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*Original Article*

# Application value of next generation sequencing technology for pathogen detection in patients with pulmonary infection and lung cancer

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**Abstract:** This study aimed to evaluate the clinical value of next-generation sequencing (NGS) in diagnosing pulmonary infection pathogens among lung cancer patients. A total of 350 lung cancer patients with pulmonary infection were retrospectively enrolled from 2022 to 2024. Sputum samples were examined by targeted next generation sequencing (tNGS) and CMT (conventional microbiological tests). The diagnostic efficacy of these two methods was compared. The tNGS positive detection rate reached 90.00%, significantly higher than 70.86% of routine tests ($P<0.05$). The top common pathogens included Mycobacterium tuberculosis, Candida albicans and Pseudomonas aeruginosa. tNGS presented shorter detection time and a markedly higher detection rate of mixed infections (50.86% vs. 18.57%, $P<0.001$). Patients with abnormal CRP or PCT levels showed distinct tNGS positive rates. The AUC of tNGS was 0.784, indicating better diagnostic accuracy than that of CMT. In conclusion, tNGS featured high positive rate, rapid detection and prominent advantages in identifying mixed infections, which is suitable for clinical etiological detection of pulmonary infection in lung cancer patients.

**Keywords:** Pulmonary infection, lung cancer, next generation sequencing, conventional microbiological test, pathogenic diagnosis

## Introduction

More than 17 million mortalities annually have been caused by infectious diseases, accounting for about 25% of total deaths worldwide [1]. It was reported that pulmonary infection was the main cause of mortality among these infectious diseases [2]. The accurate detection of pathogenic microorganisms from a respiratory tract infection is critical for precise diagnosis of infectious diseases and determination of treatment strategy [3, 4]. If the pathogens are not clearly identified in patients with pulmonary infection, it would usually make difficult targeted drug treatment, which in turn delayed the improvement of these diseases and resulted in the worsening of symptoms, or even death. Therefore, it is especially important to accurately identify the pathogen in patients with pulmonary infection.

The determination of pathogens in patients with pulmonary infection has long relied on the traditional methods such as microbial culture, polymerase chain reaction, antigen or antibody immunological methods and etc. However, the microbial culture was considered to be time-consuming and have a low detection rate. Some types of microorganisms including anaerobes, fastidious bacteria and viruses were difficult culture [5]. Although the method of antigen detection is simple and has high specificity, but the sensitivity is low. Moreover, the negative results of antigen detection could not still eliminate the pathogen infection [6]. The results of antibody immunological methods were affected by the duration of infection caused by pathogenic microorganisms [7]. It would easily lead to false negative results in patients with the immune deficiency. In additional, the detection of pathogens by antigen and/or anti-

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body immunology and polymerase chain reaction (PCR) must rely on the genetic sequence of known pathogens, and these methods could not detect the pathogens with unknown genetic sequence [8].

In recent years, the next generation sequencing (NGS) technology has undergone rapid development, enabling the rapid generation of high-throughput sequencing data. Compared with metagenomic next generation sequencing, targeted next generation sequencing (tNGS) exhibits higher detection sensitivity and lower costs, while simultaneously covering both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) detection. Additionally, it effectively mitigates the interference from host nucleic acids, thereby demonstrating greater advantages in the detection of pathogenic microorganisms with low concentrations. A retrospective study involving 35 children with traumatic brain injury or suspected postoperative intracranial infection revealed that 18 cases were confirmed through NGS detection. This finding indicates that tNGS possesses high accuracy in the diagnosis of central nervous system infections in pediatric neurosurgical patients [9]. However, clinical literature focusing on the application of tNGS detection technology in patients with lung cancer and pulmonary infection is relatively scarce, and most of the existing studies are published in the form of case reports or small-sample retrospective studies.

In the present study, we aimed to explore the accuracy and sensitivity of tNGS in pathogen detection by comparing it with traditional pathogen detection methods. The results of this study are expected to further verify the high sensitivity and accuracy of tNGS in pathogen detection, and provide a theoretical and experimental basis for evaluating its diagnostic value in pulmonary infection among patients with lung cancer.

