

Comparative Evaluation of mNGS and Traditional Culture Methods in Pathogen Detection for Pulmonary Infections

Weijun Chen^{1,*}, Ruijie Liu^{1,*}, Qiyong Qi¹, Lingen Xu², Guiqin Sun³

¹Laboratory Department, Xinchang Hospital of Traditional Chinese Medicine, Shaoxing, Zhejiang, People's Republic of China; ²Intensive Care Unit, Xinchang Hospital of Traditional Chinese Medicine, Shaoxing, Zhejiang, People's Republic of China; ³School of Medical Technology and Information Engineering, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, People's Republic of China

*These authors contributed equally to this work

Correspondence: Guiqin Sun, School of Medical Technology and Information Engineering, Zhejiang Chinese Medical University, 548 Binwen Road, Binjiang District, Hangzhou, Zhejiang, People's Republic of China, 310053, Tel +86 13868118601, Fax +86 0571-86633307, Email sunguiqin2001@163.com; Lingen Xu, Intensive Care Unit, Xinchang Hospital of Traditional Chinese Medicine, No. 188, Shijiu Feng Road, Qixing Street, Shaoxing, Zhejiang, People's Republic of China, 312500, Tel +86 13857514222, Fax +86 0575-86502818, Email xlger@163.com

Purpose: This study aimed to evaluate the diagnostic accuracy and clinical applicability of metagenomic next-generation sequencing (mNGS) in pulmonary infections by comparing it with traditional culture methods in a Traditional Chinese Medicine (TCM) hospital setting.

Methods: This retrospective cohort study enrolled 67 consecutively admitted patients with radiologically and clinically confirmed pulmonary infections from the Department of Respiratory Infectious Diseases at Xinchang Hospital of Traditional Chinese Medicine between December 2022 and September 2024. Clinical specimens included blood, bronchoalveolar lavage fluid (BALF), sputum, hydrothorax and cerebrospinal fluid (CSF). mNGS and conventional culture were performed to compare detection rates and microbial community profiles.

Results: Among 67 cases, mNGS identified pathogens in 89.55% (60/67), compared to 20.90% (14/67) by traditional culture. Of 14 dual-positive cases, only 1 (1/14, 7.14%) showed complete concordance, while most exhibited discordance or partial genus-level overlap. mNGS further detected viral co-infections in 44.78% (30/67) and identified fastidious/non-culturable pathogens such as enterovirus, human herpesvirus type 1, and *Mycobacterium tuberculosis*. Patients with chronic diseases were more susceptible to EB virus infections.

Conclusion: mNGS significantly enhances pathogen detection in pulmonary infections, supports targeted antimicrobial therapy, and holds potential for contributing to clinical outcomes and reducing antibiotic resistance.

Keywords: mNGS, pulmonary infection, pathogens, antibiotic resistance, detection

Introduction

Pneumonia is frequently observed in clinical settings and is typically caused by pathogens such as influenza A and B viruses, coronaviruses including SARS-CoV, MERS-CoV, and SARS-CoV-2, *Klebsiella pneumoniae*, *Candida albicans*, *Pseudomonas aeruginosa*, and *Mycobacterium tuberculosis*.¹⁻³ These pathogens can cause varying degrees of damage to lung tissue. If anti-infective treatments are ineffective, there is a risk of severe decline in lung function or even respiratory failure, which can be life-threatening.⁴ The reference standard for diagnosing pulmonary infections primarily relies on the isolation and culture of pathogens, complemented by antimicrobial susceptibility testing to guide therapy.^{5,6} However, this culture-based approach has several critical limitations. First, the turnaround time is lengthy, typically requiring 48–72 hours, which delays diagnosis.⁷ Second, it has limited sensitivity for detecting fungal pathogens such as *Aspergillus* spp. and *Cryptococcus* spp., making the early diagnosis of fungal pneumonia particularly challenging.⁸

