Research Paper

# Application Value of Metagenomics Next Generation Sequencing of Alveolar Lavage Fluid Metagenome in Unexplained Pulmonary Infection

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**To explore the application value of metagenomics next generation sequencing of alveolar lavage fluid metagenome in unexplained pulmonary infection. A total of 40 patients with pulmonary infection diagnosed in our hospital from January 2020 to December 2020 were collected. All patients underwent routine blood cell analysis, C-reactive protein, procalcitonin, 1,3-Beta-D glucan test, galactomannan test, sputum smear, sputum culture, blood culture+drug sensitivity, chest computed tomography and other examinations. In addition, sputum, broncho alveolar lavage fluid, blood, pleural effusion and other samples were collected to detect etiology and metagenomics next generation sequencing. Traditional laboratory methods detected pathogens in 60.00 % (24/40) samples, while metagenomics next generation sequencing detected pathogens in 87.50 % (35/40) samples. 12 cases were completely consistent with the traditional method; the results of 5 cases of metagenomics next generation sequencing were partially consistent with those of traditional methods. In these 5 cases, metagenomics next generation sequencing detected potential pathogens which were not detected by traditional methods; In 7 cases, the results of traditional methods were inconsistent with metagenomics next generation sequencing. *Chlamydia psittaci* was detected in 2 cases by metagenomics next generation sequencing. The results of etiological diagnosis showed that there was no difference in the positive rates of bacterial infection and fungal infection between metagenomics next generation sequencing and traditional methods ($p>0.05$). The positive rate of metagenomics next generation sequencing virus infection was 84.62 % (11/13), which was significantly higher than that of traditional methods 38.46 % (5/13) ($p<0.05$). In addition, 7 patients were diagnosed as atypical pathogen infection and the positive rate of metagenomics next generation sequencing was 100 % (7/7), which was significantly higher than that of traditional methods (14.29 %, 1/7) ($p<0.05$). For difficult and critical patients with infection, using metagenomics next generation sequencing to detect pathogens can improve the early detection rate of pathogens, achieve accurate and individualized treatment, shorten the length of hospital stay and reduce the mortality.**

**Key words: Pulmonary infection, second generation sequencing of metagenome, pathogen, respiratory diseases**

Pneumonia is a common high incidence rate of respiratory diseases and the infection of lung is the pathogenesis of pneumonia. With the rapid development of modern medical technology, the incidence rate of malignant tumor, acquired immune deficiency syndrome, autoimmune diseases and other diseases are increasing. Cytotoxic drugs, immunosuppressive agents, glucocorticoids and broad spectrum antibiotics have been applied more and more widely. The application of organ transplantation and hematopoietic stem cell transplantation has been more and more extensive and the spectrum of the pulmonary infection has also changed, which makes the difficulty of diagnosis increase. Traditional pathogen detection technology is mainly based on microbial culture and isolation, but these traditional detection methods rely on the vitality of pathogens, a considerable number of pathogens cannot be detected by *in vitro* culture, so the

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culture positive rate is low, the cycle is long and the accuracy is low[1,2]. Therefore, the traditional pathogen detection methods have been unable to meet the needs of diagnosis and treatment of infectious diseases. With the development of metagenomics Next Generation Sequencing (mNGS), a variety of pathogens can be detected quickly and accurately, including atypical pathogens, viruses and fungi that are difficult to cultivate[3]. The purpose of this study was to investigate the potential value of Broncho Alveolar Lavage Fluid (BALF) mNGS in the etiological diagnosis of unexplained pulmonary infection.

## MATERIALS AND METHODS

### General clinical data:

A total of 40 patients diagnosed with pulmonary infection in respiratory department of our hospital from January 2020 to December 2020 and sent to the second generation sequencing test were collected. Their baseline data were included, including gender, age, main symptoms, complications, immune status, blood oxygen saturation, inflammatory indicators, imaging examination, etc.

