Case report

Mycobacterium triplex pulmonary disease in an immunocompetent host: A case report and literature review

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A B S T R A C T

Mycobacterium triplex (M. triplex) is a bacterial species that can cause severe pulmonary diseases. Despite its clinical importance, only a few cases of M. triplex infection have been reported. Here, we present a rare case of pulmonary disease due to M. triplex in an immunocompetent patient who showed abnormal findings on chest X-ray and computed tomography scans. In this patient, the bacterium was identified by DNA sequencing analysis of the 16S rRNA and hsp65 genes. The patient was successfully treated with the appropriate antimicrobial agents. To put this case into the context of the current literature, we also reviewed other case reports of M. triplex infection.

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Introduction

Nontuberculous mycobacteria (NTM) are ubiquitous organisms commonly isolated from environmental sources, whose pathogenicity may vary according to the host's immune status [1]. NTM are most commonly associated with pulmonary infections. There has been an increase in the incidence of pulmonary infections caused by NTM in recent years, and this is an emerging public health concern [2].

Mycobacterium triplex (*M. triplex*) represents a unique species of NTM first described in 1996 [3]. This new species, a slow-growing, non-pigmented mycobacterium (Runyon group III), was named after its triple-peak cluster pattern of mycolic acid obtained on high-performance liquid chromatography. The cell morphology is short rods to coccoid, usually smooth and non-photochromogenic. *M. triplex* strain grows well at 37°C and forms mucoid opaque colonies, which are cream to buff in color [3,4]. *M. triplex* phylogenetically resembles *M. florentinum*, *M. lentiflavum*, *M. simiae*, and *M. sherrisii*, and is most closely related to *M. genavense* [5]. *M. triplex* is classified as a SAV organism based on its association with *M. simiae* and *M. avium*. SAV organisms are widely present in the marine environment and are thought to be one of

the main pathogens of fish and other marine organisms. *M. triplex* can cause opportunistic infections in both immunocompromised and immunocompetent humans exposed to environmental sources and may be fatal if the infection is disseminated. Despite its clinical importance, there are few reported cases of *M. triplex* infection because this species of mycobacterium cannot be identified by clinical methods. Here, we present a case of an immunocompetent patient with pulmonary infection due to *M. triplex*, which we identified by DNA sequencing analysis of *16S rRNA* and *hsp65*. In addition, we review other reported cases of *M. triplex* infection.

Case report

A 78-year-old HIV-negative Japanese man was admitted to our hospital for left upper meibomian gland resection of a sebaceous gland carcinoma in December 2012. He was referred to our respiratory center because of abnormal findings on chest X-ray and computed tomography (CT) scans. He had no subjective respiratory complaints. He had no past history of smoking or alcohol abuse, but had a past medical history of pulmonary tuberculosis at the age of 15 years. In 2008, some NTM were cultured from his sputum at another hospital, but the strain was not identified.

