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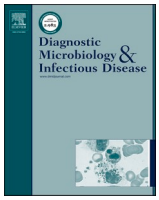

Original Article

# Comparison of targeted next-generation sequencing and traditional microbial culture in the diagnosis of pulmonary infections

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ABSTRACT

This study investigated the diagnostic potential of targeted next-generation sequencing (tNGS) for pulmonary infections. The positivity rate of tNGS was significantly higher than that of traditional microbial culture (92.6 % vs 25.2 %, $\chi 2 = 378.272$, $P < 0.001$). The proportion of two or more species of pathogens detected using tNGS exceeded that detected using microbial culture ($\chi 2 = 337.283$, $P < 0.001$). There were inconsistencies between the results of the tNGS antibiotic resistance gene and the drug susceptibility test resistance phenotype. The tNGS technique demonstrates rapid and effective capabilities in identifying bacteria, fungi, viruses, and specific pathogens, with a detection sensitivity that surpasses that of conventional culture methodologies. Microbial drug resistance genotypes detected by tNGS cannot accurately predict drug resistance phenotypes and require further improvement or integration with traditional microbial culture to establish a foundation for effective clinical treatment.

## 1. Introduction

Pulmonary infection is prevalent worldwide, causing high morbidity, mortality, and significant clinical harm, particularly in the elderly and immunocompromised populations [1–3]. When the pathogen causing a respiratory tract infection remains to be identified, it becomes challenging to develop targeted drug treatments. This, in turn, delays the patient's recovery and can lead to symptom exacerbation or even death [4]. In such cases, identifying pathogens linked to lower respiratory tract infections and developing precise treatments becomes important. However, in China, the etiological diagnosis for nearly half of patients with pulmonary infections remains unclear [5]. Identification of pathogens in the lower respiratory tract has traditionally relied on microbial culture, antigen, and antibody immunological methods. However, these methods are time-consuming and have low detection rates. Relevant literature shows that comprehensive conventional methods do not identify causative pathogens in up to 60 % of cases [6]. Additionally, they often fail to detect viruses and fastidious bacteria such as Legionella, Mycoplasma, and Chlamydia. Common specimens used for pulmonary infections include respiratory specimens such as bronchoalveolar lavage fluid and sputum [7–9]. While rapid diagnosis of pulmonary infection is important for prompt disease management and improved outcomes, accurately detecting and identifying pathogens remain a challenge.

In recent years, metagenomic next-generation sequencing (mNGS) technology has provided a promising means for pathogen-specific diagnosis, updating the diagnostic strategy for lower respiratory tract infections and making it a formidable tool in microbial molecular ecology research [10–12]. Compared to mNGS, targeted next-generation sequencing (tNGS) is more sensitive to genotyping, has fewer read segments, and offers the advantages of high sensitivity, efficiency, and economy [13–16]. tNGS has proven invaluable in identifying tens to hundreds of known pathogenic microorganisms and their virulence and resistance genes in the samples being analysed [17–19]. Owing to its low

*Abbreviations:* tNGS, targeted next-generation sequencing; mNGS, metagenomic next-generation sequencing; FOX, cefoxitin; OX, oxacillin; IMP, imipenem; MEM, meropenem; AST, antimicrobial susceptibility testing; MPA, mucoid *Pseudomonas aeruginosa*; NMPA, non-mucoid *Pseudomonas aeruginosa*.

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sequencing cost and clear pathogenic spectrum, it has received increasing attention in clinical detection [20–22]. Studies have revealed that tNGS can achieve efficacy comparable to that of mNGS in identifying pneumonia pathogens, at a quarter of the cost [19].

In this study, we conducted a retrospective analysis of patients who underwent both traditional microbial culture and tNGS detection to explore the diagnostic value of tNGS in pulmonary infections and to provide a reference for the clinical application of tNGS.