Those who were hospitalized in our department from January 2020 to December 2020 meet the diagnostic criteria for lung infection: Clinical symptoms of respiratory infection such as cough, sputum, or original respiratory disease (such as chronic obstruction) the symptoms of patients with chronic lung disease, bronchial asthma, bronchiectasis, etc.) worsen, the nature of the sputum changes from white sticky sputum to thick and purulent, chest pain (inflammation involving the pleura) or no chest pain; Abnormal blood routine, White Blood Cells (WBC)>$10\times10^9$/l or <$4\times10^9$/l, with or without nuclear shift to the left; Abnormal body temperature, often elevated; Abnormal physical examination, signs of consolidation of the lungs and auscultation tube like breath sounds or wet rales; The imaging examination is abnormal and the chest X-ray examination and/or CT examination show spot-like, strip-like or patch-like infiltration shadows or interstitial changes. Some patients Inflammation can involve the pleura and cause pleural effusion; It is sufficient to satisfy item and one of the other items; The pathogen has not been clarified by traditional laboratory testing methods; The empirical anti-infective treatment time is ≥3 d (including the anti-infective treatment time in the outside hospital) and the condition has not been relieved; The second-generation sequencing has been sent for pathogenic testing; Fiberoptic bronchoscopy has been performed. Exclude standard blood biochemical examination, pathological examination, color Doppler ultrasound, fiberoptic bronchoscopy and other examinations. Exclude autoimmune diseases (systemic lupus erythematosus, Sjogren's syndrome, etc.) caused by lung disease, pulmonary vascular disease (Wegener's granulomatosis, Sarcoidosis), pulmonary edema caused by heart failure, atelectasis caused by airway obstruction, bronchial lung cancer and other diseases.

Negative pressure suction (the general recovery rate was 40 %-60 % ) and put into sterilized silicone plastic bottles. The BALF samples were divided into two equal parts. One specimen was sent to the laboratory of our hospital for smear, culture, isolation and identification. The other specimen was immediately stored at -20° and sent to Hugo Biotech for mNGS detection.

### Test method and test procedure:

Nucleic acid extraction-300 μ Deoxyribonucleic Acid (DNA) was extracted from BALF samples using the pathogen detection kit; Synthesis of Complementary DNA (cDNA)-first, the first strand of cDNA was synthesized, then the second strand of cDNA was synthesized, then purified and quantified by Qubit fluorometer; Library construction and quality control; Computer sequencing-DA8600 gene sequencer (Illumina, Hugo Biotech) was used for sequencing.

### Data analysis:

Sequencing data split samples according to the sequencing tag, cut the connector, filter the low-quality sequence, remove the host sequence based on the alignment database, compare the pathogen database and annotate the results. The sequences meeting the following conditions are reserved for annotation: The alignment length is more than 50 bp and insertion and deletion are not allowed; The comparison Nucleotide (NT) database is the only comparison or the highest score and the evaluation is less than or equal to $1e^{-5}$. It takes 2-3 d for NGS microbial identification results.

### Interpretation of mNGS results and positive criteria:

At present, there is no unified judgment standard for the positive results of mNGS in clinic, so the final results of this study are judged by the management doctors according to the clinical history characteristics and relevant auxiliary examinations of patients to determine whether they are infected and the pathogenic microorganisms of infection.

### Statistical analysis:

All data were analyzed by Statistical Package for the

Social Sciences (SPSS) 21.0 software. The counting data were expressed by the number of cases and the rate. The paired samples were compared by paired chi square test and consistency test (McNemar test and kappa test). $p<0.05$ was considered to be statistically significant, kappa <0.4 was considered to be poor consistency and >0.7 was considered to be good consistency.

## RESULTS AND DISCUSSION

A total of 52 patients strictly followed the inclusion and exclusion criteria and finally 40 patients were included and 12 patients were excluded. 12 patients were excluded for the following reasons: 3 cases of obstructive pneumonia caused by bronchogenic carcinoma, 1 case of pulmonary vascular disease, 1 case of interstitial pneumonia caused by polymyositis, 1 case of interstitial pneumonia caused by dermatomyositis, 1 case of Sjogren's syndrome with pulmonary fibrosis and 5 cases of incomplete clinical data.