Furthermore, it cannot detect difficult-to-culture or non-culturable microorganisms.⁷ Compounding this diagnostic challenge, the widespread use of antibiotics and antifungal agents has led to annually increasing resistance rates in both bacteria and fungi.⁹ Therefore, accurately and promptly diagnosing the cause of infections and reducing unnecessary antibiotic use are crucial for the effective treatment of pulmonary infections. Metagenomic next-generation sequencing (mNGS) offers a powerful solution by providing the ability to identify a broad range of pathogens without relying on culture conditions.^{10,11}

Recent studies have highlighted both the advantages and challenges of mNGS, including its rapid turnaround time compared with culture, its ability to detect rare or unculturable pathogens, as well as concerns about high cost, data interpretation complexity, and distinguishing colonization from true infection.^{8,9} Although several studies have explored the clinical utility of mNGS in pulmonary infections, most were conducted in large tertiary hospitals. Previous studies on mNGS for pulmonary infections have been primarily conducted in Western medicine general hospitals. However, patients treated in Traditional Chinese Medicine (TCM) hospitals may present different pathogen spectra due to the distinct treatment approaches and patient characteristics. This study collected samples from 67 patients with pulmonary infections at the Xinchang Hospital of Traditional Chinese Medicine. By comparing the results of mNGS sequencing with those of conventional culture methods, this study aims to evaluate the diagnostic value of mNGS in the specific context of TCM hospitals and to determine whether differences exist in pathogen detection compared to prior research.

Materials and Methods

Study Subjects

This study was a single-center, retrospective observational study conducted at the Xinchang Hospital of Traditional Chinese Medicine between December 2022 and September 2024. Clinical data, including demographic characteristics, comorbidities, laboratory findings, and imaging manifestations, were collected from medical records. Clinical samples from patients with pulmonary infection, including sputum, cerebrospinal fluid, and bronchoalveolar lavage fluid, were respectively subjected to traditional culture methods and mNGS sequencing, and then the analysis results of the two methods were statistically analyzed.

The inclusion criteria were: (1) fever $\geq 38^{\circ}\text{C}$; (2) imaging features indicative of pulmonary infection (eg, pulmonary consolidation, ground-glass opacity, or cavity formation); (3) total white blood cell count decreased or returned to normal in the early stages of the disease, or a reduction in lymphocyte count; (4) lack of significant improvement or gradual worsening of the condition after 3–5 days of standardized antibiotic treatment.

Patients were categorized into two groups based on the presence or absence of chronic underlying diseases, including diabetes mellitus, hypertension, chronic lung disease, liver disease, kidney disease, and coronary heart disease. This stratification allowed for the analysis of pathogen profiles and infection patterns in patients with weakened immune function and those with normal immune function.

Traditional Culture Methods

Samples were inoculated onto blood agar, MacConkey agar, and Sabouraud agar plates using standardized inoculation volumes as per the guidelines of the Clinical and Laboratory Standards Institute (CLSI) and incubated at 37°C for 24 to 48 hours. Single colonies were selected to prepare bacterial suspensions with a McFarland turbidity standard of 0.50 to 0.63. Microbial (bacterial and fungal) identification was performed using the VITEK-2 Compact automated microbiology system (Mérieux, France) and its associated reagents.

Whole Genome Sequencing

For each sample, 200 ng of DNA was used for sequencing on the Illumina platform. Sequencing data underwent quality control to ensure an average genomic coverage depth of over 100X and a whole-genome coverage rate exceeding 95%. Data analysis was performed using the SAMTB platform (<https://samtb.uni-medica.com/index>) to obtain information on strain typing and gene mutations. Drug resistance mutations were identified using the resistance locus database (<https://github.com/jodyphelan/tbdb>), and resistance outcomes were predicted.

Statistical Analysis

Data were analyzed using SPSS software version 24.0. Patients were stratified into two groups based on the presence or absence of chronic underlying diseases (including diabetes, hypertension, chronic lung disease, liver disease, kidney disease, and coronary heart disease). Differences between two groups were assessed using McNemar's test. A p-value of <0.05 was considered statistically significant.

