ETIOLOGICAL DIAGNOSTIC VALUE OF TARGETED NEXT-GENERATION SEQUENCING FOR PNEUMONIA

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Abstract. Pneumonia is a common respiratory infection and a significant contributor to global mortality. Prompt identification of the pathogens responsible for pneumonia is crucial for ensuring effective treatment of patients. The study assessed the efficacy of targeted next-generation sequencing (tNGS) using bronchoalveolar lavage fluid samples from pneumonia patients (n = 338). The tNGS assay identified 70 pathogens, including 38 bacteria species, 14 types of viruses, 13 fungi species, 2 *Mycoplasma* spp, *Ureaplasma urealyticum*, *Ureaplasma parvum*, and *Chlamydia*. Among infections with a single pathogen, *Candida albicans* (12%) and *Mycobacterium tuberculosis* complex (11%) were the most common pathogens, while the fungi/viruses combination was predominant (28%) in cases of infection with multiple pathogens. Notably, tNGS method had a significantly higher positive detection rate (90%; 95% confidence interval (CI) of 86 to 93%) and higher coincidence rate (92%; 95% CI of 89 to 95%) compared to the conventional method (33%; 95% CI of 28 to 38%) and 82%; 95% CI of 76 to 85%) with p-values <0.001 and 0.008 respectively), particularly in identifying *M. tuberculosis* and atypical pathogens (*C. psittaci, Chlamydia pneumoniae, Coxiella burnetii, Legionella* spp, and *Mycoplasma pneumoniae*). Treatment in 50% of the cases was based solely on tNGS results. We conclude that tNGS is an important diagnostic tool in treating pulmonary infection, owing to its high sensitivity, specificity and rapidity in detecting causal pathogenic bacteria, atypical organisms, fungi and viruses, allowing targeted drug treatment irrespective of prior empirical medication, thereby potentially improving patients' clinical outcomes and survival.

Keywords: bronchoalveolar lavage fluid, diagnosis, pathogen, pneumonia, targeted next-generation sequencing

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INTRODUCTION

Pneumonia is a prevalent disease and remains a significant global health concern, despite advancements in diagnosis, treatment and prevention (Jin *et al*, 2022). Various microorganisms, such as bacteria, fungi and viruses, can trigger pneumonia, which may lead to acute lung injury and acute respiratory distress syndrome, potentially resulting in lasting pulmonary damage characterized by emphysematous changes or irreversible fibrosis (Bauer *et al*, 2006; Rogers *et al*, 2018). Moreover, inflammation stemming from microbial infection may contribute to the development and progression of cancer (Saus *et al*, 2019; Sun *et al*, 2023b; Bewicke-Copley *et al*, 2019). Clinical guidelines advise prompt definitive pathogen testing, especially those of unknown causes (Grief and Loza, 2018; Chinese Medical Association *et al*, 2019; Jones *et al*, 2020). Early detection of pathogens and the implementation of effective antimicrobial strategies result in reducing the risk of development of severe complications and mortality (Cilloniz *et al*, 2021). Therefore, rapid and accurate etiological diagnosis is crucial for appropriate treatment and positive outcomes for pneumonia patients.

The treatment protocol for pneumonia is based on the identification of the source(s) of infection. In general, detection of the causative microbe(s) relies on the results of bronchoalveolar lavage fluid (BALF) cultures, a reliable method for determining the microbial etiology of lower respiratory tract infections (Meyer *et al*, 2012; Escribano Montaner *et al*, 2018; Peng *et al*, 2020; Yang *et al*, 2022). However, BALF culture sensitivity is low, as shown by

numerous studies reporting low positive detection rate and delayed pathogen identification (Lebastard *et al*, 2022; Yang *et al*, 2022; Jia *et al*, 2023; Shi *et al*, 2023; Sun *et al*, 2023a). Consequently, there is an urgent need for a more sensitive, rapid and cost-effective pneumonia diagnostic method using BALF.

