Clinical Case Report

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Pulmonary infection caused by *Nocardia cyriacigeorgica* in a patient with allergic bronchopulmonary aspergillosis

A case report

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Abstract

Rationale: *Nocardia* species is known as conditional pathogenic bacteria. Among *Nocardia* species, pulmonary infection caused by *Nocardia cyriacigeorgica* associated with *Aspergillus fumigatus* complex lung disease is rarely reported.

Patient concerns: A 55-year-old female with a history of productive cough with recurrent expectoration for 20 years presented with increasing cough for 12 months. The patient presented complaining of respiratory symptoms including increasing cough with yellow phlegm, poor appetite, and generalized fatigue for a week prior to admission.

Diagnoses: *Nocardia cyriacigeorgica* pneumonia was identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and 16S rRNA gene sequencing.

Interventions: Combined treatments (trimethoprim-sulfamethoxazole and meropenem) were administered after identification of *N cyriacigeorgica*.

Outcomes: The respiratory symptoms of the patient had improved both clinically and radiologically after 4 weeks of antibacterial therapy.

Lessons: Early precise diagnosis and prompt combined therapy are of vital importance in severe *Nocardia* pulmonary infection.

Abbreviations: ABPA = allergic bronchopulmonary aspergillosis, CLSI = Clinical and Laboratory Standards Institute, MIC = minimum inhibitory concentration, *N cyriacigeorgica* = *Nocardia cyriacigeorgica*, TMP-SMX = trimethoprim-sulfamethoxazole.

Keywords: allergic bronchopulmonary aspergillosis, *Nocardia cyriacigeorgica*, pulmonary infection, 16S rRNA gene sequencing

1. Introduction

Nocardiosis is a rare opportunistic gram-positive bacterial infection that is potentially life threatening. Organisms of the genus *Nocardia* are nonmotile, aerobic, and nonspore forming, and they exhibit characteristic filamentous branching with fragmentation into coccoid or bacillary forms; the causative organism in this study comprises a group of phylogenetically diverse but morphologically similar organisms.[1] Opportunistic infections with *Nocardia* in humans occur most commonly in immunocompromised patients. Allergic bronchopulmonary aspergillosis (ABPA) is an allergic disorder that most commonly involves the respiratory system because of bronchial colonization by *Aspergillus* fungi. Here, we report the first case of infection caused by *Nocardia cyriacigeorgica* in an ABPA patient in China.

2. Case report

This case report has been approved by the ethics committee of The Fourth Affiliated Hospital, Zhejiang University School of Medicine. A 55-year-old female with a history of productive cough with recurrent expectoration for 20 years presented with increasing cough for 12 months. The patient presented complaining of respiratory symptoms including increasing cough with yellow phlegm, poor appetite, and generalized fatigue for a week prior to admission. Three days earlier, she was empirically treated with an unspecified antibiotic in another hospital. Despite this treatment, the pulmonary symptoms of the patient rapidly worsened, and in August 2017, she was admitted to the hospital for further investigation. She had a history of tuberculosis. There was no history of drug allergy or previous surgery.

A physical examination revealed that she was febrile, with a temperature of 37.4°C, a blood pressure of 135/79 mm Hg, a pulse rate of 73 beats per minute, a respiratory rate of 20 breaths per minute, and an oxygen saturation of 94% on room air. A respiratory system examination revealed bibasilar crackles, expiratory wheeze, bilateral inspiratory crackles, and bronchial

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breathing in the right infraclavicular region. On laboratory examination, the leukocyte count was normal, at 6700/μL, and the eosinophil count was 1220/μL; the ratio was 18.2%. The level of C-reactive protein was 33 mg/L (mildly elevated). The tests for hepatitis B and C viruses and human immunodeficiency virus (HIV) were negative. Computed tomography (CT) of the chest (Fig. 1 A–D) revealed enlarged right hilar and mediastinal lymph nodes, patchy shadows and central bronchiectatic changes with infection in the anterior, posterior, and apical segments of the right upper lobe, bronchial intraluminal shadows suggestive

