Case Report

Case report: co-infection of *Scedosporium* and *Mycobacterium* in lungs

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Background: There are hundreds of pathogens that cause lung infections. Compared to infections caused by a single pathogen, mixed infections account for a larger proportion of pulmonary infections and have a more severe clinical presentation, while treatment options differ between the two. We aimed to explore the advantages of metagenomic next-generation sequencing (mNGS) in the diagnosis and treatment of mixed infections.

Case Description: To investigate the specific pathogens in a 79-year-old male pneumonia patient who had recurrent cough with poor empirical treatment, we collected bronchoalveolar lavage fluid (BALF) from the patient and performed mNGS technology, along with Sanger sequencing and polymerase chain reaction (PCR) was carried out to confirm the authenticity of the pathogens detected by mNGS. The findings showed that rare pathogen *Scedosporium boydii* (*S. boydii*, reads: 18) and *Mycobacterium avium* complex (MAC, reads: 19) were detected, and the patient was subsequently transferred to another hospital for the same mNGS with the same results as the first detection. Therefore, combined treatment with voriconazole, ethambutol, azithromycin, and levofloxacin were given to the *S. boydii* and MAC for 1 week, and then patient's condition improved and discharged.

Conclusions: mNGS, a non-targeted sequencing technology, could improve the efficiency of clinical diagnosis for mixed infection of rare or atypical pathogens, bring new ideas for clinical pathogen diagnosis, and improve patient prognosis.

Keywords: *Scedosporium boydii* (*S. boydii*); *Mycobacterium avium* complex (MAC); metagenomic next-generation sequencing (mNGS); pulmonary mixed infection; case report

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Introduction

Finding pathogenic microbes is crucial to treating lung infections, however traditional diagnostic methods such as culture and smear microscopy with low sensitivity are mostly used in clinical practice for etiological diagnosis, which are prone to missed diagnosis and misdiagnosis (1). In recent years, with the development of detection technology, more and more atypical and rare pathogens such as *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila* have gradually entered people's field of vision (2). At the same time, among patients with pulmonary infection, the proportion of mixed infection can reach 65.5% (3). Compared with patients infected with a single pathogen, these patients have different antibiotic regimens and more severe clinical manifestations (3).

Because of the potential side effects of combined antibiotic therapy, the diagnosis of co-infected patients must be as precise as possible. However, the timeliness and sensitivity of the current traditional detection methods cannot fully meet the clinical requirements, for patients with atypical pathogens and mixed infections, a rapid, accurate, and full-coverage detection technology is urgently needed (4). Metagenomic next-generation sequencing (mNGS) is a non-targeted sequencing technology based on the second-generation detection platform, which has the advantages of unbiased, non-culture, and complete species coverage (5). It can determine all microbial genome sequences in clinical specimens within 72 hours, thereby identifying new pathogens or rare pathogens (6,7). Compared with traditional detection, mNGS technology can increase the pathogen detection rate of patients with lower respiratory tract infection by 46%, and significantly reduce the 28-day and 90-day mortality of patients with severe lower respiratory tract infection (8,9). This study reports the case of an elderly male patient diagnosed by mNGS with a rare co-infection of *Scedosporium boydii* (*S. boydii*) and *Mycobacterium avium* complex (MAC), who improved after antifungal and mycobacterial therapy. This case is the first international report of mixed infection by these two atypical pathogens. We present this case in accordance with the CARE reporting checklist (available at https://acr.amegroups.com/article/view/10.21037/acr-24-9/rc).

Highlight box

Key findings

- In this study, a 79-year-old male patient with pneumonia was reported. Repeated cough had a poor prognosis after empirical treatment. It was determined to be a mixed infection of *Scedosporium boydii* (*S. boydii*) and *Mycobacterium avium* complex (MAC) by metagenomic next-generation sequencing (mNGS), an emerging molecular detection technology. The patient improved and discharged after changing the drug.

What is known and what is new?

