Infection and Drug Resistance

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ORIGINAL RESEARCH

Utilizing Targeted Next-Generation Sequencing for Rapid, Accurate, and Cost-Effective Pathogen Detection in Lower Respiratory Tract Infections

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Objective: To evaluate the diagnostic performance and clinical impact of targeted next-generation sequencing (tNGS) in patients with suspected lower respiratory tract infections.

Methods: Following propensity score matching, we compared the diagnostic performances of tNGS and metagenomic next-generation sequencing (mNGS). Furthermore, the diagnostic performance of tNGS was compared with that of culture, and its clinical impact was assessed.

Results: After propensity score matching, the coincidence rate of tNGS was comparable to that of mNGS (82.9% vs 73.9%, P=0.079). The detection rates for bacterial, viral, fungal, and mixed infections were not significantly different (P>0.05). Bacterial-viral co-infection (16.7%) was the most common mixed infection detected by tNGS. tNGS showed a higher detection rate than culture (75.2% vs 19.0%, P<0.01). The positive detection rate by tNGS was not significantly different between immunocompromised and immunocompetent patients (88.6% vs 80.5%, P=0.202), but was significantly higher than that by culture (P<0.001). Moreover, 65 patients (44.5%) had their medications modified based on the tNGS results, and the majority exhibited notable improvement regardless of treatment adjustment.

Conclusion: tNGS performs comparably to mNGS and surpasses culture in detecting lower respiratory tract infections. Nevertheless, tNGS is faster and more cost-effective than mNGS, making it highly significant for guiding rational treatment.

Keywords: targeted next-generation sequencing, metagenomic next-generation sequencing, lower respiratory tract infections, diagnosis, antibiotic treatment

Introduction

Lower respiratory tract infections (LRTIs) are the fourth leading cause of death worldwide, affecting approximately 489 million people annually and causing a significant economic burden.[1,2] LRTIs are predominantly caused by various pathogens, including bacteria, fungi, viruses, atypical pathogens, and parasites. Prompt and precise etiological identification is important for effective disease management.

Traditional methods are time-consuming, have a low positive detection rate, and limited detection range, thus failing to meet clinical needs.[3,4] Clinicians often use broad-spectrum antibiotics before identifying the pathogen, which may amplify the associated negative effects, healthcare expenses and bacterial resistance.[5,6] Next-generation sequencing (NGS) offers new possibilities for precise diagnosis of infectious diseases. Our previous study underscored the significance of metagenomic next-generation sequencing (mNGS) for the accurate diagnosis and management of LRTIs.[7] However, mNGS is costly, vulnerable to human sequence interference, and cannot process DNA and RNA

Received: 4 September 2024
Accepted: 10 January 2025
Published: 17 January 2025

simultaneously.[8] In contrast, targeted next-generation sequencing (tNGS) uses multiplexed targeted amplification and high-throughput sequencing to identify numerous clinically significant pathogens and drug-resistance genes.[9] The benefits of dual DNA and RNA detection include enhanced detection speed and reduced cost.[10] The potential of tNGS has been emphasized in infectious diseases of the central nervous system, periprosthetic joints, and mycobacterial infections.[11–14] However, the diagnostic performance of tNGS for LRTIs has rarely been studied.

This study aimed to compare the diagnostic effectiveness of tNGS, mNGS, and culture in identifying pathogens, focusing on the diagnostic performance and clinical impact of tNGS in LRTIs to support its clinical application and wider adoption.

Methods

Study Design and Participants

This retrospective observational study included patients with suspected LRTIs admitted to Wuhan Union Hospital between January 2020 and November 2023. Demographic and clinical data, including age, sex, underlying diseases, clinical symptoms, laboratory and imaging results, NGS results, antibiotic treatment before and after NGS, and disease outcomes, were collected. Additionally, the CURB-65 score (respiratory rate, age, blood pressure, consciousness disturbance, and blood urea nitrogen level) was calculated upon admission to assess LRTIs severity.[15]

The inclusion criteria were as follows: 1) age ≥ 18 years, 2) suspected LRTIs, 3) NGS (mNGS or tNGS) during hospitalization, and 4) complete clinical information. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of Wuhan Union Hospital (2023–0818). Written informed consent from patients was waived due to the retrospective nature of this study and the fact that no personal identifiers were used in either the analysis or the reporting of study results.

Definition

Suspected LRTIs diagnosis was established based on (1) new or worsening focal or diffuse infiltrative lesions on chest imaging; (2) at least one of the following clinical manifestations consistent with LRTIs: ① newly developed cough, sputum production, or worsening of pre-existing respiratory symptoms, with or without purulent sputum, chest pain, dyspnea, or hemoptysis; ② fever; ③ signs of pulmonary consolidation or auscultation of moist rales; ④ peripheral blood white blood cell count $>10\times10^9$/L or $<4\times10^9$/L.[16,17]

Immunocompromised status was defined as (1) primary immune deficiency diseases; (2) HIV infection with a CD4 T-lymphocyte count < 200 cells/μL or percentage < 14%; (3) active malignant tumors; (4) undergoing cancer chemotherapy; (5) recipients of solid organ transplant or hematopoietic stem cell transplant; (6) long-term use of steroids (dose ≥ 20mg prednisone or equivalent daily for ≥ 14 days or a cumulative dose > 600 mg of prednisone) or other immunosuppressive drugs.[18]

The final LRTIs diagnosis was based on a comprehensive analysis by two experienced clinicians, considering the patient's medical history, clinical symptoms, laboratory and imaging results, conventional etiological test, NGS results, and response to antibiotic treatment.

mNGS Process

mNGS was performed on 268 specimens from 249 patients (243 bronchoalveolar lavage fluid (BALF) samples, 18 blood samples, four pleural effusion samples, two sputum samples, and one tissue sample). Firstly, the samples were subjected to cell wall lysis treatment through an internally developed method, and then 1000μL of supernatant was collected for subsequent DNA extraction. The DNA was extracted using a positive broad-spectrum enrichment kit specifically designed for pathogenic microorganisms. Secondly, sequencing libraries were prepared by DNA fragmentation, end-repair, adaptor ligation, and PCR amplification utilizing an NGS library preparation kit. The quality and concentration of the DNA libraries were assessed using an NGS library quantification kit in conjunction with a real-time fluorescent quantitative PCR instrument. The pooled libraries were sequenced on an Illumina sequencer using a single-end 50-bp sequencing strategy.