Results

General Data of Pulmonary Infection Patients

We collected samples from 67 patients diagnosed with pulmonary infection at Xinchang Hospital of Traditional Chinese Medicine from December 2022 to September 2024. The basic information of the patients with pulmonary infection was shown in Table 1. The samples included blood, sputum, cerebrospinal fluid, and bronchoalveolar lavage fluid. The cohort comprised 45 male and 22 female patients, with an average age of 65.91 years. Some patients had underlying conditions, including hypertension (29/67, 43.28%), diabetes mellitus (11/67, 16.42%), chronic kidney disease (1/67, 1.49%), and coronary heart disease (3/67, 4.48%). Imaging results showed inflammatory or infectious lesions in the lungs of patients with pulmonary infection.

Results of Traditional Culture

The detection rate using traditional culture methods was relatively low among the 67 pulmonary infection patients. The results of traditional culture was shown in Table 2. Among them, 8 cases (8/67, 11.94%) were positive in bronchoalveolar lavage fluid, 3 cases (3/67, 4.48%) were positive in sputum fluid, 2 cases (2/67, 2.99%) were positive in blood sample, and 1 case (1/67, 1.49%) was positive in pleural fluid (Table 3).

Comparison of Traditional Culture and mNGS Detection Methods

In this study, 60 out of 67 cases (60/67, 89.55%) tested positive using mNGS, whereas 14 out of 67 cases (14/67, 20.90%) tested positive using traditional culture methods (Figure 1A). Among these, 46 cases (46/67, 68.66%) were positive exclusively by mNGS. Additionally, 14 cases (14/67, 20.90%) tested positive by both mNGS and traditional culture methods. Importantly, among the 14 case that tested positive with both methods, 1 case (1/14, 7.14%) showed

Table 1 Clinical Information of Pulmonary Infection Patients

Characteristics	Patients (n=67)
Age	65.91±13.37
Female sex (%)	32.83
Length of hospitalization (days)	19.39±36.06
Biomarkers	
White blood cell count (10 ⁹ /L)	7.68±3.87
Neutrophils percentage (%)	72.33±14.38
Lymphocytes percentage (%)	17.50±10.99
C-reactive protein (mg/L)	198.86±947.37
Alanine aminotransferase (U/L)	41.22±35.70
Aspartate aminotransferase (U/L)	60.45±142.78
Creatinine (μmol/L)	82.06±74.60
D-dimer	1.79±2.95
Underlying disease	
Diabetes mellitus (%)	43.28
Hypertension (%)	16.41
Chronic kidney disease (%)	1.49
Coronary heart disease (%)	4.48

Table 2 Results of Traditional Culture

Sample Type	ID	Microorganism
Alveolar lavage fluid	PI002	<i>Aureomonas flavus</i>
	PI003	<i>Acinetobacter baumannii</i> and <i>Klebsiella pneumoniae</i>
	PI006	<i>Burkholderia cepacia</i>
	PI007	<i>Acinetobacter baumannii</i>
	PI008	<i>Aspergillus</i> spp.
	PI013	<i>Escherichia coli</i>
	PI027	<i>Penicillium</i> spp.
	PI052	<i>Aspergillus</i> spp.
Blood	PI004	<i>Bacillus cereus</i>
	PI021	<i>Escherichia coli</i>
Sputum	PI041	<i>Klebsiella pneumoniae</i>
	PI058	<i>Candida albicans</i>
	PI064	<i>Klebsiella pneumoniae</i>
Hydrothorax	PI046	<i>Oral actinomyces</i>

Table 3 Traditional Culture Result of Different Samples

Result	Blood (n)	Bronchoalveolar Lavage Fluid (n)	Sputum (n)	Hydrothorax (n)	Cerebrospinal Fluid (n)
Positive	2	8	3	1	0
Negative	19	12	13	6	3
Total	21	20	16	7	3

complete concordance, 6 cases (6/14, 42.86%) showed complete discordance, and the remaining 7 cases (7/14, 50.00%) showed partial concordance, indicating that at least one microbial pathogen overlapped (Figure 1B). A two-sided exact McNemar's test demonstrated a highly significant difference between the results of the two methods ($P < 0.001$).