On the first visit to our center, physical examination revealed piping sounds in the right lung field. Laboratory tests showed a slightly elevated inflammatory response. A chest X-ray image showed a reticular shadow in the right upper lung field and pleural calcification of the left lung along with volume reduction (Fig. 1). A chest high-resolution computed tomography (HRCT) scan revealed moderately thickened bronchial walls in the upper right bronchus,
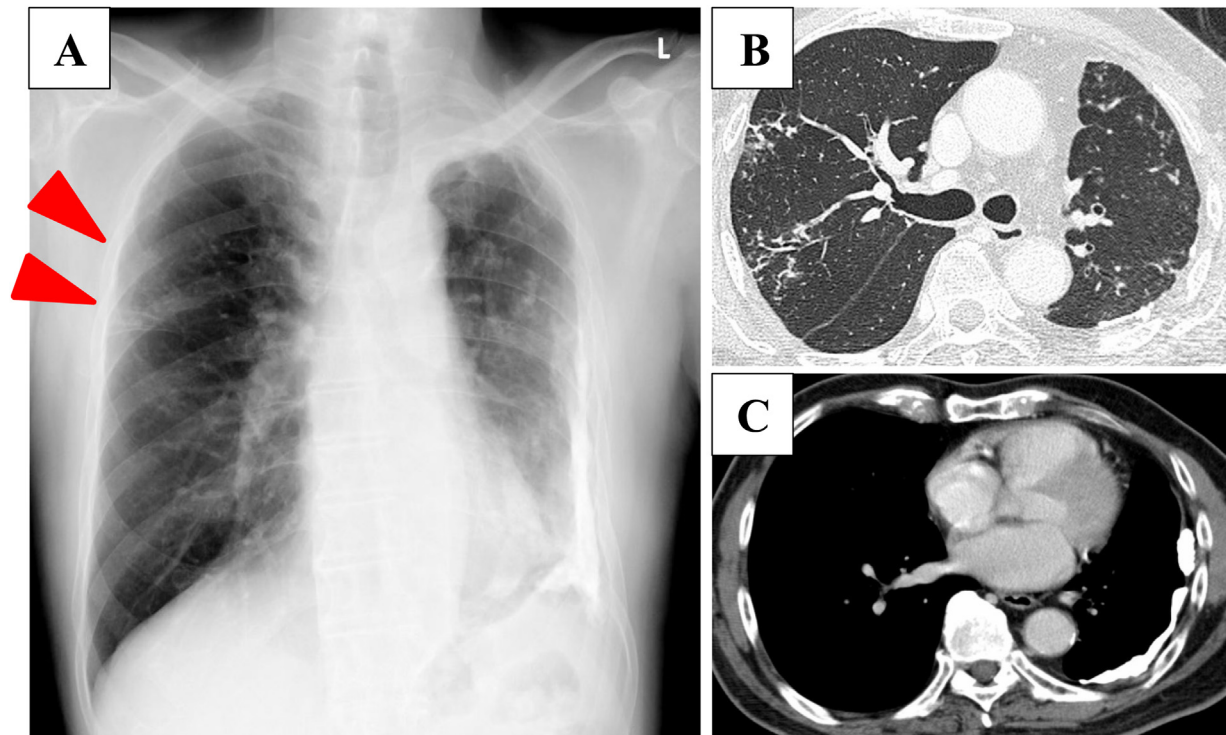
Fig. 1. Chest image of the case patient.
(A) Chest X-ray image shows a reticular shadow in the right upper lung field (arrowhead) and pleural calcification of the left lobe, along with volume reduction. (B, C) Chest high-resolution CT (HRCT) showed centrilobular micronodules in the right upper lobe and pleural calcification of the left lung, along with volume reduction.

centrilobular micronodules in the upper right lobe, infiltration in the right middle lobe, and pleural calcification of the left lung along with volume reduction (Fig. 1). Sputum smears and cultures for acid-fast bacilli (AFB) were negative, but we suspected a chronic respiratory tract infection and started the administration of a low dose of clarithromycin (CLR, 200 mg per day).

In September 2013, 9 months after the initiation of macrolide administration, his chest CT scan revealed deterioration of centrilobular micronodules and infiltration in the right lobe. He had a positive culture for AFB on an expectorated sputum sample. This isolate demonstrated slow-growing, non-pigmented, creamy and smooth colonies, consistent with the characteristics of Runyon III organisms. However, the strain could not be identified by both polymerase chain reaction (PCR) method to detect *M. tuberculosis* and DNA-DNA hybridization method (DDH Mycobacteria, Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) which is commonly used to identify 17 clinically isolated species of NTM in Japan. We also performed antimicrobial susceptibility testing of the isolates. We used BrothMIC NTM® for the broth microdilution method (Kyokuto Pharmaceutical Industrial Co., Ltd.) to determine the minimum inhibitory concentration (MIC). MICs of antibiotics for this strain were 0.06 μg/ml (CLR), 0.12 μg/ml (rifampicin), 0.03 μg/ml [rifabutin (RFB)], 0.25 μg/ml (streptomycin), ≤0.5 μg/ml (amikacin), 1 μg/ml [levofloxacin (LVX)], 4 μg/ml [ethambutol (EMB)], and 8 μg/ml [ethionamide (ETO)]. In March 2014, an expectorated sputum smear for the AFB was positive, and the patient received a higher dose of CLR (600 mg per day) and rifampicin (RIF, 300 mg per day). Ethambutol (EMB) was not administered because of his glaucoma. Eventually, the pulmonary shadow partially reduced, but expectorated sputum smear and culture for the AFB remained positive.

We amplified and sequenced the *16S rRNA* gene to identify the species of this NTM isolate. Next, we searched for homology between this sequence and various mycobacteria with the Basic Local Alignment Search Tool (BLAST®). Our results identified 2

species of NTM (*M. triplex* and *M. florentinum*) as superior candidates; however, they had the exact similarity score (Fig. 2). Therefore, we sequenced the *hsp65* gene of the NTM isolate (Fig. 3), which resulted in a similarity score of 686 for *M. triplex* and 652 for *M. florentinum*. We finally identified this bacterium as *M. triplex*.