The raw data were preprocessed to remove low-quality reads, contaminated adapters, and duplicate reads. Subsequently, host sequences were identified and filtered out by aligning them to the human reference genome (hg38 and T2T), and only high-quality sequencing data were retained. The remaining sequences were aligned to a comprehensive reference database that includes GenBank, NCBI RefSeq, and NCBI nt, and the aligned data were classified according to viruses, bacteria, fungi, and parasites. The database contains 11,027 bacteria, 1,324 fungi, 11,704 viruses, 135 mycoplasmas/chlamydias/rickettsias/spirochetes, and 229 parasites.

tNGS Process

146 specimens (consisting of 139 BALF samples, four blood samples, two sputum samples, and one tissue sample) were submitted for tNGS. Initially, 300 μL of each sample was transferred into a grinding tube preloaded with glass beads (0.1mm and 0.5mm). The tube cap was tightened, and the tube was securely attached to the adapter (Jinxin brand). The instrument was operated at 70Hz for 10 minutes. Following grinding, the supernatant was utilized for the extraction and purification of DNA/RNA using the Magnetic Bead-based Liquid Sample Pathogenic Microorganism Total Nucleic Acid Extraction Kit (Bingyuan-CJ0003).

Subsequently, a library was constructed using the Pathogeno One 400+ Library Preparation Kit (manufactured by Hebei Bingyuan Shengkang Medical Technology Co., Ltd., product code SJ0009). This process involves two rounds of PCR amplification. The sample nucleic acid and cDNA served as templates and 288 microbial-specific primers were selected for multiplex PCR amplification to enrich the target pathogen sequences. The PCR products were purified using DNA purification magnetic beads and then amplified using primers that incorporated sequencing adapters and unique barcode sequences. After purification, the quality and quantity of the resulting library were assessed and quantified using a Qubit 4.0 fluorometer.

The denatured library was sequenced on the Illumina MiSeq platform using a MiSeq Reagent Nano Kit with a sequencing read length of PE75 and an average of 0.1 million sequencing reads per sample. Following sequencing, the raw data were identified and counted through adapter recognition, followed by the filtering of low-quality reads and the identification of high-quality data with primers. Reads with correctly aligned ends were retained and compared against a pathogen database to determine the species and abundance of pathogens in the sample. The detection scope encompassed 86 drug-resistance genes, 41 virulence genes, and at least 288 pathogens, including 113 bacteria, 82 viruses, 57 fungi, 12 parasites, and 12 mycoplasmas/chlamydias/rickettsias.

Interpretation of mNGS/tNGS Results

The mNGS and tNGS results were interpreted following the methodologies stated in our previous research and the "Expert Consensus on Clinical Interpretation Pathway for Metagenomic Next-Generation Sequencing Reports in Lower Respiratory Tract Infections".[7,19]

Statistical Analysis

Normally distributed data were represented using the mean and standard deviation, while the median and interquartile range were used for non-normally distributed data. The independent sample *t*-test and Pearson's chi-square test or Fisher's exact test were used to compare continuous and categorical variables, respectively. Furthermore, to ensure that patients in the mNGS and tNGS groups had similar clinical baseline characteristics, we performed propensity score matching (PSM) with 1:1 matching and a caliper value of 0.02 on the two groups. Matching factors included sex, age, smoking history, underlying disease, and CURB-65 score. All statistical analyses were performed using SPSS software version 25.0, and $P < 0.05$ was considered statistically significant.

Results

Comparison of Demographic Information and Clinical Data

Between January 2020 and November 2023, 463 patients with suspected LRTIs consented to sample collection and NGS. Among them, three patients < 18 years were excluded, and 65 patients were excluded due to incomplete clinical data.

Ultimately, 395 patients were included in the study and categorized into infectious disease, non-infectious disease, and unknown etiology based on their final diagnoses (Figure 1).

Among the included participants, 249 (63.0%) underwent mNGS and 146 (37.0%) underwent tNGS. The proportion of patients with rheumatic immune system diseases was significantly higher in the mNGS group, while the remaining indicators, including age, sex, smoking history, and CURB-65 score, showed no statistically significant differences between the two groups. To achieve comparability between the two groups, we matched subjects from both groups using propensity analysis (1:1 matching), resulting in the successful matching of 292 patients, including 146 patients from each of the mNGS and tNGS groups. No statistically significant differences were observed in baseline characteristics between the two groups (all P>0.05, Table 1).

Comparison of Diagnostic Performance Between tNGS and mNGS After PSM

Following PSM, the overall microbial detection rate of tNGS was significantly higher than that of mNGS (86.3% vs 70.3%; P<0.05) (Table S1). Based on the composite diagnosis, the total coincidence rate (TCR) (82.9% vs 73.9%, P=0.079) and negative predictive value (NPV) (10.0% vs 2.9%, P=0.546) between tNGS and mNGS were not different. However, the positive predictive value (PPV) of mNGS was significantly higher than that of tNGS (99.0% vs 95.0%, P=0.034) (Figure 2A).

In the tNGS group, 46 microbes were identified, with bacteria accounting for 47.5% (126/265), viruses (85/265, 32.1%), fungi (49/265, 18.5%), and other pathogens (5/265, 1.9%) (Figure 2B). Conversely, the mNGS group identified 66 pathogens, including bacteria, fungi, viruses, and other pathogens, accounting for 46.2% (110/238), 24.4% (58/238), 25.2% (60/238), and 4.2% (10/238), respectively (Figure 2B). The predominant five bacteria identified in the tNGS group were *Haemophilus influenzae, Streptococcus pneumoniae, Mycobacterium tuberculosis, Pseudomonas aeruginosa*, and *Enterococcus faecium*. In contrast, the predominant five bacteria detected in the mNGS group were *M. tuberculosis, Acinetobacter baumannii, P. aeruginosa, Klebsiella pneumoniae*, and *S. pneumoniae*. The most prevalent fungi in both groups were *candida albicans, Aspergillus fumigatus, Pneumocystis jirovecii, Aspergillus flavus*, and *Candida tropicalis*. *Epstein-Barr virus* and *Cytomegalovirus* were the predominant viruses detected (Figure 2C). Subsequently, we evaluated the efficiencies of mNGS and tNGS in detecting bacterial, fungal, and viral infections; however, no significant differences were observed between the two methods in detecting bacteria, fungi, or viruses (all P>0.05) (Figure 2B).

Notably, in the tNGS group, a single potential pathogen was identified in 72 patients, two in 41 patients, and three in 13 patients. The most prevalent types were bacterial-viral co-infections (21/126, 16.7%), bacterial-fungal-viral co-

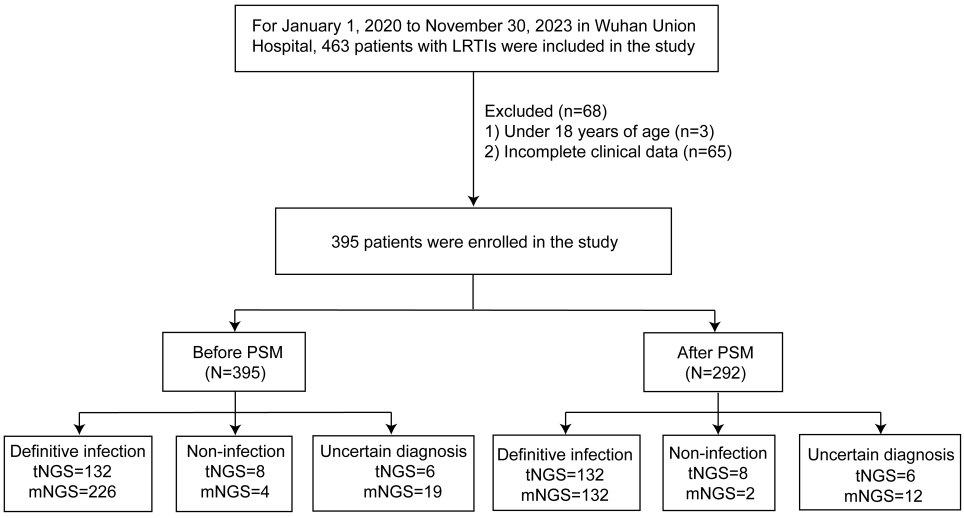

Figure 1 Flow chart of patient selection.
Abbreviations: LRTIs, lower respiratory tract infections; PSM, propensity score matching; mNGS, metagenomic next-generation sequencing; tNGS, targeted next-generation sequencing.

