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# Application value of next-generation sequencing of bronchial alveolar lavage fluid in emergency patients with infection

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**ABSTRACT**

This study was performed to explore the application value of next-generation sequencing (NGS) of bronchial alveolar lavage fluid (BALF) in emergency patients with infection. In this regard, a total of 52 patients with infection who were diagnosed and treated in the emergency department of our hospital from September 2019 to September 2021 were selected as the research objects. The BALF of the patients was analyzed by NGS, and the results were compared with the pathogen detection results of traditional microbial culture of the patients to analyze the diagnostic value of NGS in patients with infection. The results showed that among the 52 patients, 47 were positive by NGS and 13 were positive by traditional microbial culture. The pathogen detection rate of NGS was higher than that of traditional microbial culture[90.4% (47/52) VS 25% (13/52), $\chi^2$=45.539, $P$<0.001], and was able to detect viruses, fungi and other special pathogens that were difficult to be detected by traditional microbial culture, such as Chlamydia psittaci. A total of 129 pathogens were detected in the NGS test results of 47 of these patients, including bacteria, fungi, viruses and mycoplasma/chlamydia. 14 pathogens were detected in the conventional microbiological cultures of 13 patients, including bacteria and fungi. Overall, compared with traditional microbial culture methods, NGS detection has higher accuracy and can effectively detect pathogens that cannot be detected by traditional microbial culture. It has a high application value in the diagnosis of pathogens and can provide clinical guidance for the diagnosis of pathogens in patients with emergency infections.

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## Introduction

Infection is a pathological phenomenon due to the invasion and proliferation of pathogens in the body, resulting in tissue damage and different clinical manifestations. Infectious disease is a common disease in the emergency department, especially acute infection and severe infections. There are also some acute episodes of chronic infection, infectious complications on the basis of chronic diseases, and even some difficult cases such as fever of unknown origin. The disease has the characteristics of multiple pathogenic factors and rapid progression. The pathogenic agents of infectious diseases are complex. In addition to common bacteria, there are also fungi, viruses, mycoplasma and chlamydia. The etiological diagnosis of this disease is the key link in clinical treatment. However, there are still many problems to be solved urgently in the rapid identification of infectious diseases and pathogen detection. At present, routine pathogen detection methods mainly include serology, microbial culture, and immunological examination (1). However, serological tests are prone to false positives and false negatives. The microbial culture method is time-consuming and has a low positive rate. The immunological examination is difficult to meet clinical needs due to its complex procedures and high operational requirements (2). With the development of molecular biology, NGS technology has been widely used in the pathogen diagnosis of infectious diseases (3,4). NGS can obtain the genetic information of pathogens through high-throughput sequencing of gene fragments of pathogenic microorganisms, which has high sensitivity and is more advantageous for pathogens that cannot be detected by conventional detection (5,6). To analyze the BALF of patients by NGS, and to explore the value of NGS in the etiological diagnosis of patients with emergency infection, so as to provide better guidance for clinical diagnosis and treatment.

## Materials and Methods

### General information

A total of 52 patients with infectious diseases who were diagnosed and treated in the emergency department of our hospital from September 2019 to September 2021 were selected as the research objects. Inclusion criteria: (I) patients with infectious diseases presented in the emergency department; (II) Traditional microbial culture and NGS detection were performed. Exclusion criteria: (I) severe heart disease (cardiac insufficiency, acute myocardial infarction); (II) accompanied by coagulation disease, anemia. This study was approved by the Medical Ethics Committee of our hospital with the consent and knowledge of the patients and their families.

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## Research methods

### *Information collection*

Retrospective analysis was used in this study. The clinical data of the enrolled patients were collected and analyzed, including age, gender, clinical symptoms, routine laboratory test results such as white blood cell count (WBC), C-reactive protein (CRP), procalcitonin (PCT), traditional microbial culture results, and NGS results.

