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Metagenomic next-generation sequencing *vs.* conventional detection methods for detecting the pulmonary infections

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Abstract. – OBJECTIVE: The absence of proper pathogen treatment in the early stages can result in missing out on treatment chances or the overuse of antibiotics, both of which are the primary factors behind fatalities caused by lung infections. In this study, we aimed to investigate the efficacy of metagenomic next-generation sequencing (mNGS) in comparison to conventional detection methods in detecting infectious pathogens.

PATIENTS AND METHODS: In this retrospective study, the infection pathogens of 104 patients were examined, and 86 bronchoalveolar lavage fluid (BALF), eight pleural effusions, and ten sputum samples were collected. The conventional detection approaches and mNGS analysis were used to determine the infection pathogen profiles and their detection rates were analyzed.

RESULTS: Our study showed that mNGS was more sensitive (89.42%) than the conventional detection methods (56.73%) ($p < 0.001$), with a 32.69% improvement in sensitivity. The efficacy of mNGS in detecting mixed infections was significantly higher than that of conventional detection methods, with a detection rate of 85.29% compared to 17.65% ($p < 0.001$). The study demonstrated that mNGS had a higher sensitivity than the conventional detection methods when it came to diagnosing pulmonary infections, making it a potentially useful tool for clinical diagnosis.

CONCLUSIONS: Combining mNGS with other pathogenic detection techniques can be an effective way to increase the rate of detecting pulmonary infections, as well as to provide guidance for treatment adjustments. Furthermore, the timing of sample collection and antibiotic administration can influence the effectiveness of mNGS when used on BALF specimens.

Key Words:

Metagenomic next-generation sequencing, Pulmonary infection, Etiological diagnosis, Application value, Influencing factors.

Introduction

Pulmonary infection is an inflammation of the lung parenchyma caused by various pathogenic microorganisms[1]. Worldwide, respiratory infections are the most common cause of illness and death, primarily affecting the terminal airways, alveolar cavities, and interstitium[2]. The emergence of novel or rare pathogens, as well as the increased resistance of microorganisms to multiple drugs, can be attributed to the extensive use of chemotherapeutic drugs, immunomodulators, glucocorticoids, broad-spectrum antibiotics, and the transplantation of organs and hematopoietic stem cells. The prevalence of these opportunistic microorganisms has grown as a result of their capacity to cause infections in immunocompromised individuals. The inappropriate application of antibiotics and inadequate treatment approaches for pulmonary infections at early stages are the major reasons for the increased morbidity and mortality rates[3,4]. However, a limited proportion of individuals suffering from lung infections, approximately 30-40%, have been identified using available diagnostic tests[5,6]. The identification of pathogens has been traditionally done through the isolation and culturing of microorganisms[7], antigen-antibody immunological tests[8], and the utilization of polymerase chain reaction (PCR) for specific identification[9]. These techniques are limited in their capacity to locate rare and unrecognized pathogens as a result of their low positivity rate and prolonged culturing period. Furthermore, the specificity and timing of the antigen-antibody binding is not highly accurate, and PCR only has the capacity to detect a limited number of pathogens.

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Thus, there are a considerable number of infectious illnesses that have yet to be attributed to a specific source[10]. The irresponsible application of antibiotics has caused bacteria to become resistant to them, thus, making it a critical issue in terms of human health[11]. For this reason, it is imperative to be able to diagnose pathogens in a timely and effective manner in order to effectively treat infectious diseases.

Metagenomic next-generation sequencing (mNGS) is an advanced technique that allows for the identification of all microorganisms without the use of conventional microbial culturing methods. This technology is based on the idea of recognizing the DNA sequences during its production by recognizing markers at the end of a newly synthesized DNA strand, thus enabling the rapid sequencing of thousands of DNA fragments, each containing billions of nucleotides, simultaneously and independently[12]. Through the use of mNGS, it is possible to identify both known and unknown pathogens within clinical samples[13]. In 2014, Wilson et al[14] pioneered the use of this method to identify Leptospira in the cerebrospinal fluid and confirmed through PCR and serological testing, and the patient was successfully treated with penicillin G. mNGS has become a popular method for pathogen detection due to its numerous benefits, such as high throughput, high sensitivity, short turnaround time, no bias, and wide coverage. It has revolutionized the diagnosis and treatment of complex and mixed infections, which could not be identified using traditional methods. Recently, the possibility of using mNGS to identify non-sterile site specimens in the respiratory tract has been discussed, though there are still conflicting opinions[15,16].