Pathogen Types Detected by mNGS

A total of 67 cases were tested using mNGS. The results showed that the most common pathogen was the EV virus, detected in 14 cases (14/67, 20.90%), followed by *Candida albicans* in 10 cases (10/67, 14.93%), *Haemophilus influenzae* in 8 cases (8/67, 11.94%), and *Klebsiella pneumoniae* in 7 cases (7/67, 10.45%). Other detected pathogens are shown in Figure 2. The difficult or unculturable microorganisms could be detected by mNGS such as EV virus, herpesvirus type 1, and *tuberculosis bacilli*.

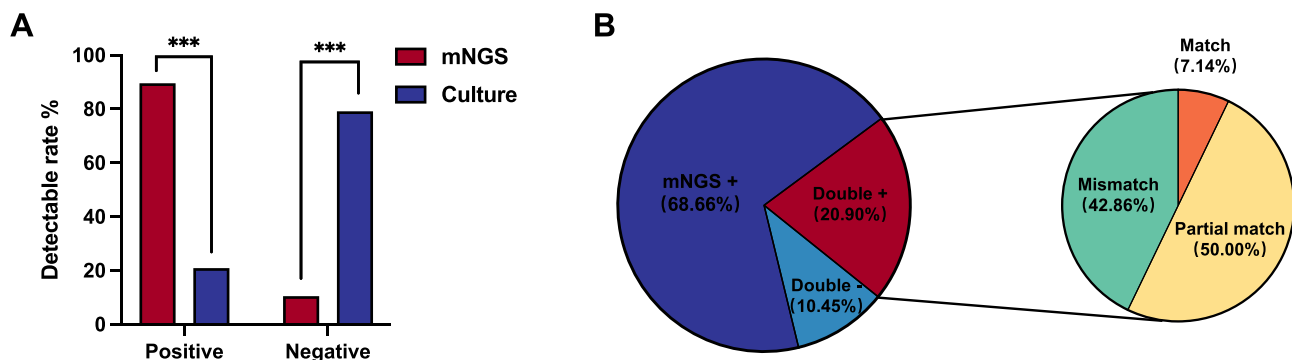


Figure 1 Detection of pathogens using mNGS and CMTs. (A) Positive mNGS and culture results in 67 patients with pulmonary infection. (B) mNGS and traditional culture methods were positive for 23 and 8 patients, respectively. ***: $P < 0.001$.