The HRCT findings of this patient were consistent with those of pulmonary NTM disease, and NTM strains were detected in 3 serial expectorated sputum samples during the clinical course. There were no other microorganisms that could cause respiratory infections detected. DNA sequencing analysis identified at least one of these NTM strains as *M. triplex*. In general clinical practice, it is extremely rare that NTM which cannot be identified with DDH are detected [6]. Therefore, it was reasonable to consider this patient had respiratory infection caused by *M. triplex*. In December 2014, levofloxacin (LVX, 250 mg per day) was started. Eventually, the pulmonary shadow reduced further, and the sputum AFB culture became negative. Fig. 4 shows the clinical course of the present case including expectorated sputum smear and culture, identification results of bacterial species, chest CT images, and treatment details.

Discussion

We described the case of an immunocompetent 78-year-old HIV-negative Japanese man with pulmonary *M. triplex* infection. Although he had several expectorated sputum smears and cultures positive for AFB, we could not identify the species by a DNA-DNA hybridization method. Further gene sequencing analysis of *16S rRNA* and *hsp65* identified the bacterium as *M. triplex*. The patient began antimycobacterial therapy with CLR, RIF, and LVX. Eventually, he showed a sputum smear and culture negative for AFB, and the abnormal shadow on his chest CT scan gradually reduced.

Over 150 species of NTM have been identified to date. In Japan, we usually identify clinical isolates of *M. avium* and *M. intracellulare* using PCR, and we use the DNA-DNA hybridization

Patient isolate	TGAGTTTTAA	GCCTTGCGGC	CGTACTCCCC	AGGCGGGGTA	CTTACTGCGT	TAGGTACGGC	60
M. triplex	· · · · · · · - ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · A · · · ·	· · · C · · · · · ·	59
M. florentinum	· · · · · · · - ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · A · · · ·	· · · C · · · · · ·	59
M. stomatepiae	· · · · · · · - ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · A · · · ·	· · · C · · · · · ·	59
M. montefiorense	· · · · · · · - ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · A · · · ·	· · · C · · · · · ·	59
Patient isolate	CGGGCCCATC	CCACACCGCA	AAAGCTTTCC	ACCACAAGAC	ATGCGTCTCG	TGGTCATATC	720
M. triplex	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	719
M. florentinum	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	719
M. stomatepiae	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · G · ·	· · C · · · · · · ·	· · · · · · · · · ·	719
M. montefiorense	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · T	719
Patient isolate	ACTCACCCAT	TCGCCACTCG	AGTACCCCCG	AAGGGG - - TT	TCCGTTCGAC	TTGCATGTGT	
M. triplex	· · · · · · · · G ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · CC · ·	· · · · · · · · · ·	· · · · · · · · · ·	838
M. florentinum	· · · · · · · · G ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · CC · ·	· · · · · · · · · ·	· · · · · · · · · ·	839
M. stomatepiae	· · · · · · · · G ·	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · CC · ·	· · · · · · · · · ·	· · · · · · · · · ·	839
M. montefiorense	· · · · · · · · G ·	· · · · · · · · · ·	· · · · · T · · · ·	· · · A · · CC · ·	· · · · · · · · · ·	· · · · · · · · · ·	839

Fig. 2. Partial alignment of *16S rRNA* sequences.
Partial alignment of *16S rRNA* sequences of *Mycobacterium triplex* (*M. triplex*), *M. florentinum*, *M. stomatepiae*, and *M. montefiorense*. Dots indicate nucleotide identity and dashes indicate deletions. Reference sequence accession numbers are as follows: *M. triplex*: AJ276890; *M. florentinum*: NR042223; *M. stomatepiae*: HM022202; and *M. montefiorense*: NR028808. The similarity scores are: 1543 (*M. triplex*), 1543 (*M. florentinum*), 1531 (*M. stomatepiae*), and 1526 (*M. montefiorense*).