Table 1 Demographic and Clinical Characteristics at Baseline for Patients With LRTIs in the Entire Cohort and the PSM Cohorts

Characteristics	Total (n=395)	Entire Cohort			PSM Cohort		
		mNGS (n=249)	tNGS (n=146)	P value	mNGS (n=146)	tNGS (n=146)	P value
Age (yr, median, IQR)	60 (50–69)	59 (50–69)	60 (50–70)	0.909	59.5 (49–68)	60 (50–70)	0.492
Sex							
Male (n, %)	133 (33.7)	88 (35.3)	45 (30.8)	0.359	48 (32.9)	45 (30.8)	0.706
Current Smoker (n, %)	133 (33.7)	83 (33.3)	50 (34.2)	0.853	49 (33.6)	50 (34.2)	0.902
Complications (n, %)							
Hypertension	95 (24.1)	59 (23.7)	36 (24.7)	0.829	37 (25.3)	36 (24.7)	0.892
Diabetes disease	41 (10.4)	23 (9.2)	18 (12.3)	0.331	19 (13.0)	18 (12.3)	0.860
Malignant tumor	64 (16.2)	37 (14.9)	27 (18.5)	0.344	23 (15.8)	27 (18.5)	0.543
Cardiovascular disease	31 (7.8)	20 (8.0)	11 (7.5)	0.859	10 (6.8)	11 (7.5)	0.821
Chronic pulmonary disease	64 (16.2)	41 (16.5)	23 (15.8)	0.853	26 (17.8)	23 (15.8)	0.638
Chronic renal disease	17 (4.3)	13 (5.2)	4 (2.7)	0.241	3 (2.1)	4 (2.7)	1.000
Chronic liver disease	13 (3.3)	9 (3.6)	4(2.7)	0.775	4 (2.7)	4 (2.7)	1.000
Rheumatic immune disease	24 (6.1)	22 (8.8)	2 (1.4)	0.003	2 (1.4)	2 (1.4)	1.000
CURB-65 (n,%)	1 (0–1)	0 (0–1)	1 (0–1)	0.143	0 (0–1)	1 (0–1)	0.123
0	194 (49.1)	126 (50.6)	68 (46.6)		75 (51.4)	68 (46.6)	
1	125 (31.6)	71 (28.5)	54 (37.0)		38 (26.0)	54 (37.0)	
2	62 (15.7)	39 (15.7)	23 (15.8)		27 (18.5)	23 (15.8)	
3	7 (1.8)	7 (2.8)	0 (0.0)		2 (1.4)	0 (0.0)	
4	7 (1.8)	6 (2.4)	1 (0.7)		4 (2.7)	1 (0.7)	

Abbreviations: LRTIs, lower respiratory tract infections; mNGS, metagenomic next-generation sequencing; tNGS, targeted next-generation sequencing; PSM, propensity score matching; IQR, interquartile range.

infections (11/126, 8.7%), and bacterial-fungal co-infections (10/126, 7.9%) (Figure 3A). Conversely, in the mNGS group, we identified a single potential pathogen in 61 patients, two in 30 patients, three in 11 patients, and four in one patient. The three most prevalent forms were viral-fungal co-infection (14/103, 13.6%), bacterial-fungal-viral co-infection (11/103, 10.7%), and bacterial-fungal co-infection (8/103, 7.8%) (Figure 3B). mNGS and tNGS showed comparable detection rates for mixed infections (40.8% vs 42.9%, P =0.751) (Figure 3C).

Diagnostic Performance of tNGS and Culture

A total of 134 patients underwent culture testing within 48h prior to and after tNGS, including 121 cases of infection, six non-infection cases, and seven cases with unclear diagnosis. The pathogen detection rate with tNGS was significantly higher than that with traditional culture (91/121, 75.2% vs 23/121, 19.0%, P < 0.01) (Figure 4A). Using a composite diagnosis as the criterion, the TCR of tNGS significantly surpassed that of culture (74.8% vs 22.8%, P<0.001). The PPV (97.8% vs 100%, P=1.00) and NPV (11.8% vs 5.8%, P=0.262) of tNGS and mNGS were comparable (Figure 4B).

Of the 134 patients, 21 (15.7%) had positive results for both tNGS and culture, 33 (24.6%) had negative results for both, 78 (58.2%) were positive only for tNGS, and two (1.5%) positive only for culture. Among the double-positive cases, three exhibited complete matching, five exhibited complete mismatching, and the remaining thirteen showed partial matching. Among the tNGS-positive/culturing-negative patients, tNGS identified pathogens that were challenging to detect under culture conditions, such as *M. tuberculosis, Mycolicibacterium fortuitum, Rhizomucor, P. jirovecii*, and *Tropheryma whipplei* (Figure 4C).

