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CASE REPORT

Overlapping infections of *Mycobacterium canariasense* and *Nocardia farcinica* in an immunocompetent patient: A case report

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Abstract

BACKGROUND

Infections by non-tuberculous mycobacteria (NTM) have become more common in recent years. *Mycobacterium canariasense* (*M. canariasense*) was first reported as an opportunistic pathogen in 2004, but there have been very few case reports since then. *Nocardia* is a genus of aerobic and Gram-positive bacilli, and these species are also opportunistic pathogens and in the Mycobacteriales order. Conventional methods for diagnosis of NTM are inefficient. Metagenomic next-generation sequencing (mNGS) can rapidly detect many pathogenic microorganisms, even rare species. Most NTM and *Nocardia* infections occur in immunocompromised patients with atypical clinical symptoms. There are no previous reports of infection by *M. canariasense* and *Nocardia farcinica* (*N. farcinica*), especially in immunocompetent patients. This case report describes an immunocompetent 52-year-old woman who had overlapping infections of *M. canariasense*, *N. farcinica*, and *Candida parapsilosis* (*C. parapsilosis*) based on mNGS.

CASE SUMMARY

A 52-year-old woman presented with a productive cough and chest pain for 2 wk, and recurrent episodes of moderate-grade fever for 1 wk. She received antibiotics for 1 wk at a local hospital, and experienced defervescence, but the productive cough and chest pain persisted. We collected samples of a lung lesion and alveolar lavage fluid for mNGS. The lung tissue was positive for *M. canariasense*, *N. farcinica*, and *C. parapsilosis*, and the alveolar lavage fluid was positive for *M. canariasense*. The diagnosis was pneumonia, and application of appropriate antibiotic therapy cured the patient.

CONCLUSION

Etiological diagnosis is critical for patients with infectious diseases. mNGS can

identify rare and novel pathogens, and does not require a priori knowledge.

Key Words: Overlapping infection; *Mycobacterium canariasense*; *Nocardia farcinica*; Metagenomic next-generation sequencing technology; Case report

Core Tip: Non-tuberculous mycobacteria (NTM) and *Nocardia* are opportunistic pathogens that can occur in immunocompromised patients who present with atypical clinical symptoms. *Mycobacterium canariasense* (*M. canariasense*) is a rare NTM species was first identified 20 years ago. We describe a patient with multiple lung nodules of unequal size with uneven internal density, and multiple small burrs at the edges of these lung lesions. The pathology results were inconsistent with malignancy, and metagenomic next-generation sequencing indicated overlapping infections of *M. canariasense*, *Nocardia farcinica*, and *Candida parapsilosis*. The anti-infective treatment was successful.

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INTRODUCTION

The non-tuberculous mycobacteria (NTM), also referred to as environmental mycobacteria, atypical mycobacteria, or anonymous mycobacteria, are ubiquitous species and potential causes of infectious diseases. Based on their growth characteristics determined from subculturing, the NTM are classified as rapidly growing mycobacteria (RGM; mature colonies in less than 7 d) or slowly growing mycobacteria (mature colonies in more than 7 d)[1]. Several RGM species have been identified as etiologic agents of bacteremia, especially in patients with low immunity, such as those with human immunodefciency virus (HIV) infections or malignant tumors. *Mycobacterium canariasense* (*M. canariasense*) is a rare species of RGM that is closely related to *M. diernhofer*[2,3], but has never been described separately in immunocompetent persons.

The genus *Nocardia* is also in the order *Mycobacteriales*, and includes at least 50 species that are aerobic Gram-positive bacilli which can invade the lungs, skin, or central nervous system, especially in immunocompromised persons. The symptoms of patients with *Nocardia* infections are often nonspecific, and include chronic cough, low-grade fever, fatigue and weight loss[4].