The purpose of this study was to evaluate the effectiveness of mNGS in diagnosing and treating pulmonary infections in comparison to conventional methods. Furthermore, the aim was to demonstrate that the combination of mNGS and conventional detection methods can enhance the rate of identifying pathogens in pulmonary infections and aid in adjusting treatment regimens.

Patients and Methods

Patients

This retrospective study included 104 adult patients with pulmonary infection who were admitted to Fujian Medical University Union Hospital from October 2020 to October 2021. Of these, 98 cases had a pathogen identified as the cause of the infection. However, 10 of these cases were associated with other pulmonary diseases, such as pneumoconiosis, interstitial pneumonia, lung cancer, leukemic pulmonary infiltrates, metastatic lung cancer, idiopathic eosinophilic pulmonary infiltrates, and mechanized pneumonia. The remaining six cases also showed pulmonary bacterial infections, but the pathogens were not identified.

A sample of 104 individuals was examined, with 59 (56.73%) males and 45 (43.27%) females. The primary symptoms observed were coughing, sputum, fever, chest pain, hemoptysis, breathlessness, chest tightness, and night sweats, either singly or in combination. The primary underlying medical conditions included bronchiectasis, chronic obstructive pulmonary disease (COPD), emphysema, post-pulmonary surgery, history of tuberculosis, lung cancer, extra-pulmonary malignancies, connective tissue disease, chronic kidney disease, hypertension, and type 2 diabetes mellitus (T2DM). The inflammatory indices of the patients were as follows: 25 patients (24.04%) had white blood cell (WBC) count > 10×10^9/L or < 4×10^9/L; 64 patients (61.54%) had a neutral ratio < 50% or > 70%; 53 patients (50.96%) had procalcitonin (PCT) level ≥ 0.05 ng/ml; and 53 patients (50.96%) had C-reactive protein (CRP) ≥ 10 mg/L. The lung CT showed that the most frequent imaging manifestation was a patchy shadow, which was observed in 75 cases (72.16%), followed by nodular shadow in 64 cases (61.54%) and solid or bronchial signs in 32 cases (30.77%). The results were summarized in Table I.

Ethics

This study was approved after a thorough review by the Medical Ethics Committee of Fujian Medical University Union Hospital (approval number: 2022KY116).

Etiological Assessment

The etiological assessment included clinical assessment, imaging, laboratory diagnosis, and pathogen detection. Data of the patients were collected, including gender, age, smoking history, underlying diseases, major symptoms, pulmonary imaging, laboratory diagnostic parameters (such as WBC cell count, neutrophil ratio, PCT, and CRP), routine pathogenic detection (sputum smear, sputum, and alveolar lavage fluid or pleural fluid culture), blood novel cryp-

Table I. Clinical features of the 104 patients with pulmonary infection.

Patient's characteristics	
Gender, n (%)	
Male	59 (56.73%)
Female	45 (43.27%)
Average age (year)	56.74±13.60
Smokers, n (%)	37 (35.58%)
Basic Diseases, n (%)	
Bronchiectasis	14 (13.46%)
COPD	6 (5.77%)
Emphysema	20 (19.23%)
Post-pulmonary surgery	7 (6.73%)
History of tuberculosis	10 (9.62%)
Lung cancer	9 (8.65%)
Extra-pulmonary malignancies	17 (16.35%)
Other chronic lung diseases	17 (16.35%)
Connective tissue disease	21 (20.19%)
Connective tissue disease	9 (8.65%)
Chronic kidney disease	6 (5.77%)
Hypertension	29 (27.88%)
Diabetes	20 (19.23%)
Symptoms, n (%)	
Cough	82 (78.85%)
Sputum	75 (72.16%)
Fever	43 (41.35%)
Chest pain	19 (18.27%)
Hemoptysis	10 (9.62%)
Laboratory inflammation indicators	
WBC ($\times 10^9$)	6.87 (5.19, 8.89)
Average neutral ratio	67.77 ± 15.56
PCT	0.058 (0.026, 0.182)
CRP	10.93 (2.03, 92.76)
Lung CT, n (%)	
Patchy shadow	75 (72.16%)
Nodular shadow	64 (61.54%)
Solid or bronchial sign	32 (30.77%)
Ground-glass opacity (GGO)	16 (15.38%)
Pulmonary cavity	10 (9.62%)
Thread net	6 (5.77%)