## 2. Materials and methods

### 2.1. Study participants

Patients with pulmonary infection who were admitted to Xixian People's Hospital between April 2022 and June 2023 and underwent concurrent tNGS detection and traditional culture detection (alveolar lavage fluid culture) were retrospectively included; the final diagnosis was established, and clinical data were complete. Inclusion criteria were as follows: (1) Clinical diagnosis of pulmonary infection with the following diagnostic criteria: 1. Pneumonia-related clinical manifestations: a) recent cough, sputum, or exacerbation of existing respiratory disease symptoms, with or without purulent sputum, chest pain, dyspnoea, and haemoptysis; b) fever; c) signs of lung consolidation and crackles; and d) peripheral blood white blood cell count $> 10\times10^9$/L or $< 4\times10^9$/L. 2. Chest imaging reveals new patchy infiltrating shadows, solid lobar or segmental shadows, ground-glass shadows, or interstitial changes with or without pleural effusion. Comply with any 1 of articles 1 and 2 [23]. (2) Both tNGS detection and traditional microbial culture were performed on the alveolar lavage fluid during hospitalisation. Collection of bronchoalveolar lavage fluid specimens: Patients underwent bronchoscopy under intravenous or local airway anaesthesia [24, 25]. Patients with incomplete clinical data or unclear diagnoses were excluded.

Finally, 404 patients were included, comprising 264 males (65.35 %) and 161 females (34.65 %), aged 10 to 90 years. The median age was 70 (10, 90) years, and the mean was 64.94 ± 15.49 years. Clinical data collection encompassed general information (name, sex, age, underlying disease, and past drug use history), results of tNGS, and other etiological detection methods used during hospitalisation (such as culture, smear, G test, GM test, and immunology test) for the enrolled patients. These data were collected through our hospital's electronic medical record system. Informed consent was waived by the Ethics Committee of the People's Hospital of Xixian due to the retrospective nature of the study. The protocol for this study was approved by the Ethics Committee of the People's Hospital of Xixian (approval number: 2003009) and was conducted in accordance with the principles of the Helsinki Declaration and its subsequent amendments.

### 2.2. Detection methods

1) tNGS detection: Collected clinical specimens were sent to the Golden Field Medical Testing Company for tNGS pathogen identification. Utilising the multi-targeted amplification and high-throughput sequencing technology (tNGS), the microbial nucleic acid sequence in the sample underwent analysis and comparison with the existing microbial nucleic acid sequence in the NCBI database (ftp://ftp.ncbi.nlm.nih.gov/genomes) for microorganism identification. The detection process encompassed nucleic acid extraction, library construction, sequencing, information analysis, and interpretation.
2) Traditional culture: a) Microbial culture method: All samples were inoculated onto Blood agar and Chocolate agar from Zhengzhou Antu Company and placed at 35 °C in a $CO_2$ incubator (Shanghai Likang) for 24–48 h for traditional culture. Microbial identification was conducted using a VITEK 2 Compact System. b) VITEK 2 Compact identification: Pure colonies isolated from the clinical strains were incubated for 18–24 h, and the corresponding identification cards were selected for identification based on colony morphology and Gram staining results.

### 2.3. Statistical analysis

SPSS 25.0 statistical software (IBM Corporation, Armonk, NY, USA) was used for statistical analysis. Counting data were presented as the number of cases and rate (%). The diagnostic efficiency of tNGS and traditional culture was compared using the paired McNemar chi-square test (paired quadruple table). Results with $P < 0.05$ were considered statistically significant.

## 3. Results

### 3.1. Clinical data

The clinical data of 404 patients with severe or refractory pulmonary infections hospitalised at Xixian People's Hospital between April 2022 and June 2023 were retrospectively analysed. Detailed demographic information is presented in Table 1.

### 3.2. Comparison of positivity rate of tNGS detection and traditional culture detection

Among the 404 BALF samples collected, 374 were tNGS-positive, resulting in a positive detection rate of 92.6 %; 102 samples tested positive through traditional microbial culture, yielding a positive detection rate of 25.2 %. The positive rate of tNGS detection was significantly higher than that of traditional culture ($\chi2 = 378.272$, $P < 0.001$) (Table 2).

