

# Application of Metagenomic Next-Generation Sequencing (mNGS) in Pathogen Detection of BALF in Patients With Acute Exacerbation of Bronchiectasis

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**Background:** Acute exacerbation of bronchiectasis (AEB) is a fatal disease in the respiratory system. Herein, we aimed to compare the detection efficacy of metagenomic next-generation sequencing (mNGS) and standard methods between high-risk and low-risk AEB patients.

**Methods:** Retrospectively, medical data of AEB patients (n = 52) were collected between March 2022 and January 2023. Based on the number of acute exacerbations in the previous year and hospitalization history, patients were categorized into a high-risk group and a low-risk group. According to the study's inclusion and exclusion criteria, a total of 52 AEB patients were included (28 high-risk and 24 low-risk). Baseline clinical data were analyzed, and mNGS of bronchoalveolar lavage fluid (BALF) samples was performed.

**Results:** Significant differences were observed between the high-risk and low-risk groups in disease duration, number of clinical visits, number of admissions, white blood cell count, neutrophil percentage, high-sensitivity C-reactive protein (hs-CRP), interleukin-8 (IL-8), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels ( $p < 0.05$ ). mNGS demonstrated clear advantages in detecting pathogens in BALF samples from patients with bronchiectasis, offering higher sensitivity and broader pathogen spectrum coverage. mNGS can effectively complement the limitations of conventional culture methods. The mNGS results showed that the infection rates of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were higher in the high-risk group compared to the low-risk group ( $p < 0.05$ ).

**Conclusion:** The mNGS proves to be an effective tool in assessing the respiratory microbiota and has the potential to improve the diagnosis and treatment of AEB patients by enabling rapid and precise pathogen detection.

**Keywords:** bronchiectasis; acute exacerbation; BALF; mNGS; pathogens

## Introduction

Bronchiectasis is a pathological and permanent dilation of the bronchial tree caused by various etiologies, leading to chronic airway inflammation with recurrent purulent infections [1–3]. The clinical manifestations of bronchiectasis include persistent or recurrent cough, expectoration, and sometimes hemoptysis, which may lead to respiratory dysfunction and chronic pulmonary heart disease [4,5].

Acute exacerbation of bronchiectasis (AEB) can further aggravate bronchial structural damage, accelerate the irreversible progression of the disease, and may induce respiratory failure and pulmonary heart disease [6]. In clinical practice, we have observed that some patients with bronchiectasis experience infrequent acute exacerbations, referred to as low-risk AEB (defined as fewer than two acute exacerbations and no hospitalization for AEB in the previous year). Conversely, patients with frequent acute ex-

acerbations are classified as high-risk AEB, characterized by two or more acute exacerbations or at least one hospitalization for AEB in the previous year. These patients are at a significantly higher risk of future exacerbations and are of particular clinical importance for targeted intervention and management.

Clarifying this risk stratification helps identify patient subgroups with poorer prognoses and provides a foundation for guiding individualized treatment strategies. In recent years, the AEB treatment has become increasingly challenging. One reasonable explanation for this issue is the recurrent infections caused by diverse microorganisms [7]. Therefore, rapid and accurate identification of infectious pathogens and the early initiation of effective antimicrobial therapy are crucial for shortening hospital stays, reducing serious complications, and lowering mortality, particularly in patients with hematologic diseases.

Currently, traditional etiological detection methods, such as culture, immunological assays, and PCR, are widely used in the clinical diagnosis of pneumonia; however, their utility is limited by poor timeliness, low pathogen coverage, and low positive detection rates [8,9]. Therefore, a large number of patients cannot receive a clear etiological diagnosis, resulting in insufficient empirical medication or treatment, which affects the clinical prognosis. Nowadays, metagenomic next-generation sequencing (mNGS) is an advanced diagnostic technology that combines high-throughput sequencing with bioinformatic analysis. It enables the rapid and simultaneous identification of diverse pathogens, including bacteria, fungi, viruses, and parasites, by sequencing DNA or RNA extracted from clinical specimens, thereby significantly reducing detection time and enhancing etiological understanding [10]. Recently, mNGS has emerged as an unbiased approach capable of theoretically detecting all pathogens present in clinical samples. It is particularly valuable for diagnosing novel, rare, or atypical infectious diseases [11]. However, the application of mNGS in the diagnosis and management of AEB remains limited, and its clinical utility continues to require further evaluation.