It can be difficult to diagnose infections by *Mycobacterium* and *Nocardia* from the conventional culture tests used in clinical practice, and delayed diagnosis may have serious adverse consequences. The rapid and efficient identification of the pathogen responsible for an infectious disease is a prerequisite for the effective treatment of these patients. Metagenomic next-generation sequencing (mNGS) does not require traditional microbial culture, and instead directly provides high-throughput sequencing of nucleic acids in clinical samples, which are then compared with a database. This method can rapidly and objectively detect many pathogenic microorganisms (including viruses, bacteria, fungi, and parasites) in clinical samples without the need for specific amplification, and is especially suitable for the diagnosis of acute and critical diseases and difficult infections. In this paper, we describe the use of mNGS to detect the rare co-occurrence of *M. canariasense* and *Nocardia farcinica* (*N. farcinica*) in an immunocompetent adult. This identification allowed administration of an effective treatment and led to patient cure.

CASE PRESENTATION

Chief complaints

A 52-year-old woman from Guangxi Zhuang Autonomous Region presented with productive cough and chest pain for 2 wk and recurrent episodes of moderate-grade fever for 1 wk.

History of present illness

The patient experienced a relapse of productive cough, persistent chest pain, and moderate-grade fever, but there were no chills, shivering, hemoptysis, or weight loss. She was diagnosed with pneumonia and treated with cefuroxime sodium (0.75 g/8 h) and levofloxacin (0.5 g/d) at a local hospital for 1 wk and experienced defervescence. However, the cough and chest pain persisted, and this affected her ability to work and study. The patient presented to another outpatient clinic for persistent cough and chest pain, with the possibility of malignancy unable to be excluded. Consequently, she presented at our hospital for further diagnosis and treatment.

History of past illness
This case had no specific history of past illness.

Personal and family history
The patient had a history of exposure to sheep feces 1 wk before symptom onset. She had no history of using steroids or other medications, no smoking, no tuberculosis, no malignant tumors or immunosuppressive diseases, and was unaware of any contact with persons with mycobacterial infections. She also reported no relevant family history.

Physical examination
No abnormalities were detected in the physical examination.

Laboratory examinations
Laboratory studies revealed leukocytosis, an elevated level of high-sensitivity C-reactive protein, and a high erythrocyte sedimentation rate (Table 1). After 5 d, multiple sets of blood and sputum cultures revealed no growth of bacteria or fungi. The culture of bacteria and fungi in bronchoalveolar lavage fluid (BALF), and acid-fast staining were also negative. An immunologic workup, which included HIV testing, was conducted based on suspicion of immunodeficiency, but all of the results were negative. Examination of autoantibodies and tumor markers also revealed no abnormalities. BALF and pulmonary nodular samples were subsequently sent for mNGS for the rapid identification of the causative pathogen(s). Sequencing results were compared with the sequences of bacteria in Gen Bank, and those with 100% agreement were accepted. Three days later, the pathogenic microbes in the nodular specimen were identified as *N. farcinica*, *M. canariasense*, and *Candida parapsilosis* (*C. parapsilosis*) (326 sequences), although the BALF only showed *M. canariasense* (Table 2).

Imaging examinations
A chest computed tomography (CT) scan showed multiple nodules of unequal size with uneven internal density, and multiple small burrs at the edges of the lesions, suggesting the infection of both lungs, but not excluding the possibility of a tumor (Figure 1A). A pulmonary nodular biopsy was performed, and the results indicated no malignancy, but there was evidence of non-necrotizing granulomatous inflammation (Figure 1B) and a yeast-like corpuscle in the alveolar cavity (Figure 1C). These findings are consistent with fungal infection.

FINAL DIAGNOSIS
The patient had a history of cough and chest pain, but anti-infection treatment at a local hospital led to no significant resolution of these symptoms. As an inpatient at our hospital, a CT examination showed nodular lesions of the lungs, but did not exclude malignant tumor. However, the results from puncture biopsy of the injured part of the lung and pathological examination were inconsistent with malignant tumor. Thus, combined with the mNGS results, the diagnosis in this patient was pneumonia due to overlapping infection by *M. canariasense*, *N. farcinica* and *C. parapsilosis*.

TREATMENT
Upon admission and during the evaluation period, empirical intravenous treatment consisted of broad-spectrum antibiotic agents [piperacillin sodium/sulbactam sodium (3 g/12 h) and voriconazole (250 mg/12 h)]. These antibiotics were maintained because they led to significant relief of the patient's clinical manifestations, including cough, sputum production, chest pain, and fever.

