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Original Article

Application value of metagenomic next-generation sequencing of bronchoalveolar lavage fluid in pathogen detection and diagnostic efficiency of acute exacerbation of bronchiectasis

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Abstract: Objective: To investigate pathogen detection performance and diagnostic efficacy of bronchoalveolar lavage fluid (BALF) metagenomic next-generation sequencing (mNGS) in patients with acute exacerbation of bronchiectasis (AE-bronchiectasis). Methods: A retrospective analysis was conducted on 78 patients with AE-bronchiectasis admitted to the First Affiliated Hospital of Guangxi Medical University from March 2020 to December 2023. Pathogen detection rates and diagnostic efficacy of conventional culture detection and BALF mNGS group were compared. Seventy-six patients diagnosed as positive by the gold standard were further stratified by bronchiectasis severity index (BSI) into mild-to-moderate and severe groups to analyze differences in pathogen profiles. Results: Compared to conventional culture, mNGS showed significantly higher detection rates for bacteria, fungi, and mycobacteria (all P<0.05), notably *Pseudomonas aeruginosa*, *Aspergillus fumigatus*, and *Mycobacterium tuberculosis* (all P<0.05). In addition, mNGS exhibited superior diagnostic accuracy (94.87%) and sensitivity (94.74%) compared to conventional culture (P<0.05), with a higher area under the ROC curve (AUC=0.974). BSI stratification showed that the detection rates of fungi and viruses were higher in the severe group than those in the mild-to-moderate group, while the detection rate of bacteria was slightly lower than that in the mild-to-moderate group. The detection rate of *Pseudomonas aeruginosa* in the severe group (51.06%) was significantly higher than that in the mild-to-moderate group (27.59%), while the detection rate of human herpesvirus 7 was significantly higher in the mild-to-moderate group (24.14%) compared to the severe group (4.26%) (all P<0.05). Conclusion: BALF mNGS demonstrates clear advantages over conventional methods in pathogen detection for AE-bronchiectasis, offering significantly better detection rates and diagnostic efficiency.

Keywords: Metagenomic next generation sequencing, bronchoalveolar lavage fluid, bronchiectasis, pathogen detection, diagnostic efficacy

Introduction

Bronchiectasis is a common chronic respiratory disease characterized by persistent cough and a large amount of purulent sputum, and some patients may also experience recurrent hemoptysis [1]. During acute exacerbation (AE), infection-induced systemic inflammatory reactions are common, including fever, elevated white blood cell count, and in severe cases, life-threatening complications such as respiratory insufficiency, respiratory failure, and cardiopulmonary dysfunction [2, 3]. These clinical challenges not only severely impair patients' quality of life but also pose a major threat to their life expectancy. Therefore, early and accurate pathogen identification is crucial for guiding individualized treatment of AE-Bronchiectasis.

The key to AE-bronchiectasis management is early and precise pathogen diagnosis. However, conventional pathogen detection methods have

certain limitations. Serological tests are prone to cross-reactivity, especially in immunosuppressed state or reinfections, which may lead to false negative results. The testing cycle of blood culture is long, and the detection success rate is low due to the abuse of antibiotics. Although bacterial culture remain a routine clinical method, its prolonged turnaround time often precludes early identification of causative pathogens, delaying targeted therapy [4-6]. These limitations highlight the urgent need for a rapid, accurate, and comprehensive diagnostic approach.

With advances in sequencing technology, metagenomic next-generation sequencing (mNGS) has revolutionized pathogen detection in infectious diseases. mNGS enables simultaneous detection of a variety of microorganisms, including bacteria, viruses, fungi, and atypical pathogens, without requiring prior nucleic acid amplification. It offers high efficiency, wide coverage, and high sensitivity [7]. mNGS has been widely used in pathogen diagnosis, demonstrating excellent performance, especially in the detection of rare and novel pathogens that are difficult to detect by conventional methods [8].

Bronchoalveolar lavage fluid (BALF), an important specimen for the diagnosis of lower respiratory tract infection, is less influenced by upper respiratory tract colonization and more accurately reflects lower respiratory tract infection [9]. Despite the promising potential of mNGS, its application in AE-bronchiectasis remains scarce. Therefore, this study employed mNGS for pathogen detection in BALF samples from patients with AE-bronchiectasis, aiming to compare the diagnostic performance of mNGS with that of conventional methods, and to analyze the differences in pathogen profiles according to disease severity. This work seeks to elucidate the value of BALF mNGS in the pathogen diagnosis of AE-bronchiectasis and provide a scientific basis for the treatment guidance of clinical patients [10].

Materials and methods

Cases selection

The clinical data of 78 patients with AE-bronchiectasis admitted to the First Affiliated Hospital of Guangxi Medical University from March 2020 to December 2023 were retrospectively analyzed. This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University.

Inclusion criteria: (1) Age ≥18 years; (2) Diagnosis of bronchiectasis according to the 2021 Chinese Expert Consensus on the Diagnosis and Treatment of Adult Bronchiectasis [11]; (3) Definition of acute exacerbation met the 2019 British Thoracic Society (BTS) Bronchiectasis Guidelines [12]: deterioration in three or more of the following symptoms - cough, sputum volume and/or viscosity, purulent sputum, dyspnea and/or reduced exercise tolerance, hemoptysis, fatigue and/or general discomfort, lasting for more than 48 hours and confirmed by clinicians as requiring treatment; (4) Complete clinical data available.

Exclusion criteria: (1) Presence of other chronic respiratory diseases, such as bronchial asthma or chronic obstructive pulmonary disease (COPD); (2) Presence of hematological diseases, severe immunodeficiency, mental disorders, or other severe comorbidities; (3) Inadequate BALF specimen quality; (4) Receipt of immunosuppressive therapy within the past 4 weeks; (5) Diagnosis of cystic fibrosis-related bronchiectasis; (6) Pregnancy or lactation.

Data collection

Data were extracted from patients' electronic medical records, including general information, laboratory parameters, and pathogen detection approach.

General information: age, sex, body mass index (BMI), past medical history (including tuberculosis, hypertension, malignant tumor, diabetes, hepatitis B, connective tissue disease, gastritis, sinusitis), smoking history, and length of hospital stay.

The relevant indicators of the Bronchiectasis Severity Index (BSI) scoring system: FEV1%pred, number of hospitalizations in the past two years, number of acute exacerbations in the past 12 months, modified Medical Research Council (mMRC) dyspnea scale, colonization with Pseudomonas aeruginosa and other microorganisms, and radiographic findings.

Pathogen detection: conventional sputum culture, conventional BALF culture, and BALF mNGS detection results.

Table 1. Basic data of patients with acute exacerbation of bronchiectasis

Characteristic	n=78
Length of stay (d)	8 (7, 11)
Age (years)	59.15±12.83
Sex [n (%)]	
Female	35 (44.87)
Male	43 (55.13)
BMI (kg/m^2)	20.05±2.98
Smoking [n (%)]	
No	58 (74.36)
Yes	20 (25.64)
Pulmonary function indicators	
FVC%pred (%)	88.75±14.12
FEV1%pred (%)	73.85±14.98

Note: BMI refers to body mass index; FVC%pred refers to Forced Vital Capacity Percentage; FEV1%pred refers to Forced Expiratory Volume in 1 second Percentage.