The medical records of AEB patients were collated and analyzed in the present study. Utilizing the advanced mNGS, the study aimed to systematically evaluate the clinical application value of mNGS technology compared with conventional culture methods in pathogen detection in bronchoalveolar lavage fluid (BALF) samples of patients with bronchiectasis.

## Patients and Methods

### *Collection of Clinical Data*

In this retrospective case-control study, medical data of AEB patients ( $n = 52$ ) were collected between March 2022 and January 2023. Based on the number of acute exacerbations in the previous year and hospitalization history, patients were classified into high-risk and low-risk groups.

**High-risk group:** patients who experienced two or more acute exacerbations in the previous year or were hospitalized for at least one acute exacerbation in the previous year.

**Low-risk group:** patients who experienced fewer than two acute exacerbations in the previous year and were not hospitalized for acute exacerbations during that period.

According to the inclusion and exclusion criteria, a total of 52 AEB patients were enrolled in this study (28 high-risk and 24 low-risk). This study was approved by the Ethics Committee of The People's Hospital of Yuhuan (No. 2022(063)), and written informed consent was obtained from all participants.

Inclusion criteria: (1) age  $\geq 18$  years; (2) chest high-resolution computerized tomography (CT) showed bronchiectasis, and the patient was accompanied by respi-

ratory symptoms such as expectoration and chronic cough; (3) patients who were in acute exacerbation, and most of them were accompanied by expectoration one month before they were enrolled in this study. The sputum was mucinous, mucopurulent, or purulent.

Exclusion criteria: (1) traction bronchiectasis caused by severe pulmonary fibrosis or emphysema; (2) bronchial asthma was not under control; (3) the patient had serious cardiovascular, liver, kidney and tumor diseases at the same time; (4) incomplete clinic information.

Diagnosis criteria for AEB [12]: the patient suddenly had more than 3 respiratory symptoms (such as dyspnea, cough, expectoration, chest pain and hemoptysis, etc.), the duration was more than 24 hours, and the treatment plan needed to be changed (such as intravenous drip or oral antibiotics).