## Material and methods

### *Subjects*

This study adopted a retrospective analysis approach to explore the diagnostic value of tNGS in patients with lung cancer and pulmonary infection. Eligible participants were patients admitted to Public Health Clinical Center Affiliated to Shandong University between December 2022 and September 2024, who were diagnosed with both pulmonary infection and lung cancer. All enrolled patients underwent both targeted next-generation sequencing (NGS, Illumina, San Diego, CA, USA) and CMT (conventional microbiological tests), with a comprehensive review conducted on their clinical data. This research project was approved by the Ethics Committee of Public Health Clinical Center Affiliated to Shandong University (Approval No.: GWLCZXEC-SOP-K-2025-151).

The inclusion criteria were defined as follows: (1) Confirmed diagnosis of pulmonary infection and lung cancer in accordance with the European Guidelines for the Diagnosis and Treatment of Adult Pulmonary Infection [10], The determination of pulmonary infections was based on clinical criteria and etiological results. clinical criteria was as follows: evidence of new or expanding pulmonary infiltration on chest films, together with two or more of the following clinical signs: abnormal body temperature (>38°C or <36°C), altered peripheral white blood cell count (>12,000/$mm^3$ or <4,000/$mm^3$), and purulent respiratory discharge. The etiological results judgment relied on an integrated analysis of clinical symptoms, seven-day microbial detection results including culture and nucleic acid testing of bronchoalveolar lavage fluid, and the efficacy of antibiotics against the suspected pathogens. The final diagnosis of patients serves as the gold standard for follow comparison; (2) Age ranging from 18 to 75 years old; (3) Completed both tNGS and conventional microbiological tests; (4) Complete clinical data available for review.

The exclusion criteria were specified as follows: (1) Refusal to provide samples for relevant testing; (2) Sputum samples failing to meet the quality requirements for tNGS detection; (3) Incomplete clinical data that could not support the research analysis; (4) Concurrent participation in other clinical research projects that might affect the study results.

### *Sample collection*

Qualified sputum specimens were collected in strict compliance with standard operating protocols. Morning oral hygiene was provided to all patients, who were then instructed to take deep breaths and forcefully expel sputum from the lower respiratory tract. All sputum samples were promptly placed into sterile contain-

ers after collection. For patients unable to produce sputum by voluntary coughing, a disposable suction catheter was used to collect sputum with the aid of negative pressure. Alternative specimen collection approaches, including sputum induction and tracheal aspiration, were also applied when needed. Approximately 3 milliliters (mL) of sputum was collected per patient and stored at -20°C within 48 hours for subsequent tNGS analysis. Furthermore, the residual sputum specimens were retained for conventional microbiological detection.

*Conventional microbiological tests*

Based on prior research findings, sputum specimens were split into multiple aliquots, each assigned to distinct detection purposes. One aliquot was utilized for fungal and bacterial smear preparation as well as culture experiments. Another aliquot was submitted for the detection of Fungal-D glucan and Aspergillus galactosidase. A third aliquot was designated for the detection of 9 antibodies targeting common respiratory pathogens, including Legionella pneumophila, Mycoplasma pneumoniae, Chlamydia pneumoniae, respiratory syncytial virus, adenovirus, Rickettsia, influenza virus, and parainfluenza virus. Additionally, DNA sequence detections were performed for a variety of pathogens, such as Streptococcus pneumoniae, Staphylococcus aureus, methicillin-resistant Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Stenotrophomonas maltophilia, Haemophilus influenzae, and Mycobacterium tuberculosis. Real-time PCR was also carried out to detect cytomegalovirus (CMV).

*Targeted next-generation sequencing*

One aliquot of sputum sample was mixed with sputum digestive buffer in a 1.5 mL centrifuge tube, followed by homogenization for 30 seconds using a vortex mixer. Subsequently, 500 μL of the homogenized mixture was subjected to DNA extraction with DNA Miniprep Kits in strict accordance with the manufacturer's protocol.

The DNA library for targeted sequencing was constructed via PCR amplification using Respiratory Pathogen Detection Kits. For complementary deoxyribonucleic (cDNA) reverse transcription, nucleic acid was first obtained, and the reaction was performed with one cycle under the following conditions: 25°C for 5 minutes, 37°C for 45 minutes, 85°C for 5 seconds, and then maintained at 4°C.