Sample collection and testing

Bronchoalveolar lavage procedure: Following preoperative evaluation and exclusion of contraindications, bronchoalveolar lavage (BAL) was performed on the second or third day after admission by qualified physicians, after obtaining informed consent from the patient and their family. Based on imaging findings and bronchoscopic examination, the bronchoscope was advanced to the affected lung segment. A volume of 20-40 ml of sterilized saline (37°C) was instilled each time, with lavage performed two to three times. Two aliquots of 5 ml of BAL fluid were collected: one was sent to the hospital laboratory for routine bacterial culture, and the other was sent to a third-party laboratory for mNGS testing.

Routine pathogen detection: Sputum and BALF specimens were collected and sent to the First Affiliated Hospital of Guangxi Medical University for routine microbiological examinations, including bacterial culture.

Interpretation of etiological results: A positive result in any pathogen detection assay was considered indicative of pathogen presence. For mNGS, microbial reports provided by the third-party testing facility were reviewed. The pathogenicity of detected microorganisms was determined by clinicians based on patients' clinical context and disease presentation (i.e., the gold standard).

Outcome measurements

Primary outcomes: Among the 78 patients who met the inclusion criteria, all underwent BALF mNGS testing and conventional pathogen testing. The detection rates of pathogens and the diagnostic efficacy between BALF mNGS testing and conventional pathogen testing (BALF culture, sputum culture testing) were compared. Secondary outcomes: Among the patients diagnosed as positive by the gold standard, patients were further stratified by the Bronchiectasis Severity Index (BSI) into a mild-to-moderate group (BSI<9) and a severe group (BSI≥9). Pathogen detection rates were compared between the two groups.

BSI scoring system [13]: The BSI score was calculated based on age, BMI, FEV1%pred, number of hospitalizations in the past two years, number of acute exacerbations in the past 12 months, modified Medical Research Council (mMRC) dyspnea scale, colonization with *Pseudomonas aeruginosa* and other microorganisms, and radiographic findings. Scores of 0-4 were classified as mild, 5-8 as moderate, and ≥9 as severe.

Statistical analysis

Statistical analysis was performed using SPSS 26.0. Measurement data were expressed as mean ± standard deviation ($\bar{x}\pm s$) or median (interquartile range, IQR) as appropriate. Count data were expressed as [n (%)] and compared using the chi-square test. A P value <0.05 was considered statistically significant.

Results

Baseline characteristics of included subjects

A total of 78 patients with AE-bronchiectasis were enrolled in this study. The age of included patients ranged from 21 to 82 years, with 35 females (44.87%) and 43 males (55.13%). Twenty patients (25.64%) were former or current smokers. Detailed baseline characteristics are presented in **Table 1**. Among the past medical history, hypertension was the most common (17 patients, 21.79%), followed by pulmonary tuberculosis (16 patients, 20.51%) (**Figure 1**).

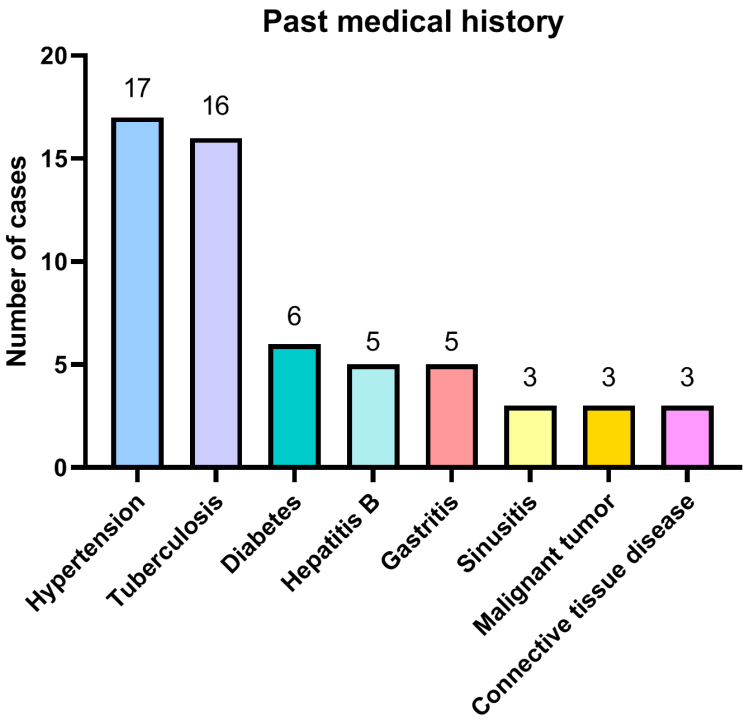

Figure 1. Frequency plot of past history of patients with acute exacerbation of bronchiectasis.

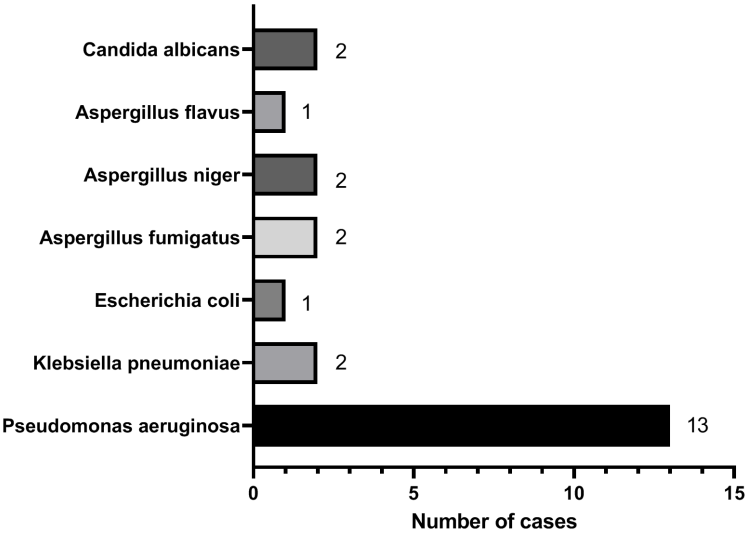

Figure 2. Distribution of pathogens in bronchoalveolar lavage (BALF) culture.

Pathogen detection analysis of mNGS and conventional detection

Among the 78 patients with AE-bronchiectasis, all underwent BALF culture, sputum culture, and BALF mNGS testing. In BALF culture, 7 pathogens were identified, including 3 bacterial species: *Pseudomonas aeruginosa* (13 case), *Klebsiella pneumoniae* (2 cases) and *Escherichia coli* (1 case); 4 fungi species: *Candida albicans* (2 cases), *Aspergillus fumigatus* (2 cases), *Aspergillus Niger* (2 cases) and *Aspergillus flavus* (1 case) (**Figure 2**). Eleven pathogens were detected by sputum culture, including 5 bacterial species: *Pseudomonas aeruginosa* (13 cases), *Klebsiella pneumoniae* (2 cases), *Stenotrophomonas maltophila* (1 case), *Enterobacter cloacae* (1 case), and *S.marcescen* (1 case). Five fungi species, including *Candida albicans* (11 cases), *Aspergillus fumigatus* (2 cases), *Aspergillus Niger* (1 case), *Trichosporon asasii* (1 case) and *Candida glabrata* (1 case). One mycobacteria species, *Mycobacterium tuberculosis* (1 case) (**Figure 3**).