### 3. 3 tNGS detection in bronchoalveolar lavage fluid and traditional culture detection of pathogen distribution

The tNGS test results of the bronchoalveolar lavage fluid of 374 patients were positive. The pathogenic species detected in these cases consisted of 32 species of bacteria, 24 types of viruses, 14 species of

**Table 1**
General characteristics of patient population.

| Characteristic | Patients, n |
|---|---|
| Gender | |
| Male | 264 |
| Female | 140 |
| Age(years) | |
| Range | 10–90 |
| Average | 64.94 ± 15.49 |
| Infection types | |
| CAP | 17 |
| AECOPD | 102 |
| AEBX | 54 |
| Lung abscess | 9 |
| Tuberculosis | 76 |
| Chronic bronchitis | 7 |
| Bronchial asthma | 8 |
| Comorbidity | |
| COPD | 128 |
| Diabetes | 46 |
| Malignancy or Immunocompromised | 69 |
| Hypertension | 122 |
| Cardiovascular | 50 |
| Chronic liver diseases | 20 |
| Renal diseases | 20 |
| Cerebral infarction | 63 |
| Cerebral haemorrhage | 14 |
| History of surgery and trauma | 93 |

AEXB: acute exacerbation of bronchiectasis; CAP: community-acquired pneumonia; COPD: chronic obstructive pulmonary disease; AECOPD: acute exacerbation chronic obstructive pulmonary disease.

**Table 2**
Comparison of positive rate between tNGS detection and traditional culture detection (n[%]).

| Detection method | Positive | Negative | Total |
|---|---|---|---|
| tNGS detection | 374(92.6 %) | 30(7.4 %) | 404(100 %) |
| Traditional culture detection | 102(25.2 %) | 302(74.8 %) | 404(100 %) |
| χ2 | 378.272 | | |
| P | 0.000 | | |

fungi, and 2 other prokaryotes, amounting to a total of 920 pathogens detected. The pathogen spectrum revealed that *Mycobacterium tuberculosis* complex (64), *Haemophilus influenzae* (59), *Klebsiella pneumoniae* (56), and *Pseudomonas aeruginosa* (51) were the leading bacterial pathogens, whereas Influenza A virus (46), Human gammaherpesvirus (31), and Epstein–Barr virus (28) were the dominating viral pathogens, followed by SARS-CoV-2_Omicron_XBB.1 (25) and Human alphaherpesvirus 1 (23). *Pneumocystis jirovecii* (48), *Aspergillus fumigatus* (37), and *Candida albicans* (30) were the predominant fungal pathogens. Other specific pathogens such as *Mycoplasma pneumoniae* (19) were also frequently detected in this study. For the traditional microbial culture method, among the 102 culture-positive cases, there were 16 species of bacteria and 5 species of fungi, with a total of 117 pathogens detected. The top five pathogens detected by traditional microbial culture were *P. aeruginosa* (34) (17 mucoid and non-mucoid *P. aeruginosa*,