tococcal podocyte antigen, blood and alveolar lavage fluid using galactomannan (GM) test, PCR detection of sputum *Mycobacterium tuberculosis* DNA, sputum for detecting antacid bacteria, TORCH for detecting toxoplasma, rubella, cytomegalovirus and herpes simplex virus, respiratory octreotide, fiberoptic bronchoscopy performance, treatment plan, specimen sampling time, disease regression, and final clinical diagnosis. Additionally, mNGS data for 104 samples were collected, including 86 samples of alveolar lavage fluid, 8 samples of pleural fluid, and 10 samples of sputum mNGS specimens. The mNGS was conducted by Jiangsu Simcere Diagnostics Co., Ltd. and Nanjing Practice Medicine Diagnostics. Co., Ltd. in accordance with standard protocols.

Diagnosis of Pulmonary Infections

No guidelines have been established to help clinicians interpret the positive results of mNGS. In this study, the diagnosis of pathogens responsible for pulmonary infection was determined by two or more experienced respiratory physicians, taking into account the clinical symptoms and other supporting test results. The conventional detection methods have been found to be inadequate in terms of their detection rate, making it difficult to measure the benefits of mNGS. Subsequently, the "final clinical diagnosis" was taken as a reference standard instead of "conventional test results". By closely monitoring the outcomes of treatments and follow-up, the incidence of misdiagnosis can be minimized.

Statistical Analysis

This study utilized SPSS 26.0 (IBM Corp., Armonk, NY, USA) software for the statistical analysis of data. The data counts were expressed numerically and as percentages. Normally distributed measures were reported as mean ± standard deviation, while skewed measures were expressed as median and interquartile spacing. McNemar's test and Cohen's Kappa test were employed to compare paired samples, while the independent samples were compared using the Chi-square test, *t*-test or rank-sum test, and multi-factor logistic regression analysis. A *p*-value lower than 0.05 was considered statistically significant. Kappa values lower than 0.4, greater than 0.7, and lower than 0 indicated poor agreement, good agreement, and inconsistency, respectively.

Results

Pathogen Status

Using mNGS, two bacterial cases - one of *Campylobacter briefus* and one of *Staphylococcus epidermidis* - and six fungal cases - five of *Candida* and one of *Fusarium acnes* - were identified as false positives. Additionally, routine testing revealed one *Staphylococcus capitis* bacterial case and two *Candida* fungal cases as false positives. These pathogens were determined to be orally colonized microbes and identified as contaminants.

The most commonly identified bacteria using mNGS were *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Stenotrophomonas malto-*

philia and *Haemophilus influenzae*. Among the fungi commonly detected by mNGS, *Candida albicans* was the most frequent, followed by *Cryptococcus neoformans*, *Yersinia pneumonia*, and *Candida glabrata*. Conventional detection methods revealed *Pseudomonas aeruginosa* as the most frequent bacterial species, with *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* following in frequency. As for fungi, *Aspergillus* was the most commonly identified using conventional methods, with *Candida albicans*, *Cryptococcus neoformans*, and *Candida tropicalis* coming in behind (Figures 1A and 1B).

In addition, mNGS revealed a total of 40 viral cases, with human herpesvirus being the most prevalent, followed by Epstein-Barr virus (EBV) and cytomegalovirus. These viruses were not identified by traditional detection methods.

Results from both mNGS and conventional detection methods showed that *Pseudomonas aeruginosa* was the most frequently detected bacterium across all four seasons. In contrast, *Streptococcus pneumoniae* infections were predominantly seen during the winter season, and *Yersinia pneumoniae* infections were more prevalent in the spring and summer seasons.

