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Diagnostic value of metagenomic next-generation sequencing combined by medical thoracoscopy surgery among infectious pleural effusion patients

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Abstract

Background Metagenomic next-generation sequencing (mNGS) is a novel method for identifying pathogens in infectious diseases. This study aimed to explored the application value of mNGS in diagnosing pulmonary infections with pleural effusion, confirmed by medical thoracoscopy.

Methods We retrospectively reviewed 25 patients with pulmonary infections and pleural effusion between July 2020 and December 2021. All patients had their diagnosis confirmed by medical thoracoscopy to obtain tissue samples for both traditional testing and mNGS. Samples included pleural effusion, successive sputum, and tissue obtained through medical thoracoscopy. We wanted to assess how effective mNGS was in accurately diagnosing these infections.

Results This study found that the positive predictive value of mNGS (76% (19/25)) was significantly higher than that of traditional testing (32% (8/25)). The most commonly identified pathogens were *Mycobacterium tuberculosis* ($n=5$), followed by *Fusobacterium nucleatum* ($n=4$), *Torque teno virus* ($n=4$), *Streptococcus intermedius* ($n=3$), *Peptostreptococcus stomatis* ($n=2$), *Porphyromonas endodontalis* ($n=2$), and *Campylobacter rectus* ($n=2$). The percentage of mNGS-positive cases was significantly higher than that from traditional testing for bacteria, but the superiority of mNGS for tuberculosis detection was insignificant. Ten cases were identified with mixed infections by mNGS, while no mixed infections were found by traditional testing.

Conclusions Our study showed that using mNGS in combination with biopsy samples obtained through medical thoracoscopy resulted in higher positive rates compared to traditional tests and provided more evidence of pathogens for patients with infectious pleural effusion.

Keywords Metagenomic next-generation sequencing, Medical thoracoscopy, Infectious pleural effusion, Diagnostic value

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Introduction

Pleural effusion is a common situation secondary to various underlying disease in clinic practice [1]. Pleural infections are the primary causes of pleural effusion and are associated with high morbidity and mortality rates globally [2]. Rapid and accurate identification of pathogens is crucial for clinical diagnosis and targeted therapies, as it can improve disease outcomes and shorten the duration of illness [3]. Therefore, enhancing definitive microbiological detection is key to improving the diagnostic rate. Empirical treatment without evidence of specific pathogens can lead to antibiotic overuse and contribute to antimicrobial resistance [4].

Conventional culture-based methods are time-consuming and have low sensitivity. Other methods, such as polymerase chain reaction (PCR), immunology, and nucleic acid hybridization, are limited to specific microorganisms [5]. Metagenomic next-generation sequencing (mNGS), a high-throughput sequencing technique, is a sensitive and rapid method for detecting multiple pathogens simultaneously in various clinical samples [6–8]. mNGS technology has shown superiority in identifying rare or novel pathogens and guiding antibiotic treatment options [9].

Medical thoracoscopy is an endoscopic technique used to diagnose and treat pleural effusions, especially in patients with pleural empyema, which has a higher mortality rate [10, 11]. Thoracoscopy surgery offers advantages for patients with encapsulated pleural effusion or empyema by allowing the breaking of adhesions, debridement, and decortication [12, 13]. Importantly, it can obtain pleural tissue samples to identify the cause of the infection. While sputum, bronchoalveolar lavage fluid (BALF), blood, and pleural fluids are often used to detect pathogens in respiratory infectious diseases [14], limited studies have assessed the diagnostic efficacy of mNGS based on pleural biopsy obtained through medical thoracoscopy for infectious pleural diseases.

Our study summarizes the mNGS results of pleural tissue obtained through medical thoracoscopy for pleural effusion patients and aims to evaluate the clinical performance and impact of combining mNGS with medical thoracoscopy in diagnosing pathogens for pleural effusion patients.

