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Case Report

mNGS-based dynamic pathogen monitoring for accurate diagnosis and treatment of severe pneumonia caused by fungal infections

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ABSTRACT

Metagenomic next-generation sequencing (mNGS) has been widely applied to identify pathogens associated with infectious diseases. However, limited studies have explored the use of mNGS-based dynamic pathogen monitoring in intensive care unit patients with severe pneumonia. Here, we present a clinical case of an 86-year-old male patient with severe pneumonia caused by a fungal infection. During the clinical treatment, four mNGS analyses were performed within two consecutive weeks. Various respiratory fungal pathogens, including *Candida orthopsilosis*, *Candida albicans,* and *Aspergillus fumigatus* were detected by mNGS of bronchoalveolar lavage fluid (BALF). Based on conventional pathogen identification and clinical symptoms, the patient was diagnosed with severe pneumonia caused by a fungal infection. The abundance of fungal species decreased gradually in response to antifungal and empirical therapies, and the fungal infections were effectively controlled. In summary, our results demonstrated that mNGS could effectively identify pathogens in patients with severe pneumonia. Additionally, dynamic pathogen monitoring based on mNGS could assist in the precise diagnosis of complex infections and may facilitate rapid induction of the most appropriate therapy.

1. Introduction

Severe pneumonia is the leading cause of morbidity and mortality in intensive care unit (ICU) patients [1]. Lung infections can be caused by various microorganisms [2]. However, serious fungal infections can have much more severe outcomes, especially in immunocompromised patients [3]. In more than half of patients with severe pneumonia, the causative agents are unclear due to limitations in sample collection and diagnostic methods, interference with antibiotic treatment, and contamination [4,5]. Traditional culture methods are widely utilized for detecting fungi and bacteria, but their efficacy is limited by a lower positive detection rate and time-consuming procedures, which make them unsuitable for meeting clinical demands. Although alternative methods, such as pathogen-specific antibodies (serology), antigenic immunological methods, and the polymerase chain reaction (PCR) can identify certain specific pathogens, they are not suitable for identifying unknown pathogens. Traditional diagnostic methods may therefore lead to misdiagnosis, missed or delayed treatment, and widespread empirical use of broad-spectrum antibiotics [6]. Moreover, the emergence of multi-drug resistant pathogens and infections caused by multiple pathogens has further complicated the identification of underlying causes of infections [7–9]. Previous studies have shown that metagenomic next-generation sequencing (mNGS) can optimize treatment strategies [10,11]. As a revolutionary diagnostic tool, mNGS can rapidly and accurately identify a wide variety of pathogenic bacteria, viruses, fungi, and parasites with high sensitivity [12,13]. Unlike traditional detection strategies, mNGS requires only two days from sample preparation to pathogen determination and is less affected by prior antibiotic treatment [11,14]. This study used mNGS to dynamically monitor changes in pathogenic microorganism abundance in

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the bronchoalveolar lavage fluid (BALF) and sputum of a patient with severe pneumonia to evaluate the potential clinical application of mNGS for identifying pathogens causing severe pneumonia.

2. Case presentation

An 86-year-old male patient was admitted with a recurrent cough, sputum production, and dyspnoea for 20 years on 15 February 2022. The patient had smoked for more than 60 years and had a 7-year history of type 2 diabetes and gout. Upon admission, the patient presented with a 38.1℃ fever, a pulse rate of 95 beats/min, and a blood pressure of 171/82 mmHg. Routine blood examination indicated a white blood cell count of 10.14 × 10^9/L (normal, 3.5 × 10^9–9.5 × 10^9/L), red blood cell count of 3.65 × 10^{12}/L (normal, 4.3 × 10^{12}–5.8 × 10^{12}/L), haemoglobin (HB) 86 g/L (normal, 130–175 g/L), platelet count (PLT) 54 × 10^9/L (normal, 125 × 10^9–350 × 10^9/L), neutrophils 89% (normal, 40%–75%), lymphocytes 5% (normal, 20%–50%), hypersensitive C-reactive protein > 10 mg/L (normal, 0–1 mg/L), C-reactive protein 137.19 mg/L (normal, 0–10 mg/L), and procalcitonin 4.39 ng/mL (normal, 0–0.05 ng/mL). The patient presented with indeterminable consciousness, weak bilateral breath sounds, and audible wet rales without pleuritic rub sounds. Chest computed tomography (CT) revealed lesions in both lungs, pleural thickening on the right side, multiple solid nodular lesions in the left lung, and left pleural effusion. The patient was diagnosed with severe pneumonia. The timeline of clinical manifestation and treatment is shown in Fig. 1A.