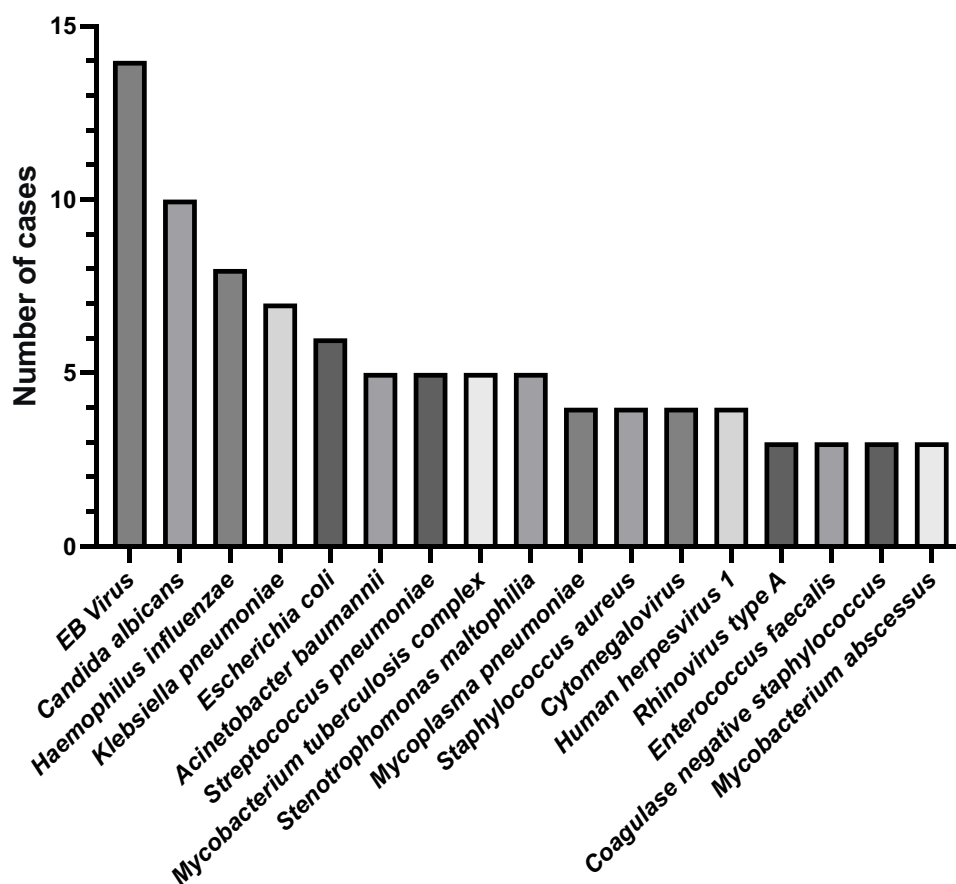


Figure 2 The pathogen spectrum of pulmonary infection patients detected by mNGS.

The Distribution of Different Groups of Microorganisms

Based on the clinical data of the patients, they were divided into two groups: the chronic disease group (35/67, 52.24%) and the non-chronic disease group (32/67, 47.76%). The pathogens detected in each group differed, as shown in Figure 3. EB virus and *Acinetobacter baumannii* were the most frequently detected pathogens in both groups. The detection rate of EB virus was significantly higher in the chronic disease group (12.22%) compared with the non-chronic disease group (6.06%). Additionally, *Elizabethkingia* was detected exclusively in the chronic disease group, possibly due to immunosuppression associated with chronic conditions. Other pathogens showed variable distributions between groups, as detailed in Figure 3, indicating potential differences in microbial profiles based on underlying health status.

Discussion

In this study, mNGS analysis was performed on clinical samples from 67 patients with pulmonary infections. Compared to traditional culture methods, the positivity rate of mNGS (60/67, 89.55%) was significantly higher than that of the culture method (14/67, 20.90%). Difficult-to-culture and unculturable pathogens, such as EB virus, Rhinovirus type A, Cytomegalovirus, and *Mycobacterium tuberculosis*, were detected, which was consistent with previous studies.^{12,13} For instance, in a study by Sun et al,¹⁴ mNGS and traditional culture were applied to blood samples from 124 patients with severe sepsis, and it was found that the positivity rate of mNGS (67.74%) was higher than that of traditional blood culture (19.35%). Zhao et al¹⁵ retrospectively analyzed the test results of various suspicious biological samples from ICU patients with high infection risk supported by extracorporeal membrane oxygenation (ECMO), and the positive detection rate of mNGS (79.6%) was also significantly higher than that of traditional culture methods (30.4%). In this study, the mixed infection rate in pulmonary infection patients detected by mNGS (36/60, 60.00%) was significantly higher than that detected by the culture method (1/14, 7.14%). Greater advantages of mNGS were observed in pathogen detection,

