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Case report

A case of pulmonary *Mycobacterium heckeshornense* infection in a healthy Japanese man

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ABSTRACT

A 72-year-old man, healthy, smoker, with long-standing cough, was referred to our hospital and his chest X-ray (CXR) revealed a cavity lesion in the right upper lobe. Direct sputum smears, but not culture in solid medium, were positive for acid-fast bacilli (AFB) without tuberculosis DNA. The preliminary diagnosis was of a non-tuberculosis infection that progressed slowly, and the CXR showed the condition to worsen daily. Four years later, a commercialized mycobacteria growth indicator tube system was used to culture the colonies of AFB successfully in liquid medium, and the species *Mycobacterium heckeshornense* was identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry. The patient responded well to triple therapy with rifampicin, ethambutol, and clarithromycin, the sputum cultures remained negative and the roentgenogram showed minor improvement over the following 6 months.

1. Introduction

A species of *Mycobacterium* (*M.*) *heckeshornense* was first described as a new isolated non-tuberculosis mycobacteria (NTM) which infects human. It was reported in a patient with pulmonary infiltrations in 2000 [1]. Except for pulmonary infectious diseases with *M. heckeshornense* [2–9], diseases such as spinal osteomyelitis and diskitis [10], lumbar spondylodiskitis [11], and lymphadenitis [12] were reported as systemic diseases. *M. heckeshornense* might be categorized as Runyon group II, as a slow-growing scotochromogenic mycobacteria [4,13], and be misdiagnosed as *M. xenopi*. The pathogenesis of infection by these two bacteria is similar, but *M. xenopi* can be distinguished by 16S ribosomal RNA (rRNA) and 16S–23S spacer gene sequences [2,6]. Today, a matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) technique was developed to easily identify bacterial species, including the species of *M. heckeshornense* [8,14]. We herein report a rare case of a healthy Japanese man with pulmonary *M. heckeshornense* infections by using modified liquid culture system and the MALDI-TOF MS techniques. Furthermore, triple anti-mycobacterial therapy with rifampicin (RFP), ethambutol (EB), and clarithromycin (CAM) was successful for the treatment of the progressive pulmonary disease.

2. Case report

A 72-year-old healthy man with chronic cough and CXR abnormalities was referred to our clinic. He was a current smoker and his smoking index was 92 pack-years. His CXR (Fig. 1A) and computed tomography (CT) (Fig. 1B) revealed infiltrations of cavity lesions at the right upper lobe. Direct sputum smears were positive for acid-fast bacilli (AFB), although the DNAs of *Mycobacterium tuberculosis* (MTB) and *Mycobacterium avium complex* (MAC) were not detected by polymerase chain reaction (PCR) techniques (SRL co., Tokyo, Japan). AFB cultures in 2% Ogawa egg slant medium (Kyokuto, Tokyo, Japan) under standard

Abbreviations: AFB, acid-fast bacilli; CAM, clarithromycin; CT, computed tomography; CXR, chest x-ray; DDH, DNA–DNA hybridization; EB, ethambutol; M, *Mycobacterium*; MAC, *Mycobacterium avium complex*; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MGIT, mycobacteria growth indicator tube; MIC, minimum inhibitory concentration; MTB, *Mycobacterium tuberculosis*; NTM, nontuberculous mycobacteria; PCR, polymerase chain reaction; RE, rifampicin plus ethambutol; RFP, rifampicin; rRNA, ribosomal RNA.

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conditions showed no growth. His CXR abnormalities were diagnosed as non-tuberculosis mycobacteria infection with unknown causes. His home doctor followed up the patient, as the chronic cough was relieved with temporary cough medicines or spontaneously.

Four years later, he revisited our clinic with cough recurrence and a weight loss of 5 kg/year with 16.4 kg/m^2 of body mass index. His vital signs were almost normal and his body temperature was 36.7 °C. His white blood cells count was 5000 cells/μL and serum C-reactive protein level was 2.26 mg/L. His chest CT revealed worsened cavity lesions with surrounding infiltrative shadows at the right upper lobe (Fig. 1C).