Methods

Patients and sample collection

We enrolled 25 patients with pulmonary infection complicated by pleural effusion from the Department of Respiratory and Critical Care Medicine at Shandong Provincial Hospital Affiliated to Shandong First Medical University between January 2020 and February 2021. The inclusion criteria were: (1) patients aged over 18 years old; (2) patients had infectious pleural effusion if the patient was diagnosed with exudative pleural effusion based on Light's criteria with or without fever; (3) patients underwent medical thoracoscopy; and (4) patients who consented to the mNGS examination. Patients who declined the mNGS test and diagnosed with simple lung cancer were excluded. Medical thoracoscopy was performed by experienced physician in our center using an Olympus rigid thoracoscope (LTF-240, Japan) under local anesthesia. The patient was in lateral decubitus and received oxygen inhalation and electrocardiograph monitoring continuously. Medical thoracoscopy was performed with a single optimal entry site by designated trocar and cannula following the sterile procedure. Systematic inspection of the pleural cavity was performed from the apex to diaphragm. In our study, pleural fine adhesions were observed in all patients and the pleural tissue was achieved for detection of pathogen and histopathological examination.

We obtained 3 to 5 forceps specimens by the rigid thoracoscopic for the detection of mNGS for each patient. The amount of each specimen was at least 5*5 mm of each forceps. mNGS and culture tests of pleural tissues were conducted for all patients. The written informed consent of mNGS detection was obtained for each participant. Additionally, sputum and pleural fluid samples were collected for conventional microbiologic tests including gram staining, bacterial and fungal culture tests, and tests for other microorganisms in our microbiological laboratory for a maximum of 5 days. Other conventional testing included *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Epstein–Barr virus*, *cytomegalovirus*, *adenovirus*, and *herpes simplex virus* serological antibody detection. Smear acid fast bacilli staining microscopy of pleural tissue and GeneXpert MTB/RIF (Xpert) detection of pleural effusion for *Mycobacterium tuberculosis* were carried out. The tissue specimens were sent to a genetic company (Hangzhou Matridx Biotechnology Co Ltd) for mNGS testing, and the results were evaluated and identified using the microbial sequences in the library.

Statistical methods

All data were presented as median and interquartile range (IQR) for continuous variables and as numbers with percentages for categorical variables. Sensitivity and specificity were calculated using 2×2 contingency tables and the McNemar's Chi-squared test. The analyses were performed using the SPSS statistical software, and a P value of less than 0.05 was considered significant.

Results

Baseline characteristics of patients

Basic information on the inclusive patients is presented in Table 1. A total of 25 patients were included,

Table 1 Basic information of the participants

Category	Median/ Percentage
Age, year	56 (24)
Gender	
Male	21/0.84
Hospitalization days	13 (9)
White blood cells×10^9/L	8.20 (5.59)
Neutrophil×10^9/L	6.00 (4.33)
C-reactive protein, mg/L	63.76 (103.95)
Procalcitonin, ng/ml	0.10 (0.24)
Albumin, g/L	34.4 (5.25)
ESR, mm/h	71 (48)
Hemoglobin, g/L	127.00 (32.50)
D-Dimer, mg/L	4.70 (9.48)
TB-IGRA	
Positive	10/0.40
Smoking history	13/0.52
Drinking history	16/0.64
Underling diseases	
Diabetes	3/0.12
Hypertension	10/0.4
Coronary disease	2/0.08
Antimicrobial therapy before thoracoscopy	22/0.88

Data are presented as n /percentage or median (interquartile range (IQR)). ESR, erythrocyte sedimentation rate; TB-IGRA, tuberculosis-interferon gamma release assays

Table 2 The diagnostic efficacy of mNGS and culture among the infectious pleural effusion

	Cases ($n=25$)		
Method	**Positive**	**Negative**	**Sensitivity**
mNGS	19	6	19/25 (76%)
Conventional test	8	17	8/25 (32%)

comprising 21 males and 4 females. The median age of the participants was 56 years. The median duration of hospitalization was 13 days. Among the patients, 10 had hypertension, 3 had diabetes, and 2 had coronary disease. Additionally, 13 patients had a history of smoking, while 16 patients had a history of drinking. There were 22 patients received antimicrobial therapy before thoracoscopy.