In the mNGS, 39 pathogens were identified, including 18 bacterial species. The top three were *Pseudomonas aeruginosa* (32 cases), *Haemophilus parainfluenzae* (12 cases), *Haemophilus influenzae* (11 cases) (**Figure 4**). Seven fungal species were detected, with the top three being *Aspergillus fumigatus* (11 cases), *Candida albicans* (4 cases), and *Pneumocystis jiroveci* (3 cases) (**Figure 5**). Six mycobacterial species were identified, with *Mycobacterium tuberculosis* (6 cases), *Mycobacterium abscessus* (3 cases), and *Mycobacterium avium* (2 cases) being the most common (**Figure 6**). Eight viral species were detected, with the top three being *human herpesvirus 5* (18 cases), *human herpesvirus 7* (9 cases), and *leptocircovirus* (9 cases) (**Figure 7**).

The results of mNGS and conventional pathogen detection showed that the pathogen

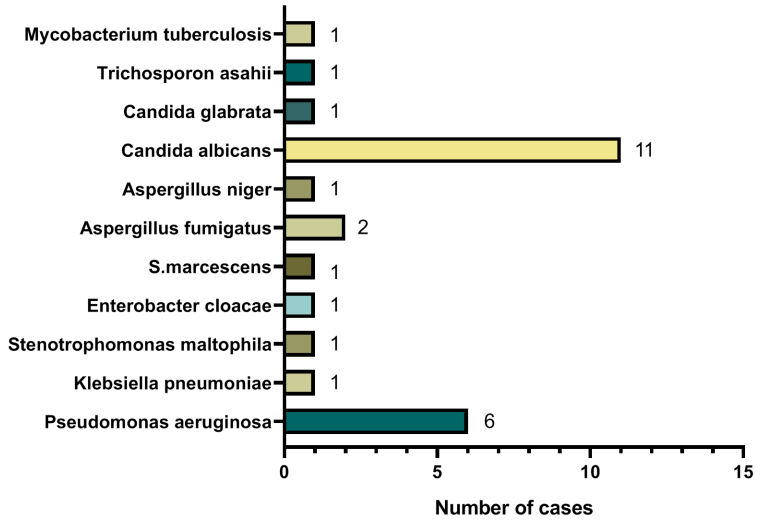

Figure 3. Distribution of pathogens in sputum culture. Note: S.marcescens denotes Serratia marcescens.

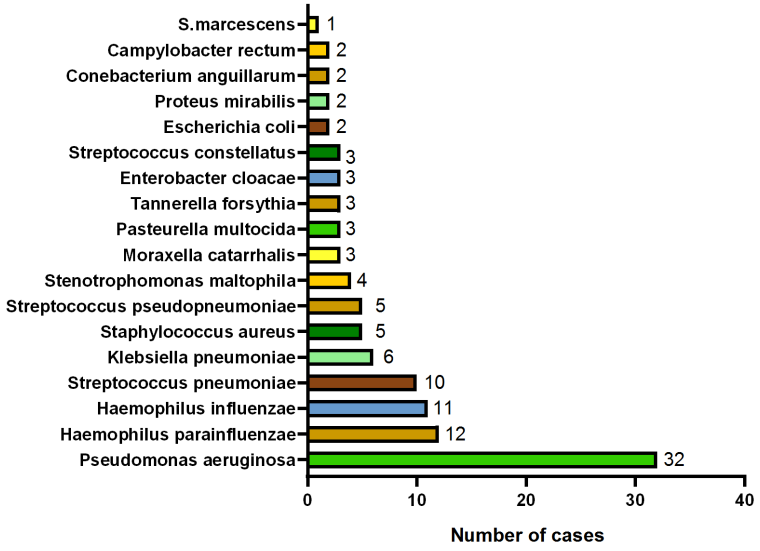

Figure 4. Distribution of bacterial species detected in metagenomic next-generation sequencing (mNGS). Note: S.marcescens refers to Serratia marcescens.

detection rate of BALF mNGS was 96.15%, significantly higher than that of BALF culture (29.49%) and sputum culture (32.05%) (P< 0.05).

Specifically, the bacterial detection rate of BALF mNGS was 79.49%, higher than 20.51% for BALF culture and 12.82% for sputum culture. The fungal detection rate of BALF mNGS was 24.36%, higher than that of BALF culture (8.97%) and sputum culture (20.51%). The mycobacterial detection rate of BALF mNGS was 15.38%, higher than that by alveolar lavage fluid culture (0.00%) and sputum culture (1.28%). All differences were statistically significant (P<0.05).

For specific pathogens, the detection rates of Pseudomonas aeruginosa, *Haemophilus parainfluenzae*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus pseudopneumoniae*, *Aspergillus fumigatus,* and *Mycobacterium tuberculosis* were significantly higher with BALF mNGS than with BALF culture and sputum culture (P<0.05). However, the detection rate of *Candida albicans* was higher by sputum culture than by BALF culture or BALF mNGS, with the difference also being statistically significant (P< 0.05) (P<0.05) (**Table 2**).

Diagnostic efficiency analysis of mNGS and conventional methods

Using the clinicians' final diagnosis as the reference standard, the diagnostic performance of mNGS and conventional detection methods was compared. Among the 78 patients, 76 patients were clinically diagnosed as pathogen-positive. mNGS identified 72 positive cases, BALF culture identified 17, and sputum culture identified 11 (**Table 3**). The diagnostic accuracy (94.87%) and sensitivity (94.74%) of mNGS were significantly higher than those of BALF culture and sputum culture (P<0.05) (**Table 4**). In addition, the area under the ROC curve (AUC) of mNGS, BALF culture, and sputum culture were 0.974, 0.612 and 0.322, respectively, indicating that mNGS had superior diagnostic value compared to conventional methods (**Figure 8**).

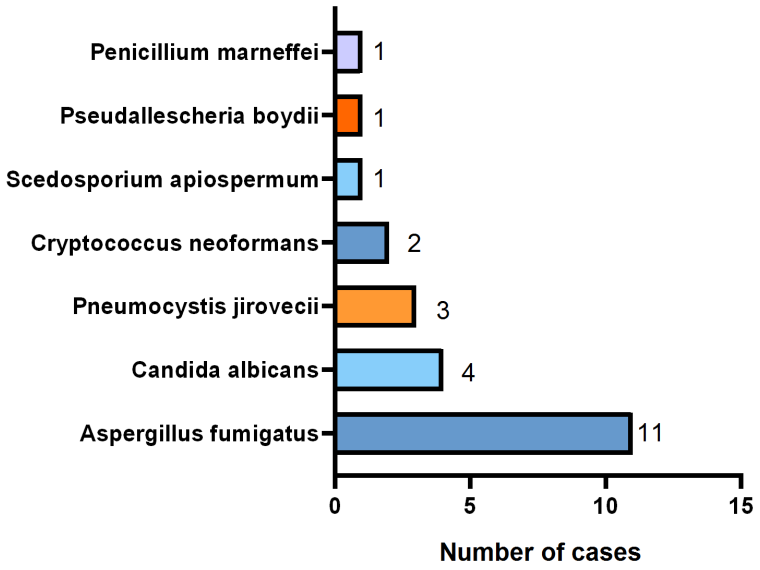

Figure 5. Distribution of fungi species detected in metagenomic next-generation sequencing (mNGS).