- *S. boydii* is a rare pathogenic fungus, which is easily confused with *Aspergillus* infection. Early and accurate diagnosis of pathogenic species is particularly important.
- This case is the first worldwide report of a mixed infection by *S. boydii* and MAC.

What is the implication, and what should change now?

- mNGS has emerged as a potent tool for detecting mixed infections caused by infrequent or unusual pathogens, hence introducing novel approaches to clinical pathogen detection and offering promising implications for enhancing patient prognosis.

Case presentation

A 79-year-old man was admitted to a local hospital because of cough, expectoration, fatigue and decreased appetite for 1 week. The patient had a history of bronchiectasis and had not received regular treatment. On admission, the patient had no symptoms such as chills, fever, chest tightness, chest pain, and palpitations. His chest computed tomography (CT) showed inflammation in the right middle lobe and right upper lobe, with symptoms of suspected pulmonary tuberculosis, pending investigation (*Figure 1A*). According to clinical experience, he was initially judged to be pulmonary infection, and then anti-infective treatment was given with mezlocillin 1.0 g q8h and etimicin 0.2 g qd. Two weeks later, the chest CT re-examination of the patient presented that the infection had not been absorbed markedly and the symptoms had not improved (*Figure 1B*). Therefore, the antibiotics were adjusted to ceftriaxone 2.0 g qd and moxifloxacin 0.4 g qd. After 10 days, the patient's cough and sputum improved slightly, while there was still chest tightness and discomfort, and the chest CT inflammation did not take a turn for the better (*Figure 1C*). Following the diagnosis by mNGS, CT of the chest 30 days after the patient took voriconazole, azithromycin, levofloxacin, and ethambutol showed significant improvement (*Figure 1D*).

The patient was then transferred to the author's hospital for further diagnosis and treatment. On admission, the patient was conscious and had normal bowel movements, but he was mentally exhausted, had poor sleep quality, and lost about 2 kg in weight compared with 1 month ago. Physical examination displayed that the patient's body temperature was 36.5 ℃, pulse rate was 88 beats/min, respiratory rate was 20 beats/min, blood pressure was 99/65 mmHg, white blood cells (WBC) count was 6.14×10^9/L, and high-sensitivity C-reactive protein (CRP) was 7.5 mg/L. Bronchoscopy was performed on the second day of admission, and demonstrated that the patient had local mucosal congestion and scattered bleeding points. Considering the possibility of tuberculosis, a tuberculosis screening test was carried out. We learned from the outcomes that bronchoalveolar lavage fluid (BALF) acid-fast staining was positive, T-cell test for tuberculosis infection was negative, and sputum Tuberculosis-Xpert was negative. At the same time, the patient's BALF was tested for mNGS to explore possible pathogens. The mNGS results