Diagnostic efficacy of mNGS and conventional test

In Tables 2, 19 cases tested positive and 6 cases tested negative using mNGS testing. In comparison, the conventional test showed 8 positive samples and 17 negative samples. The positive predictive value of mNGS was significantly higher than that of the traditional test (76% vs. 32%, $P=0.006$).

Consistency between mNGS and conventional test

In this study, we found that out of 25 cases, 7 cases (28%) tested positive for both mNGS and the conventional test. 12 cases (48%) tested positive only for mNGS, while 1 case (4%) tested positive only for the conventional test (Fig. 1). Additionally, 5 patients (20%) tested negative for both mNGS and the conventional test, but further pathology findings confirmed 2 of them were infected with *Mycobacterium tuberculosis*. Among the 7 patients who tested positive for both mNGS and the conventional test, 4 cases (57%) showed completely matching results, 2 cases (29%) showed completely mismatched results, and 1 case (14%) showed partly matched results. Figure 2 illustrated that the positive rate of mNGS for bacterial and tuberculous infections was higher compared to conventional tests.

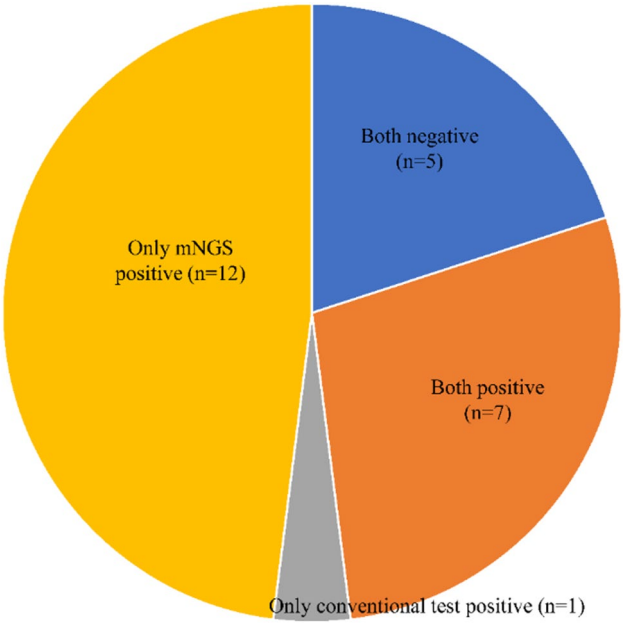

Fig. 1 Concordance between mNGS and culture. Both positive, results of mNGS and conventional test were both positive; Both negative, results of mNGS and culture were both negative; only culture+, only the result of culture was positive; only the mNGS+, only the result of mNGS was positive

Comparison of pathogen between mNGS and conventional test

Ten cases were diagnosed with mixed infections using mNGS, while no cases of mixed infection were found using conventional testing (see Fig. 3). This indicates the advantage of mNGS in detecting pathogen integrity. As shown in Fig. 3, 5 cases cases were confirmed with *Mycobacterium tuberculosis* using mNGS, while only 2 cases were diagnosed with tuberculosis using conventional tests, suggesting a higher diagnosis rate with mNGS (20% vs. 8%, $P=0.371$) in detecting *Mycobacterium tuberculosis*. Additionally, 16 patients were found to be infected with bacteria using mNGS, whereas only 6 cases were diagnosed with bacterial infection using conventional tests (64% vs. 24%, $P=0.0044$).