OUTCOME AND FOLLOW-UP
After 2-wk of antibiotic treatment, CT reexamination showed that the pulmonary nodules were significantly reduced (Figure 1D). The mNGS also detected a sequence of *C. parapsilosis*, and pathological staining revealed a yeast-like corpuscle in the alveolar cavity. Thus, oral voriconazole was administered at home for 3 months. An outpatient CT scan that was performed 1 month after the onset of antibiotic treatment showed that the pulmonary lesions had obviously disappeared (Figure 1E). On follow up, all the clinical symptoms had disappeared. There was also complete regression of the pulmonary consolidation after 3 months, based on a CT examination at a local hospital.

DISCUSSION
RGM are ubiquitous in the environment and commonly occur in water and soil. *M. canariasense* was described for the first time as the cause of a nosocomial infection in 17 patients during the period of January 2000 to September 2002 at a tertiary care hospital in the Canary Islands (Spain). This previous study reported that 15 of 17 patients had malignant diseases,

Table 1 Laboratory test results of blood samples collected at three times

Variable	Reference range	On admission	Antibiotic treatment	
			After 2-wk	After 1-month
Leukocytes (× 10^9/L)	3.69-9.16	10.34	5.23	7.12
Hemoglobins (g/L)	113-151	130	130	123
Platelets (× 10^9/L)	100-300	439	286	100
Neutrophiles (× 10^9/L)	2-7.7	8.49	3.17	5.2
Lymphocytes (× 10^9/L)	0.8-4	1.39	1.53	1.4
Monocytes (× 10^9/L)	0.12-0.8	0.4	0.39	0.38
ESR (mm/H)	0-20	92.8	41.4	26.5
hs-CRP (mg/L)	0-3	32.48	0.92	0.51

ESR: Erythrocyte sedimentation rate; hs-CRP: Highly sensitive C-reactive protein.

Table 2 Pathogenic microorganisms identified by metagenomic next-generation sequencing technology

Specimen	Genus			Species		
	Name	Sequence number	Relative abundance	Name	Sequence number	Cover degree
Nodular	*Nocardia*	1006	0.02%	*Nocardia farcinica*	393	18.87%
Nodular	*Mycobacterium*	9501	0.72%	*Mycobacterium canariasense*	3630	6.89%
Nodular	*Candida*	327	2.44%	*Candida parapsilosis*	326	0.23%
Bronchoalveolar lavage fluid	*Mycobacterium*	2260	42.34%	*Mycobacterium canariasense*	2020	1.75%

and all of them had central venous catheters (CVCs) at the time of diagnosis[3]. Subsequent reports identified this species from clinical specimens, including blood, and domestic water samples[5,6]. All previously reported cases presented with malignant diseases, especially hematologic malignancies, and most of them had a history of CVC. To our knowledge, there has been no previous report of infection by *M. canariasense* in an immunocompetent patient. A retrospective review showed *M. canariasense* was considered the etiologic agent of bacteremia in 12 of 17 cases[2]. de Miguel-Martinez *et al*[7] also reported *M. canariasense* in an oncohematological patient who had a long-term central device. In general, *M. canariasense* is a NTM that is only rarely pathogenic. In Türkiye, 90 NTM strains obtained from four different centers only identified 3 cases with *M. canariasense*[8]. A Japanese tertiary care center studied 5 patients with bloodstream infections due to RGM (4 with *M. mucogenicum* and 1 with *M. canariasense*) during a 5-month period. Another study showed that a blood isolate of *M. canariasense* from patient matched an isolate collected from a toilet in a 4-bed room[9]. Sun *et al*[10] found that the most commonly encountered NTM in China were *Mycobacterium intracellulare*, *Mycobacterium abscessus*, *Mycobacterium kansasii*, *Mycobacterium avium* and *Mycobacterium fortuitum*, and did not report *M. canariasense*. The symptoms of NTM infections are often nonspecific (chronic cough, low-grade fever, fatigue, and weight loss), but hemoptysis and chest pain are rare[4]. However, our patient had symptoms of chest pain, and we identified *M. canariasense* by mMGS of pulmonary nodules and BALF. We believe the chest pain may have been caused by the proximity of the lesion to the pleura.