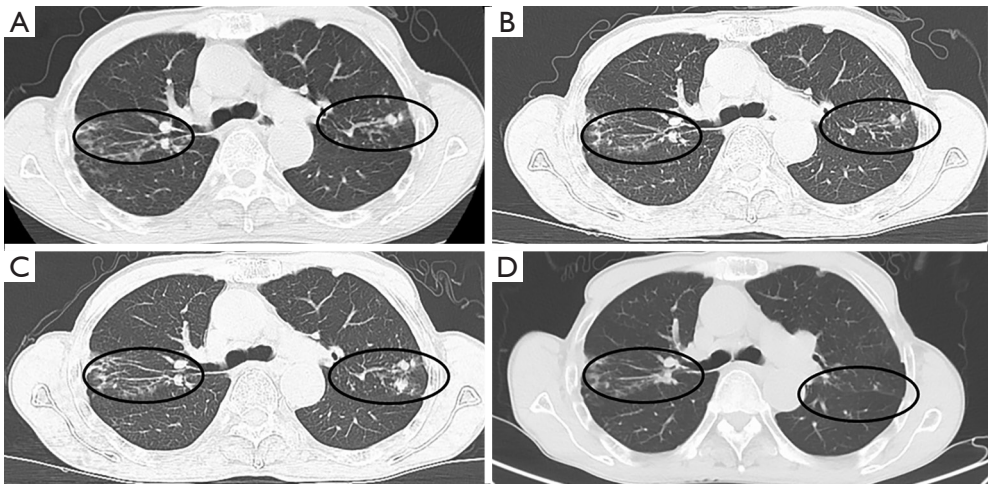

Figure 1 Chest CT scan results of the patient before and after treatment. (A-C) CT of the chest before admission. (D) CT of the chest 30 days after the patient took voriconazole, azithromycin, levofloxacin, and ethambutol. The pictures indicate the lesions, including the lesions in (A-C) and the absorption of the lesions in (D). The circles referred to the infected lesions shown on the patient's CT. CT, computed tomography.

indicated that a total of 23,126,362 single-end sequences of genomic DNA were detected in BALF specimens and 18 single-end sequences of the pathogen *S. boydii* were discovered, which relative abundance was 6.62% and the coverage was 0.0443% (*Figure 2A*). Moreover, 19 sequences were detected in the MAC with a relative abundance of 1.24% and a coverage rate of 0.0538% (*Figure 2B*). Subsequently, Sanger sequencing and polymerase chain reaction (PCR) were executed to confirm the authenticity of the *S. boydii* and MAC present in the patient's BALF. Agarose gel electrophoresis showed positive for *S. boydii* (orange arrows) (*Figure 3A*). Besides, the Ct value of *S. boydii* quantitative PCR (qPCR) detection was 30 and the amplification curve was shown in *Figure 3B*. What's more, positive for MAC as seen on agarose gel electrophoresis (shown by orange arrow) (*Figure 3C*). The Ct value of the MAC qPCR detection was 32, and the amplification curve was shown in *Figure 3D*.

Considering that a hospital in Shanghai reported a successful case of diagnosis and treatment of a pulmonary infection patient with *S. boydii* 6 months ago, the patient then was referred to the hospital for treatment. When admitted to the hospital, the patient still had cough and sputum. Afterwards, the hospital implemented bronchoscopy examination and sent the patient's right middle lobe BALF for mNGS detection again. The second mNGS results of the patient were consistent with the first results, and both *S. boydii* and MAC were detected. Therefore, based on the above test results, it was judged that the patient was a pulmonary infection caused by *S. boydii* and MAC, then the treatment option was changed to voriconazole 0.2 g q12h + azithromycin 0.25 g qd + levofloxacin 0.5 g qd + ethambutol 0.75 g qd, supplemented with diammonium glycyrrhizinate 100 mg tid to protect the liver and Aipula enteric-coated tablets 5 mg qd to protect the stomach. Patient was also instructed to keep warm and avoid cold and fatigue during outpatient follow-up visits. The patient was discharged from the hospital after taking the medicine for 1 week as instructed, and went to Jiangyin People's Hospital Affiliated to Nantong for re-examination 4 weeks after discharge. At this time, the patient's symptoms of cough and fatigue were remarkably relieved, and the re-examination of the chest CT showed that the infection was more absorbed than before (*Figure 1D*). The re-examination of liver and kidney functions was normal. Subsequently, the four drugs were continued to be taken orally for 3 months and then discontinued. The patient was free of respiratory symptoms and had a good dietary intake so far during the follow-up. The patient's treatment timeline is shown in *Figure 4*.