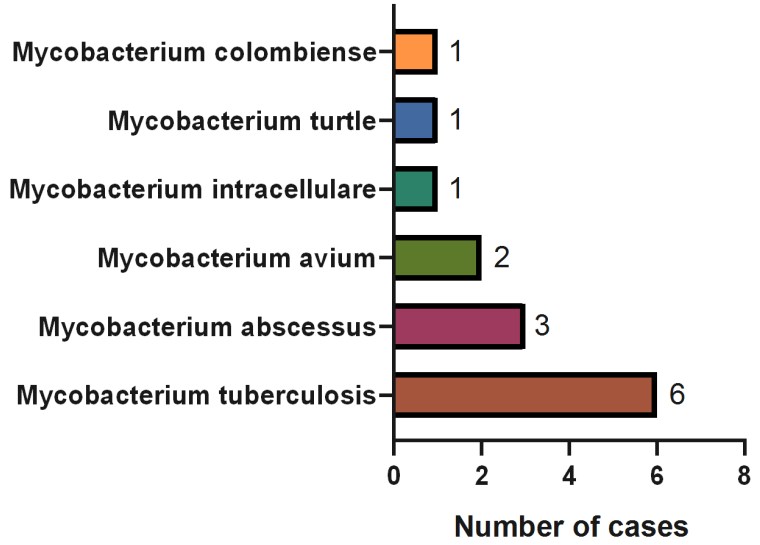

Figure 6. Distribution of mycobacteria detected in metagenomic next-generation sequencing (mNGS).

Pathogen detection rate analysis based on BSI Stratification

Previous findings demonstrated that mNGS effectively improves the detection rate of various pathogens in patients with acute exacerbation of bronchiectasis, exhibiting high diagnostic value. BSI score is a validated scoring system for assessing disease severity. To further investigate whether pathogen profiles vary with disease severity, the 76 patients who had been diagnosed as positive by the gold standard were divided into two groups based on BSI score: a mild-to-moderate group (BSI<9 points, n=29) and a severe group (BSI≥9 points, n=47).

The results showed that the total positive rate of bacterial detection in the severe group was 80.85%, which was slightly lower than that in the mild-to-moderate group (82.76%). In the mild-to-moderate group, the predominant bacteria were *Pseudomonas aeruginosa* (27.59%), *Haemophilus influenzae* (24.14%), *Haemophilus parainfluenzae* (17.24%), and *Klebsiella pneumoniae* (17.24%). In the severe group, the predominant bacteria were *Pseudomonas aeruginosa* (51.06%), *Streptococcus pneumoniae* (17.02%), and *Haemophilus parainfluenzae* (14.89%). Notably, the detection rate of *Pseudomonas aeruginosa* was significantly higher in the severe group than in the mild-to-moderate group (51.06% vs 27.59%, P<0.05) (**Table 5**).

The total positive rate of fungal detection in the severe group was 29.79%, higher than that in the mild-to-moderate group (17.24%). The main fungi detected in both groups were *Aspergillus fumigatus*, *Candida albicans,* and *Pneumocystis jirovecii*. No significant difference was observed in the fungal detection rates between the two groups (P>0.05) (**Table 5**).

The mycobacterial detection rate was 24.14% in the mild-to-moderate group and 10.64% in the severe group. *Mycobacterium tuberculosis* was the predominant species detected in both groups. There was no significant difference in the detection rate of all mycobacteria between the two groups (P>0.05) (**Table 5**).

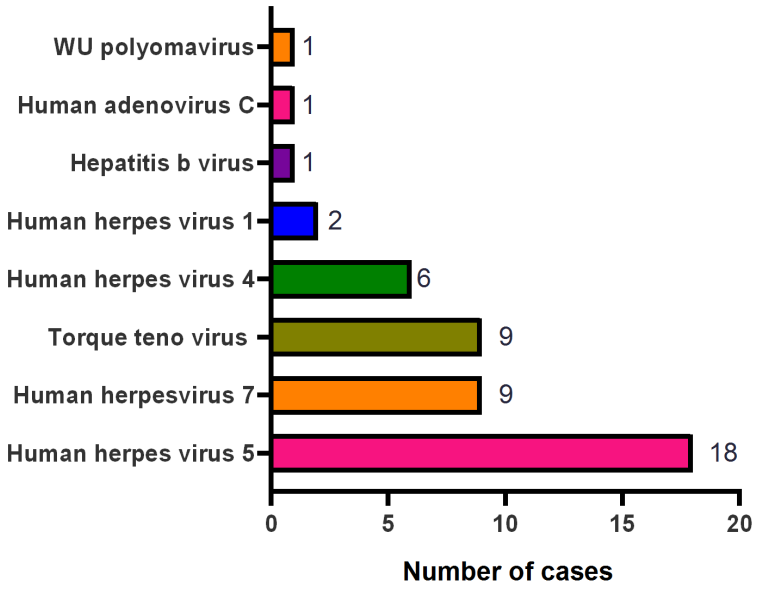

Figure 7. Distribution of viruses detected in metagenomic next-generation sequencing (mNGS).

The total positive rate of virus detection in the severe group was 48.94%, which was slightly higher than that in the mild-to-moderate group (44.83%). The main viruses detected in the mild-to-moderate group were human herpesvirus 7 (24.14%) and human herpesvirus 5 (17.24%), and the main viruses detected in the severe group were human herpesvirus 5 (27.66%) and Torque teno virus (14.89%). Among them, the mild to moderate group had a higher detection rate of human herpesvirus 7 than the severe group (24.14% vs 4.26%), and the difference was statistically significant ($P<0.05$) (**Table 5**).

Discussion

In the 78 patients with AE-bronchiectasis included in this study, a total of 7 pathogens were detected by routine BALF culture, and 11 pathogens were detected by sputum culture. Bacteria and fungi were the main pathogens, among which bacteria were few and most were common pathogens, such as Pseudomonas aeruginosa and Klebsiella pneumoniae. However, a total of 39 pathogens were detected in mNGS, including bacteria, fungi, mycobacteria, and other types of pathogens, and it also detected a variety of viruses, which were not found in conventional detection. The reasons for these differences are multifactorial. First, mNGS technology, with its high sensitivity and specificity, enables direct sequencing of pathogen DNA in BALF, allowing accurate identification of a broad range of pathogens. Second, conventional methods, such as culture method and smear microscopy, are limited by factors such as pathogen viability, culture conditions, and prolonged detection time, resulting in lower detection rate and increased likelihood of missed detection. In addition, infections during acute exacerbation of bronchiectasis are often complex and diverse, and conventional methods are less capable of detecting mixed infections [14]. Therefore, BALF mNGS technology offers significant clinical information for pathogen detection in the acute exacerbation of bronchiectasis.

In terms of the overall pathogen detection rate, the pathogen detection rate of BALF mNGS was as high as 96.15%, significantly higher than that of BALF culture (29.49%) and sputum culture (32.05%). The results suggest that mNGS provides broader pathogen coverage, which is particularly valuable in AE-bronchiectasis, when multiple or complex infections are common. The high sensitivity of mNGS allows for more comprehensive pathogen identification, consistent with the findings reported by Jin et al. [15]. This advantage may stem from the ability of mNGS to directly sequence microbial DNA in samples without prior culture, thus circumventing issues such as pathogen loss or growth inhibition associated with conventional culture methods [16]. Further analysis revealed that the detection rates of bacteria, fungi, and mycobacteria by BALF mNGS were significantly higher than that of conventional BALF culture and sputum culture; it further confirmed that mNGS has high sensitivity and specificity in identifying different types of pathogens, and can accurately identify pathogens that are difficult to detect by conventional methods, especially when detecting pathogens that are difficult to culture or grow slowly, such as *mycobacteria*, which is consistent with previous

Table 2. Pathogen detection rate of mNGS and conventional detection [n (%)]