### *Evaluation of Sample Size*

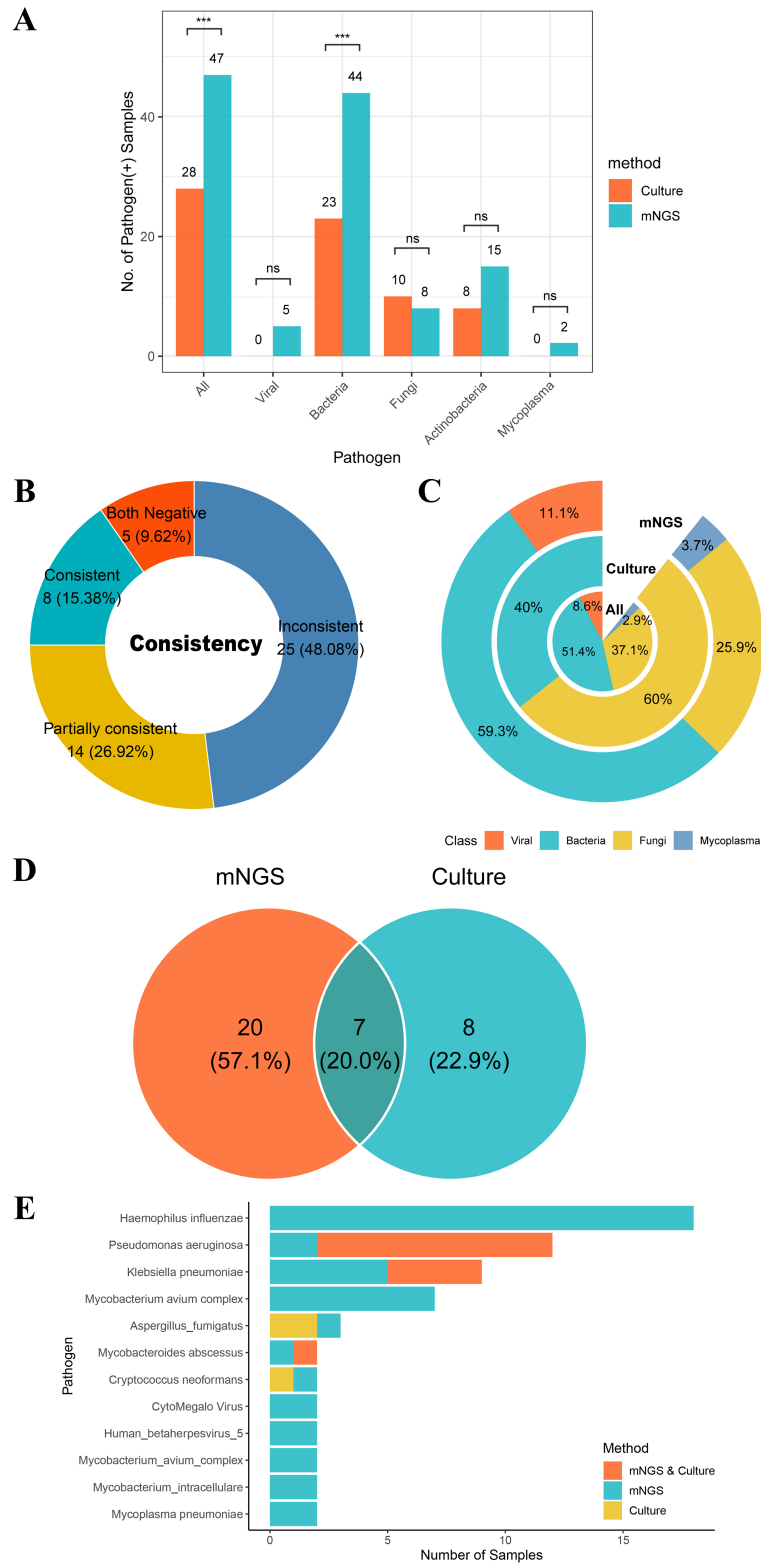
The sample size was calculated using PASS software (version 2021, NCSS, LLC, Kaysville, UT, USA), with the frequency of acute exacerbations as the primary endpoint. Assuming a statistical power of 80% and a two-sided alpha level of 0.05, the analysis indicated that a minimum of 7 and 16 patients were required for each group, respectively. To account for a potential 20% dropout rate, the minimum sample sizes were adjusted to 9 and 20 patients per group to ensure that the final analyzable sample met the statistical requirements.

### *Comparison of Basic Characteristics*

According to the patients' medical records, baseline characteristics were compared between the high-risk and low-risk groups. The analyzed variables included sex, age, body mass index (BMI), cough, cough with sputum, hemoptysis, dyspnea, wheezing, fever, and chest tightness, as well as comorbid conditions such as bronchiectasis (non-infectious), bronchiectasis (infectious), pneumonia, bronchial asthma, chronic obstructive pulmonary disease (COPD), and community-acquired pneumonia. Laboratory indicators included disease duration, number of clinical visits, number of admissions, white blood cell (WBC) count, neutrophil percentage, high-sensitivity C-reactive protein (hs-CRP), interleukin-8 (IL-8), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ).

### *Collection of Respiratory Tract Samples*

All AEB patients underwent bronchoalveolar lavage (BAL) under fiberoptic bronchoscopy. Local anesthesia of the respiratory tract was achieved using atomized 1% lidocaine solution, and 2 mg of midazolam was administered intravenously for sedation. Patients were positioned supine, and alveolar lavage was performed using a fiberoptic bronchoscope. The bronchoscope was embedded in a subsegment of the right middle lobe, and its tip was tightly fitted at the bronchial opening to prevent contamination by air-



**Fig. 1. Comparison of pathogen detection between mNGS and conventional cultures.** (A) Comparison of pathogen detection rates between mNGS and conventional cultures. (B) Comparison of pathogen detection concordance between mNGS and conventional cultures. (C) Comparative analysis of the number of pathogen species detected by mNGS and conventional cultures. (D) Venn diagram illustrating the overlap of seven pathogens detected by both mNGS and conventional cultures. (E) Number of patients infected with each pathogen as detected by mNGS and conventional cultures. \*\*\* $p < 0.001$ ; ns,  $p > 0.05$  Abbreviation: mNGS, metagenomic next-generation sequencing.

way secretions and leakage of lavage fluid. A total of 150 mL of sterile physiological saline (37 °C) was instilled in aliquots of 20–50 mL, with a total recovery rate of  $\geq 30\%$ .