Among the pathogens tested by mNGS, *Mycobacterium tuberculosis* ($n=5$) was the most frequently

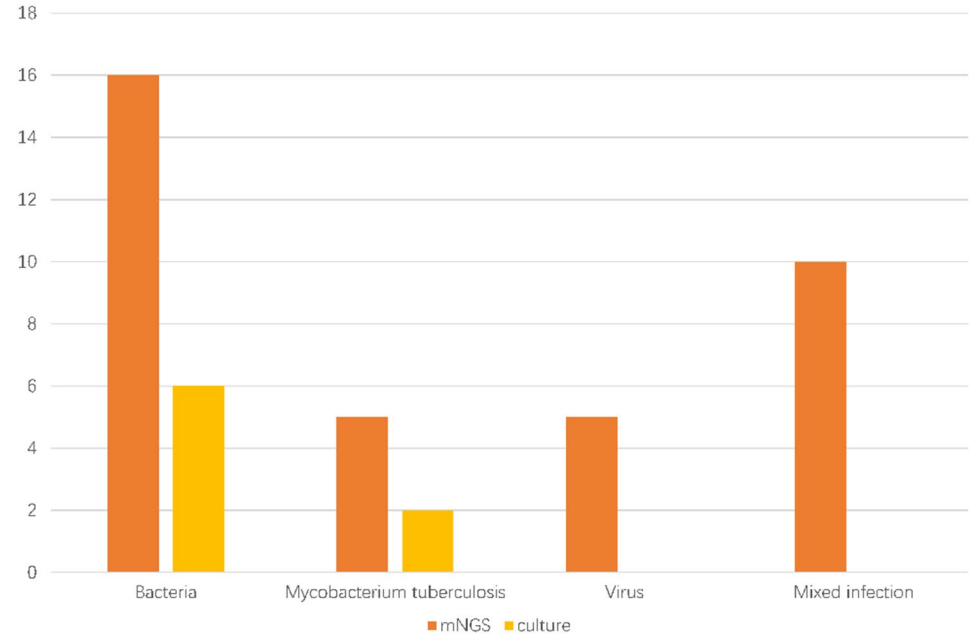

Fig. 2 The overlap of positivity between mNGS and conventional test for tuberculosis and bacteria. mNGS positive, the result of mNGS was positive; both positive, results of mNGS and conventional test were both positive; conventional test positive, result of conventional test was positive

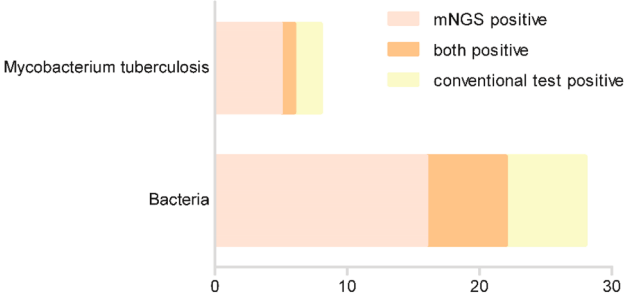

Fig. 3 Comparison of pathogen between mNGS and conventional test

detected, followed by *Fusobacterium nucleatum* ($n=4$), *Torque teno virus* ($n=4$), *Streptococcus intermedius* ($n=3$), *Peptostreptococcus stomatis* ($n=2$), *Porphyromonas endodontalis* ($n=2$), and *Campylobacter rectus* ($n=2$) (see Fig. 4A). Figure 4B shows the pathogens detected by conventional tests, with *Mycobacterium tuberculosis* being the most frequent, which is consistent with the results from mNGS.

Diagnostic value of mixed infection of mNGS

We further conducted a detailed analysis of the diagnostic performance of mNGS in detecting mixed infections. Figure 5 shows that out of 25 cases, 9 (36%) were confirmed as having a single pathogen infection through the