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki

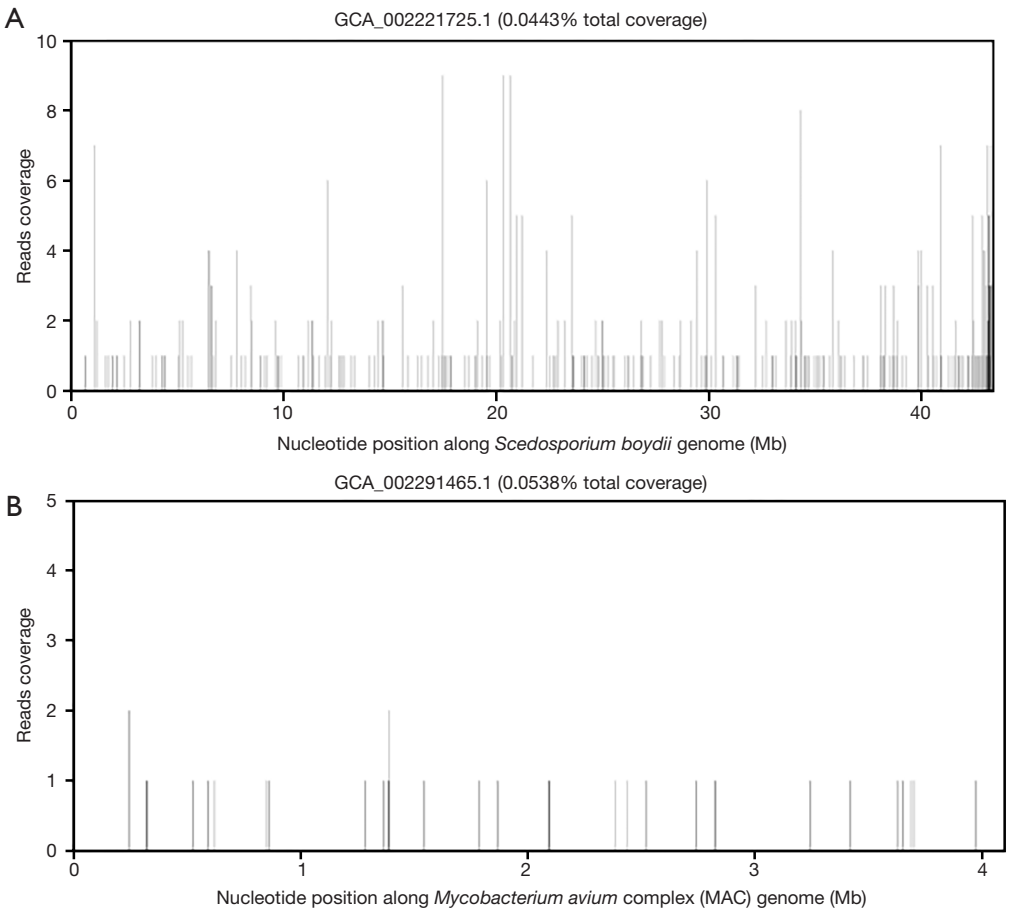

Figure 2 Coverage map of pathogen detection. (A) The coverage map of *Scedosporium boydii*. (B) The coverage map of *Mycobacterium avium* complex.

Declaration (as revised in 2013). 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 editorial office of this journal.

Discussion

S. boydii (previously named *Pseudallescheria boydii*) is commonly found in soil and sewage and can cause infectious diseases (pneumonia, osteomyelitis, arthritis, sinusitis, endocarditis, meningitis, and brain abscesses, etc.) and severe diffuse systemic disease (10). The initial symptoms of pulmonary infection often include fever, cough, sputum, chest pain, shortness of breath, etc. and its imaging (11) and pathological (12) manifestations are similar to other fungal infections, especially *Aspergillus* infections (13). Therefore, it is prone to misdiagnosis in clinical practice. One study reported a case of misdiagnosis of *S. boydii* infection as *Aspergillus* infection, and the patient eventually died (14). At the same time, the drug susceptibility of *S. boydii* and other fungi of the *Aspergillus* is different (15). To date, a few cases of have been found in several countries (*Table 1*). A study has revealed that *S. boydii* is sensitive to voriconazole, but is insensitive to other antifungal drugs such as micafungin and posaconazole, so accurate diagnosis of pathogenic species is particularly important (15). In China, there was a case of *S. boydii* confirmed by mNGS and mass spectrometry (23), but unlike this case, another atypical pathogen, the MAC, was additionally detected in this patient. MAC is one of the most common types of nontuberculous mycobacteria (NTM) (24), which is a general term for mycobacteria other than *Mycobacterium tuberculosis* and *Mycobacterium leprae*, and contains a wide range of species, so far there are more