Comparison of Detection Rate

Results from the study indicated a statistically significant difference in the detection rates between mNGS and conventional detection methods, with 89.42% and 56.73% of the pathogens detected, respectively ($p < 0.001$).

Results of the alveolar lavage fluid specimens showed a significantly higher detection rate of 88.37% with mNGS compared to 58.14% with conventional detection methods ($p < 0.001$). For the pleural fluid specimens, the detection rates of mNGS and conventional detection methods were 87.50% and 37.50%, respectively, with no significant difference ($p = 0.125$). The agreement between the two methods was slight (Kappa = 0.158 < 0.4). For this study, a single case showed a negative result for mNGS detection in the pleural fluid, while the number of nucleated cells identi-

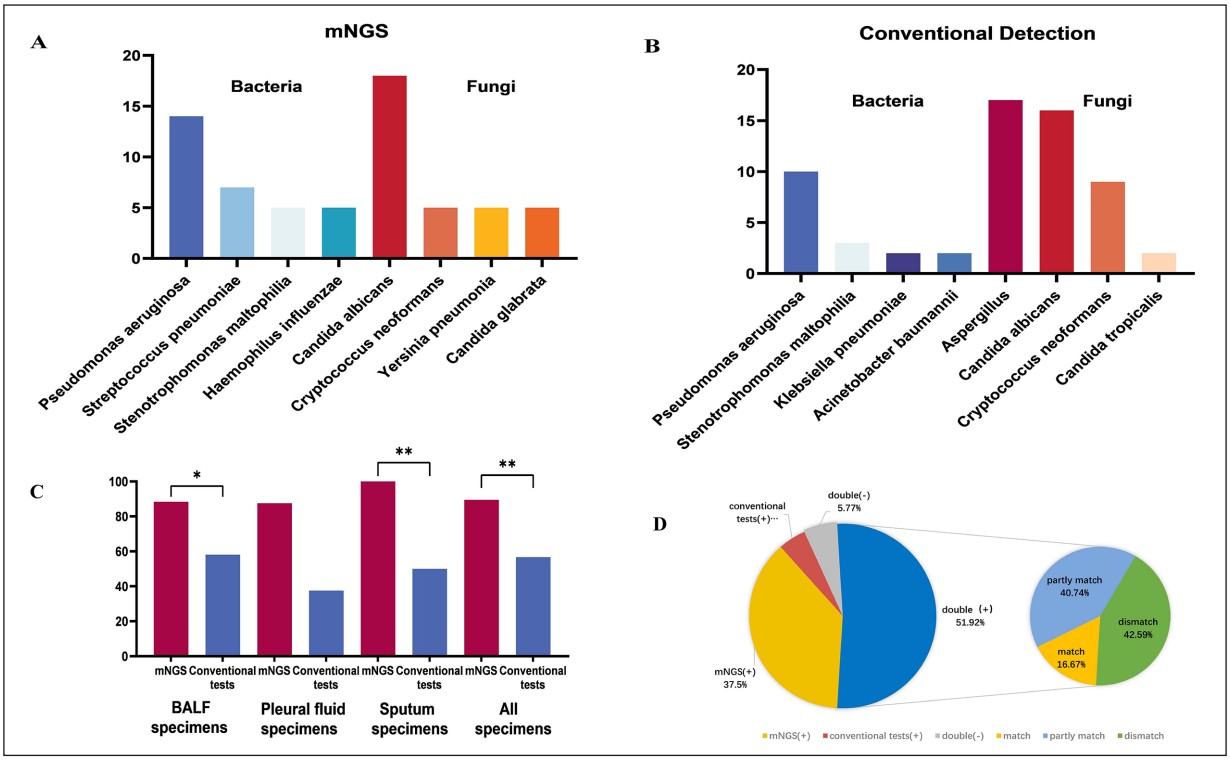

Figure 1. The four bacteria and viruses with the highest pathogenic detection rates in mNGS and conventional assays were shown in (**A-B**), respectively. Different colors indicate different pathogen categories. Comparison of different specimens and total pathogenic detection rates were shown in (**C**). *$p < 0.05$, **$p < 0.001$. Concordance between mNGS and conventional tests was displayed in (**D**). Pie chart demonstrating the positivity distribution for the detection of pathogens by mNGS and conventional test in 151 cases.

fied with conventional detection methods in the pleural fluid was much lower (553) in comparison to the other seven cases (1,993-78,451). Furthermore, the detection rates for sputa samples were significantly different between mNGS and conventional detection methods (100% and 50%, respectively), with a statistically significant result ($p = 0.015 < 0.05$) (Figure 1C).