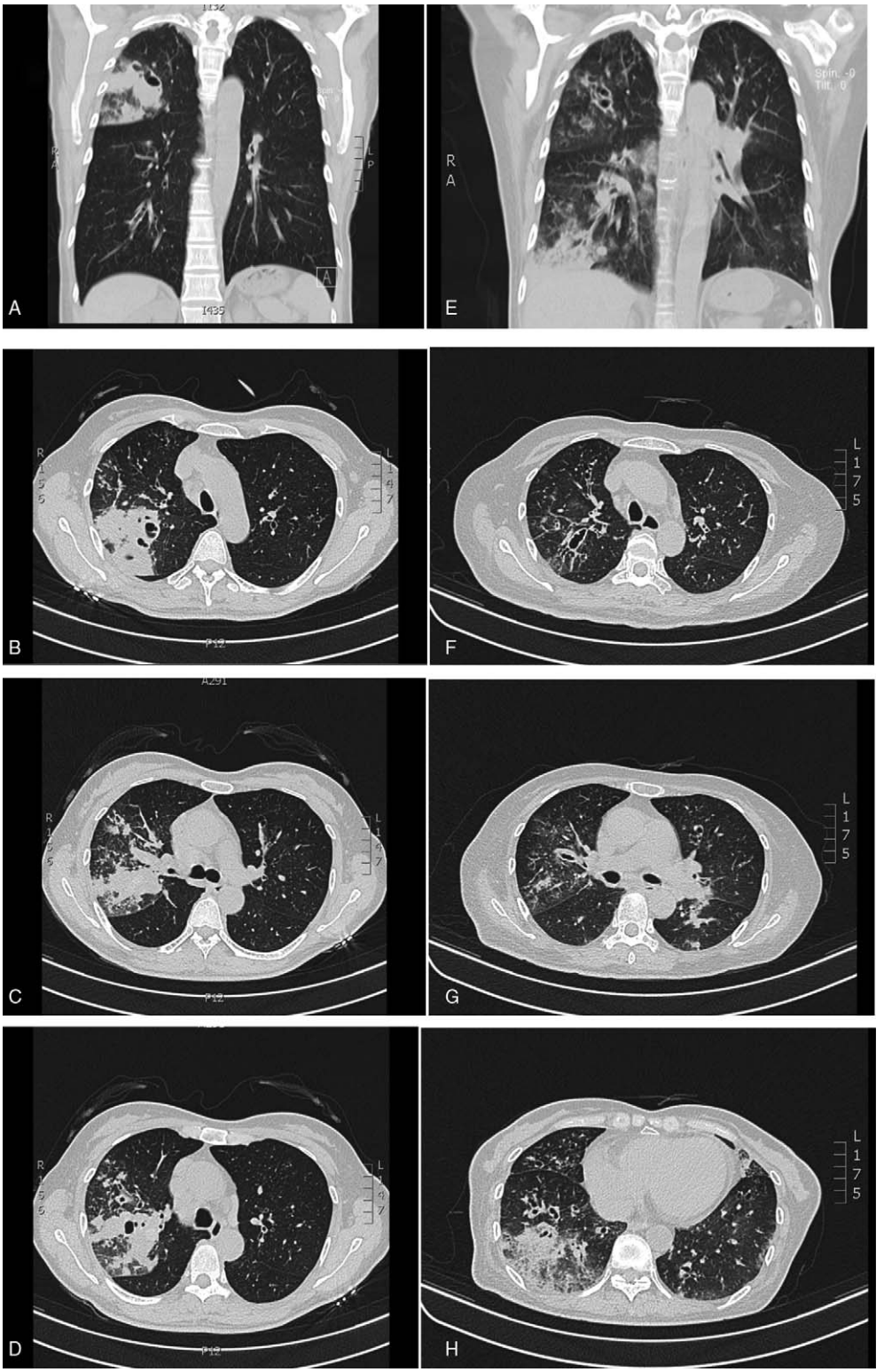

Figure 1. (A–D) Chest computed tomography (CT) findings on the day of admission. (E–H) Chest CT findings at follow-up after 6 weeks of itraconazole therapy for *Aspergillus fumigatus* infection.

of high signal attenuation, and peribronchial wall thickening in the apical segment of the left lower lobe.