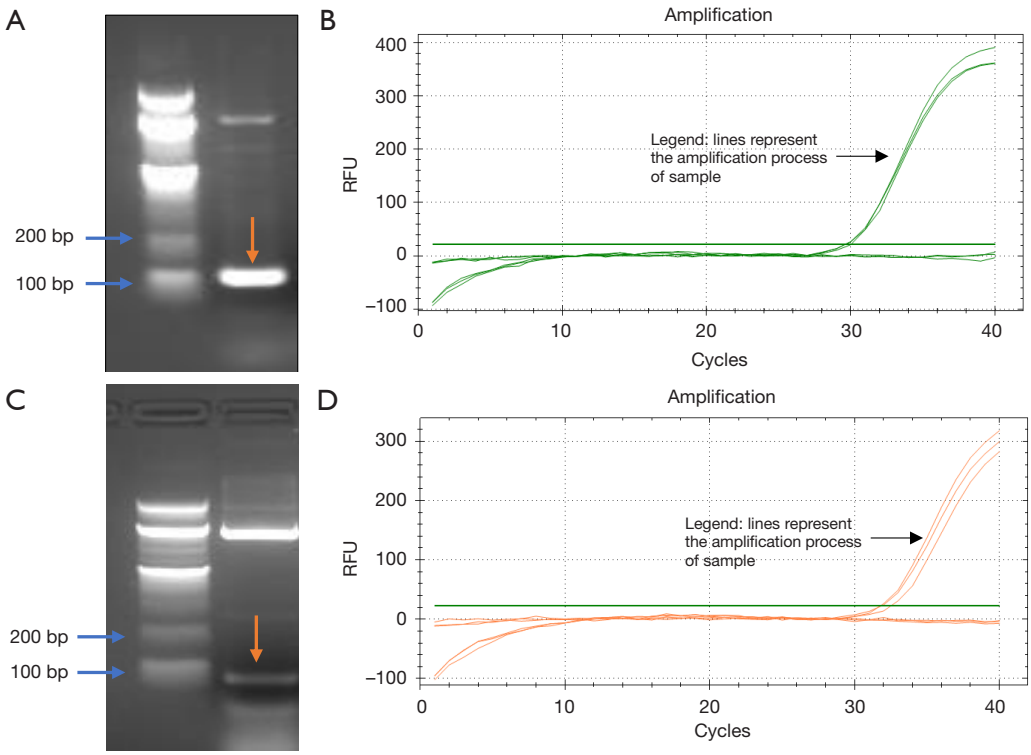

Figure 3 PCR electropherogram and qPCR analysis results of pathogen. (A) PCR electropherogram of *Scedosporium boydii*. (B) qPCR analysis result of *Scedosporium boydii*. (C) PCR electropherogram of MAC. (D) qPCR analysis result of MAC. The blue arrows represented the fragment size of the reference marker. Orange arrows showed positive detection of *Scedosporium boydii* and MAC, respectively. The curved lines in (B,D) represent the amplification process of each sample. The straight green line denotes threshold. qPCR, quantitative polymerase chain reaction; MAC, *Mycobacterium avium* complex; RFU, relative fluorescence unit.

than 190 species (25). MAC exists in the water and soil environment (24), the pathogen can cause lung infection, and people with advanced age, low body mass index, and immunodeficiency are susceptible (26,27). When the patient is infected, it can invade a variety of organs and the lung infection is the most common (24). MAC is difficult to diagnose by traditional tests due to the slow culture rate and nonspecific imaging findings of patients with pulmonary infection (28). Therefore, it is necessary to use new detection techniques such as mNGS. One study reported a patient with *Mycobacterium avium* pulmonary infection detected by mNGS but not identified by traditional testing, which provided clear information for the patient's follow-up treatment (29). The triple-drug regimens recommended in the treatment guidelines for *Mycobacterium avium* include macrolides, ethambutol and rifamycin (28), and for immunocompromised patients, nutrition and immunity should be enhanced (30).