On 16 February 2022, the patient was treated with moxifloxacin (MXF) 0.4 g once-daily (qd) in combination with imipenem and cilastatin sodium for injection (TIENAM) 1 g every 6 h (q6h). BALF was collected to conduct mNGS and conventional microbial detection. Digital chest radiography (CR) showed multiple bilateral pulmonary changes, patchy high-density shadows, increased and blurred bilateral lung markings, and decreased right lung volume. Repeat routine blood examination indicated a slight decrease in white blood cell counts to 8.99 × 10^9/L with 87.06% neutrophils. BALF smears were positive for fungi, but negative for bacteria. On 18 February 2022, BALF cultures revealed *Candida glabrata* and a small amount of pharyngeal flora. A large number of fungi and spores found in the BALF smears and positive serum galactomannan (GM) indicated that the MXF and TIENAM treatment was suboptimal. Thus, the therapy was immediately adjusted to MXF 0.4 g qd with voriconazole (VRZ) 0.2 g q12h. On 19 February 2022, a second BALF sample was collected for mNGS due to the uncontrolled pulmonary infection. On 22 February 2022, the white blood cell count decreased further to 7.99 × 10^9/L with 85% neutrophils, the procalcitonin level decreased to 1.03 ng/mL, and the C-reactive protein level increased to 157.3 mg/L. Repeat CR showed progressive lesions compared with the previous examination. TIENAM was reintroduced and a third BALF sample was collected for mNGS. On 28 February 2022, a repeat examination indicated a decreased procalcitonin level of 0.64 ng/mL, and the symptoms gradually subsided. A repeat CR revealed increased pleural effusion on the right side, while the left-sided thoracic cavities were reduced in size. As smear microscopy still revealed fungal spores, treatment was modified to caspofungin (CAS) 0.05 g qd and TIENAM 1 g q8h. A sputum sample was collected for the fourth mNGS. On 2 March 2022, BALF cultures revealed pharyngeal flora and gram-negative bacillus, but no fungi were found, indicating that the fungal lung infection was effectively controlled. A review of the BALF culture 2 days after discharge revealed *Klebsiella pneumoniae*.

During the treatment, we used dynamic mNGS to monitor the changes in microbial species in both BALF and sputum samples (Table 1 and Fig. 1B). The initial mNGS results showed that *Candida orthopsilosis*, *Candida albicans*, *Proteus mirabilis*, *Stenotrophomonas maltophilia*, and *Aspergillus fumigatus* were the co-infecting pathogens. To track the longitudinal progress of the microbial infection, mNGS was performed an additional three times. The fungal species abundance showed a gradual decrease in response to antifungal and empirical treatment, while several bacterial pathogens were detected in sputum, including *Corynebacterium striatum*, *Corynebacterium resistens*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterococcus faecium*. Comparison with previous NGS test results showed that most of these pathogens were emerging and re-emerging nosocomial drug-resistant pathogens. Supplemental Fig. 1 presents the changes in inflammatory marker levels and lymphocyte percentage in this case. These data suggest that mNGS could be a more direct method to monitor changes in pathogen load, thus aiding the monitoring of disease progression and treatment efficacy.

3. Discussion

As mNGS technology continues to evolve, mNGS-based direct and semi-quantitative surveillance has become a powerful tool for screening and comparing microbial composition and variation [15,16]. Compared with gut microbiome research, studies of the respiratory microbiome are still in the early stages, and research on the fungal microbiome is especially limited. Methodological problems such as small sample size and lack of longitudinal data have been encountered in most studies of the fungal microbiome [17]. Moreover, the pulmonary microbiome can influence disease susceptibility and may also be influenced by disease activity or treatment [18]. This longitudinal study of the lung microbiome in severe pneumonia provides new insight for the development of individualised precision medicine.