Direct sputum smears were positive for AFB, although MTB and MAC-DNA could not be detected by PCR techniques and standard AFB cultures; AFB was ultimately successfully cultured in the mycobacterium growth indicator tubes (MGIT) system (BD Biosciences, Sparks, MD, USA). A specie of *M. heckeshornense* was finally identified with the MALDI-TOF MS techniques, although no match of 18 mycobacterial species (not involving *M. heckeshornense*) were identified among the cultured AFB strains by the commercial kits of the DNA-DNA hybridization techniques (DDH Mycobacteria, Kyokuto, Japan). The progressive pulmonary *M. heckeshornense* infections were defined in the patient, because the species was isolated repeatedly from sputum samples on different days after fiberoptic bronchoscopy.

We summarized the characteristics of 11 patients (number of male, 6; median age at diagnosis, 52.6 years [range: 30–72 years]) with pulmonary *M. heckeshornense* infections based on our case and previous case reports [1–9] (Tables 1 and 2). Five patients were in the immunocompetent state, and the remaining 6 patients had some underlying diseases. Only two patients had no symptoms, although most patients had similar respiratory and general symptoms with other NTM infections. The number of patients diagnosed with lung *M. heckeshornense* infections were 7, by the 16S rRNA sequence analysis, 2 by the DNA strip assay and 2 by the MALDI-TOF MS techniques, respectively. The CXR and CT abnormalities of lung fields were limited to the right upper lobe in six patients and 6 had cavity lesions. The regimens for *M. heckeshornense* infections varied, although multidrug therapy was selected for all the patients including anti-tuberculous drugs, CAM, aminoglycosides, and some new quinolones. The limited lung lesions were successfully resected in 2 patients after anti-mycobacterial chemotherapies. Except for two patients, the number (population) of patients who had improved, not changed and worsened were 7 (64%), 2 (18%) and 1 (9%), respectively, after the treatments.

The indirect drug susceptibility of *M. heckeshornense* strain was tested according to the absolute concentration method using a microtiter technique developed by a commercial laboratory (BML. Tokyo, Japan). The minimum inhibitory concentrations (MIC) of isoniazid, RFP, EB, and streptomycin used were 0.2 g/mL, 40 g/mL, 2.5 g/ml, and 10 g/ml, respectively. According to the previous reports, *M. heckeshornense* was resistant to INH, but susceptible to RFP, EB, amikacin, CAM, and ciprofloxacin (1). Our case showed similar susceptibility to previous reports. Triple anti-mycobacterial therapy with RFP, EB and CAM at 450, 750, and 600 mg/day, respectively (his body weight was under 50 kg) was started against the progressive pulmonary *M. heckeshornense* infections based on the results of MIC and the regimens in the previous reports [1–9]. His symptoms and abnormal shadows on chest CT improved 6 months after the treatments without adverse events (Fig. 1D). Mycobacterial cultures remained negative.

3. Discussion

We experienced a rare case of pulmonary *M. heckeshornense* infection in a healthy Japanese man. Triple anti-mycobacterial therapy with RFP, EB, and CAM was successful for the treatment of progressive pulmonary *M. heckeshornense* infection. *M. heckeshornense* shows a very slow growth rate over 37–45 °C, and the differences in the growth temperature depends on the individual isolates of *M. heckeshornence* [1,3]. While we were initially unable to culture the sputum AFB on solid medium, the AFB were ultimately successfully grown using the MGIT system. According to the previous reports, isolates from 4 to 2 cases grew either in MGIT [2,5,7,8] or solid medium [3,9], respectively, while isolates from 2 additional cases grew in both conditions [3,4]. Curiously, paralleling our observation, other study reported an isolated whose culture was only achieved with MGIT, and not solid medium [5]. This is possibly because the MGIT method increased the culture-positive rate, as others reported: the culture-positive rate in smear-negative specimens was greatly increased [15]. It is possible that patients infected with *M. heckeshornense* have not been diagnosed correctly, because the MALDI-TOF MS techniques and 16s rRNA method are performed only in few laboratories. However, the use of MALDI-TOF MS techniques is becoming widespread in Japan and was helpful for the isolation of *M. heckeshornense* species [8].