### *BALF collection*

All patients underwent bronchoscopy, bronchoalveolar lavage was performed at the lesion site, and 10 to 25mL of lavage fluid was collected. Five ml of the lavage fluid was separated, refrigerated at 4 °C, and sent to the testing center for NGS within 24 hours. The rest of the lavage fluid was cultured by traditional micro-culture, that is, general bacteria and fungi culture, and bacterial smear.

The method of bronchoalveolar lavage was according to the application guide of bronchoscopy (7), and the Olympus BF260 electronic bronchoscope was selected after fasting for 6 hours before operation. Firstly, 2% lidocaine was used for local anesthesia in the throat, and fiberoptic bronchoscopy was used to enter the airway through the throat to observe the bronchial morphology. Normal saline at 37°C was used for lavage to severely inflamed lung segments, 1.0mL/kg for at least three times, and lavage fluid was obtained by random negative pressure suction. The acquired specimen was placed in a sterile disposable silicide collector, and finally, the fiberoptic bronchoscope was slowly pulled out. Oxygen inhalation was given during the operation, and blood oxygen saturation, blood pressure, and heart rate were routinely monitored. Pay close attention to the patient's breathing, lip color, and facial color.

## Next-generation sequencing

### *Sample processing and DNA extraction*

After 300μL of alveolar lavage fluid was mixed and shaken through glass beads, DNA was extracted using the TIANamp Micro DNAKit kit (DP316, TIANGENBIOTECH, Beijing) according to the instructions of the kit. The extracted DNA was used for DNA library construction (8).

### *Library generation and sequencing*

The extracted DNA was broken into 200-300 bp fragments by ultrasound. The Agilent2100 Bioanalyzer quality control library was used to insert fragments, and the Qubit dsDNA HS kit (Thermo Fisher Scientific, USA) was used to control the concentration of the DNA library. According to the detected concentration, the constructed library was cyclized to form a single-strand ring structure and then rolled to form a DNA nanosphere. DNA nanospheres were loaded into the sequencing chip and MGI2000 was used for high-throughput NGS sequencing (9).

### *Data analysis*

The raw results obtained by sequencing remove low-quality and splice-contaminated data, and the high-quality data obtained by filtering are compared on the BWA (http://bio-bwa.sourceforge.net/) website. After removing the human reference genome sequence from the sample, the remaining pathogen sequences are compared with the pathogen library. The species information and corresponding sequences of microorganisms in the samples were obtained. According to the patient's clinical information, a preliminary NGS test report was obtained.

Finally, clinicians comprehensively analyze the NGS results and issue the final test report according to the patient's condition, clinical symptoms, traditional detection and laboratory examination results. The data included in this study were microbiological data in the final test report.

## Statistical methods

Spss23.0 statistical software was used for data analysis. Measurement data were expressed as mean ± standard deviation and t-test were used for comparison between groups. Count data were expressed as the number of cases [n(%)], and the chi-square test was used for comparison between groups. $P<0.05$ was considered statistically significant.

## Results

## General information

Among the 52 patients, there were 29 males and 23 females with an average age of (62.11±19.00) years. The common clinical symptoms in all patients were fever (65.38%), cough (46.15%), sputum (32.69%), asthma (21.15%), chest tightness (9.62%), etc. Through the analysis of the laboratory examination results of 52 patients, the results showed that the common abnormal laboratory examination indicators were: 59.62% (31/52) of patients with elevated WBS; CRP was elevated in 80.77% (42/52) of patients; 40.38% of patients (21/52) had elevated PCT.

## Comparison of the detection rate of NGS and traditional microbial culture

The pathogen detection rate of traditional microbial culture was 25% (13/52), and the pathogen detection rate of NGS technology in BALF was 90.4% (47/52). The detection rate of NGS was higher than that of traditional microbial culture, and the difference was statistically significant ($P<0.05$), as shown in Table 1.