Reviewing the patient's medical history, it was found that he had the symptoms of repeated coughing and expectoration. The test results were generally consistent with clinical features and imaging findings, but the specificity was not strong. Moreover, compared with the case reported in Shanghai, the patient was older, and the etiological test displayed that the infection of the patient was more complicated, which made the follow-up treatment more difficult. Therefore, mNGS technology provides important clinical evidence for the pathogenic diagnosis of patients in time. Regarding the treatment options, for *S. boydii*, hypersensitivity voriconazole was given. And for MAC, patients with nodular/bronchiectasis MAC disease should be given a macrolide (clarithromycin or azithromycin), rifampicin or rifampin and ethambutol combined therapy, while considering that the patient was too old, it might cause adverse reactions and drug resistance, only ethambutol was given here. Although the drug susceptibility test was not performed, and the treatment plan was not completely consistent with the

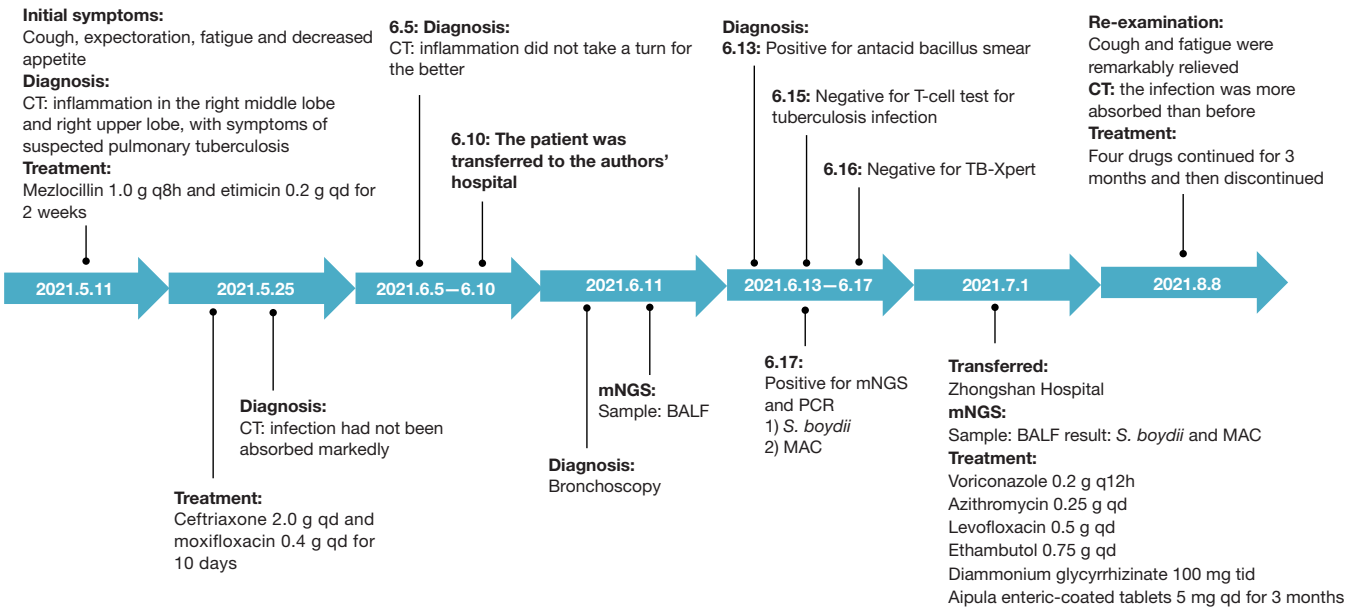

Figure 4 The treatment timeline of the patient. CT, computed tomography; BALF, bronchoalveolar lavage fluid; *S. boydii*, *Scedosporium boydii*; MAC, *Mycobacterium avium* complex; mNGS, metagenomic next-generation sequencing; PCR, polymerase chain reaction; TB-Xpert, Tuberculosis-Xpert.