When two or more pathogens are identified using either mNGS or traditional detection methods, the infection was referred to as a mixed pulmonary infection. Of the 98 patients diagnosed with lung infections, 68 (69.39%) were found to have mixed infections. The most common pattern was bacterial, fungal and viral elements (27.94%), followed by bacterial and fungal (22.06%) and mixed bacterial (14.71%). The use of mNGS demonstrated a dramatically increased rate of mixed infection detection (85.29%) when compared to conventional detection methods (17.65%) ($p < 0.001$).

A total of 72 bacterial (excluding *Mycobacterium*) infections were identified, of which 66 had clear pathogens. The detection rate of mNGS was 86.11%, significantly higher than the conventional detection methods ($p < 0.001$). The negative predictive value of mNGS was also significantly higher than that of the conventional methods ($p < 0.001$).

Analysis of 53 fungal infection cases revealed that the detection rate of mNGS was lower than that of conventional detection methods (56.60% *vs.* 77.36%), although it was not statistically significant ($p = 0.090 > 0.05$). The agreement between the two methods was low (Kappa = -0.424). Furthermore, in the 21 cases of *Aspergillus* infection, the bronchoalveolar lavage fluid (BALF)-galactomannan detection rate was higher than that of BALF-mNGS (61.90% *vs.* 19.05%, $p = 0.049 < 0.05$). These results were displayed in Table II.

An analysis of nine pulmonary *Mycobacterium* tuberculosis infections revealed that mNGS had a detection rate of 88.98%, while conventional detection methods had a rate of 55.56%. Although the difference between the two was not statistically significant ($p = 0.375 > 0.05$), mNGS showed a greater performance than conventional detection methods. Utilizing mNGS, six cases of atypical *Mycobacterium* tuberculosis and five cases of *Yersinia pneumoniae* infection were identified, which could not be identified using the existing conventional detection methods.

Consistency of MNGS With Conventional Detection Methods

In the sample of 104 lung infections, the mNGS and traditional detection methods identified 54 (51.92%) cases as positive. Of these 54 cases, 9 (16.67%) were in perfect agreement, 22 (40.74%) had some degree of concordance (one or more detected pathogens were similar) and 23 (42.59%) were completely different (Figure 1D).

Adjustment of the Treatment Plan and Disease Regression

A total of 98 cases were finally diagnosed in this study. Out of the total treatment regimens, 46 (46.94%) were adjusted in accordance with the mNGS or mNGS + conventional test results. This alteration was beneficial for 37 (80.43%) patients, as it resulted in an improvement in their disease regression. Moreover, a similar improvement was observed in 50 out of 52 (96.15%) patients, for whom the regimens were kept unchanged.

Analysis of the Factors Affecting the Detection Rate of mNGS

A total of 57 bacterial infections were identified in the alveolar lavage fluid, with the final clinical diagnosis used as the reference standard. The mNGS results were divided into two groups: 11 mNGS-negative and 46 mNGS-positive cases. Univariate regression analysis was used to compare the underlying disease symptoms, laboratory indices [WBC, neutrophil (NE), CRP, and PCT], imaging manifestations (bronchial signs, solid changes, ground glass shadow, plaques, masses, cavities, grids, and single or double lung lesions), presence or absence of abnormalities in and obvious secretions from bronchofibroscopy, duration between the sampling and starting mNGS, and duration of antibiotics administration before sampling between the two groups. The differences in the duration between the sampling and starting mNGS and the duration of antibiotics administration before sampling for mNGS were statistically significant ($p < 0.05$).

A logistic regression analysis was conducted to assess the influence of the presence or absence of abnormalities under bronchofibroscopy, the duration between the sampling and starting mNGS, and the duration of antibiotics administration before sampling for mNGS on the likelihood of positive mNGS results. The results indicated that shortening the time between the sampling and mNGS start and the duration of antibiotics administration before sampling could significantly

Table II. Comparison of the diagnostic efficacy of mNGS and conventional detection methods for bacteria and fungi.