## Overall pathogen detection results of patients

A total of 129 pathogens were detected in the NGS results of 47 patients. Fourteen strains of pathogens were detected in 13 patients with traditional microbial culture, including 8 strains of Acinetobacter baumannii, 1 strain

**Table 1.** Comparison of the detection rate of NGS and traditional microbial culture.

| Group | Number of specimens with pathogens detected | Number of specimens without pathogen |
|---|---|---|
| Traditional Microbial Culture (n=52) | 13 | 39 |
| NGS (n=52) | 47 | 5 |
| $\chi^2$ value | 45.539 | |
| *P* value | <0.001 | |

**Table 2.** Patient pathogen physical examination results.

| Pathogen | NGS | Traditional Microbial Culture |
|---|---|---|
| Acinetobacter baumannii | 18 | 8 |
| Corynebacterium striatum | 16 | 0 |
| Staphylococcus aureus | 12 | 0 |
| Pseudomonas aeruginosa | 11 | 0 |
| Enterococcus faecium | 9 | 1 |
| Candida albicans | 9 | 0 |
| Oligotrophomonas maltophilia | 8 | 0 |
| Klebsiella pneumoniae | 6 | 1 |
| Streptococcus pneumoniae | 5 | 0 |
| Pneumocystis jirovecii | 5 | 0 |
| Human herpesvirus 5 | 5 | 0 |
| Enterococcus faecalis | 4 | 0 |
| Human herpesvirus 1 | 3 | 0 |
| Haemophilus influenzae | 2 | 0 |
| Neosartorya fischeri | 2 | 0 |
| Mycobacterium arosiense | 2 | 0 |
| Serratia marcescens | 1 | 1 |
| Burkholderia cenocepacia | 1 | 0 |
| Burkholderias tabilis | 1 | 0 |
| Streptococcus pseudopneumoniae | 1 | 0 |
| Proteus vulgaris | 1 | 0 |
| Proteus mirabilis | 1 | 1 |
| Aspergillus fumigatus | 1 | 0 |
| Candida glabrata | 1 | 0 |
| Human herpesvirus 7 | 1 | 1 |
| Human herpesvirus 6A | 1 | 1 |
| Chlamydia psittaci | 1 | 0 |
| Mycobacterium tuberculosis complex | 1 | 0 |
| Total | 129 | 14 |

of Candida albicans, 1 strain of *Enterococcus faecium*, 1 strain of *Klebsiella pneumoniae*, 1 strain of Serratia marcescens, 1 strain of Aspergillus positive, and 1 strain of Proteus mirabilis. By comparison, the detection rate of pathogens by NGS was significantly higher than that by traditional detection. The specific pathogen detection results are shown in Table 2.

## Pathogen distribution and infection types detected by NGS

The pathogens of 47 NGS-positive patients were classified into four types: bacteria, fungi, viruses and mycoplasma/chlamydia. According to the above four pathogen types, the infected patients can be divided into a bacterial infection, fungal infection, etc. The specific results are shown in Table 3. NGS detected 16 bacterial species and 97 pathogens. Acinetobacter baumannii had the highest proportion, accounting for 18.56% (18/97). Five species of fungi and viruses were detected, and three species of Mycoplasma/Chlamydia were detected. The distribution characteristics of the four types of pathogenic microorganisms are shown in Figures 1-4.

**Table 3.** Types of infection in the 47 infected patients.

| Types of infection | n (%) |
|---|---|
| Bacteria | 24 (51.06%) |
| Fungus | 2 (4.26%) |
| Virus | 1 (2.13%) |
| Bacteria+Fungus | 10 (21.28%) |
| Bacteria+Virus | 5 (10.64%) |
| Bacteria+Mycoplasma/Chlamydia | 2 (4.26%) |
| Virus+Mycoplasma/Chlamydia | 1 (2.13%) |
| Bacteria+Fungus+Virus | 1 (2.13%) |
| Bacteria+Fungus+Virus+Mycoplasma/Chlamydia | 1 (2.13%) |