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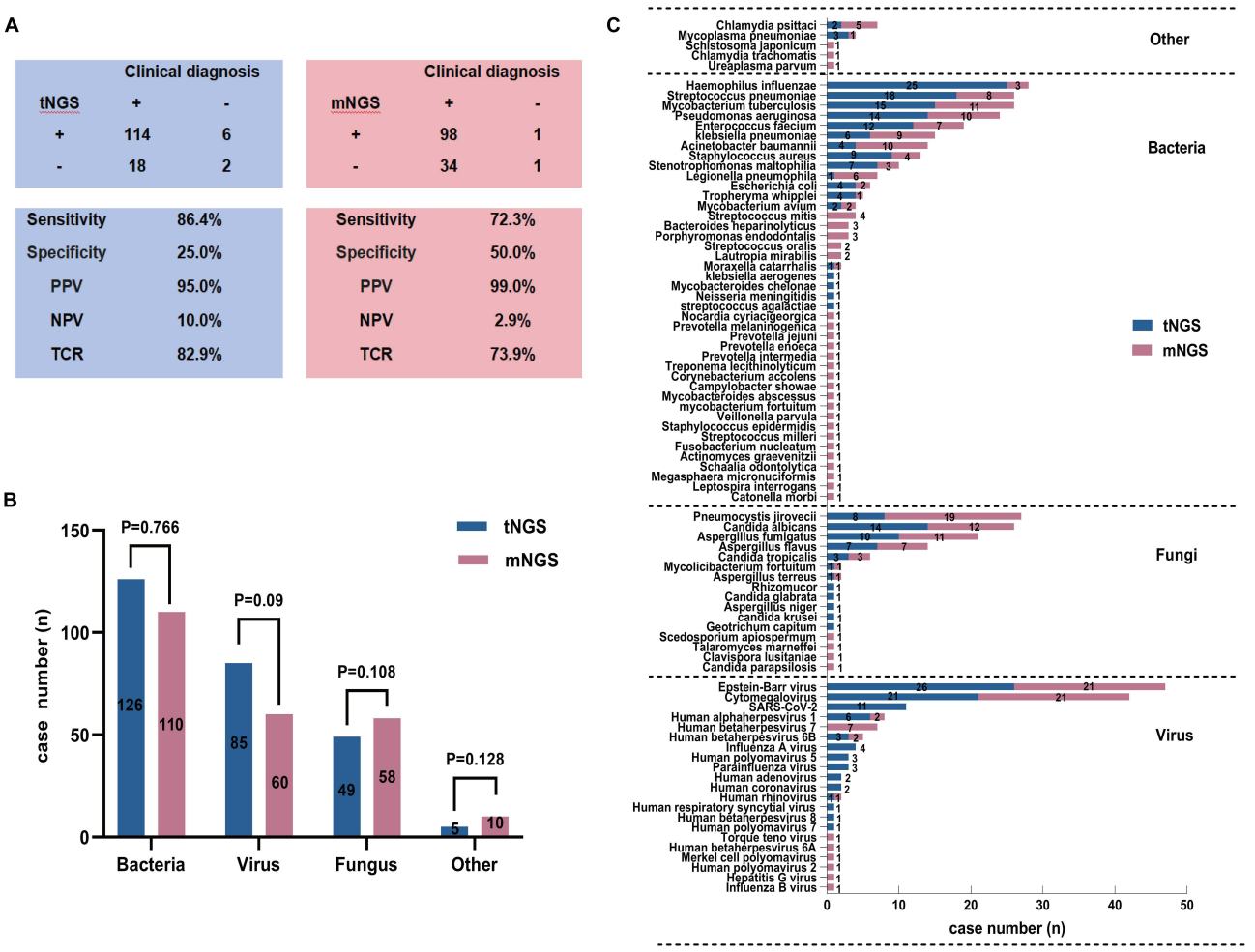

Figure 2 Comparison of diagnostic performance between tNGS and mNGS. (**A**). Contingency tables for the clinical diagnosis with tNGS and mNGS. (**B**) Comparison of the sensitivities for tNGS and mNGS in different types of pathogens. (**C**) Distribution of pathogens identified by tNGS and mNGS.
Abbreviations: PPV, positive predictive value; NPV, negative predictive value; TCR, the total coincidence rate; mNGS, metagenomic next-generation sequencing; tNGS, targeted next-generation sequencing.

Performance of tNGS in Immunocompromised Patients

To evaluate the efficacy of tNGS in immunocompromised patients with LRTIs, the tNGS group was further divided into immunocompromised (n=41) and immunocompetent (n=105) groups based on their immune states. No statistically significant differences were observed in the positivity rate of tNGS between immunocompetent and immunocompromised patients (93/105, 88.6% vs 33/41, 80.5%, P=0.202) (Figure 5A). According to the tNGS results, 17 cases (41.5%) of immunocompromised patients exhibited mixed infections, while 37 cases (35.2%) of immunocompetent patients exhibited mixed infections (P=0.484) (Figure 5A). Furthermore, among the immunocompetent patients, the most common pathogens were *H. influenzae* (18.1%), *S. pneumoniae* (16.2%), *Epstein-Barr virus* (16.2%), *M. tuberculosis* (11.4%), and *P. aeruginosa* (10.5%). In contrast, *Cytomegalovirus* (26.8%) was the most common pathogen in the immunocompromised group, followed by *Epstein-Barr virus* (22.0%), *P. jirovecii* (17.1%), *C. albicans* (17.1%), and *H. influenzae* (14.6%).

Among the 134 patients who underwent culture testing within 48h before and after tNGS, there was no statistically significant difference in the positive culture rate between immunocompromised and immunocompetent patients (8/37, 21.6% vs 15/97, 15.5%, P=0.398) (Figure 5B). Similarly, no significant difference was observed in the positivity rate of

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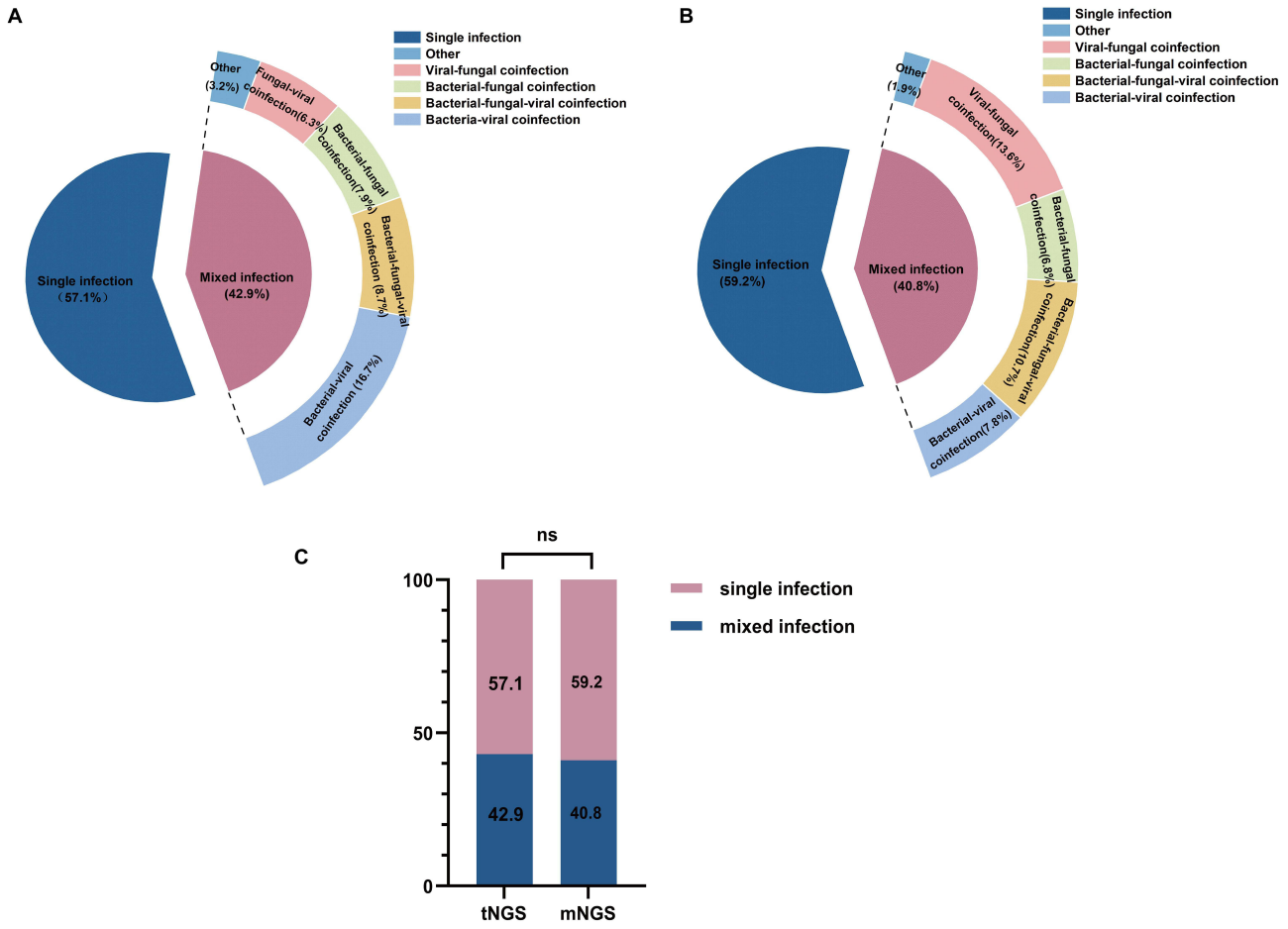