Target region enrichment of the cDNA products was carried out through PCR with the following cycling parameters: initial denaturation at 95°C for 3 minutes, followed by 28 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds; a final extension step was conducted at 72°C for 1 minute, and the reaction was then held at 4°C.

After transient centrifugation of the PCR products, purification magnetic beads were used to purify the PCR products containing the target regions. These purified PCR products were then subjected to library amplification; following another round of transient centrifugation, the library was purified using magnetic beads again.

The concentration of the constructed library was quantified using a Qubit 4.0 fluorometer. Thereafter, the diluted and denatured library was further sequenced on the KingMed MiniSeq Diagnostic-China (KM MiniseqDx-CN) platform with Illumina MiSeq Reagent Nano Kits. Sequencing data were analyzed using a data management and analysis system.

Raw data were initially processed by adapter identification, and quality control was performed based on the following criteria: paired-end reads with a length exceeding 60 bp and a Q30 value of ≥75% were retained. Primer identification was then conducted, and only reads with correct alignment at both ends were kept. The final qualified reads were aligned against a pathogen database for pathogen identification, where the database was compiled from multiple sources including the GenBank database, Nucleotide database, and RefSeq database of the National Center for Biotechnology Information (NCBI).

*Statistical analysis*

All collected data were analyzed using statistical product and service solutions (SPSS) software (version 23.0, IBM Corporation, USA). Measurement data were presented as Mean ±

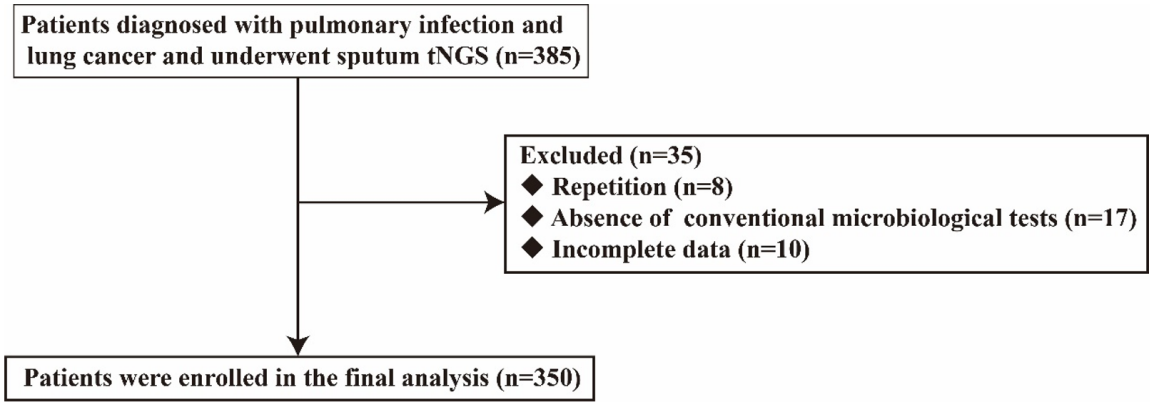


**Figure 1.** The flow diagram for the selection of patients in this study. Note: tNGS: Targeted next generation sequencing.

**Table 1.** Clinical characteristics of patients from this study

| Parameters | | Values |
|---|---|---|
| Gender (cases) | Male | 220 |
| | Female | 130 |
| Age (years) | | 64.9±7.8 |
| Expectoration (cases) | | 196 |
| Heart disease | | 16 |
| Diabetes mellitus | | 34 |
| Hypertension | | 51 |
| Cough | | 181 |
| Fever | | 87 |
| White blood cell count ($\times 10^9$/L) | | 14.58 |
| PCT (ng/mL) | | 0.54 |
| CRP (mg/L) | | 37.68 |

Note: PCT: Procalcitonin; CRP: C-reactive protein.

standard deviation (SD), while enumeration data were expressed as case number and percentage [n (%)]. The comparison of pathogen detection rates was performed by the McNemar's test. Kappa consistency analysis was adopted to evaluate the diagnostic performance of these two pathogen detection assays. Receiver operating characteristic (ROC) curve analysis was implemented, and the area under the ROC curve (AUC) was calculated to assess the diagnostic accuracy of tNGS and conventional microbiological tests. A *P* value less than 0.05 was considered to indicate a statistically significant difference.