Spectrum of pathogens	BALF mNGS	Culture of BALF	Culture of sputum	χ^2	P
pathogen	75 (96.15)	23 (29.49)	25 (32.05)	87.527	<0.001
Bacteria	62 (79.49)	16 (20.51)	10 (12.82)	88.442	<0.001
Pseudomonas aeruginosa	32 (41.03)	13 (16.67)	6 (7.69)	27.229	<0.001
Haemophilus parainfluenzae	12 (15.38)	0 (0.00)	0 (0.00)	22.331	<0.001
Haemophilus influenzae	11 (14.10)	0 (0.00)	0 (0.00)	20.091	<0.001
Streptococcus pneumoniae	10 (12.82)	0 (0.00)	0 (0.00)	17.888	<0.001
Staphylococcus aureus	5 (6.41)	0 (0.00)	0 (0.00)	7.606	0.011
Klebsiella pneumoniae	6 (7.69)	2 (2.56)	1 (1.28)	4.129	0.154
Streptococcus pseudopneumoniae	5 (6.41)	0 (0.00)	0 (0.00)	7.606	0.011
Stenotrophomonas maltophila	4 (5.13)	0 (0.00)	1 (1.28)	4.282	0.131
Moraxella catarrhalis	3 (3.85)	0 (0.00)	0 (0.00)	4.092	0.108
Pasteurella multocida	3 (3.85)	0 (0.00)	0 (0.00)	4.092	0.108
Tannerella forsythia	3 (3.85)	0 (0.00)	0 (0.00)	4.092	0.108
Enterobacter cloacae	3 (3.85)	0 (0.00)	1 (1.28)	2.925	0.328
Escherichia coli	2 (2.56)	1 (1.28)	0 (0.00)	1.843	0.775
Proteus mirabilis	2 (2.56)	0 (0.00)	0 (0.00)	2.663	0.330
Streptococcus constellatus	3 (3.85)	0 (0.00)	0 (0.00)	2.663	0.330
Conebacterium anguillarum	2 (2.56)	0 (0.00)	0 (0.00)	2.663	0.330
Campylobacter rectum	2 (2.56)	0 (0.00)	0 (0.00)	2.663	0.330
S.marcescens	1 (1.28)	0 (0.00)	1 (1.28)	1.251	1.000
Fungi	19 (24.36)	7 (8.97)	16 (20.51)	6.955	0.031
Aspergillus fumigatus	11 (14.10)	2 (2.56)	2 (2.56)	11.540	0.003
Aspergillus niger	0 (0.00)	2 (2.56)	1 (1.28)	1.843	0.775
Aspergillus flavus	0 (0.00)	1 (1.28)	0 (0.00)	1.826	1.000
Candida albicans	4 (5.13)	2 (2.56)	11 (14.10)	8.500	0.014
Pneumocystis jirovecii	3 (3.85)	0 (0.00)	0 (0.00)	4.092	0.108
cryptococcus neoformans	2 (2.56)	0 (0.00)	0 (0.00)	2.663	0.330
Scedosporium apiospermum	1 (1.28)	0 (0.00)	0 (0.00)	1.826	1.000
Pseudallescheria boydii	1 (1.28)	0 (0.00)	0 (0.00)	1.826	1.000
Penicillium marneffei	1 (1.28)	0 (0.00)	0 (0.00)	1.826	1.000
Candida glabrata	0 (0.00)	0 (0.00)	1 (1.28)	1.826	1.000
Trichosporon asahii	0 (0.00)	0 (0.00)	1 (1.28)	1.826	1.000
Mycobacteria	12 (15.38)	0 (0.00)	1 (1.28)	19.142	<0.001
Mycobacterium tuberculosis	6 (7.69)	0 (0.00)	1 (1.28)	7.491	0.018
Mycobacterium abscessus	3 (3.85)	0 (0.00)	0 (0.00)	4.092	0.108
Mycobacterium avium	2 (2.56)	0 (0.00)	0 (0.00)	2.663	0.330
Mycobacterium intracellulare	1 (1.28)	0 (0.00)	0 (0.00)	1.826	1.000
Mycobacterium turtle	1 (1.28)	0 (0.00)	0 (0.00)	1.826	1.000
Mycobacterium colombiense	1 (1.28)	0 (0.00)	0 (0.00)	1.826	1.000
Virus	36 (16.15)	-	-		
Human herpesvirus 5	18 (23.08)	-	-		
Human herpesvirus 7	9 (11.54)	-	-		
Torque teno virus	9 (11.54)	-	-		
Human herpesvirus 4	6 (7.69)	-	-		
Human herpesvirus 1	2 (2.56)	-	-		
Human adenovirus group C	1 (1.28)	-	-		
WU polyomavirus	1 (1.28)	-	-		
Hepatitis B virus	1 (1.28)	-	-		

Note: BALF refers to bronchoalveolar lavage; mNGS refers to metagenomic next-generation sequencing; S.marcescens refers to Serratia marcescens.

Table 3. Pathogen diagnosis results of mNGS and conventional detection in patients

		Clinical Diagnosis		Total
		Positive	Negative	
BALF mNGS	Positive	72	0	72
	Negative	4	2	6
Culture of BALF	Positive	17	0	17
	Negative	59	2	61
Culture of sputum	Positive	11	1	12
	Negative	65	1	66
	Total	76	2	78

Note: BALF refers to bronchoalveolar lavage; mNGS refers to metagenomic next-generation sequencing.

Table 4. Comparison of pathogen diagnostic efficacy between mNGS and conventional detection in patients

Diagnostic method	Diagnostic accuracy (%)	Sensitivity (%)	Specificity (%)
BALF mNGS	94.87	94.74	100.00
Culture of BALF	24.36	22.37	100.00
Culture of sputum	15.38	14.47	50.00
χ^2	119.514	120.804	2.182
P	<0.001	<0.001	1.000

Note: BALF refers to bronchoalveolar lavage; mNGS refers to metagenomic next-generation sequencing.

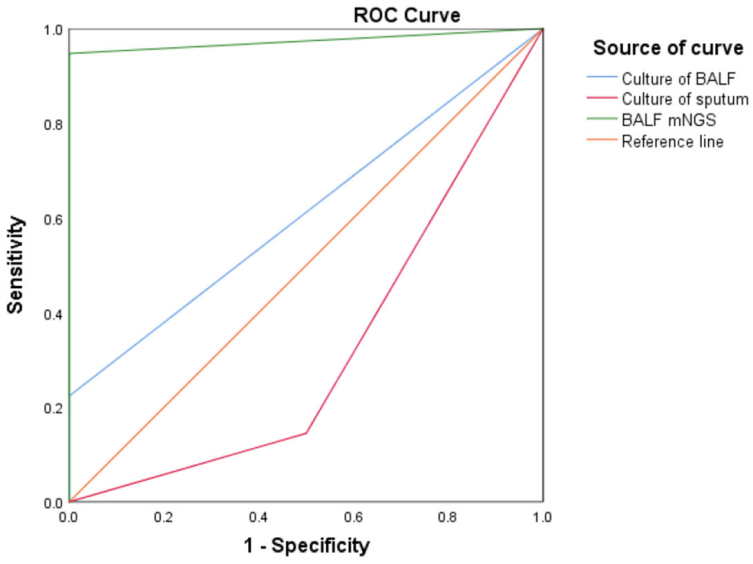

Figure 8. ROC curves of metagenomic next-generation sequencing (mNGS) and conventional detection. Note: BALF refers to bronchoalveolar lavage; mNGS refers to metagenomic next-generation sequencing.

research results [17, 18]. In terms of specific pathogens, the high detection rates of *Pseudomonas aeruginosa* and *Aspergillus fumigatus* by BALF mNGS not only highlight the key roles these pathogens play in the acute exacerbation of bronchiectasis but also demonstrate the strength of mNGS in detecting refractory or opportunistic pathogens [17, 19]. It is worth noting that the detection rate of *Candida albicans* in sputum cultures was higher than that in BALF culture or BALF mNGS. This may be attributed to the distribution characteristics of *Candida albicans* in respiratory secretions, which tends to be enriched in respiratory secretions such as sputum, whereas its content in BALF is relatively low [20]. However, this does not diminish the overall advantage of mNGS, as mNGS still exhibits higher sensitivity across a broader range of pathogens.