The three most common NTM pathogens of lung diseases are *M. gordonae* (31.8%), *M. abscessusnon* (22.4%), and *M. fortuitum* (11.8%)

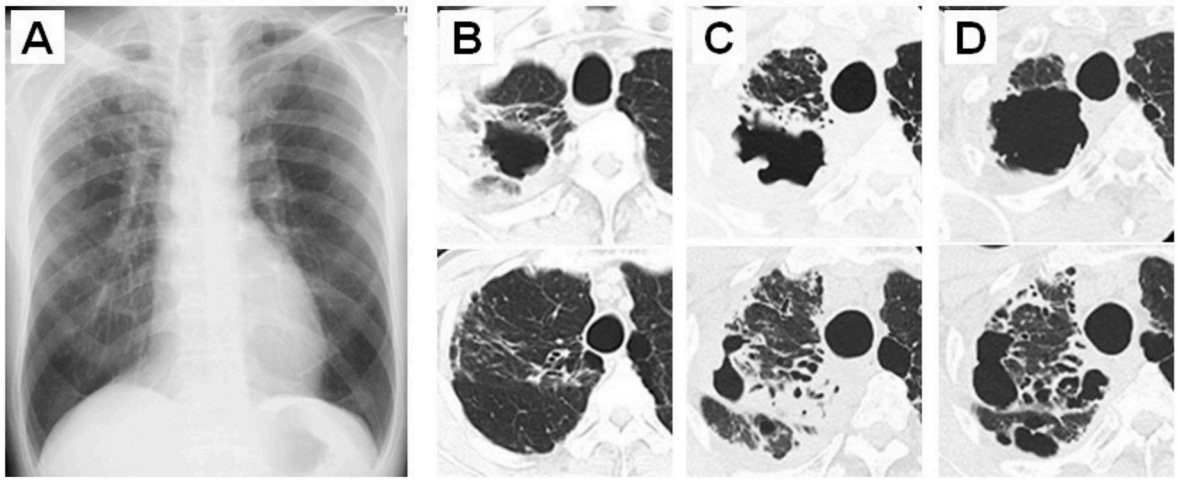

Fig. 1. Findings of chest X-ray and computed tomography
Note: A) Chest X-ray revealed infiltrations with pleural wall thickness in right upper lobe at initial visit. B) Chest CT revealed a cavity lesion with infiltration in right upper lobe at initial visit. C) Chest CT revealed worsened cavity lesions with infiltration in right upper lobe 4 years after initial visit. D) Chest CT showed that the abnormal shadows improved 6 months after treatments.
Abbreviation: CT, computed tomography.

Table 1
Summary of patients with pulmonary *M. heckeshornense* infections based on the present and previous reports.

Case no.	Report	Age and Sex	Underlying diseases	Symptoms	Specimens with identified bacilli	Radiographic findings	Location
1	2018 Our case	72 Male	None	cough and weight loss	BALF sputum	cavity with infiltrations	RUL
2	2000 Roth1)	30 Female	None	cough and fatigue	sputum	cavity with infiltrations	BUL
3	2004 Rob2)	43 Male	pneumothorax and OMI	night sweat, weight loss and fatigue	pleural effusion	infiltrations with right pleural effusion	RUL
4	2006 Kazumi3)	51 Female	Old Tuberculosis	Hemoptysis	sputum	infiltrations	BUL
5		71 Male	Pneumoconiosis	None	sputum	cavity	RUL
6	2007 Jaureguy4)	65 Female	Post RULL due to traffic accident	dyspnea on exertion, cough and weight loss	sputum	cavity and infiltrations with pleural thickness	unknown
7	2008 Hisamoto5)	68 Male	None	cough and hemosputum	sputum	infiltrations	RUL
8	2011 Morimoto6)	47 Female	None	N/A	sputum	consolidations	RUL
9	2015 Coitinho7)	53 Male	alcoholism	cough, fever and fatigue	sputum	cavity and infiltrations	BUL
10	2018 Yokoyama8)	40 Male	Behcet disease	Cough	BALF	isolated nodule	RLL
11	2018 Kurosaki9)	39 Female	None	None	TBLB	cavity and infiltrations	RUL

Abbreviation: OMI: old myocardial infarction; BALF: bronchoalveolar lavage; TBLB: transbronchial biopsy; RUL: right upper lobe; BUL: bilateral upper lobe.