## Results

*General data*

This study initially screened 385 lung cancer patients diagnosed with lung infection and receiving tNGS. 35 participants were excluded from the cohort due to unavailable routine microbiological findings, duplicated medical records and incomplete clinical data. In total, 350 eligible participants that met the inclusion criteria were selected for subsequent analyses, as seen in **Figure 1**.

Baseline characteristics of the included patients were listed in **Table 1**. The mean age of the study population was 64.9±7.8 years, with males making up 62.86% (220 cases). Regarding chronic underlying illnesses, the incidence of heart disease, diabetes and hypertension was 4.57%, 9.71% and 14.57%, respectively. Cough, sputum production and fever were the predominant clinical presentations, with detection rates of 51.71%, 56.00% and 24.86%. Laboratory tests revealed a mean white blood cell count of $14.58\times10^9$/L; meanwhile, the average levels of C-reactive protein (CRP) and procalcitonin (PCT) were 37.68 mg/L and 0.54 ng/mL.

*Pathogen detection results of tNGS*

As illustrated in **Figure 2**, the overall microbial detection rate of tNGS among the 350 enrolled patients reached 90% (315/350). For bacterial pathogens, the top three were Mycobacterium tuberculosis (32 cases), Pseudomonas aeruginosa (28 cases), and Klebsiella pneumoniae (26 cases). Among the identified pathogens, Candida albicans (29 cases) and Aspergillus (26 cases) were the most prevalent fungal species. In terms of viral pathogens, the top three were human herpesvirus type 4 (33 cases), human herpesvirus type 7 (19 cases), and rhinovirus (17 cases). Additionally, the sub-