By comparing results with the clinical diagnosis gold standard, this study systematically evaluated the diagnostic efficiency of mNGS in acute exacerbation of bronchiectasis and confirmed its significant advantages. The results showed that mNGS was markedly superior to conventional culture methods in diagnostic accuracy (94.87%), sensitivity (94.74%), with an AUC of 0.974, highlighting its high clinical application value in pathogen detection for bronchiectasis exacerbations, consistent with previous studies [9]. Conventional culture methods rely on the in vitro growth of microorganisms, which are limited by prolonged turnaround times and low detection rates for fastidious bacteria (e.g., *Haemophilus parainfluenzae*) and atypical pathogens (e.g., mycobacteria). Moreover, cultures are easily affected by prior antibiotic exposure, leading to false-negative results [15]. In contrast, mNGS can directly

Table 5. Pathogen detection characteristics in different BSI score groups [n (%)]

Spectrum of pathogens	Mild-to-moderate group (n=29)	Severe group (n=47)	χ^2	P
Bacteria				
Total positive	24 (82.76)	38 (80.85)	0.043	0.835
Pseudomonas aeruginosa	8 (27.59)	24 (51.06)	4.055	0.044
Haemophilus parainflu- enzae	5 (17.24)	7 (14.89)	0.000	1.000
Haemophilus influenzae	7 (24.14)	4 (8.51)	2.388	0.122
Streptococcus pneumoniae	2 (6.90)	8 (17.02)	0.845	0.358
Staphylococcus aureus	3 (10.34)	2 (4.26)	0.318	0.573
Klebsiella pneumoniae	5 (17.24)	1 (2.13)	3.747	0.053
Streptococcus pseudopneumoniae	1 (3.45)	4 (8.51)	0.151	0.698
Stenotrophomonas maltophila	0 (0.00)	4 (8.51)	1.178	0.278
Moraxella catarrhalis	1 (3.45)	2 (4.26)	0.000	1.000
Pasteurella multocida	3 (10.34)	0 (0.00)	2.701	0.100
Tannerella forsythia	0 (0.00)	3 (6.38)	0.611	0.434
Enterobacter cloacae	1 (3.45)	2 (4.26)	0.000	1.000
Escherichia coli	1 (3.45)	1 (2.13)	-	1.000
Proteus mirabilis	1 (3.45)	1 (2.13)	-	1.000
Streptococcus constellatus	1 (3.45)	2 (4.26)	0.000	1.000
Conebacterium anguillarum	0 (0.00)	2 (4.26)	-	0.522
Campylobacter rectum	0 (0.00)	2 (4.26)	-	0.522
S.marcescens	0 (0.00)	1 (2.13)	-	1.000
Fungi				
Total positive	5(17.24)	14 (29.79)	1.506	0.220
Aspergillus fumigatus	2 (6.90)	9 (19.15)	1.298	0.255
Candida albicans	2 (6.90)	2 (4.26)	0.000	1.000
Pneumocystis jirovecii	1 (3.45)	2 (4.26)	0.000	1.000
cryptococcus neoformans	0 (0.00)	2 (4.26)	-	0.522
Scedosporium apiospermum	0 (0.00)	1 (2.13)	-	1.000
Pseudallescheria boydii	0 (0.00)	1 (2.13)	-	1.000
Penicillium marneffei	0 (0.00)	1 (2.13)	-	1.000
Mycobacterium				
Total positive	7 (24.14)	5 (10.64)	1.548	0.213
Mycobacterium tuberculosis	4 (13.79)	2 (4.26)	1.124	0.289
Mycobacterium abscessus	2 (6.90)	1 (2.13)	0.186	0.667
Mycobacterium avium	1 (3.45)	1 (2.13)	-	1.000
Mycobacterium intracellulare	0 (0.00)	1 (2.13)	-	1.000
Mycobacterium turtle	0 (0.00)	1 (2.13)	-	1.000
Mycobacterium colombiense	0 (0.00)	1 (2.13)	-	1.000
Virus				
Total positive	13 (44.83)	23 (48.94)	0.121	0.727
Human herpesvirus 5	5 (17.24)	13 (27.66)	0.578	0.447
Human herpesvirus 7	7 (24.14)	2 (4.26)	5.020	0.025
Torque teno virus	2 (6.90)	7 (14.89)	0.466	0.495
Human herpesvirus 4	2 (6.90)	4 (8.51)	0.000	1.000
Human herpesvirus 1	0 (0.00)	2 (4.26)	-	0.522
Human adenovirus group C	1 (3.45)	0 (0.00)	-	0.382
WU polyomavirus	0 (0.00)	1 (2.13)	-	1.000
Hepatitis B virus	0 (0.00)	1 (2.13)	-	1.000

Note: S.marcescens refers to Serratia marcescens.

analyze microbial nucleic acids in BALF samples, allowing simultaneous detection of bacteria, fungi, viruses, and mycobacteria, and is less susceptible to antibiotic interference, thereby significantly reducing the missed diagnosis rate [21]. In addition, the pathological features of bronchiectasis further highlight the clinical value of mNGS. Structural destruction of the airways and impaired mucociliary clearance promote a microenvironment conducive to pathogen colonization and frequent mixed infections. Conventional culture methods typically target a single pathogen at a time and may miss polymicrobial infections, while mNGS covers the entire microbial spectrum, enabling simultaneous detection of bacterial, fungal, and viral coinfections. This comprehensive detection capability explains the significant improvement in diagnostic accuracy compared with conventional methods [22]. Notably, the diagnostic specificity of mNGS (100%) was comparable to that of BALF culture and significantly higher than that of sputum culture. This may be attributed to BALF sampling from the lower respiratory tract, minimizing contamination from upper airway flora. However, sputum culture samples are more prone to oral contamination, resulting in an increased false-positive rates, such as the overestimation of *Candida albicans* detection [20]. ROC curve analysis further confirmed the diagnostic value of mNGS, with an AUC of 0.974, significantly exceeding that of conventional methods, indicating that mNGS has higher efficiency in distinguishing pathogen infection and offers a more reliable basis for clinical decision-making [23].