A total of 40 patients with BALF mNGS samples were included, of which 10 patients received venous blood mNGS samples. Among the 40 patients, there were 24 males and 16 females, aged from 15 to 80 y (average 57.30 y). The respiratory manifestations included fever, cough, expectoration, hemoptysis, chest tightness, chest pain, dyspnea and dyspnea; with or without gastrointestinal symptoms, complications, hypertension, diabetes, gout, bird or poultry contact history, as shown in Table 1. The results of blood routine, C-Reactive Protein (CRP), Procalcitonin (PCT), 1,3-Beta-D Glucan test (G test), Galactomannan (GM) Test and chest Computed Tomography (CT) were shown in Table 2. Blood test: blood routine leukocyte count 1.34-34.71×$10^9$/l average 10.52×$10^9$/l; 31 cases had PCT >0.25 ng/l and the average value was 10.57 ng/l from 0.25 to 101.46 ng/l; CRP was 2.9-473 mg/l, with an average of 131.130 mg/l.

This study included 40 patients' traditional laboratory test results and mNGS test (including 40 BALF samples and 10 venous blood samples). The mNGS of P19, P20 and p21 samples were judged as contaminated and treated as negative. Traditional laboratory methods detected pathogens in 60.00 % (24/40) samples and mNGS detected pathogens in 87.50 % (35/40) samples. A total of 51 strains of pathogens were detected in mNGS and their distribution was shown in Table 2; a total of 30 strains of pathogens were detected by traditional methods and the distribution was shown in Table 3.

**TABLE 1: RESULTS OF ROUTINE EXAMINATION AND IMAGING EXAMINATION IN PATIENT LABORATORY**

| Number | Infiltration range of pulmonary lobes | Leukocyte ×$10^9$/l | PCT ng/l | GM | G test | CRP mg/l |
|---|---|---|---|---|---|---|
| Pl | Multiple ground glass opacity in both lungs | 11.74 | <0.25 | 0.27 | 36.8 | 10.3 |
| P2 | Exudation of lower right pneumonia | 1.89 | 034 | 0.48 | 142 | 142 |
| P3 | Multiple exudates in both lungs | 9.01 | <0.25 | 3.87 | <5.0 | 108 |
| P4 | Right upper pneumonic exudation | 2.48 | 0.28 | 1.5 | / | 26.1 |
| P5 | Multiple exudates in both lungs | 10.12 | 1.68 | 0.17 | <5.0 | 58.4 |
| P6 | Multiple exudates in both lungs | 232 | 0.25 | 0.44 | <5.0 | 191 |
| P7 | Exudation of lower right pneumonia | 6.16 | <0.25 | 0.22 | <5.0 | 6.55 |
| P8 | Multiple exudates in both lungs | 8.52 | 0.32 | 0.14 | 19.9 | 69.7 |
| P9 | Multiple exudates in both lungs | 9.73 | 24 | 0.54 | 86.5 | 473 |
| P11 | Multiple exudates in both lungs | 13.4 | 0.46 | 0.12 | <5.0 | 73.7 |
| P12 | Multiple exudates in both lungs | 2.16 | 2.38 | 0.13 | <5.0 | 287 |
| P13 | Multiple exudates in both lungs | 10.42 | 0.56 | 0.28 | <5.0 | 39.5 |
| P14 | Multiple exudates in both lungs | 6.96 | 18.03 | 0.24 | <5.0 | 75 |
| P15 | Multiple exudates in both lungs | 18.82 | 2.79 | 23 | <5.0 | 203 |
| P16 | Multiple exudates in both lungs | 20.96 | 0.38 | 0.22 | 26.71 | 156 |
| P17 | Multiple exudates in both lungs | 34.71 | 1.25 | 2.58 | <5.0 | 103 |
| P18 | Right upper pneumonic exudation | 9.87 | 1.13 | 0.73 | <5.0 | 27 |
| P19 | Right upper pneumonic exudation | 14.52 | 0.78 | 0.13 | <5.0 | 36.2 |
| P20 | Multiple exudates in both lungs | 15.23 | 1.35 | 0.67 | <5.0 | 45.7 |