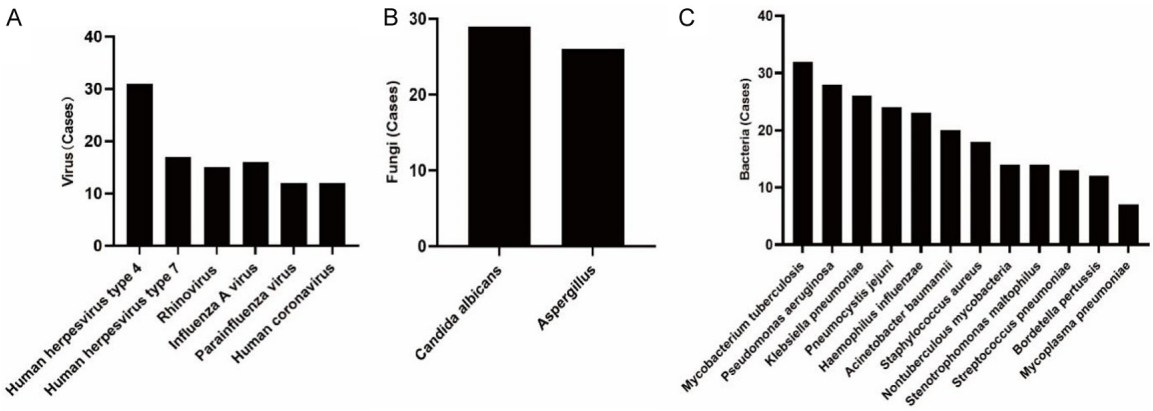


**Figure 2.** The distributive results of potential pathogens. A: Virus; B: Fungi; C: Bacteria.

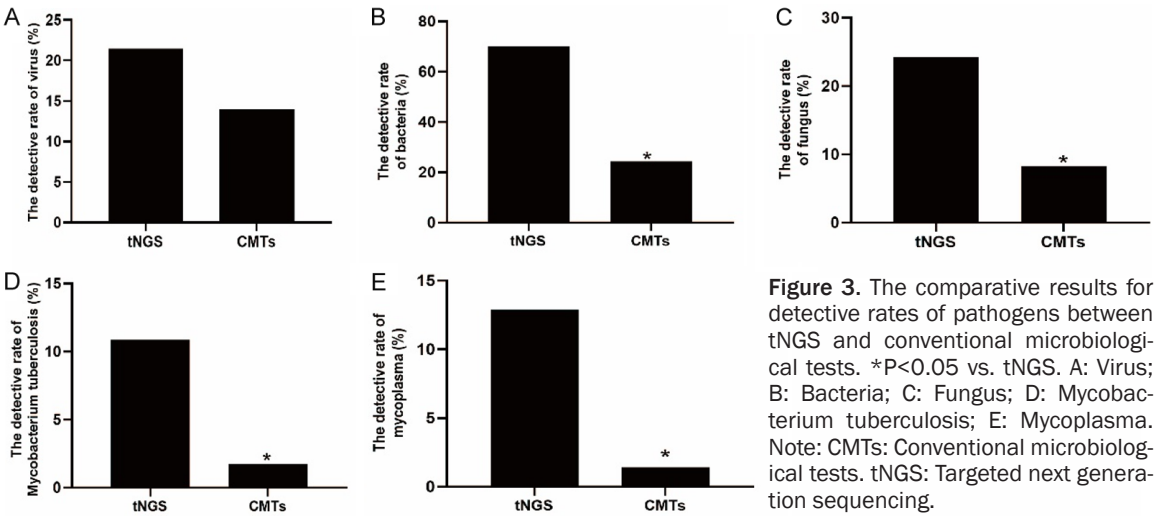


**Figure 3.** The comparative results for detective rates of pathogens between tNGS and conventional microbiological tests. *P<0.05 vs. tNGS. A: Virus; B: Bacteria; C: Fungus; D: Mycobacterium tuberculosis; E: Mycoplasma. Note: CMTs: Conventional microbiological tests. tNGS: Targeted next generation sequencing.

**Table 2.** The comparative results between tNGS and conventional mircrobiological tests

| tNGS tests (cases) | Conventional mircrobiological tests (cases) | | Total (cases) |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 238 | 77 | 315 |
| Negative | 10 | 25 | 35 |
| Total (cases) | 248 | 102 | 350 |

Note: tNGS: Targeted next generation sequencing.

sequent seven most common pathogens included Pneumocystis jirovecii (24 cases), Haemophilus influenzae (23 cases), Acinetobacter baumannii (20 cases), Staphylococcus aureus (18 cases), nontuberculous mycobacteria (14 cases), Stenotrophomonas maltophilia (14 cases), and Streptococcus pneumoniae (13 cases).

Furthermore, the detection rates of fungi, bacteria, viruses, Mycobacterium tuberculosis and mycoplasma via tNGS were 24.28%, 70.0%, 21.43%, 10.86% and 12.86%, respectively. Compared with conventional microbiological tests, tNGS exhibited significantly higher detection rates for all the aforementioned pathogen types, with statistically significant differences observed between the two groups (all P<0.05), as presented in **Figure 3**.

*Comparison of positive results between tNGS and CMT*

As seen in **Table 2**, of the 350 patients with pulmonary infection and lung cancer, 238 cases were identified as double-positive, and 25 cases as double-negative, in both tNGS and CMT. Notably, 77 cases were positive exclusively for tNGS, while only 10 cases tested positive

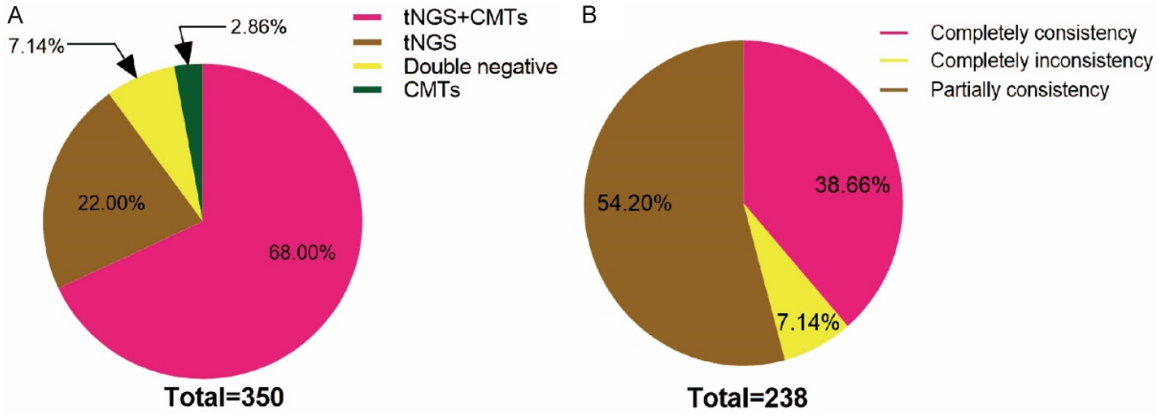


**Figure 4.** Consistency of pathogen detection results between CMTs and tNGS. Note: CMTs: Conventional microbiological tests. tNGS: Targeted next generation sequencing.

