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ORIGINAL ARTICLE

The Value of Second-Generation Metagenomic Sequencing in the Diagnosis of Respiratory Infections

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SUMMARY

Background: **This study aimed to compare the results of metagenomic next-generation sequencing (mNGS) and conventional culture detection of pathogenic bacteria in bronchoalveolar lavage fluid (BALF) of patients with respiratory tract infections and analyze the influencing factors and clinical significance of mNGS positive detection.**
Methods: **We retrospectively analyzed BALF samples from 90 respiratory infection patients at the First People's Hospital of Yongkang City from June 1, 2024, through January 28, 2025, using mNGS and conventional culture testing to compare the positivity rate, pathogen distribution, and consistency of the two methods. The relationship between mNGS detection positivity and clinical indicators of patients and patient prognosis was analyzed.**
Results: **The positive rate of mNGS detection was 77.78%, while the positive rate of conventional culture detection was 44.44%, and the difference was statistically significant ($p < 0.05$). mNGS can detect a wider variety of pathogens, mainly gram-negative bacilli, fungi, and atypical pathogens. mNGS has moderate consistency with conventional culture detection results in bacteria, fungi, and atypical pathogens, but low consistency in viruses and parasites. The positive detection of mNGS is related to factors such as patient age, underlying diseases, peripheral blood white blood cells, and C-reactive protein, which are risk factors affecting the positive detection of mNGS.**
Conclusions: **The pathogenic diagnosis of mNGS in BALF of patients with lower respiratory tract infections is superior to conventional culture detection; it can detect more and a wider range of pathogens, helping to promote rational drug use and improve patient prognosis in clinical practice.**
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INTRODUCTION

Lower respiratory tract infection refers to infectious diseases occurring in the trachea, bronchi, alveoli, and other parts below the throat. The pathogens of lower respiratory tract infections are very complex, with bacteria and fungi as the main pathogenic factors [2]. Timely and accurate identification of the pathogen causing lower respiratory tract infections is of great significance for guiding rational clinical drug use, reducing drug resistance rates, and improving patient prognosis [3]. At

present, the commonly-used methods for detecting pathogens in lower respiratory tract infections in clinical practice mainly include traditional microbial culture, molecular biology detection, and metagenomic next-generation sequencing (mNGS) [4-7]. In recent years, mNGS has gradually demonstrated its advantages and potential in the etiological diagnosis of lower respiratory tract infections [8-10]. Bronchoalveolar lavage fluid (BALF) is the surface fluid of alveoli collected after lavage of lung or sub lung segments using bronchoscopy. It is currently the most representative and accurate specimen that can reflect the distribution of pathogens in lower respiratory tract infections. Therefore, this study analyzed the pathogens in BALF of patients with lower respiratory tract infections using mNGS and conventional culture detection methods and explored the advantages, disadvantages, and consistency of the two methods, as well as the relationship between mNGS positive detection and clinical indicators and prognosis of patients, providing valuable references for clinical practice.

MATERIALS AND METHODS

General clinical data

This study included 90 patients with pulmonary infections treated at the First People's Hospital of Yongkang City from June 1, 2024, through January 28, 2025, as the research subjects. According to whether mNGS detected pathogens, patients were divided into a detection group, 70 cases, and a non-detection group, 20 cases. There was no statistically significant difference in age, gender, and underlying diseases between the two groups of patients ($p > 0.05$) (Table 1). This study was approved by the Ethics Committee of the First People's Hospital of Yongkang City (no. YKSDYRMYYEC2022-KT-HS-001-01).

Inclusion criteria

The inclusion criteria were the following:
1) Age ≥ 18 years.
2) Patients diagnosed with pulmonary infection according to the American Association for Infectious Diseases/American Chest Association (IDSA/ATS).
3) Patients who undergo bronchoscopy and were BALF samples were collected within 24 hours after admission.
4) BALF samples that underwent both mNGS and conventional culture testing simultaneously.