We proceeded with further investigations. Ziehl–Neelsen staining of the bronchoalveolar lavage fluid (BALF) was negative for acid-fast bacilli in several replicates, and culture of the bronchoalveolar lavage fluid identified *Aspergillus fumigatus*. The total serum immunoglobulin E level was significantly elevated, at 6340 IU/mL. Serum immunoglobulin M specific for *A fumigatus* was elevated (125 IU/mL). Serum immunoglobulin G specific for *A fumigatus* was elevated (>500 IU/mL). The patient was diagnosed with ABPA and was started on steroid treatment with itraconazole (0.2 g, 2 times per day) and methylprednisolone (32 mg, 1 time per day). After 2 weeks of hospitalization, her respiratory symptoms and daily activity improved. She was discharged from the hospital and advised to follow-up in an outpatient clinic.

After 6 weeks of treatment, the patient again presented to us with complaints of high-grade fever with cough, mucopurulent expectoration and increased shortness of breath accompanied by chest tightness. The patient was referred to the hospital for further evaluation. A respiratory examination revealed bilateral coarse crackles with expiratory wheezing and rhonchi at the end of inspiration in the left lung. The patient was investigated further. A complete blood count demonstrated mild anemia, a hemoglobin level of 114 g/L, neutrophil-predominant leukocytosis with 25,000 cells/μL, and an eosinophil count of 25 cells/μL; the ratio was 0.1%. The level of C-reactive protein was elevated, at 561.1 mg/L. The original level of calcitonin was elevated, at 5.33 ng/mL. CT of the lung (Fig. 1E–H) revealed that the lesion in the upper lobe of the right lung was improved; however, the lung infection in the posterior and apical segments had progressed compared with the anterior segment, with the most obvious progression in the right lower lung, and bronchiectatic changes were visible in both lungs. Ziehl–Neelsen staining of the sputum was repeatedly negative. Interestingly, on the Gram stain, gram-positive bacilli with a beaded filamentous appearance were found. In addition, modified acid-fast staining of the sputum was weakly positive. The sputum used for the fungal culture showed negligible growth, and the sputum used for the bacterial culture revealed *N cyriacigeorgica* (Fig. 2A–C). The isolate was identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF; Bruker Daltonics, Billerica, MA). The result was confirmed by DNA sequencing. Sequencing for identification was completed with a 16S rRNA bacterial identification kit and a 3730xl genetic analyzer (Life Technologies; USA). Using the BLAST search tool based on the GenBank database, the organism in the sample was identified as *N cyriacigeorgica* (100% sequence identity).

Table 1

Antimicrobial susceptibility testing results for *Nocardia cyriacigeorgica*.

Antimicrobial agent	MIC*, μg/mL	Tentative interpretation
Amikacin	0.5	S
Amoxicillin-clavulanic acid	16/8	I
Cefotaxime	2	S
Ceftriaxone	4	S
Cefepime	8	I
Ciprofloxacin	4	R
Gentamicin	1	S
Imipenem	2	S
Linezolid	1	S
Minocycline	2	I
Tobramycin	0.125	S
Trimethoprim-sulfamethoxazole	2/38	S

I = intermediate, MIC = minimum inhibitory concentration, R = resistant, S = susceptible.
* MICs were determined by the *E*-test method.

Moreover, susceptibility testing was performed with the *E*-test method according to the manufacturer's instructions. We subcultured the isolate of *N cyriacigeorgica* on sheep blood agar plates and, after 72 hours, inoculated the isolate into broth to a turbidity equivalent to the 0.5 McFarland standard as measured with a densitometer (Densimat; bioMérieux, Marcy l'Etoile, France). The inoculum was spread on Mueller-Hinton (MH) agar containing various antimicrobial agents, including amikacin, amoxicillin-clavulanic acid, ceftriaxone, gentamicin, imipenem, ciprofloxacin, minocycline, tobramycin, trimethoprim-sulfamethoxazole (TMP-SMX), linezolid, cefepime, and cefotaxime. The MICs were recorded after 72 hours of incubation at 35°C in ambient air and interpreted according the CLSI M24-A2 recommendations.[2] The MICs are shown in Table 1. After susceptibility testing was completed, the antimicrobial treatment was modified to meropenem (500 mg) thrice daily and oral TMP-SMX (80 mg/400 mg) thrice daily. Meropenem treatment was maintained for 4 weeks before suspension, and the plan was to continue itraconazole for 6 to 12 weeks because of the history of *A fumigatus* infection. The respiratory symptoms of the patient had improved both clinically and radiologically after 4 weeks of antibacterial therapy. The patient was discharged, and long-term prophylaxis with oral TMP-SMX (80 mg/400 mg) was continued for 6 weeks.