Figure 3 Percentage of patients with mixed infections for various pathogens. (**A**) Mixed infections for various pathogens detected by tNGS. (**B**) Mixed infections for various pathogens detected by mNGS. (**C**) Comparison of the detection rate of mixed pathogen infection based on tNGS and mNGS. ns: P>0.05.
Abbreviations: mNGS, metagenomic next-generation sequencing; tNGS, targeted next-generation sequencing.

tNGS between immunocompromised and immunocompetent patients (23/37, 62.2% vs 76/97, 78.4%, P=0.056). The positive rates of tNGS in both groups were significantly higher than those in culture (P<0.001) (Figure 5B).

Clinical Impact of tNGS

Prior to tNGS, empirical antibiotic therapy was administered to 86.3% of patients. Antibacterial therapy was the predominant antibiotic treatment (92/146, 63.0%), followed by antibacterial-antifungal (24/146, 16.4%), antibacterial-antiviral (6/146, 4.1%), antifungal (2/146, 1.4%), and antibacterial-antifungal-antiviral therapies (2/146, 1.4%) (Table 2). Based on the tNGS results, 13 patients (8.9%) underwent empirical antibiotic de-escalation therapy (reduction in type or scope), 52 patients (35.6%) underwent empirical antibiotic escalation therapy (addition of types or expansion of scope), and 66 patients (45.2%) maintained their current treatment (Table 2). Additionally, 15 patients (10.3%) were referred to specialized hospitals for treatment following the detection of *Mycobacterium tuberculosis* through tNGS (Table 2). Notably, the majority of cases without adjustment were related to the fact that the previously administered empirical drugs had already effectively covered the detected pathogens.

Regardless of antibiotic escalation, de-escalation, or no change, most patients showed clinical improvement, mainly manifesting as normalization of body temperature and significant improvement in respiratory symptoms, such as cough, sputum production, and dyspnea (P<0.001). In addition to lymphocyte counts, the patient's inflammatory markers, such as white blood cell count, neutrophil count, and CRP levels improved significantly (all P<0.05) (Table 3).

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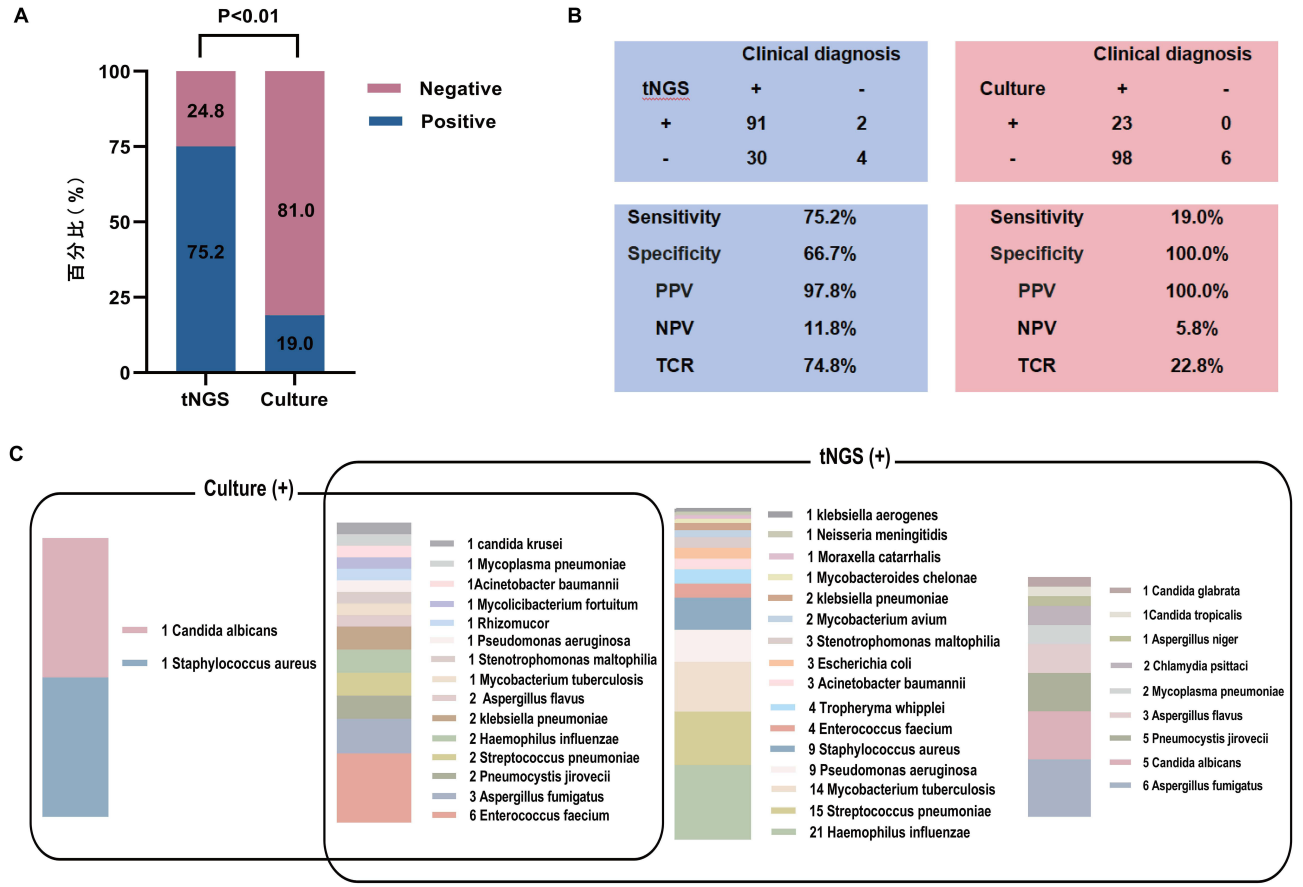

Figure 4 Performance of tNGS and culture. (**A**) Comparative analysis of the positive detection rate between tNGS and culture in patients with confirmed LRTIs. (**B**) Contingency tables for the clinical diagnosis with tNGS and culture. (**C**) Discrepant results between tNGS and culture.
Abbreviations: PPV, positive predictive value; NPV, negative predictive value; TCR, the total coincidence rate, tNGS, targeted next-generation sequencing.