Patient isolate	TACCAACGAT	GGTGTGTCCA	TCGCCAAGGA	30	GAAGACCGAC	GATGTCGCCG	GTGACGGCAC	120
M. triplex	- - - - - - - - -	· · · · · · · · · ·	· · · · · · · · · ·	22	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	112
M. florentinum	- - - - - - - - -	- - - - - - - - - -	- - - - - - - · · ·	4	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	94
M. lentiflavum	- · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	29	· · · · · · · · · ·	· · · C · · · · · ·	· · · · · · · · · ·	119
Patient isolate	GACGACGGCC	ACCGTGCTGG	CTCAGGCACT	150	CGTCAAAGAG	GGCCTGCGCA	ACGTAGCGGC	180
M. triplex	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	142	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	172
M. florentinum	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	124	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	154
M. lentiflavum	· · · · · · · · · ·	· · · G · · · · · ·	· · · · · · · · · ·	149	· · · · · · · · · ·	· · · · · · · · · ·	· · · · C · · · · ·	179
Patient isolate	GACCAAGGAG	CAGATCGCTG	CGACCGCTGG	300	TATCTCGGCG	GGCGATCAGT	CGATCGGCGA	330
M. triplex	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	292	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	322
M. florentinum	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	274	· · · · · · · · · ·	· · · · · · · · · ·	· · · · · · · · · ·	304
M. lentiflavum	· · · · · · · · · ·	· · · · · · · · · ·	· T · · · · · G · ·	299	· · · · · · · · · ·	· · · · · C · · · ·	· · · · · · · · · ·	329

Fig. 3. Partial alignment of *hsp65* sequences.
Partial alignment of *hsp65* sequences of *Mycobacterium triplex* (*M. triplex*), *M. florentinum*, and *M. lentiflavum*. Dots indicate nucleotide identity and dashes indicate deletions. Reference sequence accession numbers are as follows: *M. triplex*: AFP-000NM44; *M. florentinum*: DSM44852; and *M. lentiflavum*: FJ06136. The similarity scores are: 686 (*M. triplex*), 654 (*M. florentinum*), and 652 (*M. lentiflavum*).

Antimicrobial agents					RIF 300 mg/day	LVX 250 mg/day RIF 300 mg/day CLR 600 mg/day		
			CLR 200 mg/day		CLR 600 mg/day			
Year/month	2008	2012/12	2013/9	2014/3	2014/6	2014/12	2015/6	2015/9
Sputum AFB — Smear	?	(−)	(−)	(+)	(+)		(−)	(−)
Sputum AFB — Culture	(+)	(−)	(+)	(+)	(+)		(−)	(−)
Bacterial species	unknown		unknown by DDH	unknown by DDH	unknown by DDH ↓ *M. triplex* identified by genome sequence			

Chest CT				
Year/month	2012/12	2014/7	2015/2	2016/2

Fig. 4. Timing of diagnostic tests and clinical course of the case patient.
AFB: Acid-fast bacilli, CLR: Clarithromycin, DDH: DNA-DNA hybridization, LVX: Levofloxacin, RIF: Rifampicin.
Low-dose clarithromycin (CLR) monotherapy was started in December 2012. An expectorated sputum culture for acid-fast bacilli (AFB) became positive in September 2013, but the species could not be identified by DNA-DNA hybridization (DDH) method. In March 2014, CLR dose was increased and rifampicin (RIF) was added because sputum smear for the AFB also became positive and pulmonary shadow gradually worsened. However, the positive results persisted in both smear and culture for AFB, the species of which DDH method still failed to identify. DNA sequencing analysis could identify one of these strains as *M. triplex*. After the initiation of levofloxacin (LVX) in December 2014, both smear and culture for AFB became negative and chest CT images showed reduction of pulmonary shadow gradually.

Table 1
Summary of *Mycobacterium triplex* (*M. triplex*) infection cases and patients' main clinical features.

Case	Nation	Age (years) /Sex	Host immunity	Site of lesion	Antimicrobial agents	Response	Reference
1	Italy	40/M	Compromised (HIV infection)	Spleen Lymph node, Bone marrow	CLR + ETO	Slowly worsened	[11]
2	USA	4/F	Competent	Cervical lymph node	CLR + EMB + RFB	Healed	[12]
3	USA	13/F	Compromised (immunosuppressive agents after liver transplantation)	Pericardial effusion Ascites	No antibiotics (drainage)	Healed	[13]
4	Finland	67/M	Competent	Lung	CLR + EMB + RIF + CIP	Healed	[10]
5	Northern Ireland	47/F	Competent	Lung	NR	NR	[14]
6	France	41/M	Compromised (HIV infection)	Lung, CNS, Ascites	CLR + EMB + RIF + INH + PZA	Death	[15]
7	Italy	54/F	Competent	Lung	CLR + EMB + LVX	Slightly improved	[16]
8	USA	30/M	Competent	Lung	No therapy	NR	[17]
9	USA	82/M	Competent	Lung	CLR + EMB + RIF + CIP	Slowly improved	[18]
10	Italy	4/M	Competent	Cervical lymph node	No antibiotics (surgical excision)	Healed	[19]
11	Brazil	51/F	Compromised (HIV infection)	Lung	CLR + EMB + AMK + OFX	Healed	[20]
12 (Our case)	Japan	78/M	Competent	Lung	CLR + RIF + LVX	Slowly improved	