Metagenomic next-generation sequencing (NGS) has recently emerged as an unbiased technique for pathogen detection by directly identifying pathogens in clinical samples (Gu *et al*, 2019). By offering high sensitivity and accuracy, mNGS can detect a broad spectrum of pathogens, which is particularly beneficial for diagnosing complex infectious diseases with rare, novel, unknown, or atypical causes (Chen *et al*, 2021; Yang *et al*, 2022). Despite its versatility, mNGS poses challenges for clinicians in prompt pinpointing of the specific causative pathogen. The methodology involves handling pathogen DNA and RNA in a significant background of the patient's nucleic acids, which can lead to decreased sensitivity, especially in samples such as fluids and tissues (Chiu and Miller, 2019; Gu *et al*, 2019; Cai *et al*, 2024). This has led to the recent development of targeted NGS (tNGS), which selectively captures and sequences genetic regions of the target pathogens, enabling the detection of their DNA or RNA in clinical samples, and thereby minimizing contamination by the patient's genetic material (Zhang *et al*, 2024). This method offers a wide detection range, exceptional accuracy, customizable features, and precise subtyping capabilities, and, of importance, is cost-effective. A modified version, named probe capture-tNGS, in which integrates millions of pathogen-specific capture probes capable of eliminating host genomic DNA, has been successfully applied to the molecular diagnosis of pathogens (Li *et al*, 2020; Cai *et al*, 2024).

tNGS has not been applied for the diagnosis of etiological agents of pneumonia. Thus, we explored the utility of probe capture-tNGS in detecting suspected microbial sources of pneumonia using patients' BALF samples and evaluated its

diagnostic performance compared to the standard culture method. Our findings encompass three principal components: (1) comprehensive profiling of pathogen diversity and distribution patterns in pneumonia cases; (2) comparative analysis of gender-specific pathogen prevalence; and (3) a technical evaluation comparing detection capabilities between tNGS and traditional diagnostic approaches, with particular emphasis on pathogen spectrum identification and quantitative detection thresholds.

MATERIALS AND METHODS

Patients and samples collection

This was a retrospective study of collected clinical and etiological tests' data from patients (*n* = 338) diagnosed with pneumonia who were admitted to the emergency intensive care unit (ICU) of the Chengwu Branch, Second Hospital of Shandong University, Province of Shandong, PR China, between August 2021 and March 2024. Inclusion criteria were: (i) being ≥18 years of age; (ii) presenting a clinical diagnostic picture of pneumonia, with a sequential organ failure assessment (SOFA) score ≥2, and an acute physiology and chronic health evaluation II (APACHE-II) score indicating high severity; (iii) undergone conventional and tNGS tests for etiological pathogens; and (iv) with at least one biomarker significantly higher than the hospital-defined upper normal limit for erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, procalcitonin (PCT) level, or routine blood tests. Exclusion criteria were: (i) initial diagnosis of fever on admission due to a non-infectious disease, *eg*, tumor, autoimmune disease; (ii) unclear clinical diagnosis at discharge/death; (iii) did not undergo both conventional and tNGS tests for etiological pathogens; (iv) conventional and tNGS tests not performed within the set time frame (on the same day); and (v) incomplete medical records.

Conventional test

BALF was collected by a professional physician following the Clinical Sample Collection

Guidelines (Chinese Association of Preventive Medicine and Hospital Infection Control, 2017). Microbial identification was according to previously described (Escribano Montaner *et al*, 2018; Fang *et al*, 2020; Zhou *et al*, 2015). Bacterial number >10^4 CFU/ml is considered positive. Detection of Gram-positive and/or Gram-negative bacteria was conducted under a light microscope. Positive detection of fungi required presence in smear and culture, with the following factors taken into consideration: human host, clinical characteristics and microbiological evidence. The virus of interest was identified using a cognate immunoserological method. *Mycobacterium tuberculosis* was detected on the Lownstein-Jenson culture medium.

tNGS assay

tNGS assay was performed as previously described (Cai *et al*, 2024). Nucleic acids were extracted using an automated nucleic acids extraction system (Vazyme biotech, Jiangsu, PR China); sequencing libraries were prepared using MetaCAP Pathogen Capture Metagenomic Assay Kit (KingCreate, Guangdong, PR China); and sequencing was performed using MiniSeq sequencing system (Illumina, San Diego, CA), with an average of 106 reads per sample. The sequences were processed using fastp software (version 0.23.1) (Chen *et al*, 2018) to eliminate low-quality reads (lengths <35 bp), sequencing adapters and unidentified bases. Human sequences were filtered out using the human reference (hg38) Burrows-Wheeler Aligner (BWA) version 0.7.17-r1188 (Van der Auwera *et al*, 2013). The resulting sequences were then mapped using the Basic Local Alignment Search Tool (BLAST) search of the microbial genome database (http://ftp.ncbi.nlm.nih.gov/genomes/) of bacteria (n = 11,958), viruses (n = 7,373), fungi (n = 1,714), and parasites (n = 343) associated with human diseases. The alignment of reads with the genome of the cognate species was normalized based on reads per million, which was compared with that of the respective reference microbe spiked in a negative control sample performed in parallel.