Sample qualification criteria: (1) No contamination of lavage fluid with airway secretions; (2) recovery rate  $>40\%$ , with  $>95\%$  viable cells; (3) red blood cells  $<10\%$ , epithelial cells  $<5\%$ ; (4) intact and morphologically uniform cells without deformation.

Qualified samples were sub-packaged, centrifuged at high speed, and stored at  $-80$  °C. Simultaneously, 5–10 mL of lavage fluid was sent to the microbiology laboratory for bacterial culture.

### *mNGS Sample Processing, Sequencing, and Data Analysis*

DNA was extracted and fragmented from respiratory specimens using the Total Nucleic Acid Extraction Kit (DP419, Tiangen, Beijing, China) and the mNGS DNA Library Preparation Kit (KP141-01, Transgen, Beijing, China). The fragmented DNA underwent end repair, adapter ligation, and PCR amplification to prepare sequencing libraries. The purified sequences were used to form sequencing libraries, which were then quality-controlled using the Agilent 2100 system.

Sequencing was performed on the Illumina NextSeq 550 high-throughput sequencing platform (Illumina, San Diego, CA, USA) using a 75 bp paired-end strategy, covering 18,562 microbial species from the database. Each library generated  $\geq 2.5$  million reads (75 bp). Next, the raw sequencing data underwent quality control by mapping human host sequences to the human reference genome (GRCh38.p13) to remove low-quality reads and eliminate human host sequences. The remaining data were aligned and statistically compared to microbial genomic databases.

All pathogenic sequence reference databases were sourced from the NCBI RefSeq database (<https://www.ncbi.nlm.nih.gov/refseq/>). Sequencing results were analyzed using IngeniSeq-MG 1.0 and mNGS software (Dian Diagnostics, Hangzhou, China).

### *Statistical Analysis*

Statistical analysis was performed using GraphPad Prism 8.0 (GraphPad Software, LLC, San Diego, CA USA). Chi-square test was applied for classification variables. A rank sum test was used for grade data. Continuity variables conforming to the normal distribution were described by mean  $\pm$  standard deviation, and the comparison between groups was made by an independent sample *t*-test. The continuity variables not conforming to the normal distribution were expressed as quartile M ( $P_{25}$ – $P_{75}$ ), and a Mann-Whitney *U*-test was used for comparison between groups. Multivariate logistic regression was used for multi-factor analysis. A bilateral  $p < 0.05$  was deemed a significant difference.

## Results

### *Basic Characteristics*

As shown in Table 1, the mean age in the high-risk group ( $n = 28$ , including 10 males and 18 females) was  $62.50 \pm 9.60$  and in the low-risk group ( $n = 24$ , including 8 males and 16 females), it was  $64.25 \pm 7.47$ . The mean BMI was  $21.97 \pm 2.24$  in the high-risk group and  $21.19 \pm 2.67$  in the low-risk group. There were significant differences between high-risk group and low risk group about disease duration ( $p < 0.001$ ), number of clinical visits ( $p < 0.001$ ), number of admissions ( $p = 0.008$ ), WBC count ( $p = 0.014$ ), neutrophil percentage ( $p = 0.002$ ), hs-CRP ( $p < 0.001$ ), IL-8 ( $p = 0.029$ ) and TNF- $\alpha$  ( $p < 0.001$ , Table 1). Moreover, there were non-significant differences between groups about sex, age, BMI, cough, cough with sputum, hemoptysis, dyspnea, wheezing, fever, chest tightness, bronchiectasis (non-infectious), bronchiectasis (infectious), pneumonia, bronchial asthma, COPD, and community-acquired pneumonia (Table 1,  $p > 0.05$ ).

A multivariate regression analysis was performed on the clinical characteristics that showed significant differences between the high-risk and low-risk groups (Table 2). The results showed that levels of WBC count (OR = 0.605), neutrophil percentage (OR = 0.902), hs-CRP (OR = 0.803), IL-8 (OR = 0.960), TNF- $\alpha$  (OR = 0.903), disease duration (OR = 0.160), number of clinical visits (OR = 0.239) and number of admissions (OR = 0.243) are protective factors for low-risk patient status (Table 2,  $p < 0.05$ ).