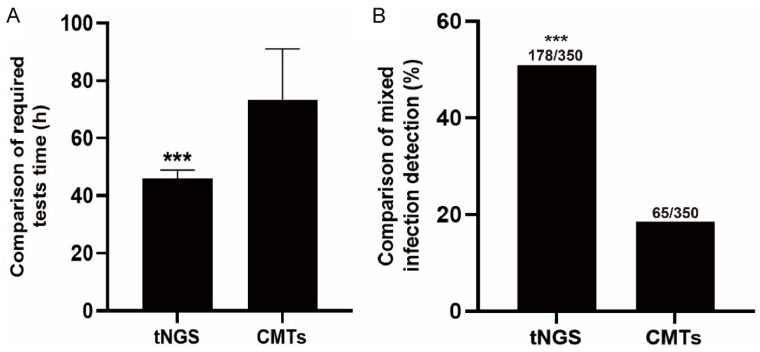


**Figure 5.** Comparative results for the required test time and the mixed infection detection between tNGS and CMTs. A: The required test time. B: The detective rate of mixed infection. ***P<0.001 vs. CMTs. Note: CMTs: Conventional microbiological tests. tNGS: Targeted next generation sequencing.

solely for CMT. Among the 238 double-positive patients, 92 cases demonstrated full consistency between tNGS and CMT, 17 cases exhibited complete inconsistency and 129 cases presented partial consistency, as depicted in **Figure 4**. The positive rate of CMT was 70.86% (248/350), which was significantly lower than that of tNGS. Furthermore, the Kappa coefficient was 0.254, indicating poor consistency between tNGS and CMT.

*Comparison of the mixed infection detection and the test time between tNGS and CMT*

As illustrated in **Figure 5**, the detection rate of mixed infections using tNGS was 50.86%, which was equivalent to 178 out of the 350 enrolled patients. This detection rate was significantly higher than that of CMT, which only reached 18.57% (65 out of 350 patients), and the difference between the two methods was statistically significant (P< 0.001). Furthermore, the average test time for tNGS was 45.84±3.15 h, while CMT required an average of 73.34± 17.68 h to complete, with a statistically significant difference observed between the two detection approaches.

*Comparison of the positive detection for tNGS between patients with different characteristics*

As illustrated in **Table 3**, these analysis findings indicated that significant differences existed in clinical parameters including CRP and PCT between the tNGS-positive and tNGS-negative study groups (all P<0.05). No statistically remarkable differences were noted in other clinical attributes among the two cohorts.

*Comparison of diagnostic values of tNGS and CMT*

The ROC curve analysis was employed to assess the diagnostic performance of tNGS and CMT. The value of AUC for CMT was 0.652 (95% confidence interval [CI]: 0.531-0.798), whereas the AUC for tNGS was 0.784 (95% CI: 0.675-0.902). Notably, the AUC of tNGS was significantly higher than that of CMT, with a statistically significant difference observed between the two approaches (P<0.05), as presented in **Figure 6**.

**Table 3.** The comparative results between true positive tNGS and true negative tNGS in different patients

| Parameters | | Positive tNGS (Cases) | Negative tNGS (Cases) | $t/\chi^2$ | P |
|---|---|---|---|---|---|
| Gender (cases) | Male | 154 | 14 | 0.743 | 0.389 |
| | Female | 84 | 11 | | |
| Age (years) | ≥60 | 141 | 16 | 0.213 | 0.645 |
| | <60 | 97 | 9 | | |
| Expectoration (cases) | Yes | 179 | 21 | 0.960 | 0.327 |
| | No | 59 | 4 | | |
| Cough | Yes | 167 | 15 | 1.097 | 0.295 |
| | No | 71 | 10 | | |
| Fever | Yes | 155 | 17 | 0.083 | 0.774 |
| | No | 83 | 8 | | |
| Diabetes mellitus | Yes | 24 | 3 | 0.090 | 0.764 |
| | No | 214 | 22 | | |
| Hypertension | Yes | 35 | 4 | 0.030 | 0.863 |
| | No | 203 | 21 | | |
| Heart disease | Yes | 17 | 2 | 0.025 | 0.875 |
| | No | 221 | 23 | | |
| White blood cell counts | Normal | 8 | 1 | 0.028 | 0.867 |
| | Abnormal | 230 | 24 | | |
| CRP (mg/L) | Normal | 4 | 2 | 4.053 | 0.044 |
| | Abnormal | 234 | 23 | | |
| PCT (ng/mL) | Normal | 2 | 2 | 7.743 | 0.005 |
| | Abnormal | 236 | 23 | | |

