In most of the cases, it has been presumed to be either *Mycobacterium celatum* or *Mycobacterium branderi* due to the presence of some of the bands, but not all, in the LPA panel.[4] However, polymerase chain reaction (PCR) followed

by direct sequencing of 16S rRNA gene and/or *rpoB* or *hsp65* has been described to be the gold standard method for the identification of *M. kyorinense* and other *Mycobacterium* species.[9,10] All the reported cases of *M. kyorinense* have sequenced either 16SrRNA gene and/or *rpoB* or *hsp65* gene for precise identification at the species level.[3-7] Campos *et al*. have attempted to speciate *M. kyorinense* through PCR restriction enzyme pattern analysis (PRA), which can be implemented in the laboratories for the identification of this rare pathogen. Amplification and restriction digestion of *hsp65* by *BstEII* and *HaeII* resulted in restriction pattern which showed the closest match towards *M. celatum type 2* in PRA site database (http://app.chuv.ch/prasite/index.html). As the restriction pattern was novel, it was deposited in the database as *M. kyorinense* based on maximal similarity in the 16SrRNA gene sequence analysis.[9] PRA can also, therefore, be implemented in diagnostic laboratories as an alternate option for sequencing to identify this bacterium. In addition to the molecular identification methods, biochemical tests such as arylsulphatase activity, tellurite reduction and heat-stable catalase production can also distinguish *M. kyorinense* from *M. celatum* and *M. branderi*.[3]

Epidemiology

M. kyorinense is a rarely reported pathogen with a handful of reports and a very few incidences globally.[3-7] Nevertheless, we can also speculate that the incidence rates may be underestimated due to difficulties in diagnosis with the available resources. *M. kyorinense* was earlier suspected to be region-specific as there was a cluster of infections with 13 cases reported only from Japan.[3] However, subsequently, there were cases reported from Brazil, Australia and Saudi Arabia, now the present case from India confirming that this bacterium is not region-specific but spread across the continents.[4,9] Molecular epidemiological facts about these strains are unknown due to the lack of typing/analysis investigations to delineate their proximity. However, the remarkable fact about the characterised strains is that the 16S rRNA gene sequences from different geographic regions differed slightly (99% similarity).

Microbiological characteristics

M. kyorinense is a non-pigmented, slow-growing organism that generally takes around 6 weeks to grow in the Löwenstein–Jenson medium and 18–20 days in Middlebrook 7H9 broth. The optimal temperature for growth ranges from 28°C to 42°C and DNA-DNA microplate hybridization analysis revealed <50% reassociation with closely related species such as *M. celatum* and *M. branderi*.[1] In the biochemical analysis, *M. kyorinense* isolates were negative for tween hydrolysis, urease activity, 3-day arylsulphatase activity, nitrate reductase, semiquantitative catalase, pyrazinamidase, tellurite reduction and niacin accumulation tests but positive for 14 days arylsulphatase activity and heat-stable catalase tests.[1,3] Mycolic acid analysis using thin-layer chromatography (TLC) and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry revealed the cell wall of *M. kyorinense* to possess alpha, keto and dicarboxymycolates.[1]

Treatment options

Although appropriate clinical guidelines for treating *M. kyorinense* are not available, existing case reports have shown significant success rates when fluoroquinolones and macrolides and/or aminoglycosides were used for treatment.[3-7] Early identification of this pathogen is also imperative for choosing the appropriate regimen for the treatment and better treatment response. From the available preceding case reports, we found that 10 patients, including ours, were treated initially with first-line anti-TB regimen containing rifampicin, isoniazid and ethambutol which turned out to be unsuccessful in all instances.[3-7] Ten patients who were administered macrolides and fluoroquinolones as the first-/second-line chemotherapy showed improvement and the infection subsided in nine patients. On the other hand, the five patients who did not receive appropriate therapy died eventually either due to the infection or associated comorbid conditions.[3-7] Minimal inhibitory concentration (MIC) assays for the *M. kyorinense* isolates in each case gave varied values. In general, almost all isolates had higher MICs for the first-line anti-TB drugs such as rifampicin, ethambutol, isoniazid and lower values for macrolides, aminoglycosides, and quinolones which constitute the treatment of choice for this pathogen. A higher level of rifampicin resistance in *M. kyorinense* is noteworthy as it is the pivotal drug for the management of *M. tuberculosis*. There is a possibility that these infections can be misdiagnosed with multi-drug resistant TB and treatment initiated for the same, delaying correct identification of *M. kyorinense*.

Genetic features

Ohtsuka *et al*. performed whole-genome sequencing of *M. kyorinense* (strain KUM060204) for the first-time using Ion PGM system (Life Technologies, USA).[11] The genome size was found to be 5.3 MB and comprised 5405 open reading frames with a G + C content of 66.9%.[11] Sequence analysis predicted Ser531Asp amino acid substitution in the *rpoB* to be responsible for rifampicin resistance. However, no substitutions could be identified in *katG*, *inhA* and *aphC* that could contribute to isoniazid resistance.[11] Overall, the sequencing results seem to suggest that the drug resistance mechanisms in *M. kyorinense* is different and not the same as that of M.tb.[11,12]

Conclusion

M. kyorinense is emerging as a clinically significant pathogen that can manifest as a varied spectrum of disease in immunocompetent individuals. Isolation and identification of this pathogen seem to be difficult with standard mycobacterial laboratory techniques, and hence, it is important to perform repeated tests and molecular analysis to identify the AFB precisely for better treatment response. In TB-endemic countries, symptoms and clinical conditions of *M. kyorinense* infection

mimic that of drug-resistant TB, especially when it involves the respiratory system. Hence, clinicians must be aware of this pathogen and have a high index of suspicion while treating intractable respiratory infection or pulmonary TB. Therapeutic options are not well defined but should include several months of treatment with a combination regimen. Of note is the fact that *M. kyorinense* is consistently resistant to the first-line anti-TB drugs, especially rifampin. Immune response of the individual should be boosted with simple host-directed therapy like good nutrition. As clinicians and microbiologists gain experience with *M. kyorinense*, further information can be obtained about the best diagnostic modality and treatment options.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Genbank accession number

The accession number for the nearly full-length 16SrRNA gene sequence of *M. kyorinense* NTM_1 strain is MH005065.1.

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Conflicts of interest

There are no conflicts of interest.

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