	Detection rate		Specificity		Positive predictive value		Negative predictive value	
	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
mNGS	86.11% (62/72)	56.60% (30/53)	94.12% (32/34)	88.68% (47/53)	96.88% (62/64)	83.33% (30/36)	76.19% (32/42)	67.14% (47/70)
Conventional test	31.94% (23/72)	77.36% (41/53)	97.06% (33/34)	96.23% (51/53)	95.83% (23/24)	95.35% (41/43)	40.24% (33/82)	80.95% (51/63)
p	< 0.001	0.090	1.000	0.219	1.000	0.165	< 0.001	0.071
Kappa	-0.035	-0.424	-0.041	0.205				

increase the odds of positive mNGS results (OR = 0.251, 95% CI 0.077-0.816, $p = 0.022$ and OR = 0.121, 95% CI 0.025-0.588, $p = 0.009$ respectively) (Table III).

Discussion

In China, epidemiological surveys of community-acquired pneumonia (CAP) have revealed *Mycoplasma pneumoniae* and *Streptococcus pneumoniae* to be the primary contributors to CAP, whereas *Pseudomonas aeruginosa* is a rare contributing factor. Gram-negative bacteria are more prevalent in the elderly and those with existing medical issues[17]. An analysis of 98 cases of CAP with obvious pathogens revealed a difference between the bacteria detected using metagenomic next-generation sequencing (mNGS) and conventional detection methods. The most commonly detected bacteria using mNGS were *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*, while those detected using conventional methods were *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. This difference may be attributed to the limitations of conventional detection methods, as *Streptococcus pneumoniae* is a causative bacterium that is difficult to detect by culturing. Additionally, the most common causative microorganism in this study was *Pseudomonas aeruginosa*, which was related to a small number of cases with underlying diseases. The pathogens causing CAP varied across regions and across seasons[18-21]. *Pseudomonas aeruginosa* was the most common bacterium in all four seasons, while *Streptococcus pneumoniae* was the most observed bacterial species in the winter, *and Yersinia pneumoniae* was the most observed bacterium in summer and spring. These results need to be confirmed in a larger sample size in future studies.

This retrospective study was conducted to evaluate pulmonary infections in 104 patients. The efficacy of mNGS for detecting pathogens associated with pulmonary infections was significantly higher than that of conventional detection methods ($p < 0.001$). Parize et al[22] carried out a multicenter prospective study, which revealed that mNGS is more effective in detecting microorganisms than the conventional methods. This finding was further supported by Langelier et al's[23] research, which concluded that mNGS could increase the rate of pathogen identification in the respiratory system. Our study demonstrated that mNGS could effectively raise the rate of detection of respiratory pathogens.

In this study, among the eight pleural fluids cases, mNGS showed negative results for only one case. Routine diagnostic methods of pleural fluid suggested a significantly lower number of nucleated cells than the other seven cases, signifying the potential of quantitative free DNA (cfDNA) signal detected by mNGS for infection surveillance[24]. Although mNGS assay technology is costly, pleural fluid routine may help in screening and narrowing down the samples for mNGS. Nevertheless, due to the limited sample size, these results require further validation in future studies.

In this study, the detection rate of mNGS for bacterial pathogens was significantly higher than that of the conventional detection methods ($p < 0.001$). However, the detection rate of mNGS for fungal pathogens showed no significant difference as compared to the conventional detection methods (56.60% *vs.* 77.36%, $p = 0.090$); this was in contrast to some of the previous findings. Miao et al[25] found that mNGS was not superior to the culturing methods for detecting bacterial pathogens but had greater efficacy for detecting fungal pathogens. Toma et al[26] also reported that the culturing method was able to identify most bacteria in bacteria-associated pneumonia as compared to sequencing. Nevertheless, Xie et al[27] and Xie et al[28] found no meaningful distinction between fungal detection using mNGS and conventional detection methods. These discrepancies may be attributed to the differences in diseases, infectious agents, specimen types,

Table III. Logistic regression analysis of the factors affecting the detection rate of mNGS to detect pulmonary bacterial infection in the alveolar lavage fluid.