Given the differences in sequencing platforms and the absence of unified criteria for interpreting tNGS results, we developed in-house positive standards for tNGS results drawing on previous reports (Bewicke-Copley *et al*, 2019; Fang *et al*, 2020). A positive result is defined as a relative abundance ≥30% of a pathogen detected by tNGS at the genus level. For a single species to be considered positive, the unique sequence detected is ≥50% compared to that of total microbial reads after normalized; for *M. tuberculosis*, a result is considered positive if at least 1 read was mapped to either the species or genus level.

Comparison between conventional and tNGS tests

Conventional and tNGS tests were performed by two different groups, with members of one group not aware of the results of the other. The results of the two types of tests were evaluated by three experienced clinicians and compared with the definitive clinical diagnosis (*viz* infection, contamination or colonization). The definitive diagnosis took into account epidemiological data, clinical manifestations, laboratory and imaging results, and responses to anti-infective therapies. Contamination is defined as the detection of environmental or symbiotic pathogen unrelated to clinical manifestations together with a positive negative control. Colonization is defined as the detection of a pathogen not related to the disease manifestations.

Conventional and tNGS tests are considered in agreement if the same microorganism is detected and the unique sequence count from a single species is ≥100. If multiple suspected pathogens are identified by the tNGS assay and correspond to the definitive diagnosis, the tNGS result is then defined as an infection resulting from multiple pathogens.

Data analysis

Means ± standard deviation (means ± SD) were reported for normal distributed metric data, and a Student's *t*-test was employed

for group comparisons. The final clinical diagnosis at discharge (clinical assessment completed within 24 hours before discharge) or death (multidisciplinary diagnosis consensus reached within 2 hours post-mortem) was the gold standard. Kruskal-Wallis rank sum test was applied to non-normal distributed data among multiple groups, and the $\chi 2$ test was used for two-category data analysis. A p-value <0.05 is considered statistically significant. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM, Armonk, NY).

Ethical considerations

The study protocol was approved by the Ethics Review Board of Chengwu Branch, Second Hospital of Shandong University (Approval No. 20250219001).

RESULTS

Demographic and clinical data

Three hundred and thirty eight recruited patients with pneumonia admitted to the Chengwu Branch, Second Hospital of Shandong University, between August 2021 and March 2024, who met the inclusion requirements consisted of 31% females (n = 105) and 69% males (n = 233), were 54 ± 19 years of age, with a body mass index (BMI) of 22.0 ± 1.63 kg/m^2 (Table 1). Sixty percent of patients (n = 203) had at least one other diagnosed disease, *eg*, kidney, neurological or cardiovascular, and 88% (n = 299) had received antibiotic treatment during the previous 48 hours before admission (Table 1). In addition, clinical severity scores demonstrated mean values (mean ± SD) of 11.2 ± 3.5 for Acute Physiology Age and Chronic Health Evaluation II (APACHE II) and 6.0 ± 2.6 for Sequential Organ Failure Assessment (SOFA). Laboratory analyses revealed white blood cell (WBC) count was 14.0 ± 0.5 ×10^9 cells/l; procalcitonin (PCT) level was 25.5 ± 14.4 ng/ml; and interleukin-6 (IL-6) level was 1.2 ± 0.5 pg/ml. These findings indicate the presence of an infection in the patient. Their median (interquartile range) length of stay in the emergency intensive care unit was 12 (10-14) days.