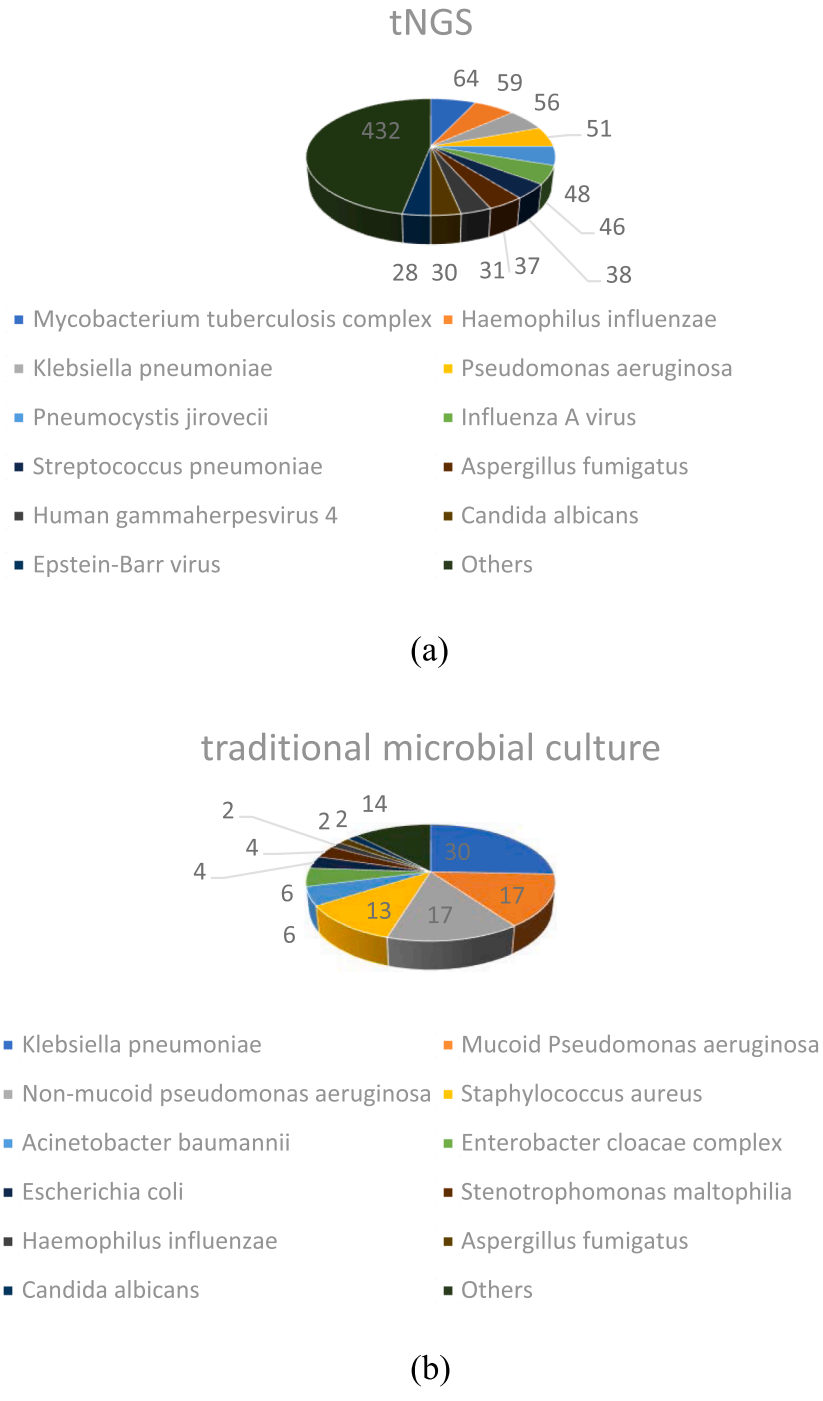


**Fig. 1.** tNGS detection and traditional microbial culture detection of pathogen distribution
**a** tNGS detection of the distribution of pathogenic bacteria. **b** Traditional microbial culture detection of pathogen distribution.

respectively), *K. pneumonia* (30), *Staphylococcus aureus* (13), *Acinetobacter baumannii* (6), and *Enterobacter cloacae* complex (6), which were significantly different from those detected using tNGS. Detailed information on the pathogens detected using tNGS and traditional culture is provided in Appendix Table S1 and Fig. 1.

### 3.4. Comparison of the distribution of tNGS and traditional microbial cultures in alveolar lavage fluid

The detection rates of bacteria, fungi, viruses, and other specific pathogens using the tNGS method were significantly higher than those using the traditional microbial culture method ($P < 0.001$) (Table 3).

### 3.5. The detection rate of mixed infection using tNGS and traditional microbial culture

Two or more species of pathogens were detected by tNGS in 65.1 % (263/404) of cases and by microbial culture in 3.7 % (15/404) of cases. The difference was statistically significant ($\chi2 = 337.283$, $P < 0.001$). Additionally, tNGS detected two or more types of pathogens in 54.0 % (219/404) of cases, while microbial culture detected this in 1 % (4/404) of cases. The difference was also statistically significant ($\chi2 = 286.304$, $P < 0.001$) (Table 4).