Nocardia is ubiquitous in the environment and occurs worldwide as a saprophytic component in fresh water, saltwater, soil, dust, decaying vegetation, and decaying fecal deposits[11]. It is a Gram-positive bacillus, and has branching hyphae that are visible by microscopy. *Nocardia* infections are usually opportunistic and occur in immunocompromised hosts; infected immunocompetent patients usually develop localized cutaneous lesions. However, Beaman *et al*[12] found that 38 of 253 infected patients had none of predisposing factors contributing to opportunistic *Nocardia* infection. *N. farcinica* is related to *Nocardia asteroides*, accounts for 6.31% of *Nocardia* infections in China, mainly occurs in Gansu Province, and is considered the most virulent species of *Nocardia*[13]. Most *Nocardia* infections are pulmonary, and are usually attributed to inhalation of airborne spores or mycelial fragments from the environment. Dry, dusty, and windy conditions may facilitate the aerosolization and dispersal of fragmented *Nocardia* cells[14]. The conditions in China's Gansu Province are typically dry, dusty, and windy, consistent with the regional distribution of *Nocardia* in China. In contrast, our patient was from a wet, rainy area in southwestern China. However, our patient reported a history of exposure to sheep feces 1 wk before the onset of symptoms. This is consistent with previous reports which found *Nocardia* in decaying fecal deposits.

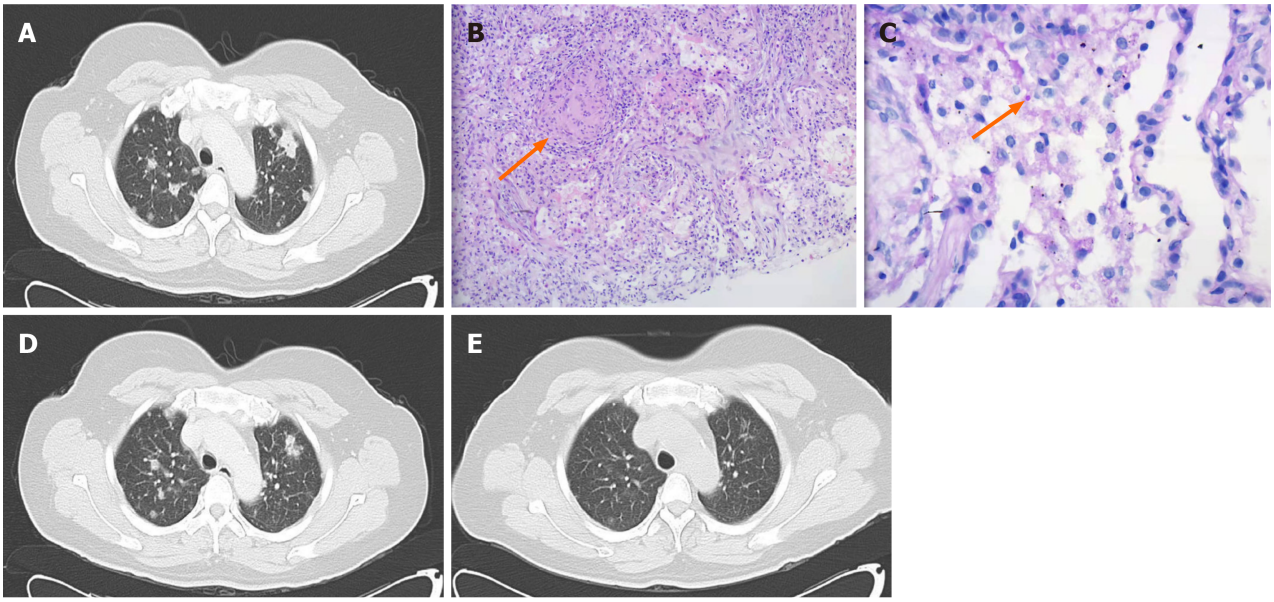

Figure 1 Changes of chest computed tomography findings and histopathology results. A: Scan at admission, revealing multiple nodules of unequal size with uneven internal density and multiple small burrs at the edges of the lesions; B: Pathology of pulmonary nodules, suggesting inflammation and granuloma formation; C: Pathology of pulmonary nodules, revealing a yeast-like corpuscle in the alveolar cavity; D: Scan after 2 wk of antibiotic treatment, showing the nodules were significantly reduced; E: Scan after 1 month of antibiotic treatment, showing the pulmonary lesions had obviously disappeared.