3. Discussion

The ABPA is an allergic disorder that most commonly involves the respiratory system because of bronchial colonization by

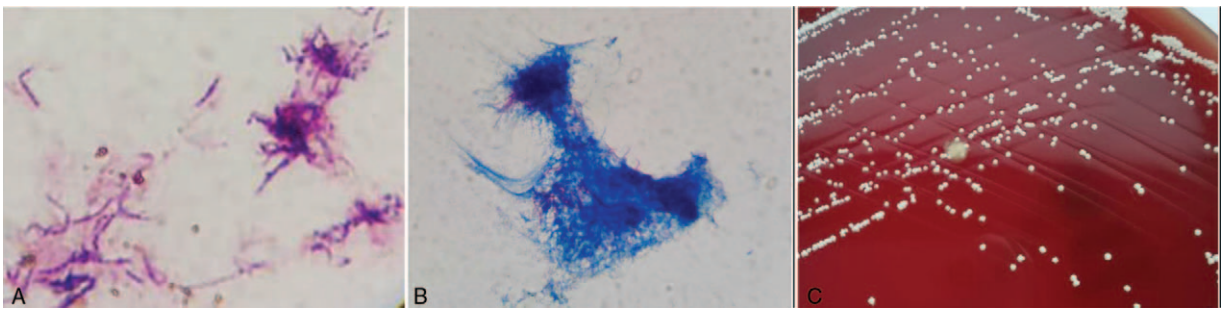

Figure 2. (A) Gram staining (magnification, ×100) of sputum. (B) Modified acid-fast staining (magnification, ×100) of sputum. (C) Colonies of *Nocardia* cultured on blood agar plates for 48 hours.

Aspergillus fungi. The diagnosis of ABPA is based on a clinical and immunologic response to *A fumigatus*. The minimal criteria required for diagnosing ABPA are as follows: asthma or cystic fibrosis with deterioration of lung function, immediate *Aspergillus* skin test reactivity, total serum IgE ≥ 1000 IU/mL, increased *Aspergillus* species-specific IgE and IgG antibodies, and chest radiographic infiltrates. Additional criteria might include peripheral blood eosinophilia, serum precipitating antibodies against *Aspergillus*, central bronchiectasis, and *Aspergillus* species-containing mucus plugs.[3] Patients with bronchial asthma and cystic fibrosis are generally predisposed to ABPA. In due course, ABPA can worsen the course of preexisting asthma or cystic fibrosis and can lead to irreversible lung damage. Delays in diagnosis or undertreatment can lead to bronchiectasis, collapse, consolidation, lung cavitations, pulmonary hypertension, and pulmonary fibrosis independent of comorbidities. Early diagnosis and treatment are essential in preventing a variety of complications. Among the antifungal drugs, itraconazole is the most commonly recommended. Studies have demonstrated that itraconazole significantly reduces total sputum eosinophils, serum IgE, and most importantly, decreases symptoms and the requirement for oral steroids.[4]