### 3.6. Performance of tNGS in antimicrobial resistance prediction

Drug resistance genes were detected in 33 patients using tNGS, among which 28 phenotypes were associated with bacterial drug resistance and 5 (26.32 %) were associated with *M. pneumoniae* drug resistance. Among them, 10 potentially related pathogens were detected during traditional culture. The drug resistance gene 23S rRNA, *A2063G*, was detected in five strains of *M. pneumoniae*. *mecA* was detected in eight specimens; the results of the drug sensitivity tests showed that three strains of *S. aureus* were resistant to cefoxitin (FOX) and oxacillin (OX), while five strains of *S. aureus* were sensitive to FOX and OX. Among the five patients with different phenotypes and drug resistance genes, three were treated with piperacillin/tazobactam, one was treated with cefotaxime and discharged, and another was transferred to a specialised hospital for treatment because of concurrent detection of *M. tuberculosis*. $bla_{NDM}$ was detected in one strain of *Escherichia coli*; the drug sensitivity test results demonstrated that the *E. coli* strains were sensitive to carbapenems. The patient was treated with piperacillin/tazobactam, and their condition improved. *M. tuberculosis* was detected in another patient, who was transferred to a specialised hospital for further treatment. The OXA-48 gene was detected in one strain of *K. pneumoniae*, and the drug sensitivity test results showed that this strain of *K. pneumoniae* was sensitive to carbapenems. The patient was treated with piperacillin/tazobactam, and their condition improved. Simultaneously, a CT scan indicated a space-occupying lung lesion, possibly a tumour. As a result, the patient was transferred to another hospital for further diagnosis. The results of the tNGS antibiotic resistance gene and resistance phenotype of drug antimicrobial susceptibility testing (AST) were inconsistent (Table 5).

**Table 3**
Comparison of tNGS and traditional microbial culture pathogens in alveolar lavage fluid (n [ %]).

| Detection condition | tNGS | Traditional culture | $\chi2$ | *p* |
|---|---|---|---|---|
| bacteria | | | | |
| positive | 273(67.6 %) | 96(23.8 %) | 156.267 | 0.000 |
| negative | 131(32.4 %) | 308(76.2) | | |
| fungus | | | | |
| positive | 144(35.6 %) | 10(2.5 %) | 144.053 | 0.000 |
| negative | 260(64.4 %) | 394(97.5 %) | | |
| virus | | | | |
| positive | 215(53.2 %) | 0 | 292.951 | 0.000 |
| negative | 189(46.8 %) | 404(100 %) | | |
| Specific pathogen | | | | |
| positive | 20 | 0 | 20.508 | 0.000 |
| negative | 384 | 404(100 %) | | |

**Table 4**
Comparison of the detection rate of mixed infection between tNGS and traditional microbial culture (n[%]).

| Pathogen | tNGS | Traditional culture | $\chi2$ | *p* |
|---|---|---|---|---|
| 2 or more species | 263(65.1 %) | 15(3.7 %) | 337.283 | 0.000 |
| 2 or more types | 219(54.2 %) | 4(1.0 %) | 283.647 | 0.000 |

## 4. Discussion

Pulmonary infection is a common clinical respiratory disease with a high incidence and is the main cause of death from infectious diseases in China and globally [2]. Particularly, with the use of immunosuppressants in clinical cancer treatment and organ transplantation, along with the increase in patients with impaired immunity owing to AIDS and other diseases, pulmonary infections have shown an increasing trend. The detection of pathogenic microorganisms is particularly important for patients with serious illness, or the ones with poor empirical treatment outcomes. Early identification of causative pathogens can significantly improve the prognosis of such patients [26]. The positivity rate of traditional detection methods is not high and cannot meet clinical needs, leading to difficulties in diagnosing and treating pulmonary infections [27,28]. tNGS technology can directly detect microorganisms in sputum, bronchoalveolar lavage fluid, and other clinical specimens, and it is an indispensable tool for detecting infectious diseases owing to its advantages of high throughput, rapid detection, and high sensitivity [29–31]. However, whether these methods can replace conventional etiological detection methods remains controversial.

The results of this study showed that the positivity rates of tNGS detection and traditional culture in bronchoalveolar lavage fluid were 92.6 % and 23.9 %, respectively, indicating that the positivity rate of tNGS detection was significantly higher than that with traditional culture. This finding is consistent with previous reports [32]. The top five pathogens detected by tNGS were *M. tuberculosis complex, H. influenzae, K. pneumoniae, P. aeruginosa, and P. jirovecii*. These findings significantly differed from those obtained using traditional microbial cultures. Owing to the limitations of traditional microbial culture detection capabilities, the detection of *M. tuberculosis* and *P. jirovecii* relies on microscopic observation and molecular diagnostic methods [32–34]. tNGS technology provides a rapid and accurate method for obtaining microbial genomic data to identify *M. tuberculosis* and *P. jirovecii*. *H. influenzae* is a human-specific organism that requires both the X and V-factor [35]. It grows on chocolate agar at 35–37° and 5 %– 10 % $CO_2$. The detection of *H. influenzae* using traditional microbial culture methods is prone to false-negative results, particularly during ongoing antibiotic treatment. Compared to other gram-negative bacteria, *H. influenzae* is relatively rare in clinical practice because of its high nutritional requirements *in vitro*. This study demonstrated that tNGS significantly improved the detection rate of fastidious bacteria, including *H. influenzae, Streptococcus pneumoniae*, and *Moraxella catarrhalis*. Additionally, this study revealed that the positive detection rate of tNGS in bronchoalveolar lavage fluid was higher than that in traditional microbial cultures for bacteria and fungi. tNGS detected a wider spectrum of pathogens than the culture method and could detect viruses and atypical pathogens.