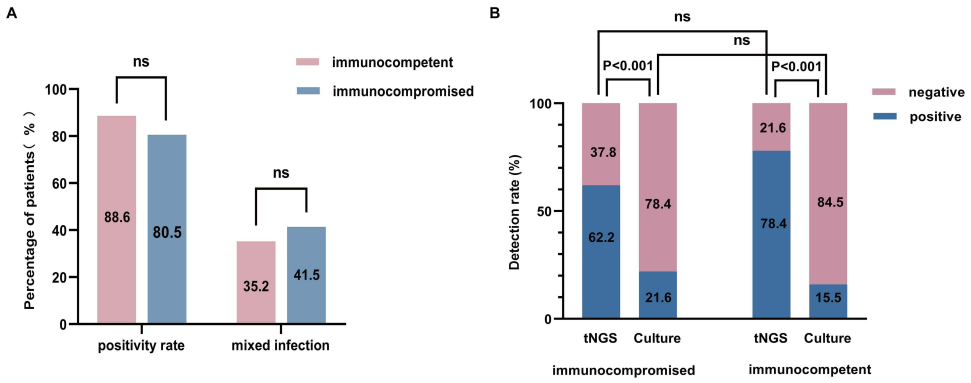

Figure 5 Diagnostic performance of tNGS in immunocompromised patients and immunocompetent patients with LRTIs. (**A**) Comparison of the positive rate and detection of mixed pathogen infection based on tNGS in immunocompetent and immunocompromised patients with LRTIs. (**B**) The positive rate comparison in immunocompetent and immunocompromised patients with LRTIs. ns: P>0.05.
Abbreviations: tNGS, targeted next-generation sequencing; LRTIs, lower respiratory tract infections.

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Table 2 The Estimated Potential Impact of tNGS Testing on the Application of Antimicrobial Agents

Characteristics	Total (n)
Pre-examination medication	
Unused	20 (13.7%)
Antibacterial therapy	92 (63.0%)
Antifungal treatment	2 (1.4%)
Antibacterial-antifungal therapy	24 (16.4%)
Antibacterial-antiviral therapy	6 (4.1%)
Antibacterial-antifungal-antiviral therapy	2 (1.4%)
Post-examination medication adjustments	
No change	66 (45.2%)
Escalation	52 (35.6%)
De-escalation	13 (8.9%)
Transfer to Wuhan Pulmonary Hospital	15 (10.3%)

Abbreviation: tNGS, targeted next-generation sequencing.

Table 3 The Symptoms and Laboratory Tests Before and After Adjusting Treatment According to tNGS

Variables	Before	After	P value	Normal range
Symptoms				
Fever (n,%)	49 (33.6)	3 (2.1)	0.000	NA
Cough (n,%)	88 (60.3)	8 (5.5)	0.000	NA
Sputum (n,%)	69 (47.3)	8 (5.5)	0.000	NA
Chest distress (n,%)	39 (26.7)	0 (0.0)	0.000	NA
Laboratory tests				
[a]WBC (g/l)	8.0 (5.1–11.4)	7.2 (4.7–8.8)	0.001	3.5–9.5
[a]NE (h/l)	5.6 (3.4–9.7)	4.7 (2.9–6.3)	0.000	1.8–6.3
[a]Lym (g/l)	1.2 (0.7–1.8)	1.4 (0.9–1.9)	0.347	1.1–3.2
[a]PLT (g/l)	238 (146–309)	265 (197–328)	0.018	125–350
[a]CRP (mg/l)	56.6 (15.2–125.9)	10.7 (4.4–32.9)	0.000	<8

Notes: [a]77 patients completed blood routine examination and CRP examination.
Abbreviations: tNGS, targeted next-generation sequencing; WBC, white blood cell; NE, neutrophil; Lym, lymphocyte; PLT, platelet; CRP, C-reaction protein.

Discussion

In this study, we conducted a retrospective analysis of the clinical data of 292 patients with suspected LRTIs from multiple campuses at Wuhan Union Hospital. Our findings revealed that tNGS is a rapid and accurate tool for the etiological diagnosis of LRTIs, exhibiting a diagnostic performance comparable to that of mNGS. However, tNGS may be more suitable for the rapid screening and diagnosis of common or known pathogens, while mNGS is more suitable for identifying unknown or rare pathogens. In addition, this study highlights the diagnostic value of tNGS in immunocompromised patients with LRTIs. Furthermore, the results of tNGS have significant value in guiding antibiotic treatment, potentially leading to significant improvements in patient clinical outcomes.

The prompt and precise identification of microorganisms in LRTIs is essential to guide antibiotic treatment, mitigate drug resistance, and improve patient outcomes.[7,20] In this study, the positive detection rate of tNGS in LRTIs was higher than that of mNGS (86.3% vs 70.3%, P <0.05), while the TCR (82.9% vs 73.9%, P=0.079) and NPV (10.0% vs 2.9%, P=0.546) of tNGS and mNGS were not significantly different. Additionally, the PPV of mNGS was significantly higher than that of tNGS (99.0% vs 95.0%, P=0.034). However. A study of 102 adult patients with pneumonia using both mNGS and tNGS to analyze LRTIs specimens revealed that the overall microbial detection rates of tNGS and mNGS were 82.17% and 86.51% (P>0.05), respectively, with no significant difference in the microbial detection rates between the two methods for different specimens.[10] Another study reported an overall accuracy of 65.6% for tNGS detection with

a PPV of 45.9% and an NPV of 85.7%. The overall accuracy of the mNGS detection was 67.1%, with a PPV of 56.6% and an NPV of 77.2%.[21] These variations may be attributed to disparities in patient populations and physicians' clinical experience. Furthermore, consistent with previous studies, mNGS and tNGS showed comparable detection rates for bacteria, fungi, and viruses, with bacteria being the most commonly detected pathogen using both techniques.[21] These findings emphasize the potential of tNGS for pathogen detection.