At initial evaluation, our patient was diagnosed with ABPA according to the criteria; therefore, we initiated symptomatic treatment based on etiology. The patient returned after six weeks with worsening symptoms. The patient was reappraised, and CT of the lung showed focal infiltrates in the upper lobe of the right lung, increased infection in the right lower lobe, bronchiectatic changes in both lungs, and evidence of infection (increased levels of inflammatory markers, fever, and progressive cough). Hence, our patient was investigated for a possible lung abscess, with tests for invasive aspergillosis and tuberculosis as differential diagnoses. Ziehl–Neelsen staining of the sputum was repeatedly negative for acid-fast organisms, and the sputum used for the fungal culture showed negligible growth. Interestingly, on the Gram stain, gram-positive bacilli with a beaded filamentous appearance were found. The sputum was cultured and revealed *N cyriacigeorgica*. Clinically, nocardiosis is a rare opportunistic gram-positive bacterial infection that is potentially life threatening. Organisms of the genus *Nocardia* are nonmotile, aerobic, and nonspore forming, and they exhibit characteristic filamentous branching with fragmentation into coccoid or bacillary forms; the causative organism in this study comprises a group of phylogenetically diverse but morphologically similar organisms.[1] Recent analyses of collections of *Nocardia* clinical isolates by mass spectrometry and gene sequencing have found that an important proportion of the isolates initially identified by conventional phenotypic and chemotaxonomic methods belongs to the novel species *N cyriacigeorgica*.[5–8] The first case was reported in 2001[9]; later, strains *N cyriacigeorgica* were described as human infectious pathogens in Greece, Turkey, Japan, Thailand, and Canada.[5,6,8,10–14] Opportunistic infections with *Nocardia* in humans occur most commonly in immunocompromised patients; however, approximately one-third of infected patients are immunocompetent. The patients at highest risk are those with a history of solid organ transplantation, hematopoietic stem-cell transplantation, malignancy, HIV infection, and chronic glucocorticoid therapy.[15] Our patient was eventually diagnosed as suffering from ABPA and was treated with steroids and antifungal drugs for 6 weeks. Nevertheless, only a few complete clinical cases of *N cyriacigeorgica* infection have been published so far. Here, we report the first case of infection caused by *N cyriacigeorgica* in an ABPA patient in China.

The isolation of *Nocardia* from any body site should always be regarded as significant in any patient.[1] The most effective isolation method for diagnosis is a noninvasive method for sputum collection. However, an aggressive diagnostic strategy that includes bronchoalveolar lavage and percutaneous lung puncture biopsy can significantly improve the outcome.[16] Staining with Gram stain and modified acid-fast stain are particularly important for providing a rapid, presumptive diagnosis while awaiting culture results.[1] Molecular techniques such as PCR, 16S rRNA gene sequencing, and restriction enzyme analysis have revolutionized the identification of specific *Nocardia* species.[1] In the current case, the isolate was identified by MALDI-TOF (Bruker Daltonics). The result was confirmed by DNA sequencing. Species identification is important because different species have different antibiotic resistance profiles.[17–19]

The rapid identification of *Nocardia* infection is important because delayed diagnosis and treatment have crucial implications for prognosis. For the treatment of nocardiosis, TMP-SMX has been the most common drug of choice for many years.[16,18–20] Linezolid is a welcome and useful addition to the treatment options for nocardiosis and is an excellent potential second-line agent.[21] In the current case, susceptibility testing was performed by the *E*-test method for amikacin, amoxicillin-clavulanic acid, ceftriaxone, gentamicin, imipenem, ciprofloxacin, minocycline, tobramycin, TMP-SMX, linezolid, cefepime, and cefotaxime. The results of the antimicrobial susceptibility testing showed that the isolate was susceptible to most of these drugs and guided us in our decision making for antibiotic treatment. With the evolution of pulmonary infection, meropenem and TMP-SMX were added to our patient's antibiotic treatment protocol. Due to differences in the antibiotic susceptibility patterns of *Nocardia* species, isolates obtained from individual patients should be evaluated for species identification and antibiotic susceptibility, and individualized treatment should be designed accordingly.[18,19]

In summary, this is the first case of pulmonary infection with *N cyriacigeorgica* occurring in a patient with ABPA in China, suggesting that *N cyriacigeorgica* plays an increasing role as an opportunistic pathogen in immunocompromised patients. This case provides a reminder that clinicians should expect the unexpected in terms of infectious agents and emphasizes the importance of microbiological diagnostic procedures.

Author contributions

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Software: Jianyong Wu.
Supervision: Yingping Wu.
Validation: Jianyong Wu.
Visualization: Jianyong Wu.
Writing – original draft: Jianyong Wu.
Writing – review & editing: Jianyong Wu,Yingping Wu, Zhiqiang Zhu.

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