Exclusion criteria

The exclusion criteria were the following:
1) Patients with other organ infectious diseases.
2) BALF samples that do not meet quality requirements, such as recovery rates below 30%, total cell counts below 10^4/mL, and neutrophil ratios above 80%.
3) BALF samples contaminated or damaged during transportation or storage.
4) The patient or their family members do not agree to participate in this study.

Methods

All patients underwent bronchoscopy within 24 hours after admission, and BALF samples were collected according to standard operating procedures. Each lung segment or sub lung segment was perfused with 20 - 40 mL of physiological saline, with a recovery rate of not less than 30%. The recovered liquid was immediately divided into two portions, one for mNGS detection and the other for routine culture detection.

mNOS test

Total nucleic acids were extracted from BALF samples using the QIAamp DNA Mini Kit (Qia gen, Germany), and a library was constructed using the NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB, USA). Double ended 150 bp high-throughput sequencing was performed using the Illumina NovaSeq 6000 platform. After quality control, removal of human source sequences, removal of low-quality sequences, and removal of duplicate sequences, the sequencing data was compared and analyzed with the NCBI database to determine the pathogen species and relative abundance based on the comparison results. The detection threshold was set to at least 5 reads per million to match the target sequence. In the pathogen composition detected by mNGS, the bacteria are *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, the fungi are *Pneumocystis jirovecii*, *Aspergillus fumigatus*, and *Aspergillus niger/Candida albicans*, and the viruses are respiratory syncytial virus, cytomegalovirus (CMV)/parainfluenza virus/human metapneumovirus.

Routine cultivation and testing

BALF samples were inoculated onto different culture media such as blood agar, chocolate agar, MacConkey agar, and potato glucose agar, and incubated in a 37°C constant temperature incubator for 24 - 48 hours to observe colony growth. Strain identification and drug sensitivity testing was performed according to conventional methods. At the same time, BALF samples were inoculated into liquid culture medium and incubated in an automatic blood culture instrument for 5 days to observe whether positive signals are generated. If positive signals are detected, strain identification and drug sensitivity testing were performed. In addition, BALF samples were prepared into smears and observed under an oil microscope for the presence of fungi or parasites.

Outcome measures

The main observation indicators are the positivity rate of mNGS and conventional culture detection, the distribution of detected pathogens, and the consistency of pathogen detection. The secondary observation indicators include clinical indicators, treatment plans, prognosis, and factors affecting mNGS positivity in patients with and without mNGS detection. Among them, clini-

cal indicators include peripheral blood white blood cells, C-reactive protein, procalcitonin, neutrophil count, and serum cytokine concentration. The prognosis includes length of hospital stay, proportion of ICU transfers, proportion of mechanical ventilation, and mortality rate.

Statistical analysis

SPSS 22.0 software was used for data analysis. The measurement data followed a normal distribution and is represented by mean ± SD. The comparison of means between the two groups was conducted using *t*-test; count data is presented in frequency and percentage, and comparison between two groups was performed using the χ^2 test. The consistency between mNGS and conventional culture detection results was evaluated using the Kappa coefficient, with Kappa values ranging from 0.01 - 0.20 indicating no consistency, 0.21 - 0.40 indicating low consistency, 0.41 - 0.60 indicating moderate consistency, 0.61 - 0.80 indicating high consistency, and 0.81 - 1.00 indicating complete consistency. The factors affecting the positive detection of mNGS were analyzed using logistic multiple regression, with $p < 0.05$ indicating statistical significance.

RESULTS

Comparison of mNGS and conventional culture detection results

The mNGS test results showed that a total of 70 pathogens were detected in the BALF of 90 patients with pulmonary infections, and no parasites were detected. The positivity rate of mNGS detection was 77.78% (70/90). The results of routine culture testing showed that a total of 26 pathogens were detected in the BALF of 90 patients with pulmonary infections, and no atypical pathogens or parasites were detected. There was a statistically significant difference in the positive rate between mNGS and conventional culture detection ($\chi^2 = 30.516$, $p < 0.001$, Table 2). After diagnosis based on the gold standard, ROC (receiver operating characteristic) curves for mNGS and routine culture test results were drawn, respectively. The ROC curve results showed that the area under the curve (AUC) detected by mNGS was 0.847, significantly higher than the AUC of routine culture (0.660), as shown in Figure 1.