The competition between different types of microorganisms makes it difficult for traditional detection methods to detect multiple pathogens simultaneously.[22] This study demonstrated that tNGS ultimately confirmed 54 cases and mNGS confirmed 42 cases of mixed infections (P>0.05). Additionally, tNGS consistently identified bacterial-viral co-infection as the predominant form of mixed infection, which is consistent with previous research findings.[7,23] In contrast, the most common mixed infection detected by mNGS and a separate investigation was viral-fungal co-infection.[24] In another study, the prevailing mixed infections consisted of bacterial-fungal and bacterial-bacterial co-infections.[25] A recent study attributes these contradictory findings in the prevalence of common mixed infection types to the changes in immunological state across various populations.[26]

Compared to traditional culture methods, tNGS can detect more pathogens, demonstrate superior diagnostic performance, and provide results promptly. Similar results have been reported in other studies. For example, tNGS demonstrated complete or partial concordance with culture in 90.9% of prospective studies on respiratory tract infections in preterm infants, resulting in a 105.9% improvement in the detection rate.[27] Another study examined sputum samples from 209 patients with confirmed LRTIs and observed that tNGS detected more potential pathogens (45 vs 23) and achieved a higher positive detection rate (96.7% vs 34.0%) compared to conventional microbiological tests.[28] Overall, tNGS improved the diagnostic efficiency for LRTIs.

Further investigations into the utility of tNGS in immunocompromised patients with LRTIs are still lacking. The data presented in this study indicated that the positive detection rate of tNGS was similar between immunocompetent and immunocompromised patients with LRTIs. Additionally, this rate was approximately three times greater than that of culture (P<0.01), demonstrating the potential of tNGS as a primary diagnostic instrument. Furthermore, immunocompromised individuals exhibited a greater prevalence of mixed infections, with the most frequently identified pathogens being *Cytomegalovirus, Epstein-Barr virus, P. jirovecii, C. albicans*, and *H. influenzae*. These findings are consistent with prior research.[29,30] In immunocompromised patients, prolonged hospital stays and an increased risk of mortality are associated with the opportunistic infections caused by *Cytomegalovirus, P. jirovecii, Candida*.[31–33] Therefore, early and accurate identification of pathogens is critical, and tNGS can potentially reduce the time required to detect opportunistic pathogen infections and mixed infections in these patients. However, our conclusions have limitations due to the limited number of immunocompromised patients included in our study. Therefore, extensive multicenter trials are needed to validate the diagnostic efficacy of tNGS in immunocompromised individuals with LRTIs.

Targeted antimicrobial therapy was predicted based on drug sensitivity and pathogen testing and pathogen results. Excessive or belated administration of antimicrobial therapy may result in extended hospital stays, increased drug resistance, escalated medical expenses, and mortality.[6,34] Culturing pathogens can take an average of 3–7 days to obtain results and drug-resistance information. Although most research highlights the diagnostic efficacy of mNGS (average of 24 hours), it is comparatively expensive (average of $440) and lacks the ability to identify drug-resistance completely.[8] In contrast, tNGS has a faster turnaround time (average 15 hours) and lower cost (average $160), and provides reliable drug resistance information.[21,35] In this study, approximately 44.5% of the patients' empirical antimicrobial therapy was modified based on tNGS results. A substantial proportion of patients exhibited noteworthy improvement after treatment modifications, whether through escalation, de-escalation, or maintaining the current regimen. Therefore, tNGS may be advantageous in facilitating targeted therapy and expediting clinical decision-making, thereby decreasing the medical burden.

This study has several limitations. Firstly, as this is a retrospective study, it is not feasible to conduct both tNGS and mNGS on the same individual, which contradicts clinical practice. Nevertheless, we have endeavored to match the two patient groups as closely as possible to ensure the comparability of our study. Secondly, antibiotic treatment was administered to the majority of patients prior to pathogen detection, which may have lowered the culture positivity rate. Lastly, general guidelines for analyzing tNGS and mNGS results are currently lacking, which may result in the misinterpretation or overinterpretation of the findings. Consequently, well-designed prospective multicenter studies with large sample sizes are needed to further evaluate the practical utility of tNGS in LRTIs.

Conclusion

In summary, compared to culture, tNGS demonstrates a higher diagnostic efficacy for LRTIs, similar to that of mNGS. However, tNGS is more cost-effective and has a quicker turnaround time than mNGS. Additionally, the expeditiousness with which tNGS completes its analyses contributes substantially to advancements in precision.

Data Sharing Statement

Demographic and clinical data of the patients which involved in this study were extracted from the patient's electronic medical record in Wuhan Union Hospital. The datasets generated and analyzed during the current study are not publicly available due to privacy or ethical restrictions but are available from the corresponding author on reasonable request.

Ethical Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of Wuhan Union Hospital (2023-0818). Written informed consent from patients was waived due to the retrospective nature of this study and the fact that no personal identifiers were used in either the analysis or the reporting of study results. All patient data were kept strictly confidential.

Author Contributions

Lisha Qin, Mengyuan Liang, Jianping Song, Ping Chen and Shujing Zhang contributed equally to this work and share first authorship. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

There is no funding to report.

Disclosure

The authors declared no competing interests in this work.

References

1. GBD 2019 LRI Collaborators. Age-sex differences in the global burden of lower respiratory infections and risk factors, 1990-2019: results from the global burden of disease study 2019. *Lancet Infect Dis*. 2022;22:1626–1647. doi:10.1016/S1473-3099(22)00510-2
2. The top 10 causes of death n.d. Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. Accessed April 26, 2024.
3. Jain S, Self WH, Wunderink RG, et al. Community-acquired pneumonia requiring hospitalization among U.S. *Adults N Engl J Med*. 2015;373:415–427. doi:10.1056/NEJMoa1500245
4. Rajapaksha P, Elbourne A, Gangadoo S, Brown R, Cozzolino D, Chapman J. A review of methods for the detection of pathogenic microorganisms. *Analyst*. 2019;144:396–411. doi:10.1039/c8an01488d
5. Tumbarello M, Viale P, Bassetti M, De Rosa FG, Spanu T, Viscoli C. Infections caused by KPC-producing Klebsiella pneumoniae: differences in therapy and mortality in a multicentre study-authors' response. *J Antimicrob Chemother*. 2015;70:2922. doi:10.1093/jac/dkv200
6. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med*. 2006;34:1589–1596. doi:10.1097/01.CCM.0000217961.75225.E9
7. Liang M, Fan Y, Zhang D, et al. Metagenomic next-generation sequencing for accurate diagnosis and management of lower respiratory tract infections. *Int J Infect Dis*. 2022;122:921–929. doi:10.1016/j.ijid.2022.07.060
8. Zheng Y, Qiu X, Wang T, Zhang J. The diagnostic value of metagenomic next–generation sequencing in lower respiratory tract infection. *Front Cell Infect Microbiol*. 2021;11:694756. doi:10.3389/fcimb.2021.694756
9. Charalampous T, Kay GL, Richardson H, et al. Nanopore metagenomics enables rapid clinical diagnosis of bacterial lower respiratory infection. *Nat Biotechnol*. 2019;37:783–792. doi:10.1038/s41587-019-0156-5
10. Li S, Tong J, Liu Y, Shen W, Hu P. Targeted next generation sequencing is comparable with metagenomic next generation sequencing in adults with pneumonia for pathogenic microorganism detection. *J Infect*. 2022;85:e127–9. doi:10.1016/j.jinf.2022.08.022
11. Flurin L, Wolf MJ, Mutchler MM, Daniels ML, Wengenack NL, Patel R. Targeted metagenomic sequencing-based approach applied to 2146 tissue and body fluid samples in routine clinical practice. *Clin Infect Dis*. 2022;75:1800–1808. doi:10.1093/cid/ciac247
12. Hong H-L, Flurin L, Thoendel MJ, et al. Targeted versus shotgun metagenomic sequencing-based detection of microorganisms in sonicate fluid for periprosthetic joint infection diagnosis. *Clin Infect Dis*. 2022;76:e1456–62. doi:10.1093/cid/ciac646