Table 1 A few cases of *Scedosporium boydii* infection reported globally

Year	Area	Disease situation	Infection site	Sample type	Patient outcome	References
2015	Germany	Six non-immunocompromised patients	Not described	FFPE or isolates	3/6 died, 2/6 no follow-up	(12)
2020	America	A patient with chronic renal failure	Systemic infection	FFPE	Died	(14)
2022	China	A systemic lupus erythematosus patient	Brain abscess	CSF	Not described	(16)
2022	America	Trimalleolar fracture	Fungal osteomyelitis	Bone tissue	Successfully treated	(17)
2023	China	Kidney transplant recipients	Central nervous system infection	CSF	Successfully treated	(18)
2023	Argentina	A heart transplant patient	Systemic infection	The isolate	Died	(19)
2024	America	A patient without any known risk factors	Systemic infection	Not described	Died	(20)
2024	China	An immunocompetent case	Systemic infection involved in lung, brain and spine	BALF	Symptoms improved	(21)
2024	Spain	An immunocompetent patient	Pulmonary infection	Lung and brain abscess cultures	Died	(22)

FFPE, formalin-fixed paraffin-embedded; CSF, cerebrospinal fluid; BALF, bronchoalveolar lavage fluid.

guidelines recommended, good results were achieved after treatment. The symptoms and imaging manifestations of the patients were prominently improved, and there were no adverse reactions such as liver and kidney function damage, indicating the accuracy and timeliness of diagnosis and treatment.

Compared to traditional methods for detecting pathogenic microorganisms, mNGS offers the primary advantage of unbiased sampling. This allows for the simultaneous identification of all potential infectious agents in samples without the need to predefine diagnostic targets (31). Furthermore, due to its high sensitivity, short turnaround time, and minimal interference from antibiotics, mNGS has found widespread application in clinical diagnostics. In order to promote the popularization and standardized development of mNGS technology, many academic consensus and technical guidelines have been published at home and abroad. To ensure the accuracy of mNGS testing, diagnostic laboratories establish quality control systems. These systems aim to reduce cross-sample contamination, eliminate host DNA interference, enhance the efficiency of microbial nucleic acid extraction methods, and improve the comprehensiveness of reference databases (32).

A number of studies have been conducted to assess the clinical impact of mNGS on pulmonary infections. Compared to traditional detection methods, mNGS has demonstrated a sensitivity of 93.5% across all clinical samples, with a sensitivity of 95.4% specifically for BALF. These findings indicate that mNGS can broadly identify clinically significant microorganisms involved in lower respiratory tract infections. In terms of clinical treatment strategies, mNGS has been shown to direct medication adjustments and prognosis in 80.7% of patients (33). BALF provides a comprehensive reflection of the overall condition of the lungs, and BALF mNGS is increasingly being utilized for the diagnosis of pulmonary infections (34).

The application process of mNGS in clinical settings is complex and faces several challenges. These include high levels of human-derived nucleic acids in specimens, difficulties in distinguishing commensal microorganisms from infectious pathogens, and high testing costs, all of which constrain the widespread adoption of mNGS. However, encouraging the innovation of the sequencing technology and the widespread of laboratory automation will contribute to the cost reduction, thereby further promote its clinical application (31).

Conclusions

The patient in this case is the world's first reported case of pulmonary *S. boydii* combined with MAC infection by mNGS method. At present, mNGS has been gradually applied to the diagnosis of clinically complex infection cases, and its sensitivity and specificity are superior to traditional culture, especially for *Mycobacterium tuberculosis*, virus and fungus (35). As a new and rapid pathogenic diagnostic tool, mNGS is faster than traditional laboratory culture methods. It can improve the efficiency of clinical diagnosis for mixed infection of rare or atypical pathogens, bring new ideas for clinical pathogen diagnosis, and improve patient prognosis.

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Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at https://acr.amegroups.com/article/view/10.21037/acr-24-9/rc

Peer Review File: Available at https://acr.amegroups.com/article/view/10.21037/acr-24-9/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://acr.amegroups.com/article/view/10.21037/acr-24-9/coif). W.W. is a current employee of Dinfectome Inc., Nanjing, China. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are 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 procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for the publication of this case report and

accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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