Note: tNGS: Targeted next generation sequencing; PCT: Procalcitonin; CRP: C-reactive protein.

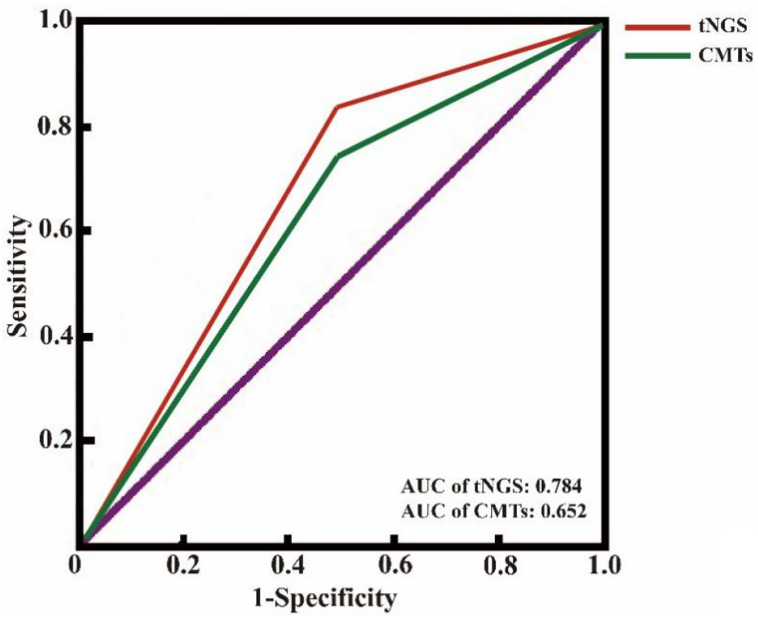


**Figure 6.** ROC curves for the diagnostic methods of CMTs and tNGS. CMTs: Conventional microbiological tests; tNGS: Targeted next generation sequencing; AUC: Area under the receiver operating characteristic curve; ROC: Receiver operating characteristic.

## Discussion

As a viable alternative to traditional microbiological assays, targeted next-generation sequencing (tNGS) has been well established for pathogen identification and the clinical etiological diagnosis of respiratory tract infections, greatly advancing the diagnostic system for infectious diseases. Accumulated clinical evidence has demonstrated that tNGS delivers superior sensitivity in detecting bacterial pathogens associated with pulmonary infections compared with conventional microbiological tests [11]. Distinct from multiplex PCR that merely amplifies target genes of suspected pathogens, tNGS integrates second-gener-