Identification of the etiology of an infectious disease plays an essential role in treatment of the patient, and laboratory culture is the traditional method for species identification. However, most patients with infections receive antibiotic treatment before sample collection, and this decreases the number of microbes and the sensitivity of culture. The mNGS technique is an unbiased method that can theoretically detect all kinds of pathogens, and is especially suitable for difficult and atypical infectious. Its main benefits are high sensitivity and rapid detection, and the results are less affected by prior use of antibiotics[15,16]. mNGS has a sensitivity rate that is approximately 15% higher than laboratory culture[16]. Less than 1% of the microorganisms identified by microscopy can be cultivated and characterized[17]. Our patient's negative culture results from blood, sputamentum, and BALF specimens may be due to the prior use of antibiotics. On the other hand, some research suggests that cultures of isolates from patients with suspected *Nocardia* infections should be held in the clinical microbiology laboratory for at least 2 wk for examination. We only monitored the culture results for 5 d, and this could be a reason for the negative results.

M. canariasense is a rare species of RGM that can be grown on Lowenstein-Jensen medium after about 4 d of culture at 37 °C, and is susceptible to most antibiotics[18]. Thus, antibiotic administration prior to specimen acquisition may be the main reason for our negative culture results. A limitation in China is that patients must pay for mNGS testing. Thus, for economic reason, our patient did not receive mGNS testing of blood and fecal samples.

Previous susceptibility testing of *M. canariasense* showed it was highly susceptible to amikacin, cefoxitin, ciprofloxacin, moxifloxacin, trimethoprim sulfamethoxazole, imipenem, doxycycline, minocycline, and linezolid, but only had intermediate susceptibility to clarithromycin[18]. The antibiotics previously used against *N. farcinica* include linezolid, amikacin, imipenem, and fluoroquinolone, and antimicrobial susceptibility testing demonstrated 100% susceptibility to linezolid[18-20]. A previous study described a patient who received sulphamethoxazole with linezolid and meropenem for nocardiosis, and subsequently recovered from clinical symptoms[18,21]. Other research in which all patients infected with *M. canariasense* received parenteral antibiotic therapy for 5-21 d showed that therapy guided by *in vitro* susceptibility testing did not improve patient outcome[2,18]. The 2020 NTM Guidelines proposed initiation of treatment (rather than watchful waiting) for patients who meet the diagnostic criteria for NTM pulmonary disease, but these guidelines do not mention the treatment of infections by *M. canariasense*[22]. Given the lack of high-quality evidence, the ideal treatment strategy for infection by this species remains unclear. Although we did not perform sensitivity testing, our anti-infective regimen showed high efficacy.

CONCLUSION

In summary, this is the first report of a patient who had overlapping infections of *M. canariasense* and *N. farcinica*. Infections by either of these pathogens are generally rare in immunocompetent hosts. The presence of lung nodules that are partially leafy and have burrs may lead to a misdiagnosis of lung cancer. We highlight the importance of identifying the causative pathogen by use of mNGS, a powerful tool for the detection of mixed infections, especially in patients with atypical symptoms who are infected by rare pathogens. The results from mNGS, combined with analysis of a patient underlying clinical status and laboratory indicators, provide clinicians with a more complete characterization of a disease and the causative pathogen. This case report also confirmed the potential role of *M. canariasense* and *N. farcinica* as

opportunistic pathogens. It is important to note that due to the rarity of overlapping infections by *M. canariasense* and another pathogen, empirical antibiotic treatment without mNGS results may not be adequate.

FOOTNOTES

Author contributions: Huang HY and Wei J substantial contribution to the conception and design of the work; Huang HY, Wei J, Bu KP and Liu JW contribution to the acquisition, analysis, interpretation of data for the work; Huang HY contribution to article writing and revising; Bu KP and Wei J agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors have read and approve the final manuscript and contributed to the conception and design of the work.

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