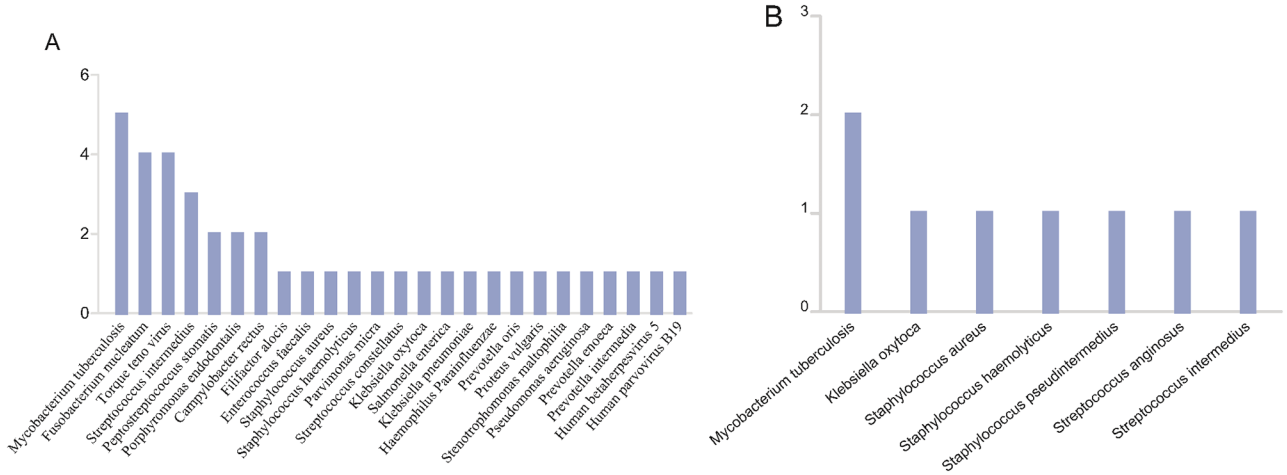

Fig. 4 Species distribution of detected pathogen by mNGS (**A**) and conventional test (**B**)