| | | | | | | |
|---|---|---|---|---|---|---|
| P21 | Multiple exudates in both lungs | 4.87 | <0.25 | 0.14 | <5.0 | 3.54 |
| P22 | Multiple exudates in both lungs | 835 | <0.25 | 0.37 | <5.0 | 75.3 |
| P23 | Multiple exudates in both lungs | 5.97 | 0.62 | 0.39 | 30.04 | 173 |
| P24 | Multiple exudates in both lungs | 7.26 | 97.11 | 2.27 | <5.0 | 448 |
| P25 | Multiple exudates in both lungs | 17.34 | 101.46 | 1.57 | <5.0 | 276 |
| P26 | Multiple exudates in both lungs | 10.28 | 0.4 | 0.22 | <5.0 | 120 |
| P27 | Multiple exudates in both lungs | 21.11 | 2.13 | 0.32 | 13.81 | 239 |
| P28 | Multiple exudates in both lungs | 13.23 | 52.19 | 0.41 | <5.0 | 118 |
| P29 | Multiple exudates in both lungs | 15.95 | 0.35 | 0.23 | <5.0 | 203 |
| P30 | Multiple exudates in both lungs | 14.83 | 1.31 | 4.88 | <5.0 | 152 |
| P31 | Multiple exudates in both lungs | 10.09 | <0.25 | 0.24 | <5.0 | 30.3 |
| P32 | Multiple exudates in both lungs | 5.17 | 0.76 | 1.38 | <5.0 | 358 |
| P33 | Multiple exudates in both lungs | 13.55 | <0.25 | 0.28 | <5.0 | 2.96 |
| P34 | Multiple exudates in both lungs | 4.77 | <0.25 | 0.29 | <5.0 | 75.6 |
| P35 | Right upper pneumonic exudation | 7.16 | 0.66 | 0.19 | <5.0 | 186 |
| P36 | Multiple exudates in both lungs | 17.47 | 10.33 | 0.17 | <5.0 | 164 |
| P37 | Interstitial changes and massive exudation in both lungs | 19.52 | 0.34 | 0.37 | <5.0 | 104 |
| P38 | Multiple exudates in both lungs | 7.42 | <0.25 | 036 | 23.78 | 93.2 |
| P39 | Multiple exudates in both lungs | 16.46 | 0.59 | 0.49 | <5.0 | 81.9 |
| P40 | Multiple exudates in both lungs | 7.21 | 1.34 | 0.23 | <5.0 | 240 |

**TABLE 2: THE DISTRIBUTION OF PATHOGENS DETECTED IN mNGS**

| Pathogen | Number of bacteria detected | Constituent ratio (%) |
|---|---|---|
| Gram negative bacteria | | |
| *Moraxella osloensis* | 5 | 9.8 |
| *Prevotella salivae* | 5 | 9.8 |
| *Veillonella dispar* | 4 | 7.8 |
| *Veillonella atypica* | 2 | 3.9 |
| *Neisseria subflava* | 1 | 2.0 |
| *Pseudomonas aeruginosa* | 1 | 2.0 |
| Gram positive bacteria | | |
| *Staphylococcus epidermidis* | 1 | 2.0 |
| *Actinomyces meyeri* | 1 | 2.0 |
| Fungus | | |
| *Candida albicans* | 7 | 13.7 |
| Virus | | |
| Human betaherpesvirus 6B | 7 | 13.7 |
| Human herpesvirus 4 | 2 | 3.9 |
| Human betaherpesvirus 5 | 1 | 2.0 |
| Human papillomavirus type 8 | 1 | 2.0 |
| Others | | |
| *Streptococcus constellatus* | 5 | 9.8 |
| *Mycobacterium tuberculosis* complex | 2 | 3.9 |
| *Nocardia abscessus* | 1 | 2.0 |
| Total | 51 | 100 |