### *Comparison of Pathogen Detection and Consistency*

To evaluate the diagnostic potential of mNGS for pathogen detection in bronchiectasis samples, sequencing analysis was performed on BALF. The primary objective was to compare pathogen detection rates and consistency between mNGS and conventional culture methods. Positivity rates for all detected pathogens—including viruses, bacteria, fungi, mycobacteria (special pathogens), and mycoplasma—were calculated for all samples using both mNGS and conventional cultures.

As shown in Fig. 1A, the pathogen detection rates for mNGS and conventional cultures were 90.38% (47/52) and 53.85% (28/52), respectively, with a significant difference between the two methods ( $p < 0.001$ ). Notably, significant differences were observed in bacterial detection between the two methods ( $p < 0.001$ ), with detection rates of 84.62% (44/52) for mNGS and 44.23% (23/52) for conventional cultures (Fig. 1A). It is worth noting that conventional cultures failed to detect viral and mycoplasma pathogens (Fig. 1A).

We compared the detection consistency between mNGS and conventional cultures by pathogen species. Each sample was categorized as follows: negative, fully consistent, partially consistent, and completely inconsis-

**Table 1. Comparison of basic characteristics.**

Indicator	High risk (n = 28)	Low risk (n = 24)	t/Z/ $\chi^2$	p
Sex (male/female)	10/18	8/16	0.032	0.857
Age (year)	62.50 $\pm$ 9.60	64.25 $\pm$ 7.47	0.724	0.472
BMI (kg/m <sup>2</sup> )	21.97 $\pm$ 2.24	21.19 $\pm$ 2.67	1.148	0.256
Symptoms				
Cough	26 (93)	21 (88)	0.033	0.856
Cough with sputum	22 (79)	15 (63)	1.626	0.202
Hemoptysis	7 (25)	2 (8)	1.479	0.224
Dyspnea	3 (11)	2 (8)	0.033	0.856
Wheezing	5 (18)	1 (4)	1.221	0.269
Fever	3 (11)	4 (17)	0.048	0.826
Chest tightness	2 (7)	3 (13)	0.033	0.856
Diagnosed diseases				
Bronchiectasis (non-infectious)	7 (25)	8 (33)	0.437	0.508
Bronchiectasis (infectious)	21 (75)	16 (67)	0.437	0.508
Pneumonia	6 (21)	1 (4)	1.990	0.158
Bronchial asthma	6 (21)	1 (4)	1.990	0.158
COPD	5 (18)	0 (0)	2.910	0.088
Community-acquired pneumonia	1 (4)	3 (13)	0.466	0.495
Disease duration [n (%)]			-4.404	<0.001
<1 year	7 (25)	20 (83)		
1-10 years	5 (18)	3 (13)		
>10 years	16 (57)	1 (4)		
Number of clinical visits [n (%)]			-3.695	<0.001
0	9 (32)	18 (75)		
1	3 (11)	5 (21)		
2	16 (57)	1 (4)		
Number of admissions [n (%)]			-2.653	0.008
0	16 (57)	22 (92)		
1	10 (36)	1 (4)		
2	2 (7)	1 (4)		
WBC count ( $\times 10^9/L$ )	9.07 $\pm$ 1.33	8.05 $\pm$ 1.58	2.537	0.014
Neutrophil percentage (%)	74.00 (72.75-77.75)	69.00 (63.75-73.25)	-3.135	0.002
hs-CRP (mg/L)	36.37 (34.74-37.64)	28.38 (25.41-30.21)	-4.589	<0.001
IL-8 (pg/mL)	91.78 (84.14-96.58)	83.35 (75.35-88.89)	-2.184	0.029
TNF- $\alpha$ (pg/mL)	45.46 (41.94-50.11)	36.78 (28.84-39.76)	-3.928	<0.001

Abbreviations: BMI, body mass index; WBC, white blood cell; hs-CRP, high-sensitivity C-reactive protein; IL-8, interleukin-8; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; COPD, chronic obstructive pulmonary disease.

tent. Samples in which acid-fast bacilli (i.e., mycobacteria) were detected by conventional culture and mycobacteria were detected by mNGS were considered the same pathogen.