Table 1

Characteristics of pneumonia patients (N = 338), Chengwu Branch, Second Hospital, Shandong University, Shandong Province, PR China, August 2021 - March 2024

Characteristic	Frequency* *n* (%)
Age (years), mean ± SD	54 ± 19
Sex	
Male	233 (69)
Female	105 (31)
BMI (kg/m^2), mean ± SD	22.0 ± 1.6
Risk behavior	
Smoking	201 (59)
Alcohol consumption	152 (45)
Condition, according to score, mean ± SD	
APACHE II	11.2 ± 3.5
SOFA	6.0 ± 2.6
Underlying disease	
Neurological disease	152 (45)
Diseases of the cardiovascular system	203 (60)
Renal disease	296 (88)
Diabetes	131 (39)
Others	79 (23)
Body temperature (^{o}C), mean ± SD	38.1 ± 0.3

Table 1 (cont)

Characteristic	Frequency* *n* (%)
Laboratory parameter, mean ± SD	
WBC (×10^9cells/l)	14.0 ± 0.5
NEUT (×10^9cell/l)	84.8 ± 3.3
LYC (×10^9cell /l)	3.3 ± 1.4
ESR (mm/hour)	59.0 ± 38.2
CRP (mg/l)	162.4 ± 70.4
PCT (ng/ml)	25.5 ± 14.4
IL-6 (ng/l)	1.2 ± 0.5
PaO_2/FiO_2 (mm Hg)	255.2 ± 59.7
Purulence secretion	254 (75)
Time on mechanical ventilation time (hours), median (IQR)	6 (4-9)
Antibiotic exposure 48 hours before tNGS	313 (93)
Time in EICU (days), median (IQR)	12 (10-14)
28-day all-causes mortality rate (%)	16

*Unless otherwise stated

APACHE II: Acute Physiology Age and Chronic Health Evaluation II;; BMI: body mass index; CRP: C-reactive protein; EICU: Emergency Intensive Care Unit; ESR: erythrocyte sedimentation rate; IL-6: interleukin 6; IQR: interquartile range; kg/m^2: kilograms per square meter; l: liter; LYC: lymphocyte count; mg/l: milligrams per liter; NEUT: neutrophil count; ng/l: nanograms per liter; PCT: procalcitonin; PaO_2/FiO_2: oxygenation index; SD: standard deviation; SOFA: Sequential Organ Failure Assessment; tNGS: target next-generation sequencing; WBC: white blood cells; ^{o}C: degree Celsius

Their median (interquartile range) length of stay in the emergency intensive care unit was 12 (10-14) days. The 28-day all-causes mortality was 16%.

BALF microbiota

Pathogens were detected in 90% of BALF samples (n = 303) using the tNGS method (unique sequences ≥50 reads), which revealed the presence of 70 types of pathogens, consisting of 54% bacteria (47 and 53% Gram-negative and -positive respectively), 20% viruses and 18% fungi (Fig 1A). Among the patients with detectable pathogens, 43% (n = 130) and 57% (n = 173) were infected with single- and multiple-type(s) respectively (Fig 1B).

Among patients infected with a single pathogen, *Candida albicans* emerged as the predominant pathogen (12%, n = 16), followed by the *Mycobacterium tuberculosis* complex (11%, n = 15), then SARS-CoV-2 (10%, n = 13) and human herpesvirus 4 (10/130, 8%, n = 10) (Fig 2A). Among the 83 male patients, *C. albicans* was the most prevalent pathogen (13%, n = 11), followed by *Pseudomonas aeruginosa* and SARS-CoV-2 (each 11%, n = 9), and then Epstein-Barr virus (10%, n = 8) (Fig 2B). Among 47 female patients, *M. tuberculosis* complex was the predominant pathogen (21%, n = 10), followed by *C. albicans* (11%, n = 5), and then *Mycoplasma pneumoniae* and SARS-CoV-2 (each 8%, n = 4) (Fig 2C).

Among 173 patients infected with multiple pathogens, fungi/viruses co-infection accounted for 28% (n = 48) of the cases, followed by bacteria/viruses (18%, n = 31), and then bacteria/fungi/viruses (16%, n = 28) (Fig 3A). Among 127 male patients, fungi/viruses co-infections accounted for 28% (n = 36), followed by bacteria/viruses (19%, n = 24, of which 58 and 42% were Gram-negative and -positive bacteria respectively), and then bacteria/fungi/viruses (16%, n = 20, of which 62 and 38% were Gram-positive and -negative bacteria respectively) (Fig 3B). Among 46 female patients, fungi/viruses co-infection was predominant (26%, n = 12), followed by different viruses or bacteria/viruses (each 6%, n = 3) (Fig 3B).