**TABLE 3: THE DISTRIBUTION OF PATHOGENS DETECTED IN TRADITIONAL METHOD**

| Pathogen | Number of bacteria detected | Constituent ratio (%) |
|---|---|---|
| Gram negative bacteria | | |
| *Moraxella osloensis* | 4 | 3.3 |
| *Prevotella salivae* | 5 | 16.7 |
| *Veillonella dispar* | 4 | 13.3 |
| *Neisseria subflava* | 1 | 3.3 |
| *Pseudomonas aeruginosa* | 1 | 3.3 |
| Gram positive bacteria | | |
| *Staphylococcus epidermidis* | 1 | 3.3 |
| Fungus | | |
| *Candida albicans* | 2 | 6.7 |
| *Aspergillus fumigatus* | 1 | 3.3 |
| *Candida tropicalis* | 3 | 10.0 |
| *Malassezia globosa* | 1 | 3.3 |
| Virus | | |
| Human betaherpesvirus 6B | 2 | 6.7 |
| Human herpesvirus 4 | 1 | 3.3 |
| Human papillomavirus type 8 | 1 | 3.3 |
| Others | | |
| *Mycobacterium tuberculosis* complex | 1 | 3.3 |
| *Streptococcus constellatus* | 2 | 6.7 |
| Total | 51 | 100 |

Of all the results, 12 cases were completely consistent with the traditional method; the results of 5 cases of mNGS were partially consistent with those of traditional methods. In these 5 cases, mNGS detected potential pathogens which were not detected by traditional methods; In 7 cases, the results of traditional methods were inconsistent with mNGS. *Chlamydia psittaci* was detected in 2 cases by mNGS.

The results of mNGS in 40 patients with pulmonary infection were compared with those in traditional laboratory by McNemar test. All 40 patients were tested by traditional laboratory etiology and mNGS, among which 22 cases were positive by mNGS and traditional laboratory test, 3 cases were negative by mNGS and traditional clinical etiology test, 13 cases were positive by mNGS but negative by traditional test and 2 cases were negative by mNGS but positive by traditional test. The difference was statistically significant ($p<0.05$), as shown in Table 4. The positive rate of mNGS test is higher than that of traditional laboratory test; But kappa=0.22<0.4, it is considered that the consistency of the two methods is poor.

According to the final results of clinical etiology, 40 patients were divided into five categories: bacteria, fungi, viruses, atypical pathogens and *Mycobacterium tuberculosis* infection (bacterial infection in simple bacterial infection and mixed infection were regarded as bacterial infection and *so on*). In view of the small sample size of each group, we used Fisher's exact test to compare the difference between the detection rate of mNGS and traditional methods. The results are shown in Table 5. There is no significant difference between the positive rate of NGS in the diagnosis of bacterial infection and fungal infection and the positive rate of traditional methods. The positive rate of mNGS was 84.62 % (11/13), which was higher than that of traditional methods (38.46 %, 5/13). The positive rate of mNGS was 100 % (7/7) in 7 patients, which was higher than that of traditional methods (14.29 %, 1/7), $p<0.01$. A total of 3 patients were infected with *Mycobacterium tuberculosis*, 1 case was detected by mNGS and 2 cases were detected by traditional methods. In addition, 11 cases of mixed infection were detected by mNGS, including double infection of bacteria and virus, triple infection of bacteria, virus and fungi. Only 2 cases of mixed infection were detected by traditional methods and 2 cases of pathogens were not detected by traditional methods.

**TABLE 4: COMPARISON OF ETIOLOGICAL DETECTION RESULTS BETWEEN mNGS AND TRADITIONAL LABORATORY**

| mNGS | Traditional laboratory | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 22 | 13 | 35 |
| Negative | 2 | 3 | 5 |
| Total | 24 | 16 | 30 |

**TABLE 5: COMPARISON OF THE EFFICACY OF mNGS AND TRADITIONAL LABORATORY TESTS IN IDENTIFYING PATHOGEN TYPES**

| Category | mNGS positive rate | The positive rate of traditional methods | p |
|---|---|---|---|
| Bacterial infection | 81.82 % | 63.64 % | 0.31 |
| Mycotic infection | 85.71 % | 50.0 % | 0.18 |
| Viral infection | 84.62 % | 38.46 % | 0.041 |
| A typical pathogen infection | 100.0 % | 14.29 % | <0.01 |
| *Mycobacterium tuberculosis* infection | 33.33 % | 66.67 % | - |