In this study, 76 patients who had already been diagnosed as positive by the gold standard were divided into mild-to-moderate group and severe group according to BSI score. The etiology of the two groups was statistically analyzed. The results showed that there was no significant difference in the detection rate of bacteria between the two groups, but there were some differences in the detection of specific pathogens. *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, and *Klebsiella pneumoniae* were the main bacteria in the mild-to-moderate group, while the detection rate of Pseudomonas aeruginosa was significantly increased in the severe group. This difference may reflect that with the increase of the degree of acute exacerbation of bronchiectasis, the microenvironment of the patient's lung changes, leading to the enhancement of the reproductive advantage of some bacteria (e.g., *Pseudomonas aeruginosa*), which is similar to the results of previous studies [17]. As a common opportunistic pathogen, the high detection rate of *Pseudomonas aeruginosa* in patients with acute exacerbation of severe bronchiectasis may be related to its strong drug resistance and colonization ability in damaged lung tissues [24]. In addition, the severe group also detected a variety of bacteria that were not seen in the mild-to-moderate group, such as *Stenotrophomonas maltophilia* and *Stenotrophomonas forsythia*, which may mean that in more severe cases, the patient's immune system function is further reduced, allowing more types of bacteria to invade and reproduce [25, 26].

In terms of fungi, although there was no significant difference in the detection rate between the two groups, the detection rate of *Aspergillus fumigatus* in the severe group was slightly higher than that in the mild-to-moderate group (19.15% vs 6.90%). Studies have shown that Aspergillus fumigatus, as a conditional pathogen, is more common in patients with reduced immunity, and the incidence of Aspergillus increases with the severity of lung disease [27], which is similar to the results of this study. This may be related to the increased risk of fungal infection caused by long-term use of broad-spectrum antibiotics, immunosuppressants and other drugs in severe patients [28]. In addition, *Pneumocystis jiroveci* is an opportunistic fungal pathogen that can cause life-threatening pneumocystis pneumonia in immunocompromised patients. *Pneumocystis jiroveci* is common in patients with acquired immunodeficiency syndrome (AIDS) and immunosuppressive therapy [29]. The imaging features of pneumocystis pneumonia are exudation of both lungs, ground-glass opacity, patchy shadows and cystic lesions. Staining microscopy and polymerase chain reaction can be used as indicators for diagnosis, but the detection rate is low. Studies have shown that mNGS has good diagnostic performance in the detection of pneumocystis [30], which is consistent with the results of this study that *Pneumocystis jirovecii* was detected in the mild-to-moderate group and the severe group. Although the difference in the detection rate of fungi between

the two groups was not statistically significant, this trend still suggests that the risk of fungal infection may increase with the aggravation of bronchiectasis, especially in patients with other risk factors (such as long-term use of glucocorticoids, broad-spectrum antibiotics), the monitoring and prevention of fungal infection should be strengthened [31]. For the detection of mycobacteria, the total positive rate (24.14%) in the mild to moderate group was slightly higher than that in the severe group (10.64%), but the detection rate of each specific mycobacterial species was not significantly different between the two groups. This indicates that the incidence of mycobacterial infection is relatively stable in patients with acute exacerbation of bronchiectasis of different severity, and there is no significant correlation between the severity of the disease. In addition, Mycobacterium tuberculosis was detected in both groups, indicating that patients with bronchiectasis cannot ignore the possibility of tuberculosis infection even in the acute exacerbation period; at the same time, the detection of other non-tuberculous mycobacteria such as Mycobacterium abscessus and Mycobacterium avium suggests that patients with severe bronchiectasis need to be alert to potential non-tuberculous mycobacterial infections, especially in individuals with reduced immunity [15, 18]. However, this study failed to reveal a direct link between mycobacterial infection and disease severity, which may be related to sample size, regional specificity, and patient background differences.

In terms of virus detection, the total positive rate of virus detection in the severe group (48.94%) was slightly higher than that in the mild-to-moderate group (44.83%), but the difference also did not reach statistical significance. Gao et al. [32] showed that respiratory viruses are usually found during the deterioration of patients with bronchiectasis, and the detection rate of respiratory viruses is higher in patients with moderate to severe bronchiectasis, which is somewhat similar to the results of our study. As an important pathogen of respiratory tract infection, the role of viruses in the acute exacerbation of bronchiectasis cannot be ignored. The presence of viruses may directly participate in the disease process or aggravate the disease by interacting with other pathogens such as bacteria and fungi [33]. In addition, the detection rate of human herpesvirus was the highest, and the detection rate of human herpesvirus 7 in the mild to moderate group was higher than that in the severe group (24.14% vs 4.26%). Studies have shown that the genome length of human herpesviruses is significantly longer than that of other viruses, resulting in a significantly higher detection rate of human herpesviruses in mNGS than other types of viruses, which may partially magnify the difference in detection rates [4].

This study still has some shortcomings [34]: (1) This study is a retrospective study and the sample size is small. Therefore, prospective, multicenter, large-scale studies are needed for repeated validation. (2) The hospital in which this study was located did not have the conditions for sequencing, and it was necessary to send the bronchoalveolar lavage fluid samples to the platform outside the hospital for sequencing, which may increase the risk of contamination and nucleic acid degradation during the storage and transportation of samples, which may lead to deviation in the results. (3) There is no unified standard for the reporting and interpretation of mNGS, and subjective bias may exist.

In conclusion, BALF mNGS is significantly superior to conventional methods in the etiological detection of acute exacerbation of bronchiectasis, which can greatly improve the detection rate of bacteria, fungi and mycobacteria, and can improve the diagnostic efficiency of pathogens. BALF mNGS also reveals the complex pathogen spectrum of patients with acute exacerbation of bronchiectasis with different severity, which provides a more comprehensive and accurate method for the etiological diagnosis of patients with acute exacerbation of bronchiectasis, which is helpful to guide clinical treatment and has high clinical application value [35].

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Disclosure of conflict of interest

None.