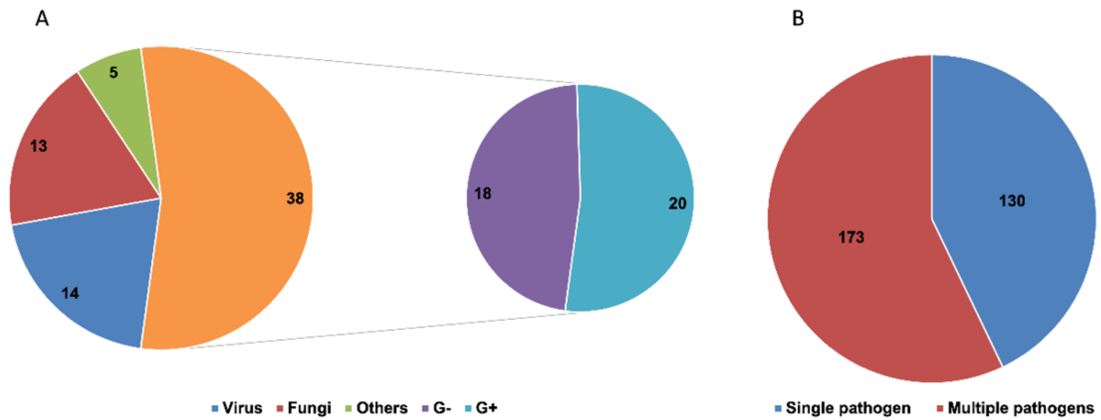

Fig 1 - Pathogens detected by tNGS in bronchoalveolar lavage fluid of pneumonia patients (*n* = 338), Chengwu Branch, Second Hospital, Shandong University, Shandong Province, PR China, August 2021 - March 2024

Note: Numeral indicates number of samples.

A: Pathogen profile; B: Single and multiple pathogenic infections

G-: Gram-negative bacteria; G+: Gram-positive bacteria; tNGS: target next-generation sequencing

Diagnostic performance of conventional and tNGS tests of BALF samples

The positive rate of conventional tests (culture and immunoserological method) for pathogens in 338 BALF samples was 33% (95% C: 28-38%), significantly lower than that of tNGS of 90% (95% CI: 86-93%) (*p*-value <0.01). Bacterial infections were detected in 48 cases using the conventional test. Among 113 pathogen-positive samples by the conventional tests, the most common microbes identified by both methods were *Acinetobacter baumannii* (11%, *n* = 13), *Enterococcus faecium* (12%, *n* = 14), *Klebsiella pneumoniae* (18%, *n* = 21), and *P. aeruginosa* (17%, *n* = 19). Concordance of the tNGS

Fig 2 - Pathogens detected by tNGS from bronchoalveolar lavage fluid of pneumonia patients (n = 130) with single pathogenic infection, Chengwu Branch, Second Hospital, Shandong University, Shandong Province, PR China, August 2021 - March 2024

A: Number and type of pathogens; B: Number and type of pathogens in male cases (n = 83); C: Number and types of pathogens in female cases (n = 47).

G-: Gram-negative bacteria; G+: Gram-positive bacteria; SARS-Co-V-2: severe acute respiratory syndrome coronavirus 2; tNGS: target next-generation sequencing