Comparison of clinical indicators and prognosis between two groups of patients

There were statistically significant differences ($p < 0.05$) in peripheral blood leukocyte count, C-reactive protein, procalcitonin, neutrophil count, and serum cytokine concentration between the mNGS detection group and the non-detection group. The prognostic indicators such as hospitalization time, proportion of patients transferred to the ICU, proportion of patients receiving mechanical ventilation, and mortality rate in the mNGS detection group were higher than those in the non-detection group, and the differences were statistically significant ($p < 0.05$) (Table 3).

Multivariate logistic regression analysis of factors affecting mNGS positive detection

Positive mNGS detection was used as the dependent variable, while age, gender, underlying disease, peripheral white blood cells, C-reactive protein, procalcitonin, neutrophil count, and serum cytokines were used as independent variables. Continuous variables (age, peripheral blood cells, C-reactive protein, procalcitonin, neutrophil count, serum cytokines) are expressed in raw values, while binary variables were gender (0 = female, 1 = male) and underlying disease (0 = none, 1 = present). The stepwise regression method was used for multivariate logistic regression analysis, and the results showed that age, underlying diseases, peripheral blood white blood cells, and C-reactive protein were risk factors affecting the detection of mNGS positivity (Table 4).

DISCUSSION

Respiratory tract infections have complex and diverse pathogens, and timely and accurate identification of pathogens is of great significance for guiding rational clinical drug use, reducing drug resistance rates, and improving patient prognosis. This study analyzed the pathogens in BALF of respiratory tract infection patients using mNGS and conventional culture detection methods, exploring the advantages, disadvantages, and consistency of the two methods, as well as the relationship between mNGS positive detection and clinical indicators and prognosis of patients and providing valuable references for clinical practice.

The results of this study showed that the positivity rate of mNGS detection was higher than that of conventional culture detection, and the difference was statistically significant ($p < 0.001$), which is consistent with relevant research results [12]. This may be related to the ability of mNGS to simultaneously detect all types of nucleic acid sequences in samples, thereby identifying multiple known or unknown microorganisms, while conventional culture detection is limited by factors such as culture conditions, culture time, antibiotic interference, and cannot detect non-cultured or difficult-to-cultivate microorganisms.

The results of this study showed that mNGS had moderate consistency (Kappa = 0.42 - 0.54) with conventional culture detection results in bacteria, fungi, and atypical pathogens and low consistency (Kappa = 0.12 - 0.25) in viruses and parasites.

Possible reasons may be related to the ability of mNGS to detect pathogens that are difficult to detect through conventional culture tests, such as non-cultured or difficult-to-cultivate microorganisms, newly-discovered microorganisms, etc.; mNGS can distinguish small differences between different bacterial genera or strains,

Table 1. Comparison of general information between two groups of patients.

	Detection group (n = 70)	Non-detection group (n = 20)	t/χ^2 value	p-value
Age (years)	50.23 ± 20.82	51.13 ± 19.78	1.726	0.166
Gender (n, %)			0.192	0.663
Male	43 (61.43)	13 (65.00)		
Female	27 (38.57)	7 (35.00)		
Underlying disease (n, %)			1.598	0.782
Hypertension	20 (28.57)	6 (30.00)		
Diabetes	11 (15.71)	3 (15.00)		
Chronic obstructive pulmonary disease	17 (26.29)	4 (20.00)		
Lung cancer	8 (11.42)	2 (10.00)		
Other	14 (20.00)	4 (20.00)		

Table 2. Comparison of positivity rate and pathogen distribution between mNGS and conventional culture detection.