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References

[1] Barbosa M and Chalmers JD. Bronchiectasis. Presse Med 2023; 52: 104174.
[2] Macfarlane L, Kumar K, Scoones T, Jones A, Loebinger MR and Lord R. Diagnosis and management of non-cystic fibrosis bronchiectasis. Clin Med (Lond) 2021; 21: e571-e577.
[3] Lee AL, Gordon CS and Osadnik CR. Exercise training for bronchiectasis. Cochrane Database Syst Rev 2021; 4: CD013110.
[4] Liu H, Zhang Y, Yang J, Liu Y and Chen J. Application of mNGS in the etiological analysis of lower respiratory tract infections and the prediction of drug resistance. Microbiol Spectr 2022; 10: e0250221.
[5] Maartens G, Griesel R, Dube F, Nicol M and Mendelson M. Etiology of pulmonary infections in human immunodeficiency virus-infected inpatients using sputum multiplex real-time polymerase chain reaction. Clin Infect Dis 2020; 70: 1147-1152.
[6] Rajapaksha P, Elbourne A, Gangadoo S, Brown R, Cozzolino D and Chapman J. A review of methods for the detection of pathogenic microorganisms. Analyst 2019; 144: 396-411.
[7] Zhang P, Chen Y, Li S, Li C, Zhang S, Zheng W, Chen Y, Ma J, Zhang X, Huang Y and Liu S. Metagenomic next-generation sequencing for the clinical diagnosis and prognosis of acute respiratory distress syndrome caused by severe pneumonia: a retrospective study. PeerJ 2020; 8: e9623.
[8] Chen W, Liu G, Cui L, Tian F, Zhang J, Zhao J, Lv Y, Du J, Huan X, Wu Y and Zhang Y. Evaluation of metagenomic and pathogen-targeted next-generation sequencing for diagnosis of meningitis and encephalitis in adults: a multicenter prospective observational cohort study in China. J Infect 2024; 88: 106143.
[9] Chen S, Kang Y, Li D and Li Z. Diagnostic performance of metagenomic next-generation sequencing for the detection of pathogens in bronchoalveolar lavage fluid in patients with pulmonary infections: systematic review and meta-analysis. Int J Infect Dis 2022; 122: 867-873.
[10] Wei Y, Zhang T, Ma Y, Yan J, Zhan J, Zheng J and Xu Y. Clinical evaluation of metagenomic next-generation sequencing for the detection of pathogens in BALF in severe community acquired pneumonia. Ital J Pediatr 2023; 49: 25.
[11] Bronchiectasis Expert Consensus Writing Group; Pulmonary Infection Assembly, Chinese Thoracic Society. Expert consensus on the diagnosis and treatment of adult bronchiectasis in China. Zhonghua Jie He He Hu Xi Za Zhi 2021; 44: 311-321.
[12] Hill AT, Sullivan AL, Chalmers JD, De Soyza A, Elborn SJ, Floto AR, Grillo L, Gruffydd-Jones K, Harvey A, Haworth CS, Hiscocks E, Hurst JR, Johnson C, Kelleher PW, Bedi P, Payne K, Saleh H, Screaton NJ, Smith M, Tunney M, Whitters D, Wilson R and Loebinger MR. British thoracic society guideline for bronchiectasis in adults. Thorax 2019; 74 Suppl 1: 1-69.
[13] Guan WJ, Chen RC and Zhong NS. The bronchiectasis severity index and FACED score for bronchiectasis. Eur Respir J 2016; 47: 382-4.
[14] Liu Z, Sun C, Xiao X, Zhou L, Huang Y, Shi Y, Yin X, Mao Z and Zhang Q. Application of metagenomic next-generation sequencing (mNGS) in diagnosing pneumonia of adults. J Infect Dev Ctries 2023; 17: 1566-1573.
[15] Jin X, Li J, Shao M, Lv X, Ji N, Zhu Y, Huang M, Yu F, Zhang C, Xie L, Huang J, Chen S, Zhu C, Lv M and Feng G. Improving suspected pulmonary infection diagnosis by bronchoalveolar lavage fluid metagenomic next-generation sequencing: a multicenter retrospective study. Microbiol Spectr 2022; 10: e0247321.
[16] Song X, Jiang H, Zong L, Shi D and Zhu H. The clinical value of mNGS of bronchoalveolar lavage fluid versus traditional microbiological tests for pathogen identification and prognosis of severe pneumonia (NT-BALF): study protocol for a prospective multi-center randomized clinical trial. Trials 2024; 25: 276.
[17] Duan JL, Li CY, Jiang Y, Liu C, Huang PR, Gao LF, Guan WJ and Cheng LL. Microbiological characteristics of the lower airway in adults with bronchiectasis: a prospective cohort study. Respir Res 2024; 25: 283.
[18] Zhang X, Chen H, Lin Y, Yang M, Zhao H, Hu J and Han D. Diagnosis of non-tuberculous mycobacterial pulmonary disease by metagenomic next-generation sequencing on bronchoalveolar lavage fluid. Infect Drug Resist 2023; 16: 4137-4145.

[19] Meng X, Liu Y, Li J, Wang L, Shi R, Chen Y, Zhu Y and Zhuang S. Metagenomic next-generation sequencing for diagnosis and efficacy evaluation of a critical case of SFTS complicated by invasive pulmonary aspergillosis. IDCases 2023; 33: e01884.
[20] Cuthbertson L, Felton I, James P, Cox MJ, Bilton D, Schelenz S, Loebinger MR, Cookson WOC, Simmonds NJ and Moffatt MF. The fungal airway microbiome in cystic fibrosis and non-cystic fibrosis bronchiectasis. J Cyst Fibros 2021; 20: 295-302.
[21] Shi XQ, Tian L, Huang ZH, Song WT, Wu JB and Chen LM. Metagenomic next-generation sequencing vs. conventional detection methods for detecting the pulmonary infections. Eur Rev Med Pharmacol Sci 2023; 27: 4752-4763.
[22] Zhang H, Shen D, Zhou J, Yang Q, Ying Y, Li N, Cao L, Wang W and Ma X. The Utility of Metagenomic Next-Generation Sequencing (mNGS) in the management of patients with bronchiectasis: a single-center retrospective study of 93 cases. Open Forum Infect Dis 2023; 10: ofad425.
[23] Guo Q, Xiao Y and Zhang S. Metagenomic next generation sequencing of bronchoalveolar lavage samples for the diagnosis of lower respiratory tract infections: a systematic review and meta-analysis. Heliyon 2023; 10: e23188.
[24] Chai YH and Xu JF. How does Pseudomonas aeruginosa affect the progression of bronchiectasis? Clin Microbiol Infect 2020; 26: 313-318.
[25] Marra R, Sgalla G, Richeldi L, Conte EG and Hill AT. Role of Stenotrophomonas maltophilia isolation in patients with non-CF bronchiectasis. QJM 2020; 113: 726-730.
[26] Solarat B, Perea L, Faner R, de La Rosa D, Martínez-García MÁ and Sibila O. Pathophysiology of chronic bronchial infection in bronchiectasis. Arch Bronconeumol 2023; 59: 101-108.
[27] Azoulay E, Russell L, Van de Louw A, Metaxa V, Bauer P, Povoa P, Montero JG, Loeches IM, Mehta S, Puxty K, Schellongowski P, Rello J, Mokart D, Lemiale V and Mirouse A; Nine-i Investigators. Diagnosis of severe respiratory infections in immunocompromised patients. Intensive Care Med 2020; 46: 298-314.
[28] Moldoveanu B, Gearhart AM, Jalil BA, Saad M and Guardiola JJ. Pulmonary aspergillosis: spectrum of disease. Am J Med Sci 2021; 361: 411-419.
[29] Bateman M, Oladele R and Kolls JK. Diagnosing Pneumocystis jirovecii pneumonia: a review of current methods and novel approaches. Med Mycol 2020; 58: 1015-1028.
[30] Chen J, He T, Li X, Wang X, Peng L and Ma L. Metagenomic next-generation sequencing in diagnosis of a case of pneumocystis jirovecii pneumonia in a kidney transplant recipient and literature review. Infect Drug Resist 2020; 13: 2829-2836.
[31] Lu D, Li C, Zhong Z, Abudouaini M, Amar A, Wu H and Wei X. Changes in the airway microbiome in patients with bronchiectasis. Medicine (Baltimore) 2023; 102: e36519.
[32] Gao YH, Guan WJ, Xu G, Lin ZY, Tang Y, Lin ZM, Gao Y, Li HM, Zhong NS, Zhang GJ and Chen RC. The role of viral infection in pulmonary exacerbations of bronchiectasis in adults: a prospective study. Chest 2015; 147: 1635-1643.
[33] Zhang XX, He JH, Pan CX, He ZF, Li HM, Lin ZH, Zhang XF, Cen LJ, Zhang RL, Shi MX and Guan WJ. Bacteria and viruses and clinical outcomes of asthma-bronchiectasis overlap syndrome: a cohort study. Clin Transl Allergy 2024; 14: e12331.
[34] Shi W and Zhu S. The application of metagenomic next-generation sequencing in detection of pathogen in bronchoalveolar lavage fluid and sputum samples of patients with pulmonary infection. Comput Math Methods Med 2021; 2021: 7238495.
[35] Chen H, Huang Q, Wu W, Wang Z, Wang W, Liu Y, Ruan F, He C, Li J, Liu J and Wu G. Assessment and clinical utility of metagenomic next-generation sequencing for suspected lower respiratory tract infections. Eur J Med Res 2024; 29: 213.