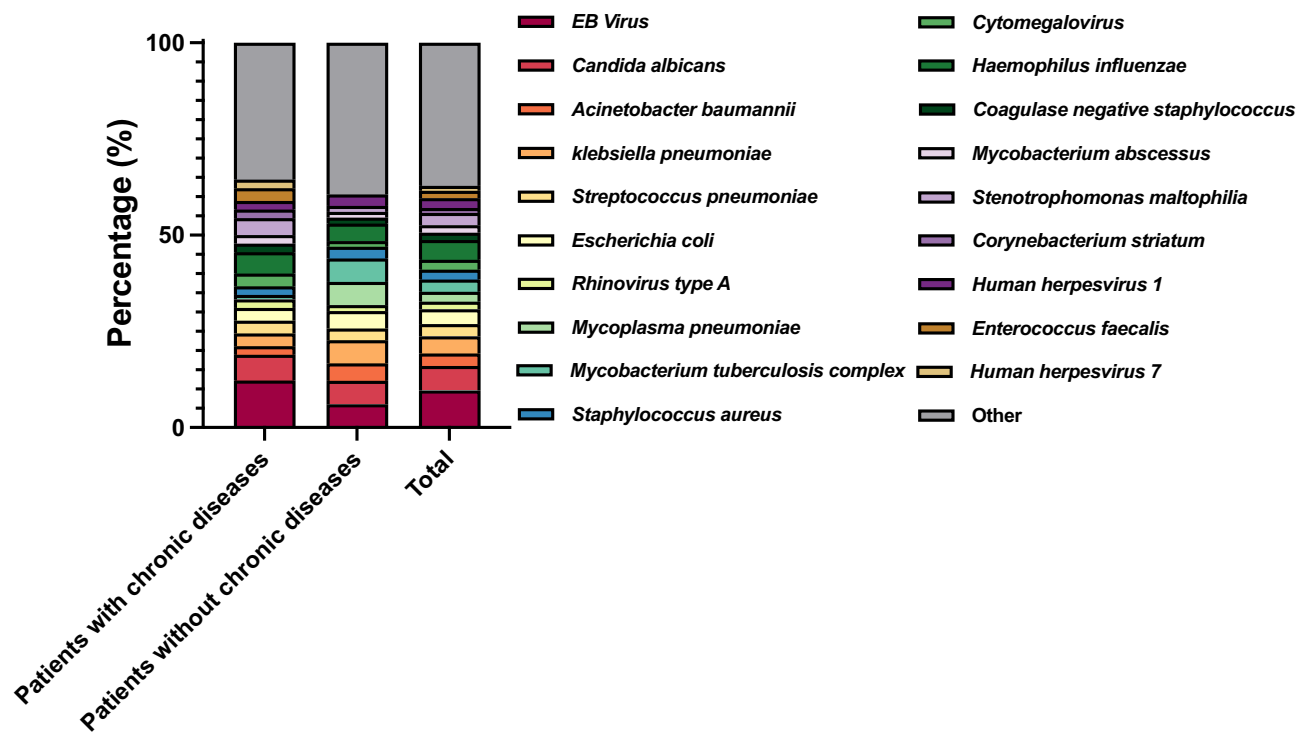


Figure 3 Proportion of the top 20 most abundant bacteria identified by mNGS.

particularly in detecting mixed infections.^{16,17} Patients with chronic underlying conditions were at an increased risk of infections caused by atypical and opportunistic pathogens.¹⁸ In this study, patients with chronic diseases such as diabetes, hypertension, coronary heart disease, and chronic kidney disease were more susceptible to EB virus infections. Additionally, one case of *Elizabethkingia* infection was detected, which may be related to the weakened immune system of chronic disease patients.