All patients were given empirical anti infection treatment according to the characteristics of medical history, clinical manifestations, relevant imaging and laboratory test results, and the treatment plan was adjusted in time after the pathogen types were identified. 7 cases were diagnosed as Nontuberculous Mycobacteria (NTM), One case was *Mycobacterium abscess* treated with azithromycin and other drugs, and other six cases were all slow-growing NTM, just follow-up observation; 2 patients were diagnosed as *Chlamydia psittaci* pneumonia and were treated with Levofloxacin; One case was diagnosed as *Mycoplasma pneumoniae* pneumonia and was treated with azithromycin; 2 patients were diagnosed as invasive pulmonary aspergillosis infection and were treated with voriconazole; *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and other infections were treated with sensitive antibiotics according to the results of drug sensitivity; Human herpesvirus, cytomegalovirus, adenovirus and other viral infections were treated with acyclovir. After individualized anti infection treatment for pathogens, supportive treatment for complications and complications, 39 patients were improved and discharged. One patient died and discharged due to multiple organ failure due to disease progression.

Pulmonary infection is one of the most common infectious diseases in clinic and has high incidence rate and mortality rate, especially in the elderly and immunocompromised population[4,5]. Rapid identification of pathogens is crucial for the treatment and prognosis of patients with pulmonary infection, but accurate detection and identification of pathogens is still challenging, especially in the host with impaired immune function[6,7]. Early identification of pathogens can be targeted to choose antibiotics, avoid abuse of antibiotics, shorten hospital stay and improve survival rate[8]. However, on the one hand, the current microbial isolation and culture technology relies on the vitality of pathogens[9] and due to the influence of the lesion surrounded by fibrous tissue and the application history of antibiotics, the positive rate of culture is low. On the other hand, histopathological analysis has no advantage in the diagnosis of other pathogens and the detection is invasive[10]. Patients with negative traditional pathogen test results often receive empirical antibiotic treatment, which may not cover the real pathogen and may lead to aggravation of infection[11,12]. The widespread use of antibiotics may promote the accumulation and spread of antibiotic resistance and multidrug-resistant pathogens[13]. Therefore, the establishment and promotion of rapid and accurate pathogen detection method is of great significance for timely, reasonable and effective prevention and treatment of infectious diseases.

Due to its advantages of high throughput, low cost, high sensitivity and no bias, mNGS has been widely used in pathogen detection[14,15]. With mNGS, only a small amount of DNA needs to be extracted from the samples to detect and identify pathogens at the same time. Due to its high positive rate in pathogen detection, mNGS has been successfully used in many clinical trials of infection diagnosis[16,17]. In addition, mNGS improved

the detection efficiency of culture negative samples[18]. In recent years, there are many high-quality studies on the application of mNGS in the etiological diagnosis of central nervous system infection in China, but there are still few reports on the diagnosis of pulmonary infection.

In this study, we retrospectively analyzed and evaluated the value of mNGS in the diagnosis of pulmonary infection pathogens. The results showed that mNGS detected pathogens in 87.50 % of the samples (35/40), while traditional laboratory detection methods detected pathogens in 60.00 % (24/40) of the samples, of which 12 cases had the same results. In addition, the detection results of 5 cases of mNGS were partially consistent with those of traditional methods. The pathogens not detected by traditional methods in these 5 cases were clinically confirmed as infectious pathogens; In 7 cases, the results of traditional methods were inconsistent with mNGS. Our study shows that mNGS has a certain value for pulmonary infection. mNGS can diagnose potential pathogens in most negative samples detected by traditional methods, but the consistency between them is poor. At present, mNGS cannot replace the traditional laboratory detection as the gold standard.