As shown in Fig. 1B, only 8 samples (15.38%) exhibited fully concordant pathogen detection between mNGS and conventional cultures, whereas 25 samples (48.08%) showed completely discordant results. We then compared the types of pathogens detected by mNGS, conventional culture, and the combined approach at the species level. mNGS, conventional culture, and the combined methods detected 27, 15, and 35 pathogen species, respectively (Fig. 1C). mNGS detected 3 viruses (11.1%), 16 bacteria (59.3%), 7 fungi (25.9%), and 1 mycoplasma (3.7%),

whereas conventional culture detected only 6 bacteria and 9 fungi (Fig. 1C). When comparing the pathogens detected by the two methods, only 7 pathogens were common, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Nocardia wallacei*, *Cryptococcus neoformans*, *Exophiala dermatitidis*, *Aspergillus fumigatus*, and *Mycobacterium abscessus* (Fig. 1D).

Finally, we summarized the number of infected patients for each pathogen. The most commonly detected pathogen was *Haemophilus influenzae*, found in 18 patients (34.62%), all of whom were identified by mNGS. Additionally, *Pseudomonas aeruginosa* was detected in 12 patients (23.08%), 10 of whom (83.33%) were identified by both mNGS and conventional cultures (Fig. 1E). *Klebsiella*

**Table 2. Multivariate regression analysis of clinical characteristics differences between high-risk and low-risk groups.**

Indicator	B	SE	Wald	<i>p</i>	OR	95% CI
WBC count	-0.502	0.217	5.352	0.021	0.605	0.396–0.926
Neutrophil percentage	-0.103	0.046	5.024	0.025	0.902	0.824–0.987
hs-CRP	-0.221	0.068	10.601	0.001	0.803	0.702–0.916
IL-8	-0.041	0.020	4.127	0.042	0.960	0.922–0.999
TNF- $\alpha$	-0.102	0.036	7.986	0.005	0.903	0.842–0.969
Disease duration	-1.830	0.486	14.183	<0.001	0.160	0.062–0.416
Number of clinical visits	-1.431	0.414	11.967	0.001	0.239	0.106–0.538
Number of admissions	-1.415	0.664	4.537	0.033	0.243	0.066–0.893

Abbreviations: SE, standard error; OR, odds ratio; CI, confidence interval; WBC, white blood cell; hs-CRP, high-sensitivity C-reactive protein; IL-8, interleukin-8; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

*pneumoniae* was detected in 9 patients (17.3%), 4 of whom were identified by both mNGS and conventional cultures (Fig. 1E).

### Pathogen Positivity Comparison Between High-Risk and Low-Risk Groups

To evaluate differences in pathogen positivity between the high-risk and low-risk groups, we analyzed BALF samples from 28 high-risk and 24 low-risk patients using both mNGS and conventional cultures. Pathogen positivity rates differed significantly between the two groups for both methods ( $p < 0.05$ , Fig. 2A).

However, the overall pathogen positivity rates between the high-risk and low-risk groups did not differ significantly (Fig. 2A). Next, we compared the positivity rates of pathogens in the high-risk and low-risk groups, performing statistical analysis on the species and genus with higher detection frequencies. At the species level, the number of *Pseudomonas aeruginosa* infections was 32% in the high-risk group, significantly higher than the 10% observed in the low-risk group ( $p < 0.05$ , Fig. 2B). The infection rate for *Klebsiella pneumoniae* in the high-risk group was 26%, significantly higher than the 5% in the low-risk group ( $p < 0.05$ , Fig. 2B). At the genus level, the infection rate for *Pseudomonas* in the high-risk group was 32%, significantly higher than the 10% in the low-risk group ( $p < 0.05$ , Fig. 2B). Similarly, the infection rate for *Klebsiella* in the high-risk group was 26%, significantly higher than the 5% in the low-risk group ( $p < 0.05$ , Fig. 2B).