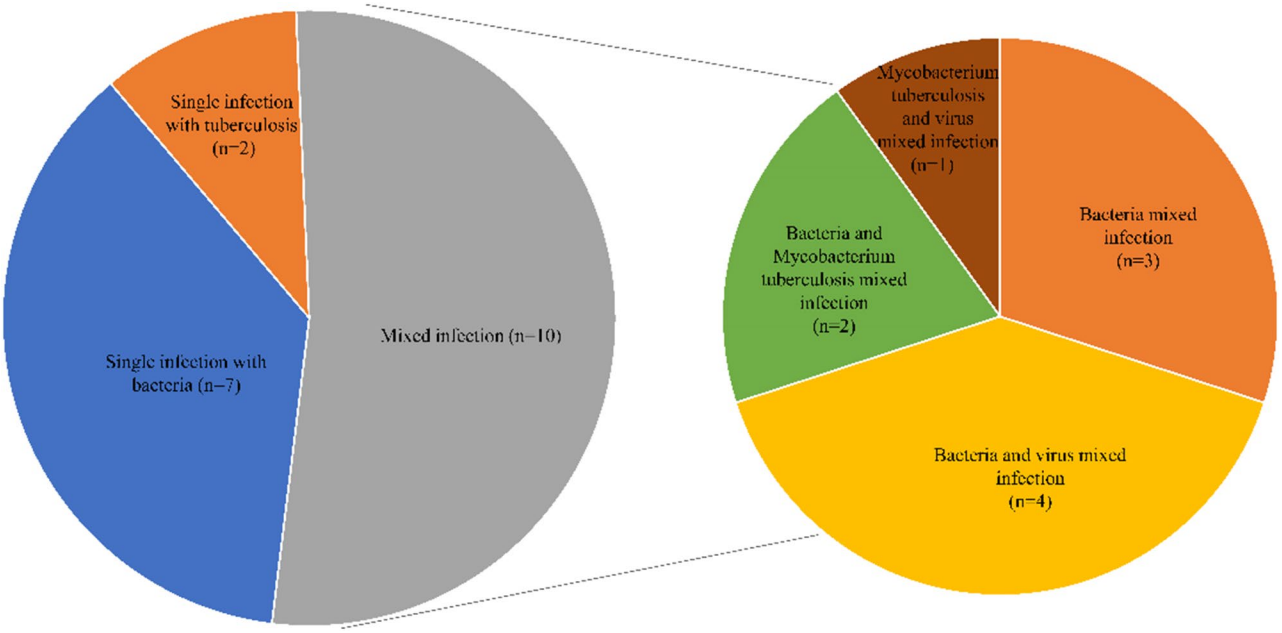

Fig. 5 Percentage of patients with single and mixed infections by mNGS

mNGS test, while 10 (40%) patients were diagnosed with mixed pathogen infections. The most common pattern of mixed infection was bacteria and virus mixed infection ($n=4$), followed by bacteria mixed infection ($n=3$), bacteria and *Mycobacterium tuberculosis* mixed infection ($n=2$), and *Mycobacterium tuberculosis* and virus mixed infection ($n=1$).

Discussion

In this study, all samples detected for mNGS were pleural tissue derived from patients undergoing medical thoracoscopy surgery. Unlike sputum and BALF, pleural tissue is considered a sterile sample, making it less likely to be affected by colonizing microorganisms. This makes it easier for clinicians to determine if the detected microorganisms are the targeted pathogens. However, the clinical significance of using pleural tissue for diagnosing infectious diseases has not been fully explored, especially when using the mNGS detection method.

Medical thoracoscopic via local anesthesia has been successfully performed for advanced stage parapneumonic effusion and empyema with advantages of being invasive, safe and effective for pleural biopsy [15, 16]. In our study, we included patients who had undergone unsuccessful thoracentesis and antibiotic treatment, necessitating medical thoracoscopy to obtain pleural tissue for further diagnosis. Research has shown that timely intervention with thoracoscopic surgery can lead to a shorter duration of antimicrobial therapy and hospital stay, as well as a reduced need for long-term administration of broad-spectrum antibiotics for patients with thoracic empyema or complicated parapneumonic effusion [17]. The findings indicated that a proper and urgent thoracoscopic surgery would contribute to a better prognosis for patients.

Identifying the pathogen is critical and essential for the precise diagnosis and optimal treatment of infectious pleural diseases. Compared with traditional microbiological diagnostic methods, mNGS offers a rapid way to detect the entire microbiome at a time and shows a higher positive rate [18]. Previous studies have shown that mNGS has higher sensitivity in detecting bacteria, fungi, and tuberculosis in samples such as BALF, blood, and sputum, compared to culture methods [19–22]. In our study, mNGS showed a sensitivity of 76% in detecting pathogens in pleural biopsy samples, which was higher than the 70% sensitivity reported in another study using pleural effusion samples [6]. This suggests that pleural tissue has a higher diagnostic value than pleural fluid in identifying infectious pleural diseases.

In our study, *Mycobacterium tuberculosis* was the most frequently detected pathogen, especially in patients with pleuritis who did not respond to empirical antibiotic treatment. Tuberculous effusion can present with various clinical features, ranging from benign effusion to complicated effusion with loculations, pleural thickening, and tuberculous empyema [23]. Our findings are consistent with previous research that identified tubercular pleural effusion as the main infectious cause of undiagnosed exudative pleural effusion [24, 25]. Although mNGS performed better in detecting bacterial infections than conventional tests, it was not superior in detecting tuberculosis pathogens. This is in line with evidence showing that the detection of tubercle bacilli in pleural effusion is

usually low and that acid-fast bacilli are detected in less than 5% of cases using microbiological tests [26]. Our research indicated that combining histopathological examination with mNGS can enhance the diagnostic sensitivity, especially for difficult-to-treat pleural infections, approaching a sensitivity of 100%.