According to the final clinical etiological diagnosis, we divided the cases into bacterial infection, fungal infection, viral infection, atypical pathogen infection (including *Chlamydia*, mycoplasma, NTM, etc.,) and *Mycobacterium tuberculosis* infection, and evaluated the detection efficiency of mNGS and traditional methods respectively. There were 22 cases of bacterial infection. There was no significant difference between the positive rate of mNGS and that of traditional methods (81.82 % *vs.* 63.64, p=0.31). In 13 patients with viral infection, the positive rate of mNGS was higher than that of traditional methods (84.62 % *vs.* 38.46 %, p=0.041). There was no significant difference between the two groups (85.71 % *vs.* 50.0 %, p=0.18). The positive rate of mNGS was higher than that of traditional methods (100.0 % *vs.* 14.29 %, p<0.01). There were 3 patients with *Mycobacterium tuberculosis* infection and the difference could not be compared because of the small sample size. All 11 cases of mixed infection were diagnosed by mNGS, of which only 2 cases were detected by traditional methods and 2 cases were not detected by traditional methods. In conclusion, the overall detection rate of mNGS was higher than that of traditional methods, especially in the diagnosis of virus, atypical pathogen and mixed infection.

In this study, the distribution of infectious bacteria was mainly bacteria, in which Gram-negative bacteria accounted for 90.0 % of all bacteria and *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* were the most common, which was basically consistent with many studies reported in China[19-21]. NTM are increasingly important opportunistic pathogens in humans. NTM cause chronic Pulmonary Disease (NTM-PD), localized infections after inoculation and disseminated infections in the severely immune compromised. Antibiotic treatment of NTM disease applies complex, toxic and long-term multidrug regimens[22,23]. Staining microscopy is the main method for the diagnosis of NTM and its sensitivity is relatively low. In this study, all 7 cases of NTM were detected by mNGS, while only 2 cases were detected by traditional methods, indicating that mNGS has certain advantages over traditional methods in the diagnosis of NTM. The main viruses were human herpesvirus (63.3 %), mainly human herpesvirus-1 (herpes simplex virus-1), human herpesvirus-4 (EB virus) and human herpesvirus-5 (cytomegalovirus). In this study, most of the viral infections were mixed with bacterial infection and fungal infection (9 cases of mixed infection including viral infection: 4 cases of simple viral infection). Studies have shown that the mixed infection of bacteria and virus is the root cause of Community Acquired Pneumonia (CAP) and Hospital Acquired Pneumonia (HAP)[24]. In this study, the detection efficiency of mNGS was higher than that of traditional laboratory methods. Among the patients infected with atypical pathogens, *Chlamydia psittaci* accounted for 62.5 %. *Chlamydia psittaci* is an obligate intracellular parasite, which has high requirements for culture and it is difficult to obtain pathogens from isolation and culture. Clinical detection mainly relies on antigen detection and Polymerase Chain Reaction (PCR) technology[20]. In this study, 2 cases of *Chlamydia psittaci* were detected by mNGS and the traditional methods were negative. We infer that mNGS has obvious advantages in the diagnosis of atypical pathogens. In all cases, only 3 cases were diagnosed as *Mycobacterium tuberculosis* infection. One case of *Mycobacterium tuberculosis* sequence was detected by mNGS and the reading was very low. Due to the small number of cases, some of them are consistent with clinical images, and there may be contamination, so it is difficult to judge the detection efficiency of mNGS for *Mycobacterium tuberculosis*.

In conclusion, mNGS has some advantages in the diagnosis of pulmonary infectious diseases, especially for viruses, atypical pathogens and mixed infection. For identification of bacteria and fungi, this study has not

shown significant difference compared with traditional methods. Considering that due to the limited number of samples included, a larger sample size study is needed. In addition, the detection time of mNGS is shorter and more efficient than that of traditional laboratory. For patients with difficult and critical diseases, the detection of pathogens with mNGS is of greater significance, which can improve the rate of early pathogen physical examination, achieve accurate and individualized treatment, shorten the hospitalization time and reduce the mortality. Therefore, mNGS can be used as an effective complementary method for traditional etiological diagnosis, and the combination of the two methods can improve the detection efficiency of the whole pathogen.

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**Conflict of interests:**

The authors report no conflicts of interest.

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