This study analyzed the diagnostic performance of mNGS in pulmonary infections. Rapid pathogen identification is crucial for accurate treatment.¹⁹ Traditional culture methods remain the reference standard for identifying many bacterial and fungal pathogens and provide antimicrobial susceptibility testing, which is essential for guiding therapy.^{20,21} However, their limitations are clear: the process is time-consuming (48–72 h or longer for slow-growing pathogens such as *Mycobacterium tuberculosis*), and sensitivity is reduced after empirical antibiotic use.¹⁵ Culture is also less effective for fungal pathogens such as *Aspergillus* spp. and *Cryptococcus* spp., where positivity rates are low. In contrast, mNGS can detect all pathogenic microorganisms present in clinical samples.²²

This study collected clinical samples such as bronchoalveolar lavage fluid, sputum, and blood samples from patients with pulmonary infection. Each sample type has specific advantages and limitations. BALF is considered highly representative of lower respiratory tract infections, but it is invasive and not always feasible. Sputum is easier to collect but may be contaminated with upper airway flora, which can complicate result interpretation. Blood culture is highly specific but has low sensitivity for pulmonary infections. By combining different sample types, our study aimed to better reflect the diagnostic value of mNGS in real-world practice.

mNGS enabled the rapid detection of a broad spectrum of pathogens, including viruses and atypical bacteria that are not detected by culture. A particular advantage lies in pathogens that are theoretically culturable but difficult or slow to grow, such as *Mycobacterium tuberculosis*, or those with inherently low positivity in culture, such as certain fungi. In these cases, mNGS can significantly shorten the time to diagnosis and improve detection sensitivity. Nevertheless, mNGS also has limitations: it cannot provide direct antimicrobial susceptibility results, interpretation may be confounded by colonization or contamination, host DNA can reduce sensitivity, and cost remains high, limiting its widespread adoption.^{19,20,23} It was particularly effective in quickly identifying rare, novel, or unknown pathogens, as well as

pathogens in complex infectious disease samples with atypical microbiological features.^{24,25} This approach can reduce the use of unnecessary broad-spectrum antibiotics. Furthermore, previous studies have shown that culture, as the reference standard for bacterial and fungal detection, is easily affected by empirical antibiotic therapy,^{15,26,27} while mNGS is less influenced by prior antibiotic treatment.

This study has several limitations. First, it was a single-center study with a relatively small sample size, which may limit generalizability. Second, while we compared positivity rates between mNGS and culture, we did not systematically analyze clinical outcomes, which restricts conclusions on patient prognosis. Third, mNGS interpretation remains challenging, and standardized criteria for clinical reporting are still evolving. Finally, antimicrobial resistance genes were not comprehensively analyzed, which has deficiencies in guiding clinical treatment.

In conclusion, we analyzed the value of mNGS in the diagnosis of pulmonary infections. While the diagnostic performance of mNGS was significantly superior to that of traditional culture methods, it should be noted that, despite the limitations of culture, positive culture results provide direct macroscopic and microscopic evidence for pathogen identification. In contrast, mNGS results may lack the ability to differentiate colonization from infection and therefore must be interpreted with caution. The clinical application of mNGS should be integrated with the patient's symptoms, imaging findings, and other laboratory results. Additionally, factors such as cost, turnaround time, and interpretation complexity may affect its widespread adoption. Larger multicenter studies are warranted to confirm whether the advantages observed in diagnostic accuracy consistently translate into improved clinical outcomes such as prognosis and mortality reduction.

Conclusion

This study demonstrates that metagenomic next-generation sequencing (mNGS) offers significantly higher pathogen detection rates than traditional culture methods in pulmonary infections. mNGS identified difficult-to-culture and unculturable microorganisms, including viruses, fungi, and atypical bacteria. These findings highlight the potential of mNGS to complement conventional methods by providing comprehensive pathogen profiles, though its results should be carefully interpreted in combination with clinical, imaging, and laboratory findings.

Data Sharing Statement

All the reported data are available within the article.

Ethics Statement

This study was approved by the Medical Ethics Committee of Xinchang Hospital of Traditional Chinese Medicine (ethical number 2024-K-75). Written informed consent was obtained from all participants. This study was performed in line with the principles of the Declaration of Helsinki. All the methods were performed in accordance with the relevant institutional ethical committee guidelines.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial funding, and no conflict of interest exists.

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