Variables	*p*	OR	OR (95% CI)
Bronchofibroscopy for abnormalities	0.335	2.410	0.043-14.389
Time between the sampling and starting mNGS	0.022	0.251	0.077-0.816
Time of antibiotics administration before sampling for mNGS	0.009	0.121	0.025-0.588

and testing conditions of the study subjects. In this study, the fungal detection rate using the conventional detection method was also relatively high.

The alveolar lavage fluid sample is an effective method for the early diagnosis of non-granulomatous invasive pulmonary aspergillosis (IPA)[29]. The sample contains galactomannan (GM), a unique component of the *Aspergillus* cell wall, which is released early in its growth. The GM level in alveolar lavage fluid has high sensitivity and specificity and correlates with the degree of invasion and clinical outcomes. In this study, the detection rate of BALF-mNGS was significantly lower than that of the BALF-GM test ($p = 0.049$). This could be attributed to the small sample size or the mode of fungal infection, as *Aspergillus* is a filamentous fungus and usually spreads on the surface of lung tissues, making it difficult to obtain using lavage[30].

This study found that, although the difference between the detection rates of mNGS and conventional detection methods for *Mycobacterium tuberculosis* was insignificant (88.98% *vs.* 55.56%, $p = 0.375$), mNGS tended to be more superior. A prospective study conducted by Zhou et al[31] revealed that the detection rate of mNGS was comparable to that of Gene X-pert detection for *Mycobacterium tuberculosis*, both of which were superior to the culture-based detection of *Mycobacterium*. This discrepancy could be attributed to the small sample size, the type of sample, the different testing institutions, and the secondary delivery of some of the samples.

In this research, mNGS was able to identify five cases of *Pneumocystis pneumonia* (PCP) and six cases of non-tuberculous mycobacterial (NTM) pulmonary infections, while none of the traditional detection methods could detect them. mNGS was able to detect PCP and NTM infections more effectively. This offers a major advantage in terms of clinical diagnosis and prognosis of PCP and NTM infections compared to the conventional detection methods. PCP is a common infection among immunocompromised patients, yet the establishment of a perfect *in vitro* culturing technique is still lacking. Hexosamine silver staining microscopy is the most frequently used detection method, yet its sensitivity is relatively low. Zhang et al[32] found that out of 13 PCP patients, only five were identified positive using conventional detection methods, while all 13 tested positive for *Yersinia pestis* using mNGS, and 11 cases of mixed infections were found. Therefore, mNGS has a higher detection rate for *Pneumocystis carinii* than conventional detection methods. The incidence of NTM pulmonary infections has been rising in recent years, and in countries with low tuberculosis epidemics, the detection rate of NTM has exceeded that of *Mycobacterium tuberculosis* complex (MTBC). As both of these infections have similar clinical presentations, imaging, and positive antacid staining, they are often misdiagnosed without further identification[33,34]. Fedrizzi et al[35] used mNGS to sequence 47 NTM strains and reconstruct and analyze the genomes of 41 previously undescribed NTMs. Furthermore, numerous cases of successful diagnosis of NTM infections using mNGS have been reported[36].

This study revealed that mNGS was more effective in detecting viruses than conventional detection methods, which is in line with most prior studies[37]. Out of the 40 viruses identified by mNGS, the most common infection was human herpesvirus, followed by EBV and cytomegalovirus, which were not detected by conventional methods. In contrast, IgM antibodies against A and B influenza, which were detected by conventional methods, were not reflected in the mNGS results. This discrepancy may be attributed to the rapid elimination of nucleic acids from the body, while antibodies remain for a longer period. Moreover, human herpesvirus and cytomegalovirus are more harmful than the influenza virus.

In this study, the detection rate of mNGS for mixed infections was significantly higher than that of conventional detection methods ($p < 0.001$), which is in agreement with the findings of Wang et al[38]. Fang et al[39] reported that the combination of mNGS and conventional methods could increase the detection rate of mixed infections from 55.6% to 58.3% compared to the mNGS alone. Thus, mNGS testing may be considered clinically beneficial for patients with possible mixed infections. This is due to its unbiased nature and wide coverage, which allows it to detect multiple lung infection patterns that cannot be identified using conventional detection methods due to the interaction and inhibition of various microorganisms. Furthermore, severe pneumonia is usually co-infected with multiple pathogens; thus, mNGS may play an important role in guiding the timely diagnosis and treatment of patients with severe pneumonia.