In this study, tNGS detected 51 strains of *P. aeruginosa*, whereas the traditional microbial culture method detected 34 strains, including 17 strains each of mucoid and non-mucoid *P. aeruginosa*. This illustrates that tNGS cannot distinguish between mucoid and non-mucoid *P. aeruginosa*. According to China's Surveillance for Bacterial Resistance (CHINET), the isolation rate of *P. aeruginosa* in comprehensive teaching hospitals in 2021 ranked fourth among all isolates and third among isolates from respiratory specimens. In this context, *P. aeruginosa*

**Table 5**
Drug resistance genes detected by tNGS and the corresponding AST by traditional microbial culture.

| Patients | Associated bacteria | MIC (ug/mL) (Traditional microbial culture) | | | | Resistance Genes (tNGS) | | |
|---|---|---|---|---|---|---|---|---|
| | | FOX | OX | IMP | MEM | mecA | blaNDM | OXA-48 |
| P29 | *Escherichia coli* | | | ≤1 | ≤1 | | + | |
| P39 | *Klebsiella pneumoniae* | | | ≤1 | ≤1 | | | + |
| P62 | *Staphylococcus aureus* | ≤4 | ≤0.25 | | | + | | |
| P64 | *Staphylococcus aureus* | ≤4 | ≤0.25 | | | + | | |
| P66 | *Staphylococcus aureus* | ≥8 | ≥4 | | | + | | |
| P67 | *Staphylococcus aureus* | ≥8 | ≥4 | | | + | | |
| P69 | *Staphylococcus aureus* | ≥8 | ≥4 | | | + | | |
| P70 | *Staphylococcus aureus* | ≤4 | ≤0.25 | | | + | | |
| P71 | *Staphylococcus aureus* | ≤4 | ≤0.25 | | | + | | |
| P72 | *Staphylococcus aureus* | ≤4 | ≤0.25 | | | + | | |

**Notes:** FOX: cefoxitin; ox: oxacillin; IMP: Imipenem; MEM: Meropenem.

infection is of particular concern. *P. aeruginosa* is one of the most common pathogenic bacteria of refractory lower respiratory tract infections and is also the most frequent pathogen causing infection in intensive care units, especially ventilator-associated pneumonia. In terms of colony morphology, *P. aeruginosa* can be divided into the mucoid (mucoid *P. aeruginosa*, MPA) and non-mucoid (non-mucoid *P. aeruginosa*, NMPA) types [36]. MPA is easily adsorbed on ducts and mucosal surfaces, forming a biofilm that serves as a diffusion barrier limiting antibiotic access to the bacterial cell. This leads to refractory infection, which is difficult to treat with conventional drugs [37,38]. Therefore, distinguishing between MPA and NMPA is crucial. Informing clinicians about this distinction, indicating it in the report, and providing a clinical basis for the timely and appropriate selection of effective antibiotics is particularly important.

Microbiological culture results usually report the dominant bacteria, usually one or two species only, and cannot present a comprehensive view of the microbes present in mixed infections. In contrast to traditional culture methods, tNGS could detect much more pathogens simultaneously. In our study, data showed that the detection rate of tNGS for two or more species of pathogens was higher than that of the microbial culture method (65.1 % vs. 3.7 %, $p = 0.000$), and the detection rate of tNGS for two or more types of pathogens was also higher than that of the microbial culture method (54.2 % vs. 1 %, $p = 0.000$). This suggests that the detection rate of tNGS for mixed infections is superior to that of traditional microbial cultures. Furthermore, tNGS holds advantages over traditional microbial culture in characterising polymicrobial ecosystems, illustrating the microbial distribution and dominant strains in patients' respiratory tract.