13. Zhang G, Zhang H, Zhang Y, Hu X, Tang M, Gao Q. Targeted next-generation sequencing technology showed great potential in identifying spinal tuberculosis and predicting the drug resistance. *J Infect*. 2023;87:e110–2. doi:10.1016/j.jinf.2023.10.018
14. Li J, Zhang L, Yang X, et al. Diagnostic significance of targeted next-generation sequencing in central nervous system infections in neurosurgery of pediatrics. *Infect Drug Resist*. 2023;16:2227–2236. doi:10.2147/IDR.S404277
15. Lim W, van der Eerden MM, Laing R, et al. Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. *Thorax*. 2003;58:377–382. doi:10.1136/thorax.58.5.377
16. Kalil AC, Metersky ML, Klompas M, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the infectious diseases society of America and the American thoracic society. *Clin Infect Dis*. 2016;63:e61–111. doi:10.1093/cid/ciw353
17. Metlay JP, Waterer GW, Long AC, et al. Diagnosis and treatment of adults with community-acquired pneumonia. an official clinical practice guideline of the American thoracic society and infectious diseases society of America. *Am J Respir Crit Care Med*. 2019;200:e45–67. doi:10.1164/rccm.201908-1581ST
18. Ramirez JA, Musher DM, Evans SE, et al. Treatment of community-acquired pneumonia in immunocompromised adults: a consensus statement regarding initial strategies. *Chest*. 2020;158:1896. doi:10.1016/j.chest.2020.05.598
19. Chinese Thoracic Society. Consensus of clinical pathways of metagenomic next-generation sequencing test in diagnosis of lower respiratory tract infections in China. *Zhonghua jie he he hu xi za zhi*. 2023;46:322–335.
20. Yue R, Wu X, Li T, Chang L, Huang X, Pan L. Early detection of legionella pneumophila and aspergillus by mngs in a critically Ill patient with legionella pneumonia after extracorporeal membrane oxygenation treatment: case report and literature review. *Front Med Lausanne*. 2021;8:686512. doi:10.3389/fmed.2021.686512
21. Gaston DC, Miller HB, Fissel JA, et al. Evaluation of metagenomic and targeted next-generation sequencing workflows for detection of respiratory pathogens from bronchoalveolar lavage fluid specimens. *J Clin Microbiol*. 2022;60:e0052622. doi:10.1128/jcm.00526-22
22. Geng S, Mei Q, Zhu C, et al. Metagenomic next-generation sequencing technology for detection of pathogens in blood of critically ill patients. *Int J Infect Dis*. 2021;103:81–87. doi:10.1016/j.ijid.2020.11.166
23. Peng J-M, Du B, Qin H-Y, Wang Q, Shi Y. Metagenomic next-generation sequencing for the diagnosis of suspected pneumonia in immunocompromised patients. *J Infect*. 2021;82:22–27. doi:10.1016/j.jinf.2021.01.029
24. Legoff J, Zucman N, Lemiale V, et al. Clinical significance of upper airway virus detection in critically ill hematology patients. *Am J Respir Crit Care Med*. 2019;199:518–528. doi:10.1164/rccm.201804-0681OC
25. Xie G, Zhao B, Wang X, et al. Exploring the clinical utility of metagenomic next-generation sequencing in the diagnosis of pulmonary infection. *Infect Dis Ther*. 2021;10:1419–1435. doi:10.1007/s40121-021-00476-w
26. Wu X, Li Y, Zhang M, et al. Etiology of severe community-acquired pneumonia in adults based on metagenomic next-generation sequencing: a prospective multicenter study. *Infect Dis Ther*. 2020;9:1003–1015. doi:10.1007/s40121-020-00353-y
27. Dai Y, Sheng K, Hu L. Diagnostic efficacy of targeted high-throughput sequencing for lower respiratory infection in preterm infants. *Am J Transl Res*. 2022;14:8204–8214.
28. Deng Z, Li C, Wang Y, et al. Targeted next-generation sequencing for pulmonary infection diagnosis in patients unsuitable for bronchoalveolar lavage. *Front Med*. 2023. doi:10.3389/fmed.2023.1321515
29. Hardak E, Avivi I, Berkun L, et al. Polymicrobial pulmonary infection in patients with hematological malignancies: prevalence, co-pathogens, course and outcome. *Infection*. 2016;44:491–497. doi:10.1007/s15010-016-0873-3
30. Pan T, Tan R, Qu H, et al. Next-generation sequencing of the BALF in the diagnosis of community-acquired pneumonia in immunocompromised patients. *J Infect*. 2019;79:61–74. doi:10.1016/j.jinf.2018.11.005
31. Azoulay E, Timsit J-F, Tafflet M, et al. Candida colonization of the respiratory tract and subsequent pseudomonas ventilator-associated pneumonia. *Chest*. 2006;129:110–117. doi:10.1378/chest.129.1.110
32. Green ML, Leisenring W, Xie H, et al. CMV viral load and mortality after hematopoietic cell transplantation: a cohort study in the era of preemptive therapy. *Lancet Haematol*. 2016;3:e119–27. doi:10.1016/S2352-3026(15)00289-6
33. Chen H, Liang Y, Wang R, et al. Metagenomic next-generation sequencing for the diagnosis of pneumocystis jirovecii pneumonia in critically pediatric patients. *Ann Clin Microbiol Antimicrob*. 2023;22:6. doi:10.1186/s12941-023-00555-5
34. Kanj SS, Bassetti M, Kiratisin P, et al. Clinical data from studies involving novel antibiotics to treat multidrug-resistant Gram-negative bacterial infections. *Int J Antimicrob Agents*. 2022;60:106633. doi:10.1016/j.ijantimicag.2022.106633
35. Wu X, Liang R, Xiao Y, et al. Application of targeted next generation sequencing technology in the diagnosis of Mycobacterium Tuberculosis and first line drugs resistance directly from cell-free DNA of bronchoalveolar lavage fluid. *J Infect*. 2023;86:399–401. doi:10.1016/j.jinf.2023.01.031