In this study, the majority of diagnoses were made using mNGS, though some of the empirical treatments did not cover all pathogens. This

concurs with a study by Miao et al[25] that showed mNGS had the potential to guide treatments, yet was not significantly more advantageous than the traditional methods due to the preference for more economical treatments in the clinic. Of the 98 patients, 46.94% (46/98) had their regimens adjusted according to mNGS results, with 49.46% (46/93) being mNGS-positive. In comparison, 66.10% (39/59) of patients tested positive with traditional methods had their regimens adjusted accordingly. Of those whose regimens were adjusted based on mNGS or mNGS plus conventional detection results, 80.43% saw improved conditions. This was higher than the 96.00% of patients whose regimens were adjusted using conventional detection methods alone. While mNGS results did not seem to significantly improve prognosis, this may be due to most cases being of patients with multi-drug resistant pathogens and poor treatment.

It was observed that shortening the time between sampling and initiating mNGS, as well as the duration of antibiotic administration prior to sampling for mNGS, increased the likelihood of bacterial detection using mNGS (OR = 0.251, p = 0.022; OR = 0.121, p = 0.009). This finding was consistent with the previous study by Miao et al[25], which suggested that mNGS was less affected by prior antibiotic use compared to conventional detection methods[24,40]. Li et al[41] also reported a correlation between the duration from disease onset to sampling and the species within the airway microbiota. Thus, it is hypothesized that the timing of specimen sampling and antimicrobial drug use may influence the results.

Limitations

This study had certain limitations which must be taken into consideration. Firstly, it was a single-center retrospective study with a small sample size, which may have caused recall bias, selection bias, and limited representativeness. Additionally, further research is needed to determine the efficacy of mNGS in different sample types, such as BALF, pleural effusions, and sputum, in order to provide a higher diagnostic value in clinical settings. Moreover, a quantitative assessment of the abundance of each infectious pathogen would significantly improve the diagnostic power for pulmonary infections. Lastly, the mNGS tests were dispatched to two commercial laboratories, which may have resulted in a decrease in accuracy due to the extended turnaround time and the disparity between experimenters.

Conclusions

In summary, mNGS has demonstrated its efficacy in patients with complex clinical presentations, as it is capable of detecting rare or atypical pathogenic infections that may not be identified through conventional detection methods, ineffective standard anti-infective therapy, or a high likelihood of mixed infections. The selection of mNGS or conventional detection methods should be based on the patient's morbidity characteristics, ancillary tests, and other clinical manifestations, as well as the epidemiology and clinical characteristics of each pathogen, in order to avoid misdiagnoses or wasting of resources. By taking into account the timing of sampling and antibiotic administration, mNGS can be used as a supplementary method to conventional detection methods, thereby shortening the confirmation time of pathogens, improving the efficiency of overall pathogen detection, promoting targeted antimicrobial therapy, and ultimately improving the patient prognosis.

Ultimately, while mNGS cannot replace other detection methods, it can be an effective supplement to pathogen detection, thus increasing the rate of pathogen identification in pulmonary infections and providing treatment guidance. Furthermore, the time of sample collection and administration of antibiotics can affect the efficacy of mNGS in BALF samples.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

L.-M. Chen and X.-Q. Shi designed the paper and guided the implementation. X.-Q. Shi and L. Tian drafted the manuscript. X.-Q. Shi, L. Tian, Z.-H. Huang, W.-T. Song, and J.-B. Wu collected the cases and summarized the data. X.-Q. Shi and L. Tian processed the data and did the statistical analyses. All the authors approved the final manuscript as submitted and agree to be accountable for all the aspects of the work.

Data Availability

All data generated or analyzed during this study are included in this article and its supplementary material.

Ethics Approval

Approval was granted by the Ethics Committee of Fujian Medical University Union Hospital (Date: 2022-06-17).

Informed Consent

As a retrospective study, this study only involved consulting patient's case data and did not intervene in patient treatment, so informed consent was waived.

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