Fungal pathogen infections are a growing public health concern worldwide [19,20]. In February 2023, the World Health Organization (WHO) published its first list of fungal priority pathogens which include *Cryptococcus neoformans*, *Candida auris*, *Aspergillus fumigatus*, and *Candida albicans* [21]. In recent decades, the incidence of pulmonary fungal infections has increased significantly in immunocompromised patients [22]. A study of the distribution of oral fungal communities in healthy populations found that 36.1% of fungal species are indetectable using traditional microbiological culture methods [23]. In contrast to fungal cultures, which are often time-consuming and challenging, mNGS is an unbiased and efficient method for rapid pathogen identification and may therefore overcome current diagnostic limitations [24]. mNGS has unique advantages in detecting pulmonary fungal infections. According to Miao et al., mNGS exhibits better performance in detecting fungi compared with conventional methods (OR, 4.0 [95% CI, 1.6–10.3]; $P < 0.01$) [14]. Current pulmonary fungal studies mostly focus on patients with cystic fibrosis (CF) [25], and fungi such as *Aspergillus fumigatus* and *Candida* are important pathogens responsible for the development of lung infections in patients with CF [26]. The medical history of the patient in this case study suggests relatively poor immunity and therefore increased susceptibility to opportunistic pathogen invasion of the lungs. The first BALF samples from the patient in this case study revealed multiple fungi. While *Candida albicans* is the most common fungal pathogen in the ICU [27]and is responsible for more than half of all candidemia cases [28], the first mNGS analysis in this case showed that *Candida orthopsilosis* comprised 92.6% of the *Candida* species. The incidence of non-*Candida albicans* infections has been increasing in recent years, and research on non-*Candida albicans* is becoming increasingly important [29]. Additionally, mNGS also detected *Aspergillus fumigatus* in the first BALF specimen. *Aspergillus fumigatus* is one of the most common fungal pathogens [30] and invasive pulmonary aspergillosis remains a leading cause of death in immunocompromised patients [31]. A significant decrease in fungal abundance was observed during antibiotic treatment, consistent with the laboratory findings. In summary, mNGS exhibits promising clinical value for the accurate detection of pulmonary fungal infections.