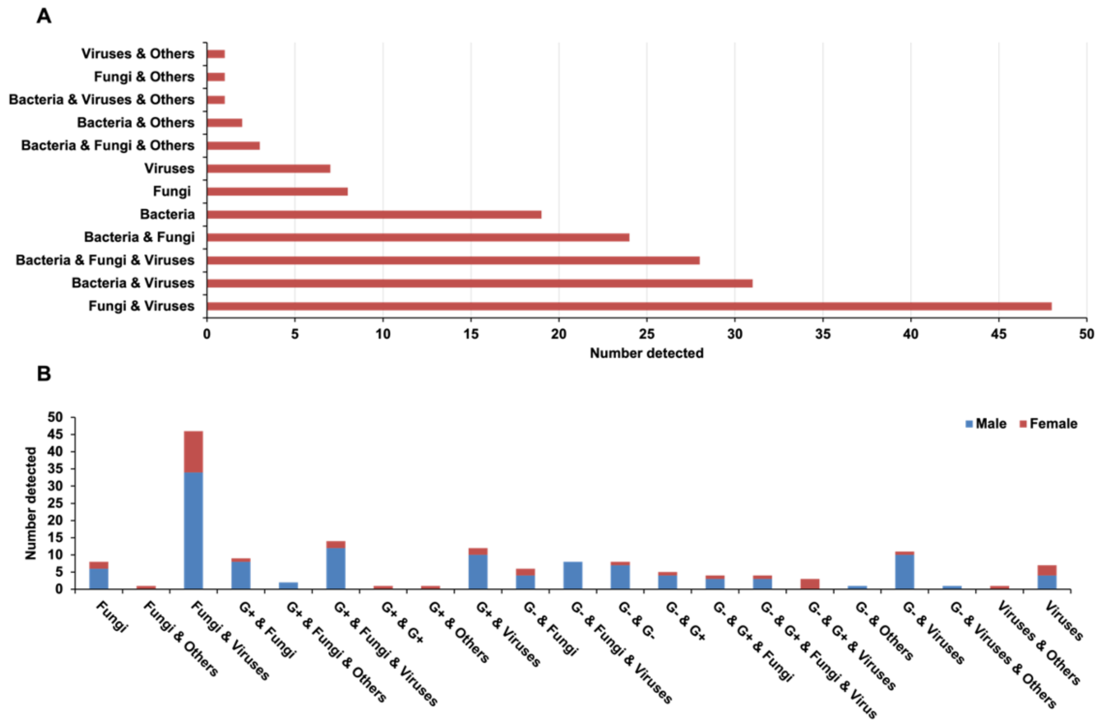

Fig 3 - Pathogens detected by tNGS from bronchoalveolar lavage fluid of pneumonia patients (n = 208) with multiple pathogenic infection, Chengwu Branch, Second Hospital, Shandong University, Shandong Province, PR China, August 2021 - March 2024

A: Pathogen profile; B: Number and type of pathogens in females and males

G-: Gram-negative bacteria; G+: Gram-positive bacteria; tNGS: target next-generation sequencing

test with the final diagnostic result was 92% (95% CI: 89-95%), significantly higher than that of conventional tests of 83% (95% CI: 76-85%) (p-value <0.01).

Optimization of treatment strategies based on tNGS results

Among the 338 enrolled pneumonia patients, 86% (n = 291) of the cases were diagnosed based

on tNGS results only, most of whom were infected with *M. tuberculosis* (10%, *n* = 29) and/or atypical pathogens (58%, *n* = 169). The choice of drug treatment in 50% (*n* = 168) of the patient was directly guided by the tNGS results. Of note, prior treatment of 31% (*n* = 106) of the patients coincided with that recommended from the tNGS results. Only 47 cases were diagnosed and treated using signs, symptoms, medical history, and computed tomography (CT) results. The turnaround time (time from receipt of sample to the identification of potential pathogens) of the tNGS test was ~ 2 days compared to 3-5 days using the conventional tests that, as described above, were also less sensitive. Overall, 75% (*n* = 254) of the patients were cured and 13% (*n* = 43) had improved conditions. However, there was 16% (*n* = 55) mortality due to severe underlying diseases.

DISCUSSION

Pneumonia remains a major health problem and is associated with high morbidity and mortality in all age groups worldwide (Morris *et al*, 2014; Torres *et al*, 2021). A large variety of pathogens, *viz* bacteria, respiratory viruses and fungi, are probably the etiological agents of pneumonia, with substantial geographical variability in their prevalence. In addition, the emergence of drug-resistant pathogens is a growing challenge to the development of innovative treatments and preventive measures. Thus, prompt identification of the causal pathogen(s) in patients and early initiation of therapy targeted against the identified pathogen(s) are essential for the effective treatment of pneumonia.

BALF is commonly employed in various diagnostic tests to identify the causative pathogen(s) responsible for pneumonia (Yang *et al*, 2022). We also relied on BALF specimens for conducting tNGS tests, which reduced the time for detection of the causative pathogens from 3-5 days by conventional tests to 2 days. Notably, targeted treatment regimens for 50% of the patients were based on the tNGS

results. Implementation of tNGS has aided clinicians in optimizing antibiotic regimens and minimized the empirical use of broad-spectrum antibiotics (Cai *et al*, 2024).