## Discussion

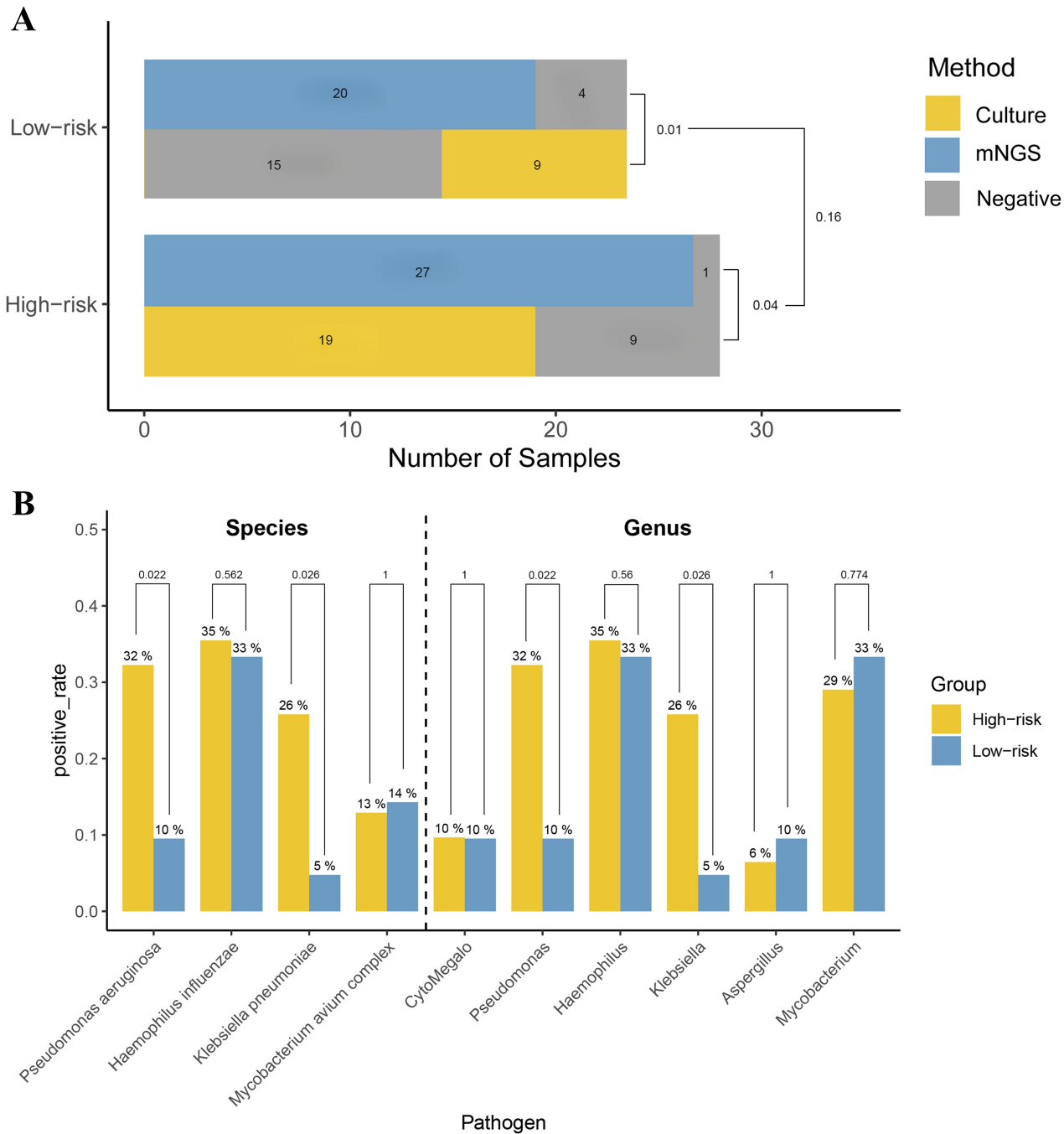
The occurrence of AEB is closely associated with multiple factors, including bacterial infection, air pollution, comorbid asthma, and alterations in bacterial metabolic activity [13–16]. During AEB episodes triggered by bacterial infection, WBC count and neutrophil percentage are typically elevated, accompanied by neutrophilic airway inflammation [7,17–19].

In the present study, we further explored factors influencing AEB development. Our data indicated significant differences between high-risk and low-risk AEB patients in disease duration, number of clinical visits, number of admissions, WBC count, neutrophil percentage, hs-CRP, IL-8 and TNF- $\alpha$ , with higher values observed in the high-risk group. These abnormal characteristics may serve as predictive indicators for identifying patients at high risk of AEB.

The most prominent finding of this study is that the overall pathogen detection rate of mNGS is significantly higher than that of traditional cultures. This result is highly consistent with the conclusions of previous studies [20].