Prior research has demonstrated that tNGS offers specific benefits in the identification of pathogenic microorganisms present at low levels, particularly in the detection of their virulence and/or drug resistance genes [39,40]. We also investigated the consistency between antibiotic-resistance genes and AST results. Four resistance genes were detected, namely 23S rRNA, *A2063G, mecA, bla*$_{NDM}$, and *OXA-48*. Mutation of the A2063G gene locus in 23S rRNA reduces the affinity of macrolide molecules and ribosomes, leading to *M. pneumoniae*'s resistance to macrolides. *mecA*, commonly found in *S. aureus,* encodes a special penicillin-binding protein that reduces its affinity for beta-lactam antibiotics. This leads to resistance to amoxicillin, methicillin, cefepime and other beta-lactam drugs. *bla*$_{NDM}$ encodes a class B metallic beta-lactam enzyme that hydrolyses a broad spectrum of beta-lactam antibiotics, including penicillin, cephalosporins, and carbapenems. OXA-48 expresses oxacillinase and hydrolytic effects on carbapenems, resulting in drug resistance to carbapenems like imipenem and meropenem. In the present study, the resistance genes *mecA, bla*$_{NDM}$, and OXA-48 detected using tNGS were inconsistent with the resistance phenotypes observed in the antimicrobial susceptibility tests. The genotypes and phenotypes of microbial drug resistance vary. Even if tNGS resistance gene test results are positive, it cannot be confirmed that the microbes are resistant to the corresponding drug. These were likely harboured by members of the resident microbiota that did not meet the thresholds for inclusion in the reported data. This further demonstrates the challenges faced in directly testing resistance genes from specimens obtained from non-sterile sites. This is consistent with the findings of previous studies [41]. Therefore, when a drug resistance gene is detected by tNGS, it should be further confirmed in combination with traditional microbial culture, and the results of antimicrobial AST should be given precedence.

A rapid and accurate pathogenic diagnosis is crucial for effective antimicrobial treatment and improved patient outcomes. Through ultra-multiplex PCR amplification and high-throughput sequencing, tNGS can identify dozens to hundreds of known pathogenic microorganisms, including their virulence and resistance genes. This is achieved with a high positivity rate and detection speed. Collectively, we presented the first data on comparing the test efficiency of tNGS and traditional culture methods detection in respiratory samples. The present study demonstrates that the tNGS's diagnostic efficacy surpassed conventional assays. The tNGS test offers a more comprehensive pathogen spectrum for pulmonary infections than traditional culture methods. Research indicates that tNGS is a valuable tool for rapid and accurate pulmonary infection diagnosis and a powerful supplement to the diagnosis process. This helps overcome the limitations of traditional microbial cultures, which have a low positive rate and narrow coverage. However, tNGS test results alone cannot be the gold standard for diagnosing pulmonary infections. Instead, they should complement other indicators in a synergistic diagnosis approach. Additional larger-scale sample studies, detailed and comprehensive detection procedures, and standardised interpretation criteria are needed to optimise its clinical application. tNGS demonstrates high diagnostic accuracy in detecting severe or refractory lung infections. This study shows that the tNGS method is fast and efficient in detecting bacteria (especially fastidious bacteri and *M. tuberculosis*), fungi, viruses, and special pathogens, and its detection sensitivity is superior to traditional culture methods. It also has apparent advantages in detection time, which can compensate for the shortcomings of low positive detection rate, narrow coverage, and long cycle of traditional pathogen microorganism culture. However, tNGS cannot distinguish mucoid *P. aeruginosa*, and the positive results of tNGS drug resistance genes cannot confirm that the microorganism has a certain resistance to the corresponding drug. This needs to be further improved or combined with traditional cultural methods to provide a basis for effective clinical treatment.

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## CRediT authorship contribution statement

**Yongyan Liu:** Writing – original draft, Formal analysis, Data curation. **Ruijie Wang:** Visualization, Investigation, Data curation, Conceptualization. **Youhua Yuan:** Validation, Supervision, Software, Methodology, Data curation. **Chen Zhao:** Validation, Supervision, Data curation. **Qian Wang:** Validation, Data curation. **Yujie Wang:** Investigation, Data curation. **Xi Zhang:** Validation, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Baoya Wang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

The authors declare that they have no conflicts of interest.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2024.116534.

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