When it comes to bacterial infection, the main pathogens are *Fusobacterium nucleatum*, *Streptococcus intermedius*, *Peptostreptococcus stomatis*, *Porphyromonas endodontalis*, and *Campylobacter rectus*. The most commonly detected virus was *Torque teno virus*. Our findings partly aligned with previously published data [6]. In contrast to the pathogen results of pulmonary infection [27], we did not find any fungal infections in our study. We observed that only 2 cases had completely different results between mNGS and traditional detection methods. The consistency rate in our study (57%) was higher than that reported in other reported studies (4.17% and 14.3%) [19, 28]. Another retrospective study [29] analyzed the diagnostic value of mNGS in suspected infectious diseases, and the completely matched cases were only 2%, which was lower than our rate. We believed that the higher consistency rate in our results was due to the biopsy pleural sample, which increased the reliability of mNGS results. Therefore, our results supported the idea that using a sterile sample for mNGS would be beneficial in distinguishing causative pathogens from microbial colonizers.

Another advantage of mNGS is its efficacy in diagnosing mixed infections. In our study, conventional microbiologic tests did not find any cases of mixed infection. Wang et al. [30] found that the diagnostic sensitivity of mNGS was 97.2% among patients with mixed pulmonary infections. In our results, the sensitivity of mNGS for mixed infection was 40%, which was close to that from another study [6]. We suggested the lower positive rate in this study may be correlated with the detected sample and the inclusion criteria. Patients with mixed infections often suffer from underlying diseases and compromised immunity [31]. In our study, patients who were eligible for medical thoracoscopy were excluded if they had severe underlying diseases or were in poor physical condition. The most common pattern of mixed infection in our study was bacterial and viral mixed infection, followed by bacterial mixed infection. Our results indicated the advantage of mNGS in detecting viral pleuritis comparing with the conventional tests including specific antibody detection and nucleic acid PCR. The clinical diagnosis of viral pleural effusion was challenged among conventional testing limited by difficulties in virus separation and culture. Published studies had indicated that pleural effusion could be a poor prognostic factor for patients with virus infection [32]. Therefore, early pathogen diagnosis by means of mNGS would be beneficial for the prognosis of virus pleurisy, especially considering the state of mixed infection [33]. *Torque teno virus*, *Human parvovirus B19* and *Human betaherpesvirus 5* are the detected virus pathogen in our study, which indicated the state of immune suppression among mixed pathogen infected patients [34]. However, subjective judgement of NGS results from experienced physician would be necessary to judge whether the detected virus was the suspected pathogen in terms of the disadvantage of reduced specificity in exchange for its superior sensitivity. Carefully interpretation of the results should be needed combining with the patient's clinical characteristics. In critically ill hematology patients, the most common mixed infection pattern was also bacterial and viral [35], while Xie et al. observed the most frequent pattern was bacterial and fungal [28]. The inconsistency may result from the detected sample, study population and even demographic characteristics.

There are several limitations in our study. First, the sample size of study population was small. Second, mNGS is relatively expensive and not covered by medical insurance, which may introduce a selection bias. Third, because there is no unified standard for unbiased detection of mNGS and there are difficulties to distinguish the colonizing and environmental pollutant microorganism from infectious pathogen, subjective judgement of NGS results from experienced physician would be also important. This is a challenge for future clinical research. Prospective and randomized controlled trials in the future should be conducted.

Conclusions

In summary, mNGS showed better performance in detecting pathogens compared to conventional microbiologic tests, especially for identifying bacteria and mixed infections. When combined with biopsy samples obtained during medical thoracoscopy, mNGS could improve diagnostic accuracy and provide more consistent results for infectious pleural effusion. Therefore, mNGS is valuable for identifying pathogens and can contribute to improving disease prognosis.

Abbreviations

PCR	Polymerase chain reaction
mNGS	Metagenomic next-generation sequencing
BALF	Bronchoalveolar lavage fluid
IQR	Interquartile range

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-10806-9.

Supplementary Material 1

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Author contributions
NG and DL conceived and designed the study. NG and SF performed the data analyses. NG, XY, JZ, ZY and YW collected the data and interpreted the results of the data analyses. NG and DL wrote the manuscript. All authors read and approved the final version of manuscript.

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Data availability
All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Declarations

Ethical approval
This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Shandong Provincial Hospital Affiliated to Shandong First Medical University. Informed consent was obtained from all individual participants included in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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