Studies have shown that the most frequently isolated bacteria from airway secretions of patients with bronchiectasis include *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Cattamora*, *Streptococcus pneumoniae*, and *Staphylococcus aureus* [21]. In the present study, we found by mNGS sequencing that the most frequent infections were *Haemophilus*, followed by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, which aligns with the findings from Zhang *et al.* [22]. *Haemophilus* was the pathogen with the highest detection rate in this cohort, but it was completely overlooked by traditional culture. The high sensitivity of mNGS has revealed the real and potentially underestimated microbial load in the airways of patients with bronchiectasis, especially these difficult-to-culture bacteria, providing a new perspective for understanding the chronic inflammatory state of the disease.

Another important finding of ours is that the infection rates of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in the high-risk group were significantly higher than those in the low-risk group. These two pathogens are well-recognized for their association with bronchiectasis severity, frequency of acute exacerbations, and mortality [23,24]. mNGS can more sensitively detect colonization by these “high-risk” microorganisms, making it a potentially valuable prognostic tool. Early identification and intervention targeting these pathogens—through strategies such as targeted eradication therapy or intensified monitoring—may help interrupt disease progression and improve long-term



**Fig. 2. Comparative analysis of pathogen detection in high-risk and low-risk groups.** (A) Comparison of pathogen positivity between mNGS and conventional cultures in high-risk and low-risk groups. (B) The number of pathogens detected at the species and genus levels in high-risk and low-risk groups. Abbreviation: mNGS, metagenomic next-generation sequencing.

outcomes. This approach provides a novel microbiological foundation for risk-stratified management of bronchiectasis.

The mNGS results confirm that the infection rates of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in the high-risk group are significantly higher than those in the low-risk group. The above outcomes presented that they were closely associated with the attack of AEB disease.

This suggests that *Pseudomonas aeruginosa* may play a more significant role in the pathogenesis of AEB in high-risk patients, potentially contributing to more severe disease outcomes or frequent exacerbations.

Moreover, mNGS provides a more comprehensive and detailed characterization of the respiratory tract microbiome. This technique may facilitate early identification of high-risk patients, allowing for more targeted therapeutic

tic strategies and potentially improving clinical outcomes. However, the composition of the respiratory microbiota can be influenced by various confounding factors, including patients' comorbidities and medication use. Although we attempted to adjust for some known confounders in our statistical analysis, the possibility of residual confounding remains, which represents an inherent limitation of observational studies.

Several limitations should be acknowledged. Due to the high cost of mNGS, the current sample size in our study remains limited. Further expansion of the cohort is necessary to validate and refine the present findings. Additionally, the regional and ethnic specificity of our study population indicates that our microbiome results require confirmation in other populations, and the absence of an external validation cohort represents a notable limitation. Therefore, our results should be considered exploratory and hypothesis-generating, warranting confirmation through larger-scale, prospective, multicenter studies. For future research, leveraging metabolomics technologies, we plan to conduct a multicenter study to strengthen the clinical relevance of our findings for the diagnosis, treatment, and prognosis of AEB.

In summary, we found that the occurrence of high-risk AEB is closely associated with disease duration, number of clinical visits, WBC count, neutrophil percentage, hs-CRP, IL-8, and TNF- $\alpha$ . This study also demonstrated that, in patients with bronchiectasis, mNGS significantly increased the detection rate of pathogens in BALF compared with conventional culture. Notably, mNGS showed a clear advantage in detecting bacteria, viruses, and mycoplasma, while revealing a more complex and comprehensive pathogen spectrum.

## Conclusion

This study identified key risk indicators of high-risk AEB, including disease duration, inflammatory markers (such as hs-CRP, IL-8, and TNF- $\alpha$ ), and specific microbiota abnormalities. mNGS can serve as an important complementary tool for the etiological diagnosis of patients with bronchiectasis. When combined with conventional cultures, it significantly enhances pathogen detection, improves understanding of disease-associated microbiology, and can ultimately guide more effective clinical management strategies.

## Availability of Data and Materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

## Author Contributions

YJ and JD performed the research. XJ analyzed the data. XJ drafted the manuscript. All authors contributed to critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

This research was approved by the Ethical Committee of The People's Hospital of Yuhuan (No. 2022(063)) and in accordance with the Declaration of Helsinki. Written Informed consent was obtained from all participants.

## Acknowledgment

Not applicable.

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## Conflict of Interest

The authors declare no conflict of interest.

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