ation sequencing techniques to generate high-throughput biological information comprehensively [12]. In routine clinical settings, tNGS can serve as an effective adjunct diagnostic tool to complement conventional microbiological tests. Compared with metagenomic sequencing, this novel approach achieves higher diagnostic sensitivity at a lower economic cost [13]. It has also been documented that conventional NGS strategies depend heavily on predicted microbial spectra, thereby limiting their capacity to identify previously unknown pathogens [14]. Multiple clinical investigations have validated the promising clinical value of NGS in etiological diagnosis of infectious diseases [15]. In the present research, we conducted a comparative analysis focusing on the clinical performance of sequencing-based assays for pulmonary infection diagnosis. Prior studies have extensively explored the utility of targeted NGS in oncological detection [16]. Building on these findings, the current study was designed to systematically evaluate the clinical efficacy of NGS in patients with pulmonary infections, and further compare its diagnostic performance with that of routine microbiological examinations. Additionally, a targeted NGS panel covering 350 pathogens was adopted to assess its detection efficiency in clinical sputum specimens. Among enrolled patients, NGS identified a broader spectrum of potential pathogens, covering prevalent and clinically significant respiratory microorganisms, including viruses, bacteria, fungi, and atypical pathogens. Relative to conventional microbiological tests, NGS significantly improved the pathogen detection yield for lower respiratory tract infections and yielded an elevated positive rate, with 90.0% versus 70.82%, respectively. These findings indicated that numerous patients might have undetected causative pathogens that failed to be identified by routine microbial testing. The above results were consistent with the conclusions reported by Pham et al. [17]. Furthermore, a total of 238 patients presented positive findings in both tNGS and conventional microbiological tests, with an overall full or partial concordance rate of 92.86% between the two detection strategies. This finding indicates the robust clinical application value of tNGS in such patient cohorts, which is consistent with the findings previously reported by Yang et al. [18]. In terms of mixed infections, the pathogenic spectrum of pulmonary infectious diseases is generally complex and heterogeneous, and mixed infection is clinically prevalent in this condition. Traditional microbiological techniques are limited in the simultaneous detection of multiple pathogens within a single clinical specimen. In contrast, NGS exhibits distinct superiority and plays a critical role in the accurate identification of mixed infections. Previous research has verified that targeted sequencing of pathogenic genomes in mixed samples exerts no obvious influence on genome coverage. Notably, the genome coverage of two viral pathogens in two clinical samples reached 95%-100% in a prior study [19]. In the present study, the positive detection rate of mixed infections via NGS was calculated to be 50.86%, which was markedly higher than that of conventional microbiological tests. Consistently, Chung et al. also demonstrated that NGS possesses prominent advantages over conventional microbiological tests in the identification of mixed infectious diseases [20].

Accumulating evidence has confirmed that potentially pathogenic microorganisms ubiquitously colonize the respiratory tract, as this anatomical site is not completely sterile [21]. Sputum samples collected from the lower respiratory tract via non-invasive or minimally invasive approaches are highly susceptible to contamination by indigenous flora of the upper respiratory tract in clinical patients [22]. Accumulating research has also verified that several pathogenic microbes belong to the commensal respiratory microflora [23], thereby complicating the interpretation and clinical analysis of sputum-based tNGS results. Several studies have indicated that relying solely on sputum specimens to confirm a certain microorganism as the causative pathogen of infection is clinically impractical [24]. Distinguishing between pathogenic infection and commensal colonization of such microorganisms generally depends on comprehensive evaluation, including NGS findings, patients' clinical manifestations, and clinicians' professional experience. Under such circumstances, tNGS exhibits superior performance in identifying pathogenic information compared with traditional microbiological examinations. Given its capacity to simultaneously detect a wide range of pathogens in a single test, tNGS substantially improves diagnostic efficiency. Furthermore, this technology facilitates differential diagnosis and

accurate identification of mixed infections in clinical practice.

Several limitations of the present study should be acknowledged. Firstly, the sample size was relatively small, which might compromise the overall efficacy and detection accuracy of tNGS. Secondly, the sensitivity and specificity of tNGS could not be calculated owing to the absence of standardized reference criteria for etiological diagnosis, restricting the comprehensive evaluation of its diagnostic value. Thirdly, it remained challenging to differentiate microbial infection from commensal colonization, especially for upper respiratory tract colonization contamination in sputum specimens. This issue arose from the lack of unified pathogenic diagnostic criteria for current tNGS detection. Fourthly, a positive tNGS result in this research was simply defined as the detection of pathogen-related reads. Fifthly, it fails to distinguish whether tNGS detects live bacteria, dead bacteria, or nucleic acid fragments, resulting in insufficient clinical explanatory power.

To summarize, the current research elucidates the clinical utility of sputum-based NGS in etiological testing for patients with pulmonary infections. Data from this investigation verified that NGS outperforms traditional microbial detection methods in both positive diagnostic yield and detection efficiency. This approach enables early recognition of causative microorganisms, including low-colonization strains and highly virulent pathogens that are difficult to identify via routine examinations. Furthermore, targeted NGS delivers unique strengths for detecting complex mixed infections. Collectively, our observations solidify the clinical prospects of targeted NGS as a reliable auxiliary modality for etiological analysis and comprehensive diagnosis of lower respiratory tract infectious diseases.

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## Disclosure of conflict of interest

None.

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