In a clinical setting, positive respiratory tests are of limited use as diagnostic tools for such infections due to their low sensitivity (50%), specificity (20-70%, depending on the patient's immune status) and long turnaround time (Ioanas *et al*, 2001; Meersseman *et al*, 2007; Samuel, 2023). Novel molecular diagnostics, predominantly nucleic acid-based methods, have significantly transformed the employment of BALF for diagnosis by markedly enhancing specificity, sensitivity and speed allowing the identification of a single pathogen or combination of pathogens (Qin *et al*, 2024).

Here, in a small cohort of 338 patients with a clinical picture of pneumonia, the tNGS assay of BALF samples identified 70 different suspected causal pathogens of this respiratory illness in 90% of the patients, of whom 43% were infected with a single pathogen and the remaining with multiple pathogenic agents (bacteria, fungi and viruses). Cultures, recognized as the gold standard method for bacteria detection, are predisposed to prior antibiotic treatment (Jadavji *et al*, 1997; Zhang *et al*, 2020). Among the recruited patients, 92% had received empirical antibiotics prior to entry into the program. tNGS assay appeared less impacted by the preceding antibiotic treatments; 50% of the patients had their treatment protocol modified in light of the tNGS results and, fortunately, 31% required no change in drug treatment. The final diagnosis and treatment adjustments in most cases relied on a combination of tNGS results, laboratory findings, CT scans, and clinical features. Diagnosis for pneumonia based on tNGS results reached 92% agreement with the final medical diagnosis, compared to 83% by conventional tests.

tNGS assay identified *M. tuberculosis* and atypical pathogens (*C. psittaci, Nocardia, Legionella,* and *Mycoplasma*) that were missed by conventional tests.

Viruses also play a crucial role in the etiology of unexplained pulmonary infections in hospitalized patients (Garbino *et al*, 2004). Diagnostic PCR significantly enhances the diagnosis of virus infections but faces limitations due to the vast diversity of virus types and subtypes (Garbino *et al*, 2004). mNGS can detect DNA and RNA pathogens but the complexity of the technique and associated costs are constraining factors for its adoption in a clinical setting (Cai *et al*, 2024). On the other hand, tNGS inherently detects both DNA and RNA pathogens and offers a more convenient and cost-effective method. With the tNGS technique, we were able to identify RNA pathogens, including viruses and *Mycoplasma*, in 37% of the BALF specimens, underscoring its broad detection capabilities and high specificity in effectively tackling challenges posed by viral pneumonia.

Nevertheless, 10% of the cases whose etiological pathogens could not be detected by the tNGS method. There are two possible reasons: i) the patients exhibited mild symptoms of pneumonia resulting in a low pathogen abundance in BALF, and ii) constraints inherent to tNGS technology, which, at present, lacks a globally accepted standardization of the methodology, such as guidelines on indications, sampling timing, quality control, sequencing platforms, data analysis, and interpretation of results. Another drawback is the difficulty in distinguishing between colonization and infection. In addition, due to an individual patient's unique clinical presentation of the pneumonia syndrome, it may not be practical to rely on a set of fixed criteria to define the exact causal pathogen(s), particularly in infection with multiple pathogenic agents. Furthermore, we did not include a no-intervention group due to ethical constraints and established medical protocols. The final diagnosis of pneumonia was determined by three medical experts based on the patient's complete clinical conditions and laboratory findings. Future research should address the limitations of tNGS technology to

enhance its translational utility.

In conclusion, we conducted a retrospective cross-sectional analysis of the diagnostic effectiveness of tNGS compared to conventional tests on bronchoalveolar lavage fluid (BALF) from patients with presumptive pneumonia upon admission to a hospital emergency intensive care unit. As expected, the tNGS assay outperformed conventional methods in sensitivity and specificity of detecting causal pathogens, such as *M. tuberculosis*, atypical pathogens, fungi, and viruses. These properties together with a shorter turnaround time make the tNGS technique a valuable tool for prompt diagnosis and more targeted treatment of the responsible pathogens, thereby enhancing patient prognosis and reducing mortality. Furthermore, the tNGS detection system will help decrease the empirical overuse of wide-spectrum antibiotics, thereby curbing the development and spread of microbial drug resistance, a serious concern in the current lack of new antibiotics entering clinical use.

CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflict of interest.

AVAILABILITY OF DATA AND MATERIALS

The datasets analyzed in the current study are available from the corresponding author upon reasonable request.

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