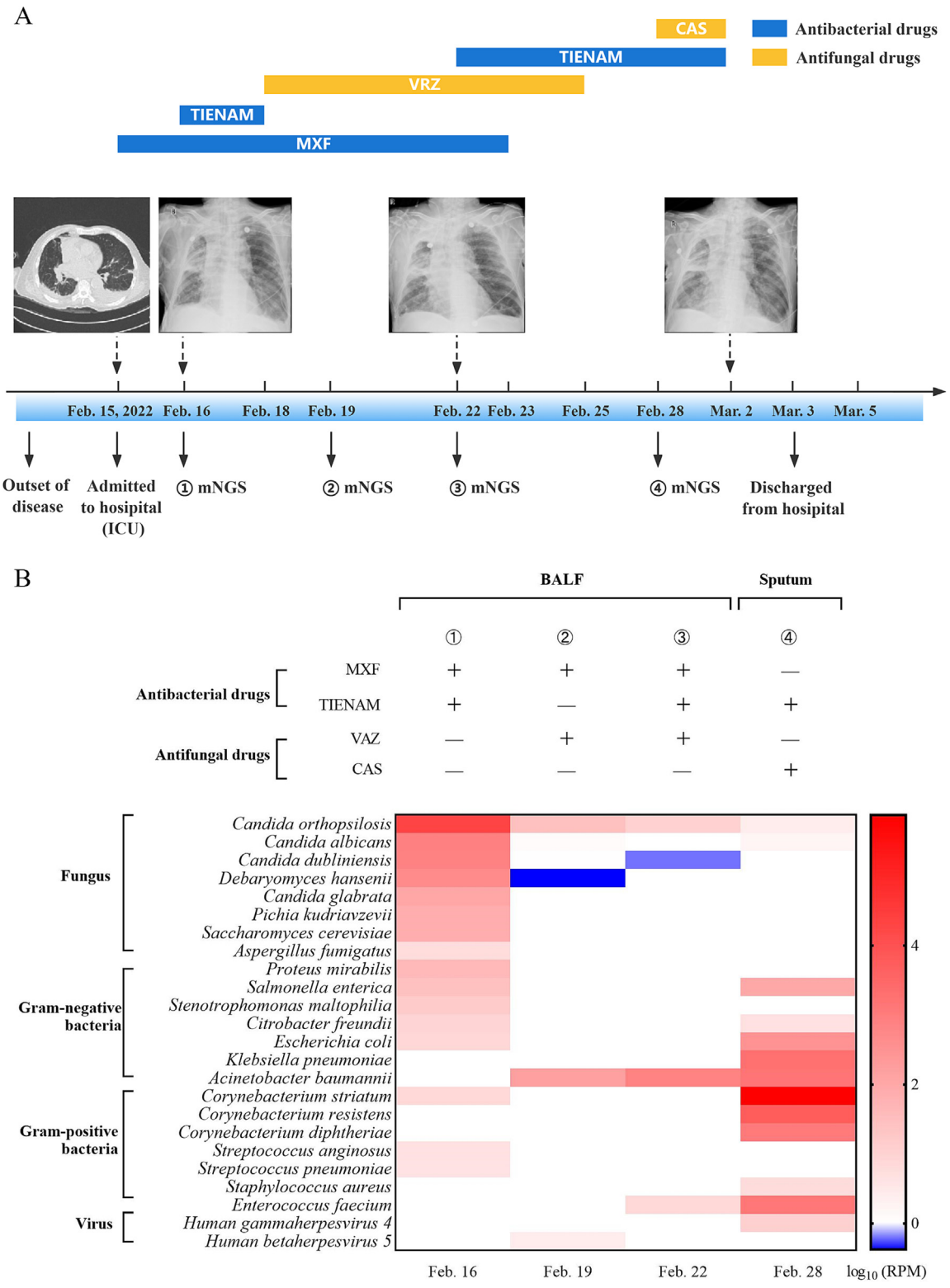

Fig. 1. Clinical course and dynamic pathogen monitoring. A) Timeline of diagnosis and treatment. MXF, moxifloxacin; TIENAM, imipenem, and cilastatin sodium for injection; VRZ, voriconazole; CAS, caspofungin; mNGS, metagenomic next-generation sequencing. B) Changes in microorganism abundance in respiratory samples following antibiotic treatment. RPM, reads per million mapped read; BALF, bronchoalveolar lavage fluid.

Several studies have shown that delayed or insufficient use of antibiotics in patients with community-acquired pneumonia can increase the risk of respiratory failure and sepsis [32]. However, inappropriate antibiotic use has led to an intractable problem of opportunistic pulmonary infections in immunocompromised patients. Infections caused by multi-drug resistant (MDR) pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), multi-drug resistant *Streptococcus pneumoniae* (MDRSP), and multi-drug resistant

Table 1
Abundance of pathogens detected by metagenomic next-generation sequencing (mNGS) in consecutive samples.

	BALF* (reads)			Sputum (reads)
	2022-2-16	**2022-2-19**	**2022-2-22**	**2022-2-28**
Fungus	*Candida orthopsilosis* (465,334) *Candida albicans* (19,634) *Candida dubliniensis* (17,555) *Debaryomyces hansenii* (10,670) *Candida glabrata* (2,484) *Pichia kudriavzevii* (1,796) *Saccharomyces cerevisiae* (1,721) *Aspergillus fumigatus* (138)	*Candida orthopsilosis* (782) *Candida albicans* (35) *Candida dubliniensis* (29) *Debaryomyces hansenii* (12)	*Candida orthopsilosis* (320) *Candida dubliniensis* (18)	*Candida orthopsilosis* (32) *Candida albicans* (20)
Bacteria	*Proteus mirabilis* (933) *Salmonella enterica* (653) *Stenotrophomonas maltophilia* (379) *Citrobacter freundii* (225) *Escherichia coli* (192) *Corynebacterium striatum* (181) *Streptococcus anginosus* (115)	*Acinetobacter baumannii* (4,218)	*Acinetobacter baumannii* (20,511) *Enterococcus faecium* (227)	*Corynebacterium striatum* (9,180,733) *Corynebacterium resistens* (66,584) *Klebsiella pneumoniae* (23,673) *Acinetobacter baumannii* (18,366) *Enterococcus faecium* (15,144) *Corynebacterium diphtheriae* (13,245) *Escherichia coli* (3,726) *Salmonella enterica* (1,207) *Tannerella forsythia* (114) *Staphylococcus aureus* (77) *Citrobacter freundii* (60)
Virus	/	*Human betaherpesvirus 5* (75)	/	*Human gammaherpesvirus 4* (141)

*BALF = bronchoalveolar lavage fluid.