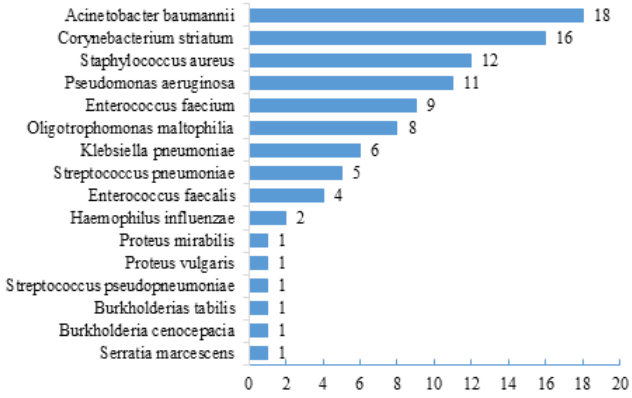


**Figure 1.** Bacterial species distribution of 47 NGS-positive patients.

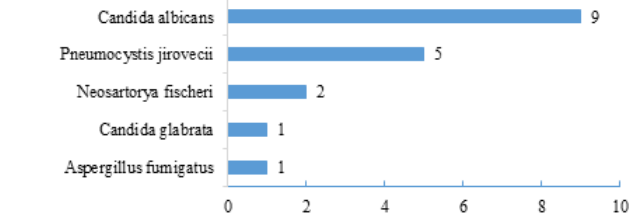


**Figure 2.** Fungal species distribution in 47 NGS-positive patients.

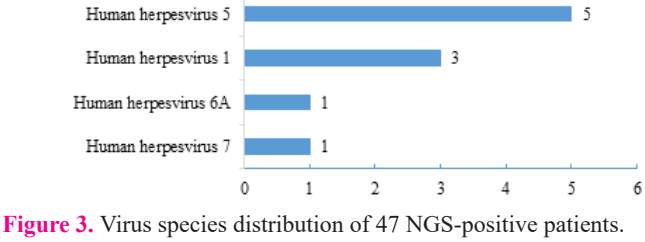


**Figure 3.** Virus species distribution of 47 NGS-positive patients.

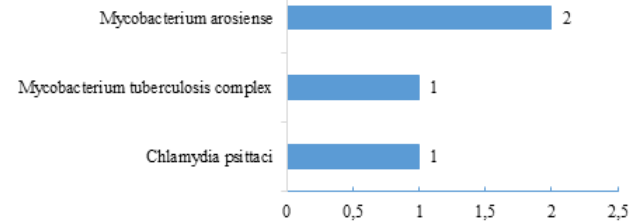


**Figure 4.** Distribution of Mycoplasma/chlamydia species in 47 NGS positive patients.

## Discussion

Infectious diseases are one of the most common diseases in the emergency department. The disease involves

various systems, such as central nervous system infection, respiratory system infection, digestive system infection, etc. If the infection is not effectively controlled, the disease may further progress to severe infection and life-threatening. Therefore, the etiological diagnosis of infected patients is the most critical link in the diagnosis and treatment of infectious diseases. However, patients with emergency infections are often complicated with multiple pathogens at the same time and the pathogens can not be diagnosed, resulting in unsatisfactory treatment effects and delayed best treatment time.