AMK: Amikacin, CIP: Ciprofloxacin, CLR: Clarithromycin, CNS: Central nervous system, EMB: Ethambutol, ETO: Ethionamide, HIV: human immunodeficiency virus, INH: Isoniazid, LVX: Levofloxacin, NR: Not reported, OFX: Ofloxacin, PZA: Pyrazinamide, RFB: Rifabutin, RIF: Rifampicin.

method for other NTM. The latter method can also distinguish NTM from *M. tuberculosis*. This method involves a DNA-DNA hybridization between parts of the DNA sequence from a standard strain stabilized on microplates and those of the isolated strain [7]. DNA-DNA hybridization enables the quantitative evaluation of the similarity between both DNA strands by reheating the generated hybrid and measuring the temperature at which the molecules separate to form single-stranded DNA. This method is simple and rapid; however, it can only identify 17 species of NTM: *M. avium*, *M. intracellulare*, *M. kansasii*, *M. gordonae*, *M. chelonae*, *M. abscessus*, *M. scrofulaceum*, *M. fortuitum*, *M. marinum*, *M. simiae*, *M. szulgai*, *M. gastri*, *M. xenopi*, *M. nonchromogenicum*, *M. terrae*, *M. triviale*, and *M. peregrinum*. Disadvantages of this method include the inability to distinguish between 2 genetically close species and the possible misidentification of a species that cannot be identified as another species [8].

Recently, information on the whole-genome sequence of a variety of bacteria has become available, and we can distinguish between closely related species through the identification of specific, preserved DNA sequences. DNA sequencing of housekeeping genes, such as *16S rRNA*, *rpoB*, *recA*, and *hsp65*, is used for microbial analyses [9]. Housekeeping gene sequencing analysis can identify more than 110 species of mycobacterium with a high degree of accuracy by combining multiple analyses of gene sequences. In the present case, we could not identify *M. triplex* by the DDH method. However, DNA sequencing analysis of the housekeeping genes, *16S rRNA* and *hsp65*, resulted in the correct diagnosis and enabled the administration of a successful medication regimen.

Table 1 summarizes the main clinical features of the current patient and the 11 previously published cases of *M. triplex* infection. Most *M. triplex* infections have been reported from the United States or Europe. Of these, 4 cases involved immunocompromised hosts, whereas 7 cases were found in immunocompetent patients.

Although extrapulmonary lesions are often observed in immunocompromised hosts, only the lungs and lymph nodes are involved in immunocompetent hosts. Clinical and radiographic features of *M. triplex* pulmonary infection resemble those of tuberculosis and other NTM. The primary symptoms are cough,

hemoptysis, and fatigue. Radiographic studies most often note pulmonary nodules, followed by lung infiltrates, multifocal bronchiectasis, and cavitation. All reported cases were definitively identified by analysis of housekeeping gene sequencing. Antimicrobial susceptibility of *M. triplex* is similar to that of *M. avium* complex. Most *M. triplex* cases are susceptible to CLR and RIF and resistant to isoniazid. Most patients with *M. triplex* infection received 2–4 antimicrobial agents: EMB, RIF, CLR, and ciprofloxacin [10]. Of the 11 cases, 8 showed improvement after antimicrobial treatment. However, it should be noted that drug susceptibility testing of NTM isolates is difficult to interpret because there are some discrepancies between in vitro and in vivo clinical outcomes [2]. This finding is particularly true for NTM species that are rarely identified in clinical practice.

We reported a case of *M. triplex* pulmonary disease in a 78-year-old immunocompetent male. The patient received successful treatment with the appropriate antimicrobial agents after definitive diagnosis by housekeeping gene analysis. However, little remains known and further investigations are necessary to better describe *M. triplex* infection.

Funding

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Author contribution

MS was involved in data collection, analysis, interpretation and manuscript writing. AT was involved in data collection, analysis and interpretation. SM and MF were involved in data analysis and interpretation. All authors approved the final version of the manuscript.

Ethics approval and consent for publication

All authors meet the ICMJE authorship criteria. Written informed consent was obtained from the patient for publication of this case report and accompanying images.

Declaration of Competing Interest

All authors have no conflicts of interest directly relevant to the content of this article.

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