Acinetobacter baumannii (MRAB), may not respond to conventional antibiotic therapy, resulting in unsatisfactory clinical outcomes [8,33,34]. Therefore, the use of proper drugs for timely treatment is crucial for the best prognosis [35,36]. The fourth sputum specimen revealed a high abundance of *Acinetobacter* and *Corynebacterium*. Globally, 44.3% of *Acinetobacter baumannii* isolates were classified as MDR [7]. *Acinetobacter baumannii*, an opportunistic pathogen responsible for morbidity and mortality in immunocompromised patients [37,38], was detected in three consecutive mNGS. In the last mNGS, *Corynebacterium striatum* was the predominant species. The relative abundance of *Corynebacterium striatum* increased during antibiotic treatment, possibly due to its drug resistance. Although *Corynebacterium* spp. are considered normal flora and are often disregarded when isolated from clinical specimens [39], *Corynebacterium striatum* has recently emerged as an MDR pathogen capable of causing nosocomial outbreaks [40]. *Klebsiella pneumoniae* is also a nosocomial pathogen frequently found in ICU patients [41]. Patients with longer ICU stays are more susceptible to ICU-acquired infections, necessitating the rapid identification of pathogenic bacteria and their antibiotic susceptibility to treat severe pneumonia promptly and shorten hospitalization and mechanical ventilation time.

The patient in this case was diagnosed with a lung co-infection of fungi and bacteria based on a comprehensive assessment of clinical features, laboratory tests, and imaging results. A previous study reported that 40.3% of patients with fungal lung infections had mixed fungal and bacterial infections [42]. Immunocompromise is a major risk factor for bacterial and fungal co-infection [43], and inflammatory pulmonary lesions also increase susceptibility to co-infection. Moreover, the use of multiple antibiotics suggests the presence of more complex infections. Long-term use of antibiotics (≥14 d) not only increases drug resistance but also disrupts the normal flora, leading to dysbiosis of the microbiome. Therefore, the identification of mixed fungal and bacterial infections is crucial for appropriate clinical management [42].

This study had some limitations. Firstly, it was a retrospective study. Secondly, the patient was treated with antibiotics before the collection of BALF and sputum samples, which may have impacted the accuracy of the traditional culturing results. It is worth noting that mNGS technology has certain inherent limitations, including high cost, susceptibility to interference by host nucleic acids and background pathogenic microorganisms, as well as a lack of standards for pathogenic microorganism report interpretation [12]. Nonetheless, it can be used as a complementary diagnostic method [44].

In summary, this case report indicates that mNGS can help identify pathogens quickly and effectively, increase diagnostic accuracy, and improve treatment outcomes and prognosis. Furthermore, mNGS can provide clinicians with the information required for the treatment of severe infectious diseases [45]. Consistent with previous studies [18,46], we observed that changes in the respiratory microbiome were linked to disease progression in severe pneumonia. Our results suggest that mNGS-based dynamic pathogen monitoring is useful for the precise diagnosis and treatment of severe pneumonia.

Ethics statement

This study involving a human participant was reviewed and approved by the National Institute for Viral Disease Control and Prevention Ethics Committee, China CDC (No. IVDC2022-001). The patient provided written informed consent to participate in the study.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Zhen Li: Data curation, Writing – original draft. **Changcheng Wu:** Data curation, Writing – original draft. **Li-An Tang:** Data curation, Writing – original draft. **Yinjie Liang:** Investigation, Writing – review & editing. **A. Ruhan:** Investigation, Writing – review & editing. **Debin Huang:** Investigation, Writing – review & editing. **Chuanyi Ning:** Conceptualization, Methodology, Writing – review & editing. **Wenling Wang:** Conceptualization, Methodology, Writing – review & editing.

Wenjie Tan: Conceptualization, Methodology, Writing – review & editing.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bsheal.2023.04.004.

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