	mNGS (n = 100)	Routine culture (n = 40)	A + B + (n)	A - B - (n)	A + B - (n)	A – B + (n)	Kappa value
mNGS positive	77.78 (70/90)	44.44 (40/90)					
Bacteria (n)	45 (27/8/8/2)	34 (23/7/1/1)	25	43	19	3	0.541
Fungus (n)	16 (10/4/2)	4 (2/1/1)	2	71	14	3	0.418
Virus (n)	7 (3/1/3)	2 (2/0/0)	1	80	6	3	0.246
Atypical pathogen (n)	2 (1/1)	0	0	89	1	0	0.122
Parasite (n)	0	0	0	90	0	0	-

A mNGS test, B routine culture, bacterial species gram-negative bacilli/gram-positive cocci/anaerobic bacteria/other bacteria, fungal species Candida/Aspergillus/other fungi, virus types respiratory syncytial virus/*Mycoplasma pneumoniae*/other viruses, atypical pathogens *Legionella*/ *Chlamydia pneumoniae*.

Table 3. Comparison of clinical indicators between two groups of patients.

	Detection group (n = 70)	Non-detection group (n = 20)	t value	p-value
WBC (x 10^9/L)	12.5 ± 3.7	7.8 ± 2.7	4.28	< 0.001
C-reactive protein (mg/L)	97.5 ± 30.1	63.1 ± 26.8	3.051	0.003
Procalcitonin (ng/mL)	12.6 ± 7.8	7.0 ± 3.6	3.132	0.0033
Neutrophil count (x 10^9/L)	9.7 ± 3.9	6.3 ± 2.8	4.101	< 0.001
IL-6 (pg/mL)	76.4 ± 32.4	48.2 ± 25.9	3.765	< 0.001
IL-8 (pg/mL)	102.6 ± 41.6	68.4 ± 30.9	36.959	< 0.001
TNF-α (pg/mL)	37.9 ± 12.6	25.1 ± 11.9	3.484	0.001
Hospital stays (days)	18.1 ± 6.9	12.6 ± 11.8	3.346	0.001
Transfer rate to ICU (n, %)	22 (31.4)	13.3 ± 4.8	15.354	< 0.001
Mechanical ventilation ratio (n, %)	20 (28.6)	4 (20.0)	14.451	< 0.001
Case fatality rate (n, %)	11 (15.7)	1 (5.0)	7.653	< 0.001

IL-6 interleukin 6, IL-8 interleukin-8, TNF-α tumor necrosis factor-α.

Table 4. Multivariate logistic regression analysis of factors affecting mNGS positive detection.

	Regression coefficient	Standard error	Wald X2 value	p-value	OR value	95% CI
Constant term	-1.212	0.052	522.075	< 0.001	0.292	0.232 - 1.038
Age	0.034	0.011	9.5843	0.002	1.028	1.005 - 1.061
Gender	-0.121	0.341	0.125	0.722	0.891	0.441 - 1.789
Underlying disease	0.935	0.371	6.344	0.011	2.561	2.244 - 5.169
WBC	0.172	0.045	12.658	0.001	1.182	1.065 - 1.305
C-reactive protein	0.021	0.012	8.846	0.004	1.025	1.006 - 1.042
Procalcitonin	0.013	0.011	5.102	0.057	1.005	1.007 - 1.015
Neutrophil count	0.087	0.062	2.084	0.113	1.102	0.976 - 1.226
Cytokines in serum	0.056	0.022	3.615	0.066	0.995	0.995 - 1.002