Table 2
Summary of patients with pulmonary *M. heckeshornense* infections based on the present and previous reports.

Case no.	Methods of identification	Regimens of treatment	Drug susceptibility								Prognosis
			INH	RFP	EB	SM	KM	LVFX	CPFX	CAM	
1	MALDI-TOF MS	CAM + RE	I	S	S	S	S	S	–	–	improved
2	16S rRNA gene sequence	HRE/PTH/CPFX and RUL lobectomy	–	–	–	–	–	–	–	–	N/A
3	16S rRNA gene sequence	HREZ + RE	I	S	I	S	I	–	S	S	not changed
4	16S rRNA gene sequence	RE/KM	–	–	–	–	–	–	–	–	dead
5	16S rRNA gene sequence	None	–	–	–	–	–	–	–	–	improved
6	DNA strip assay	HRE→HREZ/OFLX→RE/CAM/MFLX	–	S	S	–	–	–	S	S	improved
7	16S rRNA gene sequence	HREZ→HRE	I	S	S	S	–	S	–	–	not changed
8	16S rRNA gene sequence	MEPM→MFLX	–	–	–	–	–	–	–	–	improved
9	DNA strip assay	HREZ→HRE/LVFX/CAM	–	–	–	–	–	–	–	–	improved
10	MALDI-TOF MS	HRE/STFX	–	S	S	S	S	S	–	S	improved
11	16S rRNA gene sequence	HREZ→RE/CAM and RUL lobectomy		S	S					S	improved

Abbreviation: 16S rRNA: 16S ribosomal ribonucleic acid gene sequence; AMK: amikacin; CAM: clarithromycin; CPFX: ciprofloxacin; DNA: deoxyribonucleic acid; EB: ethambutol; HRE: isoniazid + rifampicin + ethambutol; HREZ: isoniazid + rifampicin + ethambutol + pyradinamide; INH: isoniazid; KM: kanamycin; LVFX: levofloxacin; MALDI-TOF MS: Matrix assisted laser desorption ionization-time of flight mass spectrometry; MEPM: meropenem; MFLX: moxifloxacin; N/A: not available; OFLX: ofloxacine; OMI: old myocardial infarction; PTH: prothionamide; RE: rifampicin + ethambutol; RFP: rifampicin; RLL: right lower lobe; SM:; streptomycin; STFX: sitafloxacin; S: susceptible; I: intermediate; R: resistant.

except for MAC and *M. kansasii* in Japan [16]. To our knowledge, the pulmonary *M. heckeshornense* infections are rare in Japan as well as worldwide (Table 1). The pathogenesis of *M. heckeshornense* is still unclear, although the species seems to infect both immunocompromised and immunocompetent patients [1–12].

With these clinical, radiologic, and microbiologic findings, the patient met the American Thoracic Society/Infectious Diseases Society of America diagnostic criteria of nontuberculous mycobacterial lung disease [13]. The DNA-DNA hybridization techniques did not cover *M. heckeshornense* in commercial kits in Japan. *M. heckeshornense* species could be isolated by the MALDI-TOF MS techniques in our patient. The MALDI-TOF MS techniques may be a useful method for identifying rare species of NTM, such as *M. heckeshornense* [8,14].

The recommended treatments have not been established for lung *M. heckeshornense* infections [1–13,16,17]. We carefully selected the initial regimen of triple therapy with RFP, EB, and CAM, by reference to previous results of drug susceptibility in isolated *M. heckeshornense* strains [1–9]. We also referred to previous therapeutic outcomes and basic research of *M. xenopi* infections, because of the similar pathogenicity in *M. heckeshornense* and *M. xenopi* [1,17–21]. Our selected regimen led to good responses in the patient's respiratory and general symptoms, maintained a negative culture result, and showed improvements in CXR abnormalities over the following 6 months (Fig. 1D). However, attention should be paid to the prognosis and timing of ending treatments in the future.

In conclusion, we experienced a rare case of pulmonary *M. heckeshornense* infection and summarized the previous reported cases. The widespread use of MALDI-TOF MS techniques and triple therapy with RFP, EB, and CAM may contribute to early diagnosis and good controls of *M. heckeshornense* infections.

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Declaration of competing interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.rmcr.2020.101093.

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