At present, the main method for clinical diagnosis of pathogens is traditional microbial culture. Although the positive results detected by this method have clear significance, it also has limitations, such as a low positive rate, a long culture period, and limited accuracy (10). According to the results of this study, among 52 patients with emergency infection, only 13 patients (25%) detected pathogens by the traditional microbial culture detection method. However, NGS technology can directly extract the nucleic acid of samples for sequencing without microbial culture, determine the pathogen within 24-36 hours, and simultaneously detect bacteria, fungi, viruses, mycoplasma, parasites, etc, with the characteristics of wide coverage (11-13). Zhang et al. (14) studied patients with bacterial meningitis and found that the sensitivity and specificity of NGS in the diagnosis of the disease were 70.3% and 93.9%, respectively, and the positive predictive value and negative predictive value were as high as 81.4% and 91.3%. Takeuchi et al. (15) also pointed out that NGS can be used for the diagnosis and detection of pathogens in bronchoalveolar lavage fluid of patients with respiratory tract infections. A number of studies have shown that NGS technology can detect rare pathogens that cannot be detected by conventional methods, and has guiding significance for the use of anti-infective drugs in patients with severe pulmonary infection, which can benefit patients in clinical treatment (16,17). Among the 52 infected patients in this study, the positive detection rate of NGS by collecting bronchoalveolar lavage fluid was 90.4%. A total of 97 strains of 16 kinds of bacteria were detected. There were 5 species of fungi (18 strains). There were 5 kinds of viruses (10 strains). There were 3 species of Mycoplasma and Chlamydia, with a total of 4 strains. NGS technology has certain advantages in the diagnosis of bacteria, fungi, viruses and other pathogens. This result is consistent with the results of LIU et al (18). LIU (18) used next-generation sequencing of bronchoalveolar lavage fluid to study 32 children with severe pneumonia, and 32 cases were detected with pathogens, with a positive rate of 94.1%. Compared with traditional microbial culture detection methods, NGS significantly improved the accuracy of pathogens. Clinically, NGS reports can be interpreted according to the diagnostic results of NGS, and targeted antibiotics can be selected for treatment according to the pathogen, so as to improve the treatment effect of patients.

Infectious diseases are more likely to occur in immunocompromised people and are prone to co-infection with multiple pathogens. If the early diagnosis and anti-infection treatment are not timely, the patient is prone to respiratory failure, which is life-threatening. NGS technology can quickly, accurately and comprehensively determine the gene sequence of microorganisms in specimens, especially some special and rare pathogens. Clinically, NGS results can be used to comprehensively evaluate the condition of patients, select the best treatment plan, and improve the prognosis of patients (19). In addition, Saeb (20) pointed out that NGS testing can not only diagnose pathogens but also investigate the epidemiology of infectious diseases and find resistance genes, so as to avoid empirical treatment failure. According to the expert consensus on the application of NGS in severe infections (21), it is recommended to perform NGS on suspected infection sites in addition to traditional laboratory tests and microbiological tests for patients with clinical infection who are critically ill, critically ill, or immunocompromised. At the same time, as an invasive examination method, bronchoalveolar lavage fluid is not suitable for some patients who are severe or intolerant to bronchoscopy. Therefore, for patients who do not tolerate bronchoscopy, peripheral blood NGS testing may be considered instead. By studying the diagnostic value of peripheral blood NGS for pneumocystis pneumonia, Gu Peng et al. (22) showed that peripheral blood NGS also has high sensitivity and specificity, and its accuracy is significantly better than that of traditional methods.

## Advantages and limitations

Numerous studies have shown that NGS can quickly and accurately detect and identify microbial pathogens (23-25). However, NGS also has limitations. First of all, although NGS has a good detection effect in pathogen detection, there is no standard interpretation of NGS reports at present, and due to the differences in personal experience and knowledge level of doctors, the interpretation of the results may be different. Therefore, clinical analysis should be based on the patient's condition and other clinical data. Second, depending on the location of the lesion, the obtained specimen may not contain valuable pathogenic DNA, leading to false negative results. Finally, due to the high cost of NGS testing, the patient's financial burden is greater than that of traditional testing methods, which limits the promotion of this technology. With the development of technology, the cost of NGS technology is expected to be reduced, which will further promote its clinical application.

## Conclusion

In conclusion, the detection rate of pathogenic microorganisms in the NGS of BALF is higher, which can improve the accuracy of pathogenic diagnosis in infected patients. NGS is more rapid, comprehensive and accurate than traditional microbial culture methods. NGS has a high application value in the clinical diagnosis of infection. The use of NGS is helpful for the targeted use of antibiotics, reducing the occurrence of drug resistance and shortening the course of the disease.

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