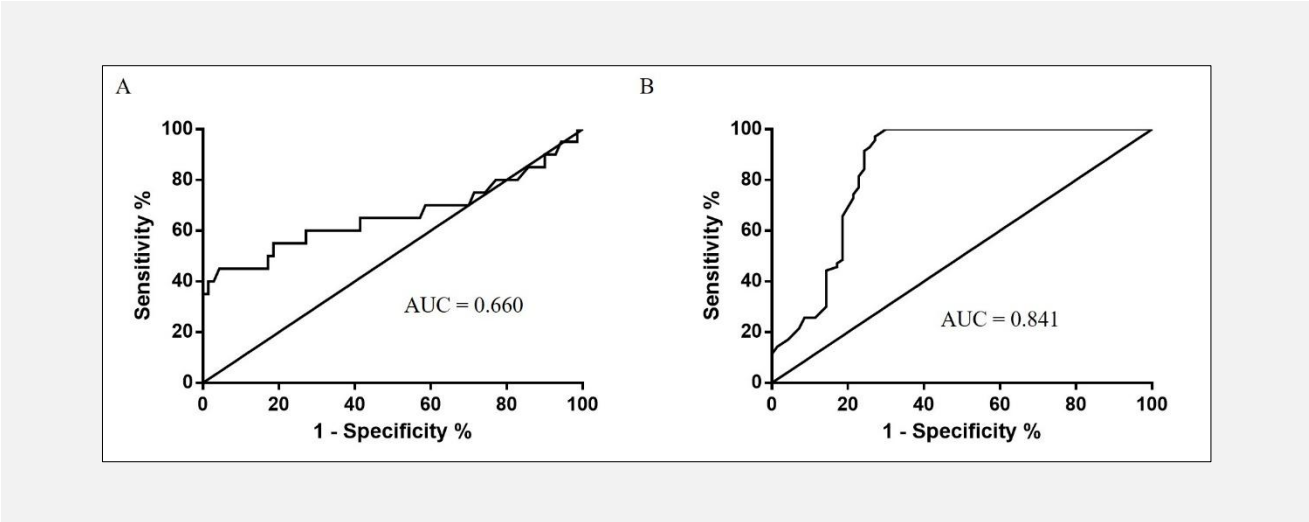

Figure 1. The ROC curve of routine culture and mNGS tests.

A) The ROC curve of routine culture test.
B) The ROC curve of mNGS test.

while conventional culture testing may classify them into the same category; mNGS may detect some non-pathogenic or low pathogenic microorganisms, such as normal microbiota in the skin or mouth, while conventional culture testing may ignore their presence; mNGS may detect residual nucleic acid from some dead or inhibited microorganisms, while conventional culture testing can only detect active microorganisms [13,14]. Therefore, there are certain differences between mNGS and conventional culture detection results; they cannot completely replace or negate each other and should be combined with clinical comprehensive judgment.

The results of this study showed that the positive detection of mNGS is related to factors such as patient age, underlying diseases, peripheral blood white blood cells, and C-reactive protein, suggesting that mNGS can be used as an auxiliary tool for assessing the condition and prognosis of respiratory infection patients. The inflammatory indicators such as peripheral blood white blood cells, C-reactive protein, procalcitonin, neutrophil count, and serum cytokines in the mNGS detection group were higher than those in the non-detection group, and the differences were statistically significant ($p < 0.05$), indicating that positive mNGS detection reflects a strong inflammatory response in the patient's body [15,16]. Positive mNGS detection suggests poor prognosis for patients [17,18]. Multivariate logistic regression analysis showed that age, underlying diseases, peripheral blood white blood cells, and C-reactive protein were risk factors affecting mNGS positivity, indicating that these factors were related to the pathogen load and severity of infection in patients [19].

This study has the following limitations: it is a retrospective controlled study, and there is a possibility of selection bias and confounding factors; the small number of patients included in this study may affect the stability and credibility of the results; this study did not further validate the non-pathogenic or low pathogenic microorganisms detected by mNGS, which may lead to overinterpretation or misunderstanding of the results.
In summary, mNGS has high sensitivity and broad spectrum in the pathogenic diagnosis of BALF in patients with lower respiratory tract infections and can detect pathogens that are difficult to detect by conventional culture tests. It is of great significance for guiding rational clinical drug use and improving patient prognosis. There are certain differences between mNGS and conventional culture detection results; they cannot completely replace or negate each other but should be combined with clinical comprehensive judgment.

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Ethical Approval Statement:
This study has been approved by the Ethics Committee of the First People's Hospital of Yongkang (no. YKS DYRMYYEC2022-KT-HS-001-01).

Declaration of Interest:
None.

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